696 POSTER

A potent enantiomer of gossypol, AT-101: Screening of anti-angiogenic protein targets in glioblastoma multiforme cells

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Background: Glioblastoma multiforme (GBM) is the most common malignant glioma in adults with extremely high morbidity and mortality. Angiogenesis in cancer strongly correlates with the risk of invasion and metastasis. Inhibition of angiogenesis by bevacuzimab shows modest activity in highly-vascular GBMs, but more therapeutic options are needed. AT-101, the (−)- enantiomer of gossypol, is a natural polyphenolic compound extracted from the cotton plant with potent cytotoxic effects on various tumor types. In the present study, we investigated the anti-angiogenic protein targets of AT-101 in GBM cell lines (U-87MG and T98G).
Material and Methods: Real time monitoring of cell proliferation was assessed by xCELLigence system in U-87MG and T98G cells after AT-101 (1−40 μM) exposure. Changes in angiogenesis-related protein expressions were investigated by human angiogenesis antibody array. Changes in protein levels were accepted as significant if there was at least a 1.5-fold change in expression when compared to untreated control.
Results: AT-101 inhibited cell proliferation in a dose and time dependent manner in tested cell lines. The IC_{50} values of AT-101 in U-87MG and T98G cells were 2.4 and 2.7 μM, respectively, at 20 h. The exposure of AT-101 in both cell lines resulted in significant inhibition of expression of angiogenesis-related protein levels which are known to have pivotal roles in invasion, angiogenesis and metastasis.
Conclusions: We found out that AT-101 potently inhibited angiogenesis-related cytokines in GBM cells. AT-101 shows preliminary but promising results for the future treatment strategies for GBM.
No conflict of interest.

697 POSTER

The influence of metformin on the breast cancer phenotype

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Background: Metformin is frequently used in the treatment of diabetes mellitus (DM) type 2. To get insight whether the therapy of diabetes or hyperglycemia with metformin might induce the changes in biology of cancer cells, we determined the prevalence of breast cancer patients with enhanced HER-2, estrogen and progesterone receptor expression on tumor cells and compared it with those found in general breast cancer population.
Patients and Methods: In this study 768 patients in the period November 2011-December 2012 with surgically removed tumors were included. From this group 42 patients had DM type 2 or hyperglycemia and were pretreated (not less than one month) with metformin alone or sometimes in combination with other antidiabetic drugs. In 37 out from these 42 patients malignant tumors were found (three of them were with bilateral tumors), while 5 out from 42 patients were with benign breast disease. It needs to be mentioned that 14 of 37 additionally were treated with some sulfonylurea derivatives, while 3 of 37 used additionally insulin as the antidiabetic therapy. Receptor status was assessed analyzing tumor cells obtained at diagnosis by immunohistochemistry. Estrogen and progesterone receptor expression was scored from 0 to 8; scores 3 and above were considered positive. Intensity of HER-2 expression were graded from 0 to 3+; scores 3+ and 2+ (after confirmation of HER-2/neu amplification by additional analysis, chromogenic in situ hybridization) were considered as positive.
Results: In 30 out from 37 patients' tumor enhanced ER/PR positive protein expression was found (81.1%). Score 3+ of HER-2 receptor expression was not found on examined patients tumor cells, even more, tumors with HER-2 expression 2+ (found in five patients) were without HER-2 amplification. Frequency of patients with ER/PR positive tumors in general breast cancer population in Institute of Oncology and Radiology of Serbia (from data obtained analyzing 1410 patients' tumors in the period November 2011-December 2012) was 85.5%. HER-2 expression score 3+ was found in 16.6%. Obtained data show that metformin used alone, or in combination with other antidiabetic drugs could modify biology of breast cancer regarding downregulation of HER-2 amplification and/or expression.
Conclusions: Results from this work show that metformin might influence changes in the biology of malignant breast cancer cells downregulating HER-2 expression.
No conflict of interest.

698 POSTER

Gene expression profiles of circulating tumor cells (CTCs) in patients with metastatic breast cancer (MBC) treated with aromatase inhibitors (AI)

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Background: Enumeration of CTCs can be used to assess prognosis in MBC and to evaluate treatment response. Besides enumeration, molecular characterization of CTCs is a promising tool to develop a more personalized treatment approach. In this study, we evaluated the association between mRNA expression of 96 measurable genes in CTCs and response to first-line AI in MBC patients with estrogen receptor (ER)+ primary tumors.
Materials and Methods: CTCs were isolated and enumerated from the blood of 25 MBC patients prior to start of first-line therapy with an AI. Fourteen patients received a non-steroidal AI (8 letrozole, 6 anastrozole) and 11 patients were treated with exemestane. mRNA expression levels of 96 genes were measured by quantitative RT-PCR as previously described (Sieuwerts et al. Clin Cancer Res. 17:3600–3618, 2011). Expression levels of these genes were studied for their association with time to progression (TTP) after start first-line AI.
Results: Median TTP was 338 (range 14–1239) days and median baseline CTC count for the 25 patients was 14 (range 0–753). In this relatively small cohort, the clinically relevant cut-off level of $\geqslant$5 CTCs in association with TTP did not reach statistical significance (Hazard Ratio [HR] 4.76, 95% Confidence Interval [CI]: 0.59–38.22, $P = 0.14$). For type of AI, when comparing steroidal with non-steroidal AI, the measures in Cox univariate regression analysis were HR 2.54 (95% CI: 0.67–9.64), $P = 0.17$. A 10-gene CTC predictor was constructed based on the Wald statistics of the contribution of the individual genes in univariate Cox regression analysis of TTP. To identify patients with good and poor outcome, the Wald corrected sum of the 10 genes was used to dichotomize the continuous 10-gene predictor (HR 12.87 [95% CI: 1.60–103.56], $P = 0.016$). In multivariate analysis, corrected for the clinically relevant variables type of AI and CTC count, only the 10-gene CTC predictor was an independent factor associated with TTP (HR 12.46 [95% CI: 1.29–120.08], $P = 0.029$).
Conclusions: A 10-gene CTC expression profile was constructed which distinguishes good and poor outcome to first-line AI in MBC patients. This profile is currently being validated in an independent group of patients.
Conflict of interest: *Corporate-sponsored research: Veridex*

699 POSTER

Nanofluidic digital PCR for improved selection of metastatic colorectal cancer patients to anti-EGFR therapies

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Background: Concomitant detection of mutations in downstream signalling molecules of the EGFR pathway (*KRAS*, *BRAF*, *PIK3CA* and *NRAS*) has been suggested to improve the selection of candidate metastatic colorectal cancer (mCRC) patients that will respond to anti-EGFR therapy. In addition, *EGFR*(S492R) point mutation has been associated with acquired resistance to cetuximab. We assessed the feasibility of a nanofluidic digital PCR array platform to simultaneously detect hotspot mutations with high sensitivity.
Methods: 26 primary tumor FFPE tissues from patients (15M/11F; 3 stages I−II; 23 stages III−IV) with chemotherapy-refractory mCRC treated with cetuximab plus chemotherapy in the pre-*KRAS* selection era (between 1997 and 2006) were included in the mutational analysis. Digital PCR was performed using the Digital Array Chip. Conventional genotyping was

performed using LightCycler 480. A panel of 17 hotspot mutations were assessed: all codon 12, G13D, Q61H and A146T9 *KRAS*, V600E *BRAF*, M1043I, H1047R, H1047L and H1047Y in exon 20 of *PIK3CA,* Q61K and Q61R *NRAS* and S492R *EGFR.*
Results: Analytical sensitivity of digital PCR for mutant alleles was 0.05%-0.1% whereas LightCycler detected 1−5%. Eight of 26 (31%) patients were positive for at least one mutation with the Light Cycler. Digital PCR increased this number to 11/26 (42%) confirming all positives. Digital PCR identified multiple mutant alleles in 5 cases. Digital PCR reclassified as mutant 1 of the 5 cases with progressive disease. The case with a G13D mutation identified showed partial response (Table).
Conclusions: Digital PCR provides a robust and highly sensitive detection of EGFR-pathway hotspot mutations that may result in better classification prior to anti-EGFR treatment.
No conflict of interest.

Patient	Stage	FLUIDIGM panel	LightCycler panel	Response
Digital PCR mut only				
7	IV	MUT (G13D)	wt	PR
16	IV	MUT (G12V/G12D)	wt	NE
21	IV	MUT (G12V)	wt	PD
Digital PCR and LC mut				
1	III-B	MUT (G12D/G12S)	MUT (G12D)	SD
4	I	MUT (G12V/H1047Y)	MUT (H1047Y)	SD
9	III-B	MUT (G12D)	MUT (G12D)	SD
11	IV	MUT (A146T)	MUT (A146T)	PD
12	II-A	MUT (G12D)	MUT (G12D)	SD
20	IV	MUT (G12D)	MUT (G12D)	SD
22	IV	MUT (H1047R/H1047Y)	MUT (H1047R)	SD
25	IV	MUT (G12C/Q61H)	MUT (Q61H)	NE
Panel *wild type*				
2	IV	wt	wt	PD
3	IV	wt	wt	SD
5	IV	wt	wt	SD
6	IV	wt	wt	PD
8	II-B	wt	wt	NE
10	III-B	wt	wt	SD*
13	III-B	wt	wt	PD
14	III-C	wt	wt	CR
15	III-B	wt	wt	PD
17	IV	wt	wt	PR
18	IV	wt	wt	PR
19	IV	wt	wt	PR
23	IV	wt	wt	PR
24	III-B	wt	wt	SD
26	II-A	wt	wt	NE

PR: partial response, CR: complete response, PD: progressive disease, SD: stable disease, NE: not evaluable.
*prolongued SD.

700 POSTER

18F-misonidazole positron-emission tomography (FMISO-PET) as an early biomarker of vascular normalization in response to antiangiogenic therapy

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Background: Vascular normalization (VN) is a mechanism by which antiangiogenic drugs (AD) improve vessel function and oxigenation in tumors, enhancing chemotherapy effect. A non-invasive VN marker would allow clinical decisions in patients receiving AD. We evaluated 18F-misonidazole (FMISO) positron-emission tomography (PET) imaging of tumor hypoxia as an early marker of VN, using the AD dovitinib (DOV) in pancreas tumografts (TG).
Methods: Two pancreas TG with characterized gemcitabine (GEM) response (Panc286-resistant; Panc215-sensitive) were implanted in nude mice. Tumor perfusion, hypoxia and glucose uptake before/after 4-day DOV course were explored as potential VN parameters using perfusion computed tomography (P-CT) (HU in 4 ROIs/mice), FMISO-PET (tumor SUV mean), and fluorodeoxyglucose (FDG)-PET (tumor SUV max). Tumor samples at the same timepoints were collected for VN microscopical analysis. Microvessel density and tumor hypoxia were quantified with CD31-pimonidazole co-staining. Perfusion was assessed measuring tumor extravasation of a fluorescent 10KDa-dextran. Whether DOV improved GEM efficacy was studied comparing tumor growth in DOV+GEM *vs* GEM treated animals. Stats: t-test for pairwise comparisons, ANOVA for tumor growth comparisons. Minimum number of tumors per conditionant parameter: 10. All shown data $p < 0.05$.
Results: Total animals in study: 194. In GEM-resistant TG Panc286, DOV+GEM caused a tumor growth inhibition (TGI) of 35.1% *vs* GEM alone at 59 days of treatment ($p = 0.044$). 4-day-DOV course significantly lowered FMISO uptake in Panc286 tumors (SUV mean DOV 0.60, Vehicle 1.16 $p < 0.001$) and tumor perfusion by P-CT (HU mean DOV 123, vehicle 249; $p < 0.001$). Microscopical changes mirrored image findings, as tissue hypoxia was 10-fold decreased and 10KDa-dextran clearance in tumors was 5-fold increased *vs* controls after 4-day DOV course in preliminary analysis. In GEM-sensitive TG Panc215, DOV+GEM also caused a TGI of 54.1% *vs* GEM alone at 116 days of treatment ($p = 0.005$). However, no changes in FMISO-PET and P-CT were observed after 4-day DOV course, neither changes in tissue hypoxia and vascular perfusion in microscopical analysis.
Conclusion: FMISO-PET can tract hypoxia evolution after a short course of DOV. In tumors in which AD reverse chemoresistance by means of VN, FMISO-PET could be an early marker of AD efficacy. However, as evidenced by Panc215 results, other mechanisms than VN may play a role in AD response.
No conflict of interest.

701 POSTER

Erlotinib in EGFR wild type platinum resistant NSCLC: now a predictor factor

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Background: Erlotinib (Erl) is effective in first-line treatment in patients affected by metastatic NSCLC harboring an EGFR mutation. Erl is also approved in the subsequent lines of therapy regardless of EGFR mutational status, although its use is debated in patients with NSCLC EGFR WT. In a retrospective clinical study, we previously demonstrated that EGFR overexpression is associated with a remarkable effectiveness of Erl in second line therapy after a platinum-based treatment in patients with NSCLC EGFR wt. The aim of this *in vitro* study is to investigate whether the exposure to Cisplatin (CDDP) may result in EGFR overexpression, thus determining the acquisition of sensitivity to Erl.
Materials and Methods: We used two EGFR-WT cell lines (A549 and H460) and two EGFR-MT cell lines (H1650 and HCC4006). For each cell line we assessed the sensitivity to CDDP and to Erl performing a MTT-test and the expression of EGFR, pEGFR, c-MET, IGF1-R using PCR and Western Blot.
Results: Among the EGFR WT cell lines, H460 resulted sensitive to CDDP and resistant to Erl. This cell line was subsequently made resistant to CDDP by a continuous exposure to increasing concentrations of the cytotoxic agent (CR-H460). A new MTT-test was then performed on CR-H460, confirming the acquisition of resistance to CDDP and sensitivity to Erl (OR: H460-CR vs H460 0.44, 95% CI 0.27–0.71, $p < 0.001$). CR-H460 showed an increased EGFR and pEGFR mRNA and protein expression compared to the parental cell line (OR: H460 vs H460-CR, 95% CI 3.87–9.30, $p < 0.001$). This increased EGFR expression in H460-CR correlated with the increase of sensitivity to Erl ($r -0.957$, r^2 0.916, $p = 0.043$).
Conclusions: Our *in vitro* model suggests that Erl efficacy in patients affected by NCSCLC EGFR wild-type and previously treated with chemotherapy may be related to the induction of EGFR expression subsequent to the exposure to platinum derivatives. Therefore, a new evaluation of EGFR and pEGFR expression after first-line platinum-based therapy could be a predictor of response to Erl in clinical practice.
No conflict of interest.

702 POSTER

Endoxifen and fulvestrant regulate gene expression of estrogen receptor alpha and its co-activators DEADbox5 and 17

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Background: Application of anti-estrogens remains a standard approach as endocrine treatment of erstrogen-receptor alpha (ERα) -positive breast cancer. ERα antagonists trigger a competitive blockade of the receptor. Tamoxifen, with its active metabolite endoxifen, acts as a selective receptor down-modulator (SERM) by inhibition of activating function 1 (AF-1) of ERα. Fulvestrant acts as a selective receptor down-regulator (SERD) via an increased ERα degradation created by inhibition of both ERα activating functions (AF-1, AF-2). In some cases, both active agents show an inefficacy by lack of the expected therapeutic effects or therapy-resistance occurs due to gain of ERα-independency accompanying tumor progression. The two RNA helicases p68 (DEADbox5, DDX5) and p72 (DEADbox17; DDX17) act as co-activators of several tumor-associated proteins, such as ERα. Overexpression of both factors could be demonstrated in various malignant tumors. DDX17 expression correlates to decreased Her2/neu levels, extended relapse-free periods, and an increase in overall survival rates. In contrast, DDX5 expression is associated with increased Her2/neu levels and higher tumor grading, but no correlation with relapse-free or overall survival became significant so far. This study aimed for the investigation of potential regulatory effects of endoxifen and fulvestrant on the expression of ERα and its co-activators DDX5 and 17.
Material and Methods: Four ERα-positive and one ERα-negative breast cancer cell line underwent 24 hrs treatment with endoxifen or fulvestrant, respectively, mimicking therapeutic concentrations. In parallel, a negative control, treated with solvent DMSO only, was included in analysis. mRNA and protein levels of ERα, DDX5 and DDX17 were analyzed by RT-PCR and Western blot.
Results: Both ERα antagonists created a significant decrease of mRNA and protein expression levels of all target genes. DDX5 and 17 expression levels generally decreased, whereat endoxifen treatment triggered a stronger effect than fulvestrant. While both ERα antagonists caused a uniform decrease in ERα protein levels, DDX protein levels were differentially affected. Fulvestrant triggered a uniform downregulation of DDX5 and 17. In contrast, endoxifen stimulation resulted in an up-regulation of DDX5 and 17 protein levels in some ERα-positive cell lines.
Conclusion: Both ERα antagonists show regulatory effects on ERα, DDX5 and 17 mRNA and protein expression. However, differing effects could be observed on protein levels in different cell lines. The obtained *in vitro* data might explain individual therapeutic efficacy or the occurrence of resistance against endocrine therapy dependent on cellular context. Furthermore, the elucidation of DDX status might serve as a useful prognostic tool to estimate efficacy of anti-estrogen treatment in breast cancer therapy.
No conflict of interest.

703 POSTER

Monitoring cancer treatment responses using cancer-testis antigen microarrays

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Background: There is increasing evidence that the aberrant expression of cancer-testis (CT) antigens − a family of approximately 150 proteins that are both (auto)immunogenic and overexpressed in tumours in various types of human cancers − makes them potentially attractive immunotherapy targets, as well as possible cancer diagnostic markers. More specifically, since none of the CT antigens appear to be cell surface antigens, they are currently considered potential cancer vaccine targets rather than targets for antibody-based therapy. The underlying hypothesis of our study is that there are measurable differences in autoantibody repertoires between pre- and post-vaccinated cancer patient samples, potentially augmented by prior chemo- or radiotherapy, which will correlate with likelihood of response of individual patients to a given therapeutic treatment.
Material and Methods: A novel protein microarray platform containing 123 cancer antigens of interest was developed, optimised and tested, with the intent of allowing the quantification of broad cancer-related autoantibody profiles of cancer patient serum samples collected pre- and post-vaccination, chemotherapy or radiotherapy, and to correlate that data with patient responder phenotypes (Ethical consent: LICR HREC number 2003/01660, UCT HREC number 240/2011). An efficient bioinformatic pipeline of data extraction, filtering, graphing and analysis was also developed, as a means to facilitate and automate processing and analysis of the large volumes of generated microarray data.
Results: Using our CT antigen microarray platform, we have developed a robust, sensitive, high-throughput and highly multiplexed means to assay patient autoimmune responses to an experimental treatment. Our data suggests a limit of detection of 10–100pg/ml − which is competitive with Luminex assays − as well as linearity over 3 orders of magnitude, which is strongly encouraging for future quantitative analyses. Using this platform in preliminary assays on a cohort of melanoma patient sera, we have observed robust, reproducible and relevant patient autoimmune profiles.
Conclusions: A glimpse of the clinical utility of our new array tool is evident, with possible applications in monitoring therapeutic responses to an experimental treatment. However, studies involving larger patient cohorts are necessary to explore these preliminary results in more depth.
No conflict of interest.

704 POSTER

Predictive efficacy of low burden EGFR mutation detected by next-generation sequencing on response to EGFR TKIs in non-small-cell lung carcinoma

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Background: Direct sequencing is the standard method for the detection of epidermal growth factor receptor (EGFR) mutations in lung cancer; however, its relatively low sensitivity limits its clinical use. Therefore, this study was conducted to investigate sensitivity of PNA-LNA PCR clamp and Ion Torrent PGM compared to direct sequencing and predictive value of those sequencing techniques for EGFR-TKI efficacy.
Material and Methods: EGFR mutational status were assessed by direct sequencing, PNA-LNA PCR clamp and Ion Torrent PGM in 57 NSCLC patients who undergone lung resection. We evaluated predictive efficacy of PNA-LNA PCR clamp on the EGFR-TKI treatment in 36 patients with advanced NSCLC retrospectively.
Results: Compared to direct sequencing (16/57, 28.1%), PNA LNA PCR clamp (27/57, 47.4%) and Ion Torrent PGM (26/57, 45.6%) detected more EGFR mutations. Among the EGFR mutant patients from PNA-LNA PCR clamp, EGFR mutant patients had significantly longer PFS (14.31 vs. 21.61 months, P = 0.003) than EGFR wild patients. However, there was no difference in response rate (75.0% vs. 82.4%, P = 0.195), overall survival (34.39 vs. 44.10 months, P = 0.422) between EGFR mutant by direct sequencing and PNA-LNA PCR clamp.
Conclusions: Our results demonstrate firstly that patients with EGFR mutations were detected more sensitively by PNA-LNA PCR clamp and Ion Torrent PGM than direct sequencing. EGFR mutations detected by PNA-LNA PCR clamp may be as a predicative factor for EGFR TKI response in NSCLC patients.
No conflict of interest.

705 POSTER

Angiogenic marker associated with resistance to neoadjuvant chemoradiotherapy in rectal cancer

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Purposes: The ability to achieve pathologic down staging after neoadjuvant chemoradiotherapy (CRT) is correlated with improved survival. However, there is no effective method of predicting which patients will response to neoadjuvant CRT. Neoadjuvant CRT can change the expression of angiogenic factors. However, little is known about its possible changes in response to preoperative CRT. We examined the expression of angiogenic factors in rectal cancer tissues before preoperative CRT and after surgery.
Materials and Method: Fifty five patients with locally advanced rectal cancer were studied. All patients were given preoperative CRT of 5040 cGy for 5−6 weeks with concurrent administration of 5-fluorouracil and leucovorin. Surgical resection was performed 6−8 weeks later in all patients. Immunohistochemical staining for angiogenenic markers (vascular endothelial growth factor [VEGF], placenta growth factor [PLGF], hypoxia inducible factor 1α [HIF 1α], stromal cell derived factor [SDF 1α]) were performed on specimens obtained before preoperative CRT and after surgery. A semiquantitative-immunohistochemical score established from the extension and intensity of the angiogenic factors was used for analysis.
Results: The positive expression rate of VEGF, PLGF, SDF 1α, and HIF 1α was 56.4% (31/55), 65.5% (36/55), 70.9% (39/55), and 47.3% (26/55), respectively. The expression rate of VEGF, PLGF, SDF 1α, and HIF 1α was increased by 3.6% (2/55), 7.3% (4/55), 30.9% (17/55), and 1.8% (1/55)

after neoadjuvant CRT, respectively. Expression of VEGF, PLGF, and HIF 1α protein was downregulated after neoadjuvant CRT in the rectal cancer tissues (P <0.001, P = 0.001, P = 0.044, respectively). However, SDF 1α was upregulated after neoadjuvant CRT (P <0.001). And also, upregulated expression of SDF 1α after neoadjuvant CRT was significantly associated with resistance to CRT (P = 0.035). However, SDF 1α showed no correlation with other clinical factors (age, sex, clinical stage).
Conclusion: Expression of SDF-1α was increased in the rectal cancer tissue after neoadjuvant CRT, as well as has been associated with CRT resistance. Our data suggests that SDF 1α should be evaluated as new target for antiangiogenic therapy.
No conflict of interest.

706 POSTER

Differential molecular analysis of non small cell lung cancer by laser capture microdissection of formalin-fixed, paraffin-embedded tissue

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Background: The introduction of molecular analysis and targeted therapy significantly improved the therapy of lung cancer. In the targeted treatment of lung cancer, monoclonal antibodies such as cetuximab and small molecules such as iressa have shown to be effective subtypes of lung cancer harboring certain molecular characteristics. The predictive biomarkers for the effectiveness of these therapies are mutations in the corresponding targets or in pathway related molecules. In order to accurately determine the molecular signatures of a histological heterogeneous cancer such as lung cancer, microdissection of tumor tissue compartments is mandatory to reveal tumor specific alterations. However, the recovery of good quality RNA from FFPE sections can be challenging due to fixation processes.
Material and Methods: The protocol for Laser Capture Microdissection was implemented on an Axiovert 200/PALM R MICROBEAM IV instrument. Slides were deparaffinized and stained with cresyl violet to enable a betterevaluation of cell morphology. Tumor areas of a homogenous cell type as well as stromal areas were microdissected. RNA as well as DNA was isolated from these different compartments using the Qiagen Allprep Kit and quality control was performed using the Agilent 2100 bioanalyzer. Isolated DNA was used for mutation analysis of hot spot regions of three clinical relevant genes: KRAS, BRAF, and EGFR.
Results: In this study, we have successfully isolated tumor and stromal compartments from five formalin fixed and paraffin embedded NSCLC biospecimen. Total RNA was extracted with overall yields ranging from 1.0 to 279 ng. Extracted RNA was suitable for subsequent RT-PCR. Furthermore, hot spots regions of three genes KRAS, BRAF, and EGFR have been successfully sequenced even with very small amounts of extracted DNA of less than 1 ng. Therein, an EGFR mutation was detected in case 2 and a KRAS mutation in case 3. Mutations were only found in microdissected tumor samples and not in stromal samples, confirming the accuracy of microdissection.
Conclusion: Human tissues, in particular, tumor tissues, are complex structures composed of heterogeneous mixtures of morphologically and functionally distinct cell types. The described workflow is suitable for preparing RNA and DNA from very small amounts of microdissected FFPE tissue samples of <2 mm^2 in sufficient quality and quantity for further applications such as mutation analyses and RT-PCR for gene expression analysis.
No conflict of interest.

707 POSTER

HPV16 detection in HNSCC and correlation with p16 expression and overall survival

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Background: We sought to determine the presence of Human papillomavirus type 16 (HPV16) in tumor samples from patients (pts) with locally advanced HNSCC through E1, E6 and L1 viral fragments detection.
Our aim was to establish their prognostic role in oropharynx tumors (OT) and in non-OT, then to correlate positive (pos) or negative (neg) samples for each fragment with p16 expression.
Materials and Methods: We analyzed 206 samples (OT/non-OT: 66/140) from pts treated with CRT between 1997 and 2011 (175M/31F; median age 59.6, range 20.6–85.6).
E1, E6 and L1 fragments were detected by PCR on DNA extracted from PFFE tissues using specific primer pairs; DNA of pos and neg control cell lines was added at each session. Amplicons were visualized on 2% agarose gel. p16 expression was analysed by IHC.
Results: Although we found a different % of pos samples for each fragment studied, OT showed, overall, a significantly higher % of pos samples vs non-OT: E1 pos was 19.7% in OT and 4.3% in non-OT (p < 0.001), while E6 pos was 68.2% in OT and 50.7% in non-OT (p = 0.02) and L1 was pos 45.5% in OT and 20.7% in non-OT (P < 0.001).
When PCR positivity was correlated to OS, we observed a significant correlation in the OT population with E1 (p = 0.016; median OS = 161.8 in pos *vs* 15 months in neg).
Neither E1 in non-OT (p = 0.145) nor E6 nor L1 in OT and non-OT (p = 0.189 in OT and p = 0.242 in non-OT for E6; p = 0.426 in OT and p = 0.97 in non-OT for L1) reached any difference in overall survival (OS).
p16 pos was 68% in OT and 50% in non-OT (p < 0.007). We have previously demonstrated that p16 high positivity (>50%) confers a survival advantage in patients with OT, while in the non-OT the same pos values correlate with a non significant negative prognostic effect.
A significant correlation between E1 pos samples and p16 high expression was found in OT (p < 0.001). This correlation was not seen in non-OT with E1 neither with E6 nor L1 in the whole population. We identified 3 OT pts E1 pos but p16 neg and 35 pts E1 neg but p16 pos. Analysis of OS suggested E1 pos to be a stronger prognostic marker in OT then p16 pos (p = 0.005).
Conclusions:
1. E1 positivity by PCR may be of clinical relevance in OT. Discrepancies seen with E6 and L1 should be further investigated considering the biological cycle of HPV16.
2. E1 positivity has an even stronger effect as p16 high pos (>50%) in OT. Moreover, where both determinations were not consistent, E1 positivity seems to correlate with OS better than p16.

No conflict of interest.

708 POSTER

Wiskott-Aldrich Syndrome protein (WASP) and WASP interacting protein (WIP) are tumor suppressor in ALK-mediated lymphomagenesis

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Background: Anaplastic Large Cell Lymphoma (ALCL) is a T-cell lymphoma that frequently carries the t(2;5) translocation that fuses the ALK gene to Nucleophosmin (NPM1) gene. NPM-ALK transforms lymphocytes by inducing different signaling pathways that control cell proliferation and survival.
Material and Methods: We performed gene expression profiling (GEP) analysis on different lymphomas samples and immunohistochemistry with WASP and WIP antibodies on human samples of ALK+ALCL. To investigate signaling, we infected ALK+ ALCL cell lines (TS and SU-DHL1) with a specific shRNA against ALK and against Stat3 or CEBP/β. To measure WIP and WASP mRNA and protein levels, we used qRT-PCR and western blot analysis, respectively. Finally, we crossed mice deficient for WASP or WIP gene ($WASP^{-/-}$ or $WIP^{-/-}$) or conditionally deficient for Cdc42 (Cdc42f/f) with NPM-ALK Tg mice to check for lymphoma development and overall survival.
Results: ALK+ ALCL had significantly lower expression of WIP and WASP proteins than normal T cells or other T cell lymphomas, as determined by GEP, immunohistochemistry and WB on cell lines and primary tumor samples. In ALK+ ALCL cell lines, we demonstrated that ALK inhibition resulted in up-regulation of WIP and WASP, thus indicating that ALK directly repressed WIP and WASP expression. Such regulation was dependent on a Stat3 and CEBP/β-mediated transcriptional repression.
In mouse models, we showed that WASP protein levels were strongly reduced in lymphomas from NPM-ALK Tg/$WIP^{-/-}$ mice. Remarkably, WIP and WASP worked as tumor suppressors, as either $WASP^{-/-}$ or $WIP^{-/-}$ backgrounds significantly accelerated NPM-ALK lymphomagenesis. Haploinsufficiency of Cdc42 in NPM-ALK Tg/ $WASP^{-/-}$/$Cdc42^{f/-}$ mice restored normal lymphoma incidence, thus suggesting that WASP deficiency accelerated lymphomagenesis by deregulating downstream Cdc42 activity.
Conclusions: In the present study we demonstrated that the expression levels of WASP and WIP are down-regulated by oncogenic ALK in lymphoma. Remarkably, reduced levels of WASP and WIP have key roles in lymphomagenesis as they accelerate NPM-ALK lymphoma progression in *in vivo* mouse models. Thus, for the first time we demonstrate WASP and WIP as tumor suppressors in lymphoma. Our data implicate that lymphoma arising in Wiskott-Aldrich syndrome (WAS) patients, where WASP function

is impaired, could be better explained by an intrinsic tumor suppressor function of WASP rather than a general immunosuppression of the patients.
No conflict of interest.

709 POSTER

Bevacizumab exposure is accompanied by EGFR activation in colorectal cancer (CRC) models providing a rationale for combinations of bevacizumab and erlotinib in the GERCOR DREAM phase III trial

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Background: Combinations of EGFR and VEGF(R)-targeted agents have consistently shown at least additive activity in preclinical CRC models when the targeted agents were administered alone (*Larsen et al., Pharmacol Therap 131:80, 2011; Poindessous et al., Clin Cancer Res 17:6522, 2011*) paving the way for the GERCOR DREAM-OPTIMOX3 phase III trial, an optimized chemotherapy + bevacizumab strategy ± erlotinib in metastatic CRC patients (*Tournigand et al., ASCO 2012, A 3500*). Currently, the mechanistic basis for the additive activities of the two types of targeted agents is not well understood. Although use of EGFR-directed mAbs are counter-indicated in CRC patients with mutant *KRAS*, the situation is less clear for EGFR-targeted TKIs like erlotinib.
Material and Methods: Three human CRC xenograft models expressing wt *KRAS*/*BRAF*, mutant *KRAS* or mutant *BRAF* were established in nude mice. Animals were treated with bevacizumab and erlotinib, alone or in combination, and the influence on tumor growth, viability and the presence of phosphorylated ErbB/HER family members was determined. Treatment-related toxicity was estimated by weight loss.
Results: Combinations of bevacizumab and erlotinib were significantly more active than either agent alone for all three xenograft models although the advantage of combining the two agents was particularly striking for the *KRAS*/*BRAF* wt xenograft model. Unexpectedly, erlotinib alone showed strong antitumor activity in the *BRAF* mutant HT-29 xenograft model. The bevacizumab plus erlotinib combination was less toxic, as determined by weight loss, compared to erlotinib alone. Interestingly, IHC analysis showed that bevacizumab activates EGFR in all three xenograft models which is attenuated in the presence of erlotinib. Erlotinib also attenuates the active phosphorylated form of HER3/ErbB3, in particular when combined with bevacizumab.
Conclusions: We here report that bevacizumab and erlotinib combinations are significantly more active than either agent alone in CRC models with different *KRAS* and *BRAF* status. We further demonstrate that bevacizumab activates EGFR signaling similar to what has been described for irinotecan and ionizing radiation. Although bevacizumab selectively recognizes human VEGF, this is unlikely to influence our findings, since murine VEGF is believed to play a relatively minor role in CRC xenograft models. Taken together, our findings suggest that mutant *KRAS* and *BRAF* have lesser influence on the sensitivity to EGFR-targeted TKIs than is the case for the anti-EGFR mAbs and provide a mechanistic basis for the increased activity of the bevacizumab and erlotinib combination.
Conflict of interest: *Advisory board: AdG. Corporate-sponsored research: AKL, CT, TA, AdG. Other substantive relationships: AS*

710 POSTER

DOK7 expression in colorectal cancer cells and association with patient survival

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Background: The downstream of tyrosine kinase (DOK) protein family is presently known to have seven members, so called DOK1−7. The precise role of the DOK proteins is not entirely clear; some authors have suggested a potential tumour suppressor role for these proteins whilst others have shown an association between expression of these proteins and cell migration. Our study aimed to determine the expression profile of DOK7 in human colorectal cancer cell lines and its association with clinical and prognostic outcome.
Material and Methods: Three human colorectal cancer cell lines (HRT18, HT115 and RKO) were analysed using polymerase chain reaction (PCR) to determine DOK7 expression. Primary colorectal cancer tissue collected at operation from 94 patients was examined by a consultant pathologist. Anti-DOK7 transgenes and expression constructs for human DOK7 were prepared and used for transfection and creation of sublines with differential expression of DOK7. Frozen sections of each tissue sample were used to extract RNA and this was used to generate cDNA which was analysed using quantitative transcript analysis to determine DOK7 expression. Patients were routinely followed up clinically and radiologically after surgery and the median follow up period was 65 months. The expression profile was then analysed against the clinical, pathological and outcome data.
Results: DOK7 transcript expression was highly positive in HRT18 cells. HT115 and RKO cells on the other hand were negative for DOK7 expression. Knockdown in HRT18 cells (HRT18$^{\Delta DOK7}$) resulted in reduced expression of DOK7. The reduction of DOK7 in the cells resulted in a reduced rate of growth compared to wild-type cells and those transfected with control vector. Analysis of clinical data revealed that DOK7 expression was significantly negatively correlated with grade of tumour differentiation, TNM stage and Dukes stage. Furthermore, DOK7 expression was inversely correlated with patient survival ($p = 0.011$).
Conclusions: DOK7 expression was higher in non-aggressive tumour cells (HRT18) compared with aggressive tumour cells (HT115). However, DOK7 expression was also found to be negatively associated with patient survival suggesting a diverse role for this protein in malignant tumours. Further work is necessary to further elucidate the effect of DOK7 expression on cell function and cell migration response to mitogens and motogens.
No conflict of interest.

711 POSTER

In vitro studies on irradiation and Akt inhibition in human malignant glioma

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Background: Glioblastoma multiforme (GBM) is the most common, invasive and deadly primary type of malignant brain tumor. The Phosphatidylinositol-3-Kinase-Akt pathway is commonly overexpressed in GBM and has been associated with resistance to therapy. The aim of the study was to investigate the cytotoxic and radiosensitizing effects of the Akt inhibitor MK-2206 on human malignant glioma cells and spheroids in vitro.
Materials and Methods: Experiments were performed on a panel of five GBM cell lines (U251, T98, D384, U87, VU122). Cells were treated with the allosteric Akt inhibitor MK-2206 alone and in combination with irradiation (0−8 Gy). Endpoints: cell survival (clonogenic assay), cell invasion (transwell Boyden chamber technique) and expression of the proteins PTEN, Akt and pAkt (Western blot). U87 multicellular spheroids were analysed in a growth − volume − assay following the combination treatment of MK-2206 (1microM), fractionated irradiation (5 x 2 Gy) and repeated administration of temozolomide (TMZ; 5 x 5microM).
Results: MK-2206 reduced the expression of the phospho-Akt key protein of the PI3Kinase-Akt pathway. The drug was cytotoxic for all glioma cells in the dose range between 1 and 10mM for 24 hours, but no radiosensitizing effect was found on clonogenic cell survival. The invasion capacity was assessed at doses between 1 and 10 microM MK-2206 for 16 hours. A dose-dependent inhibition of invasion was observed for all but one of the cell lines. Irradiation (4 Gy) alone increased the expression of pAkt, which was inhibited (30min, 1 h, 2 h and 4 h) following pre-incubation with MK-2206 (1microM and 10microM for 1 h). When the drug was administered additional to irradiation, a further inhibition of cell migration and invasion was observed, which was not found after irradiation alone. The radio-enhancing effect of MK-2206 was most pronounced in inhibition of the growth of glioma spheroids in the fractionated irradiation regimen.
Conclusion: Targeting of the PI3K-Akt pathway enhanced the effect of radiation and TMZ in a series of *in vitro* assays, in particular regarding cell invasion and migration, and on spheroid growth. Taken together, Akt pathway inhibition yields promising perspective in the therapy of GBM patients.
No conflict of interest.

712 POSTER

Smads expression is changed following receptor-like protein tyrosine phosphatase kappa (PTPRK) knockdown in prostate cancer cell

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Background: Smad-dependent pathway is part of TGF-β pathway which regulates different cell function such as proliferation, adhesion and migration. Recently, it has been reported that PTPRK is up-regulated by TGF-β and is probably involved in TGF-β dependent anti-proliferation and migration effects in keratinocyte. Furthermore, PTPRK has been indicated as a potential tumour suppressor in primary central nervous system lymphomas. Our recent works have shown certain implications of PTPRK in both breast cancer and prostate cancer. However, the role played by PTPRK in the Smad dependent signalling and the influence on epithelial mesenchymal transition (EMT) of prostate cancer cells remains largely unknown. Present study aimed to study the effect of PTPRK knockdown on Smad signalling and EMT of prostate cancer cells.
Material and Methods: Ribozyme transgenes were constructed to knock-down PTPRK expression in PC3 cells, following verification of the knockdown was carried out using RT-PCR, real time q-PCR and Western blot. The expression of Smads and relevant EMT markers have been assessed using both PCR and Western blots.
Results: Knockdown of PTPRK resulted in alterations of SMADs expression. SMAD1 and SMAD3 expression were significant decreased at both mRNA and protein levels following PTPRK knockdown; nevertheless, SMAD4 expression was increased at both mRNA and protein levels. Other Smads expression was not affected by the PTPRK knockdown. Furthermore, expression of Snail and Slug were reduced in PTPRK knockdown cells; however, there were no effects on other EMT markers including uPA and Vimentin.
Conclusions: Knockdown of PTPRK can reciprocally regulate the expression of certain Smads in prostate cancer cells and may be involved in the EMT triggered by Smad signaling. However, the underlying mechanism is yet to be investigated.
No conflict of interest.

713 POSTER

Association between c-Met and lymphangiogenic factors in patients with colorectal cancer

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Background: Lymphangiogenesis plays an important role in cancer metastasis. Although animal models show a strong relationship between lymphangiogenesis and lymph node metastasis and survival, the clinical significance of lymphangiogenesis in colorectal cancer (CRC) remains uncertain. The goal of this study was to evaluate the association between c-Met and lymphangiogenic factors and to elucidate their prognostic significance for patients with CRC.
Methods: A total of 379 tissue samples were obtained from surgically resected specimens from patients with CRC in Soonchunhyang University Cheonan Hospital between January 2002 and December 2010. The expressions of c-Met, vascular endothelial growth factor (VEGF)-C, VEGF-D, VEGF receptor (VEGFR)-3, and podoplanin were examined by immunohistochemistry. The expression of each marker and clinical factors were analyzed.
Results: Three hundred and one of 379 (79.4%) tissues had c-Met expression. High expression of c-Met in tumor cells was significantly associated with high expression of VEGF-C ($P < 0.01$) and VEGFR-3 ($P = 0.01$). But, there was no statistically significant association with podoplanin (P =. 587) and VEGF-D ($P = 0.96$). Of the 103 evaluable patients, expression of c-Met in tumor cells was significantly associated with advanced clinical stage ($P = 0.20$), positive lymph node status ($P = 0.38$), and high expression of VEGF-C ($P = 0.20$). But, there was no statistically significant association with podoplanin (P =. 518), VEGFR-3 ($P = 0.85$), VEGF-D (P =. 203), and overall survival (P =. 360).
Conclusion: Our results provide indirect evidence for an association and possible regulatory link of c-Met with the lymphangiogenic factors. But, c-Met expression in patients with CRC are not prognostic indicator for overall survival in this retrospective study.
No conflict of interest.

714 POSTER

Improving cell based models through viral vector technology − chances for target research and screening approaches

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Overexpression (Oex) and knockdown (KD) studies are indispensable tools for basic and clinical research in functional genomics and target research. Here, we demonstrate a systematic method for generation of cell models with near perfect inducible KD rates.
Commercially available and in-house platforms for inducible RNAi were compared and a screening platform for highly active shRNAs developed. The screening platform was adapted to both, RNA Polymerase III- and RNA Polymerase II-dependent shRNA expression. Our fine-tuned combination of shRNA validation and viral vector design enable us to translate high knockdown rates (near 100%) into stable cell lines as well as primary cells, even in inducible systems.
These novel cell systems are likely to leverage cell-based models for target research and screening applications. As a case study we present the potent, inducible knockdown of a G-protein coupled receptor in HEK293 cells.
No conflict of interest.

715 POSTER

MAPK and PI3K activation in esophageal carcinomas

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Background: MAPK (Mitogen-Activated Protein Kinase) and PI3K (Phosphatidyloinositol 3- kinase) pathways play significant role in cell survival and have been implicated in various types of cancer including esophageal carcinomas (EC). The aim of our study was to investigate the possible prognostic significance of MAPK and PI3K pathways in EC in the Greek population.
Material and Methods: Forty four samples from patients with EC were screened for the presence of activating mutations at exons 18, 19, 20, 21 of EGFR gene, codons 12 and 13 of K-RAS gene, exon 15 of B-RAF gene, exons 9 and 20 of PIK3CA gene as well as exon 4 of AKT1 gene by High Resolution Melting Analysis and Pyrosequencing. In 29 cases immunohistochemistry was performed in order to evaluate expression levels of pERK (Extracellular − signal Regulated Kinase) and pAKT.
Results: The analysis of genomic DNA from 44 esophageal samples revealed no mutation in the examined genes except of a somatic K-RAS mutation at codon 12, which was detected in one laser microdissected squamous cell carcinoma. Elevated nuclear as well as cytoplasmic pERK (100% and 62% of cases) and pAKT (90.5% and 52% of cases) expressions were observed. Increasing pERK nuclear and cytoplasmic expression along with the intensity of nuclear staining was found to be significantly correlated with tumor grade in univariate and multivariate statistical analysis. In adenocarcinomas subgroup pAKT cytoplasmic expression was negatively correlated with stage.
Conclusions: Our current study demonstrates the presence of activated ERK and AKT despite the absence of upstream alterations (except one K-RAS mutation). ERK activation is a rather late event, contributing to the acquisition of a more aggressive phenotype in esophageal cancer while AKT activation appears more crucial during early stages in esophageal adenocarcinomas.
No conflict of interest.

716 POSTER

Tumor infiltrating B cells in primary cutaneous T-cell lymphomas correlate with disease progression and might represent a potential target for therapy

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B cells have been recently described to mediate tumor biology but so far their role as tumor promoting or tumor repressing lymphocyte population remains controversial. Mycosis fungoides (MF) and other primary cutaneous T cell lymphomas (CTCL) are characterized by an indolent course in early stages. However, advanced stage MF (⩾ EORTC Stage IIB) and the follicular MF subtype (FMF) as well as Sézary syndrome

(SS) show a more aggressive pattern with a median survival of less than two years. The pathogenesis of these more aggressive courses is still incompletely understood. Anecdotal reports have previously described CD20 positive cells in CTCL but further characterization of these cells have not been performed. We systematically analyzed the B cell infiltrate in paraffin samples of CTCL patients by immunohistochemistry (CD20 and CD79a) and correlated these data with the stage, subtype and clinical course. Advanced stage MF, FMF and SS samples contained significantly increased numbers of infiltrating B cells per lymphoma infiltrate. Moreover, time to progression showed a significant inverse relationship with the density of the B cell infiltrate. Based on our results, we hypothesized that infiltrating B cells might be a therapeutic target. In a 77-year old patient suffering from advanced stage FMF with a significant B-cell infiltration and progression after standard treatments, intralesional B-cell depletion with the anti-CD20 monoclonal antibody rituximab resulted in a sustained local tumor regression. In summary, we present first evidence on the potential tumor promoting role of infiltrating B cells in CTCL which warrants further study as a potential therapeutic strategy.
No conflict of interest.

717 POSTER

Spontaneous canine mast cell tumour as a model to study the correlations between infiltrating c-Kit positive cells and angiogenesis: possible translation for human cancer

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Background: Canine cutaneous mast cell tumour (CMCT) is a c-Kit driven tumour that share similar c-Kit mutations found in human Gastro-Intestinal Stromal Tumour (GIST) and other human malignancies. CMCT is a common cutaneous tumour in dog, with an higher incidence than in human. It is classified in three subgroups, well and intermediately differentiated (G1 and G2), corresponding to a benign disease, and poorly differentiated (G3), corresponding to a malignant disease which metastasize to lymph nodes, liver, spleen and bone marrow.
Materials and Methods: In this study, we have evaluated c-Kit expression status, microvascular density (MVD), mast cell granulated and degranulated status density (MCGD and MCDD) and in a series of 97 CMCTs and we have correlated these parameters each to other, by means of histochemistry, immunohistochemistry double staining and image analysis system.
Results: Date show that diffuse cytoplasmic and focal paranuclear (Golgi-like) immunostaining c-Kit expression correlates with high MVD, G3 histopathological grade and MCDD. On the other hand, cell membrane c-Kit expression status correlates with low MVD, G1-G2 histopathological grade and MCGD.
Conclusion: We suggest that these findings may play a role as highlight the key role of c-Kit in the biopathology of canine MCTs indicating a link between aberrant c-Kit expression, increased angiogenesis and higher histopathololgical grade. Finally CMCT seems to be a useful model to study the role of c-Kit activated MCs in tumour angiogenesis and inhibition of MCs degranulation or activation by mean of novel c-Kit tyrosine kinase inhibitors might be a useful anti-tumour and anti-angiogenic strategy worthy to further investigations.
No conflict of interest.

718 POSTER

Continuous low-dose topotecan treatment selectively induces premature senescence in MYCN-amplified neuroblastoma cells in vitro and in vivo

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Recurrent disease is a major challenge in high-risk neuroblastoma (NB), demanding new strategies for consolidation treatment. Previous reports have indicated that DNA-damage inducing drugs trigger a cellular program called premature senescence, a state of proliferative arrest, assumed to limit tumor growth *in vitro* and *in vivo*. We have shown that continuous, low-dose treatment with the chemotherapeutic drug hydroxyurea (HU) leads to senescence in primary NB-cell lines *in vitro*. In this study, we have explored, whether senescence can be induced by other low-dose chemotherapeutic drugs in primary *MYCN*-amplified NB-cell lines and in a xenograft mouse model and whether this will limit tumor growth and aggressiveness.
We found that camptothecin (CPT), a topoisomerase I-inhibitor, triggered apoptosis and senescence within 2−3 weeks when added to cultured primary NB-cells at a low concentration of 3−5 nM. CPT-treated NB-cells were G1/0-arrested and stained positive for the senescence-associated-beta-galactosidase. Importantly, HU- and CPT-treated senescent cells secreted less angiogenesis-, metastasis- and inflammation-associated factors, such as VEGF, MMP-9 and MCP-3, compared to the positive control, BrdU-treated NB-cells. However, HU- and CPT-treated cells expressed the favorable CD44, MHCI and activating NK/NKT-cell receptor ligands, which are absent on non-treated cells. For confirmation *in vivo*, topotecan (TPT), a CPT derivative, was injected i.p. at a clinically low dose of 0.1 mg/kg/d over 2 weeks daily in xeno-transplanted nude mice. Preliminary data suggest a higher frequency of senescent tumor cells, a reduction of tumor size and vascularization in TPT-treated mice. Furthermore, analysis of the gene expression profile of 3 CTRL and 3 TPT-treated tumors, revealed up-regulation of p21, CD44 − both up-regulated in senescent NB-cells *in vitro* - and ATRX, which has been associated with favorable outcome in NB patients. These *in vitro* and *in vivo* studies shall enable future clinical application of tumor cell senescence as therapeutic strategy.
No conflict of interest.

Proffered Papers Session (Mon, 30 Sep)
Drug Development

800 ORAL

A phase 1 study of S-222611, an oral reversible dual tyrosine kinase inhibitor of EGFR and HER2, in patients with solid tumours

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Background: S-222611 is a novel, oral, reversible tyrosine kinase inhibitor of EGFR, HER2 and HER4 with potent and long lasting *in vitro* and *in vivo* pre-clinical activity. A dose-escalation study in patients (pts) has been completed and an expansion phase is on-going.
Material and Methods: Doses from 100 to 1600 mg daily were tested in the dose escalation in previously treated pts with solid tumours expressing EGFR or HER2 with expansion in selected tumours at 800 mg. Pharmacokinetics (PK) and pharmacodynamic assessment of serial tumour biopsies, were included. Responses (RECIST) were assessed by imaging at 8-week intervals.
Results: A total of 33 patients (24 male; aged 25−80 y) were included in the dose-escalation. S-222611 was generally well tolerated with two dose-limiting toxicities (rash at 1200 mg; diarrhoea at 1600 mg). A maximum tolerated dose was not defined. To date 17 patients (6 male; aged 32−75 y) have been included in the expansion phase. Diarrhoea was the most frequent toxicity in the 50 pts, but was rarely worse than grade 1/2. Nausea, rash, anorexia, vomiting and fatigue were also seen. Bilirubin rises with normal transaminases were observed.
Plasma concentrations increased with dose up to 800 mg, which was selected for the expansion. Steady state values of C_{max} and AUC_{0-24} at this dose are in the effective range of concentrations in mouse models. Average $t_{1/2}$ of 33 h is consistent with once daily dosing.
Tumour responses were seen over the full dose range tested (100–1600 mg). Of the 50 treated pts, there were 10 tumour responses, of which one was a clinical complete response (a pt with HER2 positive breast carcinoma previously treated with trastuzumab and lapatinib), 3 were partial responses (PRs) confirmed on repeat scans 2 months later, and 6 were unconfirmed PRs. The confirmed PRs were in HER2 positive breast and EGFR positive renal and oesophageal tumours and the 6 unconfirmed PRs were in breast and oesophageal tumours. An additional 3 pts with vaginal, pancreatic and gastric tumours showed stable disease for $\geqslant$26 weeks. Four pts have received treatment for more than 80 weeks.
Conflict of interest: *Other substantive relationships: Donaldson, Posner and Kawabata are all employees of Shionogi Ltd. Other authors have received funding to cover clinical trials costs only*

Table (abstract 801).

	CAP	TAB		TAB			
	400 mg BID (cont)	400 mg BID (cont)	300 mg BID (cont)	200 mg TID (cont)	250 mg TID (int)	400 mg BID (int)	400 mg QD (cont)
Evaluable for safety, n	18*	17*	18*	16	15	16	15
Grade ⩾3 events, n (%)							
Anaemia	4 (22)	5 (29)	4 (22)	1 (6)	1 (7)	0	1 (7)
Vomiting	1 (6)	0	0	2 (13)	0	1 (6)	0
Dose modifications, n (%)							
Reductions	3 (17)	11 (65)	4 (22)	2 (13)	2 (13)	4 (27)	2 (13)
Interruptions	6 (33)	10 (59)	8 (44)	10 (63)	8 (53)	10 (67)	5 (33)
Evaluable for antitumour effect, n	20†		13	15	14	16	15
Least-squares mean change in tumour size at wk 8,‡ %	−19.0	NA§	−19.9	−14.4	−5.4	−14.7	−3.3

*Includes ovarian and breast cancer pts with a germline *BRCA1/2* mutation.
†Includes pts from a second previously reported group.
‡Adjusted for baseline characteristics; §Dose not considered tolerable in a prior data review so analysis of antitumour effect not performed

801 ORAL

Administration of continuous/intermittent olaparib in ovarian cancer patients with a germline BRCA1/2 mutation to determine an optimal dosing schedule for the tablet formulation

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Background: Initial studies with the oral PARP inhibitor olaparib identified 400 mg BID as a tolerable and efficacious dose requiring 16 capsules (CAP)/day. We report final results of a study aimed at identifying an optimal dose of the more practical tablet (TAB) formulation, for which we previously reported a dose-response relationship. An expansion cohort was recruited to this multistage Phase I trial to evaluate alternative administration schedules (NCT00777582; sponsor, AstraZeneca).
Materials and Methods: In this expansion cohort, patients (pts) with *BRCA1/2* mutation and relapsed ovarian or primary peritoneal cancer were randomized 1:1:1:1 to one of two continuous (cont; 200 mg TID, 400 mg QD) or two intermittent (int; 250 mg TID: 2 wks on/1 wk off; 400 mg BID: 1 wk on/1 wk off) schedules. Primary objective was safety and tolerability. Secondary objectives included antitumour effect. Results were compared with previously reported groups receiving 400 mg CAP BID, 400 mg TAB BID or 300 mg TAB BID (all cont; Molife *et al* ASCO 2010, 2012).
Results: 62 patients were randomized. Median lines of prior chemotherapy was 3 (range: 1−11). 6 pts (10%) discontinued treatment due to toxicity. The table shows key safety and antitumour effect data.
Conclusions: 400 mg BID TAB (int and cont) were not considered tolerable for long-term use. 250 mg TID (int) and 400 mg QD (cont) failed to match the efficacy seen in prior cohorts. 200 mg TID did not improve tolerability vs 300 mg BID, so 300 mg BID (cont) is the recommended tablet dose for Phase III studies in *BRCA1/2*-mutant ovarian cancer, including the maintenance therapy setting. These findings simplify olaparib administration from 16 CAP to 4 TAB per day.
Conflict of interest: *Ownership: A. Fielding & K. Bowen are employees of AstraZeneca and own AstraZeneca stock. Advisory board: C. Gourley has been a consultant for Roche, Boehringer-Ingelheim, Schering-Plough, GlaxoSmithKline and Chugai. Corporate-sponsored research: C. Gourley has received research funding from AstraZeneca. Other substantive relationships: C. Gourley has received honoraria and other remuneration from Roche, Boehringer-Ingelheim, Schering-Plough, GlaxoSmithKline,Chugai and Pharma-Mar*

802 ORAL

MEDI4736, an anti-PD-L1 antibody with modified Fc domain: Preclinical evaluation and early clinical results from a phase 1 study in patients with advanced solid tumors

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Background: Tumors can evade immune detection by expressing the programmed cell death ligand 1 (PD-L1). Blockade of PD-L1 may help overcome immunosuppressive effects and restore T-cell activity against tumors. In nonclinical experiments, inhibition of PD-L1 was shown to produce durable antitumor activity as a single agent and in combination with other therapies. MEDI4736, a human monoclonal antibody that binds specifically to PD-L1, was engineered with a triple mutation in the Fc domain to abrogate Fc-mediated effector function, and is currently being evaluated in Phase 1 studies.
Materials and Methods: The properties of MEDI4736 were determined via *in vitro* assays, and anti-mouse PD-L1 was used in murine models of cancer to explore pharmacodynamic markers and combinations with other therapies. An ongoing Phase 1, multicenter, open-label study is evaluating the safety profile, pharmacokinetics, biomarkers, and antitumor activity of MEDI4736 administered IV in subjects with advanced solid tumors.
Results: MEDI4736 demonstrated potent and specific binding to PD-L1 with picomolar affinity. It blocks the interaction of PD-L1 with PD-1 and CD80 resulting in increased T-cell activation *in vitro*, but does not trigger cytokine release in whole blood assays. Three point mutations in the Fc domain have abrogated Fc-mediated effector function *in vitro*. Treatment of tumor-bearing mice with anti-mouse PD-L1 resulted in changes in peripheral immune markers and antitumor responses in a subset of mice. The combination of anti-mouse PD-L1 and CTLA-4 antibodies significantly enhanced this activity, leading to tumor regression in all mice treated.
As of 15 April, preliminary results from the Phase 1 study include 8 subjects (med. age 65 yrs, range 46−71), ECOG 0−1, with a median of 4 (3−10) prior treatments, received a median of 6 doses (1−13) of MEDI4736. No dose limiting toxicitites or drug-related grade ⩾3 adverse events were reported (no pneumonitis or colitis of any grade). An early signal of clinical activity was observed as evidenced by RECIST-based responses and prolonged disease stabilization in different tumor types, including patients with extensive disease. Tumor shrinkage was observed as early as 7 weeks and was sustained at subsequent time points.
Conclusions: Preliminary safety and clinical activity data for MEDI4736 are encouraging at the initial dose levels explored and warrant further investigation of this molecule alone and in combination with other therapies.
Conflict of interest: *Corporate-sponsored research: MedImmune sponsored the study. SK, JL, NS, SA, JW are investigators and received research funding for the conduct of the study. ABH, RS, PR, AS, RI are employees of MedImmune. Other substantive relationships: ABH, RS, PR, AS, RI own stock/stock options in Astrazeneca*

803 ORAL

Evaluation of tolerability and anti-tumor activity of GDC-0980, an oral PI3K/mTOR inhibitor, administered to patients with advanced solid tumors or non-Hodgkin's lymphoma (NHL)

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Background: The PI3K-AKT-mTOR signaling pathway is dysregulated in a wide variety of cancers. GDC-0980 is a potent and selective oral dual inhibitor of class I PI3K and mTOR kinases that demonstrates broad activity in various xenograft cancer models.
Materials and Methods: A phase I dose-escalation study, PIM4604g, was conducted in 2 Stages: Stage 1 evaluated oral, daily (QD) doses of 2−70 mg GDC-0980 in a 3+3 design. Stage 2 evaluated 30 mg and 40 mg GDC-0980 QD. Safety and tolerability of GDC-0980 was assessed, as well as pharmacokinetics (including proton pump inhibitor (PPI) interaction), pharmacodynamic (PD) assessment of PI3K pathway inhibition and PIK3CA mutations, and anti-tumor activity.
Results: 113 patients were enrolled, 57 in Stage 1 and 56 in Stage 2. The MTD was 50 mg QD. DLTs were Grade 3 rash and symptomatic Grade 3 hyperglycemia at 70 mg QD. Grade ⩾3 pneumonitis was observed in 3 patients in Stage 1 at doses of ⩾40 mg, including 1 mesothelioma patient with Grade 5 pneumonitis. Based on Stage 1 tolerability data, a recommended phase 2 dose of 40 mg QD was evaluated in Stage 2 for all tumor types, with the exception of malignant pleural mesothelioma (MPM) where the recommended dose was 30 mg QD. The most frequent Grade ⩾3 drug-related adverse events (AEs) at 30 mg and 40 mg GDC-0980 were hyperglycemia (15%), rash (12%), diarrhea (8%) fatigue and abnormal LFTs (7%), and pneumonitis (6%), and 17% of the patients discontinued due to an AE (4% at 30 mg, 24% at 40 mg). The exposure of GDC-0980 was dose-proportional and no interaction with the PPI rabeprazole was detected at 40 mg GDC-0980. RECIST anti-tumor activity was observed in Stage 1 at GDC-0980 doses of 8−50 mg, with 2 PRs (MPM patients, 1 at 50 mg and 1 with a PIK3CA mutation at 8 mg). At the recommended phase 2 dose, 2 PRs for MPM patients at 30 mg and 1 PR for a head and neck cancer patient with a PIK3CA mutation at 40 mg were observed. GDC-0980 doses of ⩾16 mg demonstrated significant PI3K pathway inhibition (pAKT and insulin/glucose levels). Additionally, pathway inhibition by FDG-PET responses was observed in 50% of the patients.
Conclusions: GDC-0980 was generally well-tolerated at the recommended phase 2 doses of 30 mg and 40 mg. Significant PI3K pathway inhibition was observed in PD assays at doses of ⩾16 mg. Anti-tumor activity has been observed at the recommended phase 2 doses. Updated data on clinical outcomes and biomarker correlates will be presented.
Conflict of interest: *Ownership: Genentech, Roche. Advisory board: Genentech, Roche. Board of directors: none. Corporate-sponsored research: Genentech, Roche. Other substantive relationships: Advisor: GSK, AstraZeneca, Pfizer, Exelixis, Merck, Novartis, Arno Therapeutics*

804 ORAL

Phase Ib study of oral pan-PI3K BKM120 in combination with the oral MEK1/2 inhibitor GSK120212 in patients with selected advanced solid tumors and RAS / BRAF mutations

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Background: MAPK and PI3K/AKT/mTOR pathways regulate proliferation, differentiation and cell death in different human cancers. A cross-talk interaction between these two pathways provides the rationale for combining PI3K and MEK inhibitors.
Methods: Primary objective of this phase Ib trial is to determine the maximum tolerated dose (MTD) and/or recommended phase II dose (RP2D) for daily oral administration of the PI3K inhibitor BKM120 with the MEK inhibitor GSK1120212. Secondary objectives include safety, tolerability, pharmacokinetics and efficacy.
In dose escalation part, patients (pts) with *RAS*/*BRAF* mutated (mt) solid tumors, pancreatic cancer or triple negative breast cancers were enrolled. A Bayesian logistic regression model with overdose control guided dose escalation was utilized. In dose expansion part, pts with RAS/BRAF mt ovarian, pancreatic and non-small cell lung cancer (NSCLC) were enrolled.
Results: As of June 2012, 75 pts were treated with BKM120 + GSK1120212 and have been evaluated for safety, 66 in dose escalation and 9 in dose expansion part. The MTD was reached at 70 mg BKM120 + 1.5 mg GSK1120212. The dose was later reduced due to adverse events (AEs) to 60 mg BKM120 + 1.5 mg GSK1120212, the RP2D. Most frequent AEs (>25%) irrespective of relationship, were diarrhea (60%), dermatitis acneiform (57%), nausea (42%), vomiting, CK elevation (39% each), asthenia (33%), reduced appetite (31%), pyrexia, stomatitis (29% each), maculopapular rash (27%), hyperglycemia and rash (25% each). The most common grade 3/4 AEs (>5%) irrespective of relationship were CK elevation, maculo-papular rash (12% each), ALT increase (9%), AST increase, thrombocytopenia (8% each), stomatitis (7%), diarrhea, acneiform rash, macular rash and acute renal failure (5% each). No deaths were related to treatment. 28 (37%) pts had treatment discontinuation and 48 (64%) dose reductions/delays dues to AEs. As of February 2013, 21 KRAS/ BRAF mt ovarian cancer pts have been treated and 19 were evaluable (⩾1 post-treatment tumor assessment) for response. 7/19 (37%) evaluable pts achieved a best overall response (BOR) of partial response (confirmed in 6 of them) and 9 had a stable disease (SD). 19/24 pancreatic pts were evaluable for efficacy and 12/19 achieved a BOR of SD. For the 17 NSCLC pats, 11 pts were evaluable and 7 had a BOR of SD.
Conclusions: BKM120 and GSK1120212 can be safely combined. Promising clinical activity has been observed in pts with KRAS/BRAF mt ovarian cancer.
Conflict of interest: *Ownership: GlaxoSmithKline (Le). Advisory board: Trovagene (Janku), Novartis (Bedard). Corporate-sponsored research: Novartis (Janku), Roche (Janku), Transgenomic (Janku), Biocartis (Janku) Trovagene (Janku), Novartis (Bedard), GlaxoSmithKline (Bedard) Research funding paid by novartis to institution (Van Cutsem). Other substantive relationships: Speaker at ECC 2013 * travel covered by meeting organizers (Bedard) Novartis employee and stock owner (Zubel)GSK employee and a GSK stock owner (Le)*

805 ORAL

Final results of a first-in-human clinical trial of OSI-027, a small molecule dual mTORC1/mTORC2 inhibitor in patients with advanced malignancies

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Background: OSI-027 is an orally available selective inhibitor of mTORC1 and mTORC2. We report a phase I trial to evaluate its safety, tolerability, pharmacokinetics (PK), maximum tolerated dose (MTD) and pharmacodynamics (PD) in tumor and surrogate tissues. (NCT00698243; sponsor Astellas Pharma Inc).
Material and Methods: Patients (pts) with refractory malignancies, ECOG performance status ⩽2 and adequate organ function were enrolled in one of 3 administration schedules (S); S1: once daily for 3 of every 7 days (qd 3/7d), S2: once weekly (qwk) or S3: continuous qd, in 21-day cycles. Dose limiting toxicities (DLT) were assessed in cycle 1 (CTCAE v3). Dose escalation was pursued for each schedule with a 3+3 design. Safety and PK were studied after single and multiple dosing. PD analyses were performed in expansion cohorts using paired pre/post-dose tumor biopsies.
Results: 123 pts were treated (S1−54pts, S2−39pts, S3−30pts). Main tumor types were colorectal (n = 32), melanoma (12) and renal (11). Doses ranged from 10–480 mg per week (S1:10–160 mg qd 3/7d; S2:10–240 mg qwk; S3:5−50 mg qd). DLTs were grade (G) 3 fatigue (n = 4), G2 elevated

serum creatinine (2), G3 cardiomyopathy (1), G2 decreased left ventricular ejection fraction (1), G3 hyperglycemia (1), G3 rash (1) and G3 bone pain (1). The MTD for S1 is 120 mg qd 3/7d. Dose escalation in S2 was limited due to high burden of capsules required; no MTD was identified. The MTD for continuous dosing (S3) is 35 mg qd; however, schedule was halted due to renal toxicity, showing insufficient PD inhibition at tolerable dose-levels. G $\geqslant$3 adverse events (AE) included G3 fatigue (n = 6), G3 diarrhea (2), G3 nausea/vomiting (2), G4 myocardial infarction (1) and G5 acute renal failure (1). Expansion cohorts were initiated for S1 at 90 mg and 120 mg based on safety data. 5/13 (38%) pts in S1–120 mg required a dose-reduction or discontinuation due to AE. C_{max} and AUC were dose-linear. PD inhibition was associated with drug exposure, with the most substantial inhibition in tumor biopsies seen at the highest doses. Stable disease (RECIST 1.0) after 24 weeks was seen in 5 pts, including 1 pt with GIST (exons 11 and 17 KIT mut) who was on treatment 45 weeks.
Conclusions: OSI-027 inhibits mTORC1/2 in a dose-dependent manner. The most significant AEs were fatigue, renal and cardiac events. Intermittent schedules were better tolerated. S1–120 mg qd 3/7d achieved substantial target inhibition but was not tolerated by a proportion of patients.
No conflict of interest.

Poster Session (Sun, 29 Sep)
Drug Development

806 POSTER

A new approach to integrate the grade of toxicity and later cycles in the analysis and reporting of phase I dose finding trials

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Background: In oncology, the maximum tolerated dose (MTD) is commonly defined as the dose of a given treatment associated with a certain level of dose limiting toxicities (DLT), evaluated during a predefined time window, typically one or two cycles of treatment. Adverse events (AE) are usually assessed sequentially, using NCI-CTCAE grading criteria. Our working hypothesis is that the longitudinal assessment of AEs can provide relevant additional information for defining the optimal dose and schedule of a new treatment. To illustrate our approach, we retrospectively reanalysed three phase I clinical trials of anticancer agents.
Material and Methods: We developed a new dose-finding method that uses all the data collected at the end of the trials. A mixed effect proportional odds model was used to account for repeated measurements. We collected individual patient data from two continual reassessment method phase I clinical trials (aviscumine in solid tumours and erlotinib with radiotherapy in brainstem gliomas). A third trial was included (classical 3+3 design of DoxLipeg + cyclophosphamide in ovarian carcinoma). The outcome of interest was the worst grade (G) of toxicity in each cycle, using three grading categories (G0−1/G2/G3−5). We estimated the probability of G2−5 and G3−5 toxicity per cycle for each evaluated dose level. We defined the dose associated with a per cycle probability of severe toxicity close to 20% as the recommended phase II dose. The risk of toxicity over time was also investigated.
Results: In the three trials a total of 83 patients were included and treated at 14, 3 and 5 dose levels, respectively. In the first two trials, four and two DLT occurred respectively; 94 and 96 cycles were administered (worst grade: 38 G2, 22 G3 AEs; 19 G2, 7 G3−5 AEs, respectively). No increased risk of toxicity was detected with time for the first two trials. We could not disentangle late toxic AEs from cumulative AEs as time is confounded with cumulative dose. The per cycle risk of G3−5 toxicity was slightly lower with the mixed effect proportional odds model analysis, as compared to an analysis restricted to the first cycle. Analysis of the third trial is in process.
Conclusions: Dedicated methods, whose operating characteristics were evaluated elsewhere, allows for analysing toxic adverse events from all cycles of treatment. They should be integrated in recommended phase II dose assessment.
No conflict of interest.

807 POSTER

Investigating the antiproliferative activity of high affinity DNA aptamer on cancer cells

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Background: The purpose of the study is to investigate the antiproliferative activity of SL_2-B aptamer on cancer cells.
Materials and Methods: *Surface plasmon resonance (SPR)*: The binding affinity of SL_2-B aptamer sequence was investigated using SPR, where $VEGF_{165}$ acted as ligand. The binding analysis was carried out with aptamers at different concentrations and sensorgrams were analysed in BIAevaluation software to calculate the equilibrium dissociation constant K_d.
Antiproliferative activity: SL_2-B aptamer was incubated with Hep G2 cells for 3 days in hypoxia conditions. The antiproliferative effect was determined using MTT assay. For flow cytometry, after 3 days of aptamer treatment, the cells were incubated with anti-human Jagged-1 fluorescein antibody for 1 hr and analysed using flow cytometry.
Results: *Binding analysis by SPR*: Due to presence of nucleases, the unmodified aptamer exhibits low structural stability in the cellular conditions. To alleviate this problem, the SL_2-B aptamer was chemically modified with phosphorothioate (PS) linkages at 5′ and 3′-terminus. The K_d value for the PS-modified SL_2-B was found to be 0.56nM, which is similar to K_d for unmodified. Introducing PS-modification does not appear to affect the binding affinity of the aptamer.
Antiproliferative activity: Lower cell proliferation was observed at 15μM modified SL_2-B concentration after 72 hrs of aptamer treatment (52±2.1%). On the contrary, the unmodified sequence did not exhibit significant inhibitory activity on cellular proliferation. This could be due to the degradation of the unmodified sequence by nuclease enzymes in the media before pronouncing its effect. The incubation of cells with scrambled sequence showed minimal decrease on the cell proliferation, confirming that the inhibitory effect by modified SL_2-B was sequence specific. Due to the crosstalk between VEGF and notch signalling pathways in tumour progression, the effect of PS-modified SL_2-B aptamer was tested on Jagged-1, which is one of the notch ligands via flow cytometry. Compared to the untreated sample, modified SL_2-B exhibited decrease in the fluorescent signal indicating the downregulation of Jagged-1 expression in Hep G2 cells. In western blotting, the modified aptamer appears to induce lower expression of the Jagged-1 protein in Hep G2 cells as compared to the scrambled sequence. This confirms the sequence specific inhibition of the aptamer. Based on these results, it can be concluded that the binding of modified SL_2-B to VEGF exhibits its antiproliferative activity in Hep G2 cells not only by inhibiting VEGF pathway but also the interconnected notch signalling pathway.
Conclusions: From the data, we conclude that post-modification, the PS-modified SL_2-B aptamer retained its binding affinity and exhibited sequence specific antiproliferative activity on Hep G2 cancer cells. Hence, it appears that chemical modification can be a useful approach in prolonging the half-life of SL_2-B aptamer in the *in vitro* conditions.
No conflict of interest.

808 POSTER

Capillary morphogenesis gene 2 is a potential target for anti-angiogenic therapy

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Background: Capillary morphogenesis gene 2 (CMG2) was identified as a gene being up-regulated in capillary morphogenesis. It is also known as a receptor of anthrax toxin. It has been shown to be involved in the cell adhesion and motility of various cell types, including epithelia and endothelia. Present study aims to examine to investigate the therapeutic potential of targeting CMG2 to prevent tumour related new vasculature.
Materials and Methods: Full-length of human CMG2 gene and different fragments of the same gene were amplified and constructed into a mammalian expression plasmid vector. The effect of CMG2 and its different fragmented protein products on functions of vascular endothelial cells was examined using various *in vitro* and *ex vivo* angiogenesis models, and *in*

vivo tumour growth which including tubule formation of endothelial cells, aorta ring assay and xenograft mouse model.
Results: The overexpression of CMG2 enhanced the adhesion of endothelial cells to extracellular matrix, but was negatively associated with cell migration. Over-expression of certain fragments (extracellular domains) inhibited the tubule formation and migration of endothelial cells. Small peptides mimicking the amino acid sequence of the fragments potently inhibit the *in vitro* tubule formation and *ex vivo* angiogenesis. Tests of certain small peptides showed an inhibitory effect on in vivo tumour growth of cancer cells which we have examined.
Conclusion: CMG2 is a potential target for treating tumour related angiogenesis. Small peptides mimicking the extracellular domain of CMG2 can potently inhibit the in vitro and ex vivo angiogenesis, which may contribute to its inhibitory effect on in vivo tumour growth. The mechanisms underlying require further investigation.
No conflict of interest.

809 POSTER

Safety and efficacy of bevacizumab as front-line treatment of brain metastases from solid tumors

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Background: B is approved for the treatment of advanced colorectal, lung, kidney, breast cancers and high-grade gliomas. Despite the large employment of B in the treatment of brain primitive tumors, there is only a very limited experience with BM and none in the treatment of previously untreated secondary brain lesions.
Material and Methods: We have treated the patients (PTS) with BM not suitable for local treatment with a B-based therapy associated with chemotherapy or INF-alfa as indicated for the primary cancer type.
Results: From March 2010 to June 2012 we collected 18 PTS with BM mostly from lung and renal adenocarcinoma and the majority of patients had a treatment-naive brain disease (see table). B has proved to be safe and effective: RR was 82% of PR with 18% of SD. PFS was 14 months (95% CI: 3.0–25.0) and OS was 15 months (95% CI: 3.7–26.3). Moreover, B has a high capability to give clinical benefit in PTS with BM, mostly reducing perilesional edema, sometimes with a long lasting effect. The general toxicity we detected was the same as known in clinical practice: no cerebral hemorrhagic events were reported, even if two cases of cerebral ischemia and 1 of gastric perforation were recorded.
Conclusion: B for BM is feasible and safe and the efficacy data are very promising.
No conflict of interest.

Pt no.	Gender/Age/ Primary tumor	Prior therapy for brain mts	Treatment	CNS response	Extra-CNS response	PFS	OS
1	Fe/62/NSCLC	None	Bev+Cis+Gem	Near CR	PR	31.1+	31.1+
2	Ma/41/NSCLC	None	Bev+Cis+Gem	SD	SD	11.4	15.9
3	Ma/70/kidney	NSurg	Beva+Inf-alfa	CR	PD	18.4+	18.4+
4	Fe/58/lung	None	Bev+Cis+Gem	PR	PR	0.9+	3.6
5	Ma/56/kidney	None	Beva+Inf-alfa	PR	PR	20.3	33.2
6	Ma/73/kidney	SRS	Beva+Inf-alfa	PR	PR	20.7+	20.7+
7	Ma/71/Kidney	None	Beva+Inf-alfa	SD	SD	6.5	12.3
8	Ma/69/NSCLC	None	Bev+Cis+Gem	SD	PD	7.6+	8.2
9	Ma/65/NSCLC	None	Bev+Cis+Gem	SD	PD	1.9	3
10	Ma/70/lung	WBRT	Bev+Cis+Gem	SD	PD	6.8	9.6
11	Ma/50/NSCLC	None	Bev+Cis+Gem	SD	PR	7.2+	9.9
12	Ma/67/NSCLC	None	Bev+Cis+Gem	PR	PR	14.6+	14.6+
13	Fe/75/NSCLC	None	Bev+Cis+Gem	PR	PR	9.6+	9.6+
14	Ma/67/NSCLC	None	Bev+Cis+Gem	PR	PR	8.2+	8.2+
15	Fe/67/endometrial ca	None	Bev+Cis+Gem	PR	PR	17.7+	17.7+

SRS: sereotactic surgery; WBRT: whole brain radiotherapy; NSurg: neurosurgery; Bev: bevacizumab; Cis: cisplatin; Gem: gemcitabine; Inf-alfa: interferon-alfa.

810 POSTER

Phase 1b study of multiple dosing schedules of pazopanib in combination with epirubicin or doxorubicin in patients with advanced solid tumors

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Background: Pazopanib (PAZ) is an oral, multitargeted inhibitor of VEGFR-1/2/3, PDGFR-α/β, and c-Kit. Optimal dose and schedule for combining PAZ with epirubicin (EPI) and doxorubicin (DOX) need to be established.
Methods: Part 1 of the VEG109603 trial explored PAZ+EPI in dose-escalation cohorts to define the optimally tolerated regimen (OTR) for 3 concomitant dosing schedules (21-day [d] cycles): Arm A (PAZ d1−21, EPI d3); Arm B (PAZ d1−8, EPI d3); Arm C (PAZ d14−21, EPI d1). The OTR of each schedule was further evaluated in 3 concomitant cohorts of up to 12 patients (pts) to select the regimen for Part 2, which combined PAZ+DOX (Arm D) based on safety and tolerability results. Blood samples were obtained for pharmacokinetic and pharmacodynamic analysis.
Results: Overall, 111 pts were treated, including 65 pts in the OTR cohorts. The OTRs were PAZ 400 mg/EPI 75 mg/m^2 (Arm A), PAZ 800 mg/EPI 90 mg/m^2 (Arms B and C). Dose-limiting toxicities (DLTs) were evaluated during the first and subsequent cycles to assess prolonged feasibility. In the dose-escalation cohorts, DLTs included grade (Gr)4 neutropenia, Gr3 nausea/vomiting/dehydration, and Gr3 deep vein thrombosis. In the PAZ+EPI OTR cohorts, DLTs included Gr4 neutropenia, Gr4 pulmonary thrombosis, and Gr4 febrile neutropenia. The most common adverse events (AEs; any Gr) in all cohorts were neutropenia, nausea, and asthenia, and the most common Gr⩾3 AEs were neutropenia and leukopenia. Tolerability of PAZ+EPI worsened with repeated doses. Based on the safety profile of Part 1, the Arm B dosing schedule was selected for PAZ+DOX in Part 2, which defined the OTR as PAZ 800 mg/DOX 60 mg/m^2. DLTs in Arm D included Gr4 neutropenia, Gr5 febrile neutropenia, and Gr1 LVEF decrease. Neutropenia and leukopenia were the most common Gr⩾3 AEs.
Pharmacokinetic analysis showed that EPI may increase exposure to PAZ ($AUC_{(0-24)}$ and C_{max} were ~18% greater when PAZ was administered concomitantly with EPI in Arm A). PAZ did not significantly interfere with EPI disposition. In the PAZ+EPI OTR cohorts, 11 evaluable pts had best response as partial response: 2 (13%) in Arm A; 3 (16%) in Arm B; and 6 (38%) in Arm C. In the PAZ+DOX OTR cohort (Arm D), there was 1 complete (7%) and 1 partial response (7%).
Conclusions: The OTRs of PAZ+EPI and PAZ+DOX were PAZ 800 mg d1−8 plus EPI 90 mg/m^2 or DOX 60 mg/m^2 d3 of a 21-d cycle. These regimens showed an acceptable safety profile and should be considered for further evaluation.
Sponsor: GlaxoSmithKline.
Conflict of interest: *Ownership: (BS + HT) GlaxoSmithKline stock ownership. Advisory board: (LG) Roche, Genentech, GSK, Novartis, Pfizer, Boehringer Ingelheim, Astra Zeneca, Celgene, BioScience, Tahio (KH) Stemergie Biotechnology. Board of directors: (LG) Roche, Genentech, GSK, Novartis, Pfizer, Boehringer Ingelheim, Astra Zeneca, Celgene, Bio-Science, Tahio. Corporate-sponsored research: (LG) Roche, Genentech, GSK, Novartis, Pfizer, Boehringer Ingelheim, Astra Zeneca, Celgene, BioScience, Tahio (KH) will have in the second half of 2013 by Servier. Other substantive relationships: (BS + HT) Employed by GlaxoSmithKline*

811 POSTER

Omega-3 polyunsaturated fatty acids-derived drugs as potential anti-angiogenic treatment for solid tumours

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Background: Polyunsaturated fatty acids (PUFA) can be divided into n-6 and n-3 PUFA depending on the position of the first double bond. Although n-3 PUFAs are found to be cancer-suppressing, n-6 PUFAs are disease-promoting. Mechanisms of such effects are still unknown. Both n-3 and n-6 PUFA undergo bio-conversion by two major enzymes: cyclooxygenases and cytochrome P-450s. Potential reason for different activity of n-3 and n-6 PUFA-derived metabolites could be related to the observed conformational differences between these two fatty acid types and mediators derived from them.
Angiogenesis (creation of new blood vessels from the existing vasculature) is very important process in tumour growth. In this work we have assessed effects of two major classes of n-3 PUFA metabolites: a) cyclooxygenase-2 (COX-2)-derived and b) cytochrome P-450, subgroup 2J2 (CYP2J2)-derived products. PUFA products of these two enzymes have been implicated in regulation of angiogenesis.
Material and Methods: Four types of MDA-MB-468 (human breast cancer cells) clones were constructed by permanent transfection with either control or plasmids expressing COX-2, CYP2J2 or both. These clones were incubated with n-3 PUFA (Eicosapentaenoic acid) and medium or extract from these cells was tested for suppression of two angiogenic processes *in vitro* using human umbilical vein endothelial cells (HUVEC): 1) Tube formation assay and 2) Migration assay. Tube formation assay consisted of observing formation of vascular precursors or 'tubes' after plating HUVEC on the layer of artificial extracellular matrix (Matrigel). HUVEC migration was investigated using our unique 'Matrigel droplet' assay.
Results: Our findings suggest that both types of mediators derived from n-3 PUFA inhibit angiogenesis. COX-derived metabolites suppress both EC

migration and tube formation whereas CYP2J2-derived mediators suppress only tube formation.
Conclusions: Our results provide evidence that both COX-2 and CYP2J2 derived n-3 PUFA metabolites suppress angiogenesis *in vitro* and new molecules derived from them could serve as future anti-cancer therapeutics.
No conflict of interest.

812 POSTER

Phase 1 dose-escalation study of E7820, a novel anti-angiogenic agent, administered orally twice-daily to patients with advanced, refractory solid tumors

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Background: E7820 is a novel sulfonamide derivative with potent anti-angiogenic activity based on the inhibition of endothelial cell proliferation and tube formation. The antitumor effects of E7820 were associated with inhibition of integrin alpha2 expression in endothelial cells *in vitro*. E7820 MTD for single daily administration was achieved at 100 mg. PK/PD analysis integrating preclinical and clinical data showed that twice-daily (BID) dosing results in a greater reduction of integrin alpha2 expression. BID dosing may also ameliorate toxicity associated with high maximum plasma concentration (Cmax) and may allow a greater total daily dose and drug exposure. This Phase 1 study was performed to determine MTD, safety and PK of E7820 following BID dosing.
Patients and Methods: Patients (pts) with advanced solid tumors, ECOG 0−1, ⩾18 years (yrs) and adequate organ function were eligible. E7820 was administered orally, BID continuously in 28 day cycles. Blood samples for PK analysis were collected on Day 1 and Day 8 over 12 hr post dose.
Results: 24 pts (M/F: 18/6; median age 57 yrs (range 38−77) were treated at 50 and 60 mg. Tumor types were colorectal (n = 8), renal (n = 3), GIST (n = 2), and others (n = 11). DLTs were observed in 2 pts at 60 mg BID and included Gr 3−4 leukopenia, Gr 3 neutropenia and neutropenic sepsis and Gr 3 fatigue. MTD was determined to be 50 mg BID. Frequently occurring adverse events ([AEs] all grades with ⩾10% incidence) were fatigue (42%), constipation (33%), diarrhea, nausea and vomiting (29% each), abdominal pain (25%) and dyspepsia (17%). E7820 exposure was dose-related with Cmax observed 0.5−5 hr post-dose. BID E7820 dosing resulted in 1.6−3.8 fold accumulation on multiple dosing. The highest E7820 exposure and highest accumulation (R=3.31, 3.38) was observed on day 8 in subjects who experienced DLTs. Cmax values in patients who had DLTs were comparable to those in other subjects in this dose cohort. The best overall response observed was stable disease.
Conclusions: E7820 at an MTD of 50 mg BID has manageable toxicity. DLTs were associated with high E7820 exposure, but not with Cmax. Twice-daily dosing of E7820 does not appear to offer a better toxicity profile compared to daily administration.
Conflict of interest: *Other substantive relationships: Eisai employees: L. Reyderman, B. de las Heras, D. Verbel, B Ink.*

813 POSTER

Allosteric inhibition of FGFR2 affects angiogenesis and cancer cell proliferation

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Background: The recent identification of fibroblast growth factor receptor 2 (FGFR2) overexpression or mutations in different cancer types has generated an opportunity for a novel target-based therapy. Here we explore the effects of allosteric inhibition of FGFR2 on angiogenesis and cancer cell proliferation.
Material and Methods: To assess the efficacy of RPT835, novel extracellular allosteric inhibitor of FGFR2 (RusPharmTech, LLC) on FGF-mediated cell proliferation, endothelial cells (SVEC-4−10), human umbilical vein endothelial cells (HUVEC), and breast cancer (T47D), gastric cancer (Kato III), lung cancer (A549) FGFR-expressing cells were incubated in a 96-well microculture plate and were treated with serially diluted RPT835. Brivanib was used as a control. Basic FGF was added at a concentration of 25 ng/ml. Control wells were left untreated. Cell growth inhibition was determined using Promega's Cell Titer-Glo® assay. SVEC-4−10 cell migration was evaluated in the Boyden Chamber assay. In vivo angiogenesis was measured with subcutaneously implanted Matrigel plugs containing bFGF (100 ng/ml) or bFGF (100 ng/ml) + RPT835 (15 mg/kg). Negative control group was without stimulation and treatment. Number of endothelial cells/vessels was calculated.
Results: Basic FGF significantly increased proliferation of the HUVEC, SVEC-4−10 and cancer cells in untreated control group (P = 0.001). RPT835 significantly inhibited FGF-triggered endothelial cell proliferation when compared with control (P<0.001) or brivanib (P<0.001, IC50=289 nmol/L) with IC50 of 11 nmol/L (HUVEC) and 10 nmol/L (SVEC-4−10). RPT835 significantly decreased proliferation of A549 (IC50=10 nmol/L) as well as T47D (IC50=0.97 umol/L) cells. There was no impact on Kato III cells (IC50 >10 umol/L). In Boyden Chamber assay, RPT835 reduced endothelial cell migration up to 60%. In vivo, bFGF induced proliferation of endotheliocytes and mature vessels formation (P<0.001). There were no vessels in FGFR2 inhibitor and negative control groups.
Conclusions: Allosteric inhibition of FGFR2 affects endothelial and cancer cell proliferation, migration as well as mature vessel formation.
No conflict of interest.

814 POSTER

The pterocarpanquinone LQB118 alters the subcellular localization of Nrf2 and reduces XIAP expression sensitizing acute myeloid leukemia cells to apoptosis

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Background: Despite advances in chemotherapy, the five-year survival rate for patients with acute myeloid leukemia (AML) is about 20%. There have been efforts in recent years to establish new antileukemic drugs with decreased side effects and lower toxicity to healthy cells of AML patients. Pterocarpanquinone LQB118 [2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone] has emerged as a promising molecule for therapeutic applications. Recently, we have shown that LQB118 is effective in inducing apoptosis in cells from patients with AML and chronic myeloid leukemia (CML). Our partner group has shown that LQB118 increases the reactive oxygen species (ROS) levels in a leukemia cell line, suggesting a possible role of this pathway in its mechanism of action. Nrf2 is a transcription factor that controls the expression of various genes that encode proteins involved in detoxification, transport of xenobiotics, apoptosis inhibition, which are all involved in the response to oxidative/electrophilic stress to preserve cell life. XIAP is a member of the inhibitor of apoptosis family of proteins (IAP) which, in addition to being a caspase inhibitor, may be up-regulated under oxidative/electrophilic stress. Our objective is to elucidate the molecular mechanism of LQB118.
Material and Methods: The effect of LQB118 (3 and 6 μM) and idarubicin (0.01 μM), a chemotherapeutic agent used in AML therapy, on XIAP gene expression by real-time PCR and subcellular localization of Nrf2 transcription factor by immunofluorescence was evaluated in the AML cell line, Kasumi-1.
Results: In previously published reports, we have shown that LQB118 sensitizes Kasumi-1 cells to apoptosis and induces DNA fragmentation. XIAP mRNA expression was decreased by LQB118, whereas idarubicin increased said expression. Thus, after incubation with both concentrations of LQB118 for 24 and 48 h, the subcellular localization of Nrf2 was cytosolic as compared to untreated and also with idarubicin-treated Kasumi-1 cells, where Nrf2 localization was predominately nuclear.
Conclusions: In agreement with literature data, we found that a decrease in XIAP expression may contribute to sensitization of AML cells to ROS, after treatment with LQB118. In addition, Nrf2 cytosolic localization is a mechanism of maintenance of oxidative homeostasis, guaranteed by an efficient molecular complex. However, in AML there is constitutive nuclear activation of Nrf2, which contributes to the resistance to chemotherapy. Together, these findings may contribute to understand chemotherapy resistance in AML. Further investigation is currently in progress.
Financial support: Programa de Oncobiologia, INCT, FAPERJ, CNPq, Ministério da Saúde.
No conflict of interest.

815 POSTER

Antitumor activities and drug resistance overcoming of novel nogalamycins

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Background: *Streptomyces nogalater* Lv65 is a producer of an anticancer antibiotic nogalamycin. Its derivatives are used in chemotherapy of tumors. Certain aspects of the regulation of nogalamycin production have been characterized. However, most enzymatic reactions of nogalamycin biosynthesis have not been studied. The mechanisms controlling formation of the sugar moieties of nogalamycin and their enzymatic modification, particularly methylation, are not known.
Material and Methods: The anticancer activity of modificated nogalamicine was compared with doxorubicine after 72 h treatment in 12 cancer cell lines. To identify the alterations in cell cycle distribution induced by the nogalamicine, PI staining was performed. Changes in the expression levels of diverse cell regulatory proteins were investigated by Western Blotting.
Results: Bioinformatic analysis of the *snogM,* gene revealed that the product of these gene was involved in methylation of the nogalose moiety of nogalamycin. Disruption of the *snogM* gene in the chromosome of *S. nogalater* Lv65 resulted in *S. nogalater* strains ΔsnogM. The fact of gene disruption was confirmed by DNA−DNA hybridization. Inactivation of the *O*-methyltransferase genes had no affect on morphological features of the recombinant strains. One of the main goals of our work was also verify activity of this new modification of nogalamicine against several tumor cell lines. We have found that IC50 all used cell lines is between 4.3 nM (SW1573 lung adenocarcinoma cell line) and 15.21 nM (MCF-7 brest cancer cell line). By the way, the IC50 of famous anticancer drug doxorubicin is between 31.74 nM (SW1573 lung adenocarcinoma cell line) and 79.31 nM HCT116 p53 −/− human colorectal carcinoma cell line). Also nogalamicine was very efectiv against MRP1-overexpressing sublines HL60/adr and SW1573/2R120, which are srong resitence to the action of doxorubicine. Interesting, HCT116 cells with deleted p53 gene are almost 2 times more sensitive to the new nogalamicine as its wild type cell line. And in case of doxorubicine, human colorectal carcinoma HCT116 cells with deleted p53 −/− gene are more resistance, as its wild type cell line. This data show that such kind of modification of nogalamicine can be a new word in overcoming of p53 dependent drug resistance.
Conclusions: Genetic manipulations with the *snogM* gene of the nogalamycin biosynthetic gene cluster is a potentially valuable tool for generation of novel anthracycline antibiotics which can permit overcoming resistance of various human tumor cells.
This work was supported by WUBMRC grant (to D. Klymyshin and Y. Senkiv).
No conflict of interest.

816 POSTER

Trichosanthin, a type I ribosome-inactivating protein, inhibits lymphoma cell growth by promotion of apoptosis

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Background: Trichosanthin (TCS) is a 27 kDa type I ribosome-inactivating protein purified from the root tuber of a traditional Chinese medicine herb *Trichosanthes kirilowii*, which has been reported to have an anti-tumor activity. The aim of this study was to explore the growth inhibition effect of TCS on lymphoma cells and its possible mechanism. A total of 9 non-Hodgkin's lymphoma (NHL) cell lines were included in the experiment, including 5 diffuse large cell lymphoma (DLBCL) and 3 Burkkit lymphoma and one T cell lymphoma cell lines.
Methods: After different doses of TCS were added to the cultured cells, MTT assay, flow cytometry and Western blotting methods were used to investigate the effects and mechanism of TCS on the growth of lymphoma cells.
Results: The results showed that TCS could inhibit the proliferation of all NHL cells especially DLBCL cells. And the growth inhibition activity was associated with the expression levels of Mcl-1, Bcl-2 and Puma. Higher expression of Bcl-2 and Puma and lower expression of Mcl-2 were associated with higher efficacy. Flow cytometric analysis disclosed that TCS mainly induced apoptosis in those cell lines. Furthermore, the TCS-induced apoptosis was attributed to the activations of caspased-3 and PARP-1.
Conclusions: Therefore, TCS can inhibit NHL cell growth through inducing apoptosis, and the expression of Mcl-1, Bcl-2 and Puma maybe predicts its efficacy (JCYJ 20120613113228732, NSFC 81171154, GJHS 20120621153317134).
No conflict of interest.

817 POSTER

Autophagy modulation with mTOR inhibitor sirolimus and anti-EGFR monoclonal antibody cetuximab: phase I study in patients with advanced cancers

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Background: EGFR kinase activates PI3K/mTOR and MAPK pathways and EGFR nonkinase function is important for SGLT glucose transport. Preclinical data suggest that knocking down EGFR increases autophagy, which in combination with mTOR inhibition can lead to autophagic cell death. In addition, PI3K/mTOR activation can mediate resistance to EGFR targeting therapies, which can be abrogated by simultaneous mTOR inhibition.
Methods: Patients (pts) with refractory advanced cancers received IV cetuximab (day 1, 8, 15, 22) with oral sirolimus (day 1−28) in 28-day cycles. Doses were escalated in a 3+3 schema. Endpoints were maximum tolerated (MTD) or phase 2 recommended dose (RP2D), safety, response, and PD/PK analyses.
Results: To date, 112 pts were treated in 8 dose levels and 4 expansion cohorts at RP2D of sirolimus 6 mg and cetuximab 400 mg/m^2 followed by 250 mg/m^2 since MTD has not been reached. Dose limiting toxicities included grade (G) 3 mucositis (n = 3), and grade 4 thrombocytopenia with bleeding (n = 1). Other significant drug related toxicities included G3 mucositis (n = 1), G3 acneiform skin rash (n = 4), $\geqslant$ G3 hypersensitivity reaction (n = 3), G3 proteinuria (n = 1), G3 hyperglycemia (n = 1), G3 hyperlipidemia (n = 1), G4 lymphopenia (n = 5) and G4 thrombocytopenia (n = 3). Shrinkage per RECIST of more than 20% tumor was observed in 7 pts (head and neck squamous cell [HNSCC, n = 4], non-small cell lung [NSCLC, n = 2], and parathyroid cancer [n = 1]) including 4 partial responses (5 received prior EGFR therapies). PK data (n = 31) shows dose proportional increase in sirolimus weekly trough levels and the median trough level at RP2D was 15.7 ng/mL. PD data showed adequate mTOR inhibition as measured by pS6K activity in PBMC (ELISA) in 25 tested pts ($p < 0.001$) and decreased accumulation of autophagosomes as measured by % of LC3 positive PBMCs (flowcytometry) in 23 tested pts ($p < 0.001$). Some spots are still open in the expansion cohorts. Updated clinical, PK and PD data (including pre- and post-treatment biopsies) will be presented.
Conclusions: Cetuximab and sirolimus is well tolerated and demonstrates early antitumor activity in patients with refractory HNSCC, NSCLC, and parathyroid carcinoma.
Conflict of interest: *Corporate-sponsored research: Filip Janku has research funding from Biocartis, Novartis, Roche, Transgenomic, Trovagene*

818 POSTER

4,4'-dimethoxybenzophenone thiosemicarbazone: Underlying mechanism of action of a compound with selective proapoptotic novel activity in human leukemia cells

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Thiosemicarbazones have shown wide pharmacological versatility and their application as antibacterial, antiviral and anticancer agents make them valuable candidates for the development of new drugs.
In the search for new potential anti-leukemic drugs, a family of benzophenone thiosemicarbazones were synthesized and tested for antiproliferative activity in human acute leukemia U937 cell line. From this initial screening the 4,4'-dimethoxybenzophenone thiosemicarbazone (T44Bf) was identified as the most potent. Based on that, the aim of the present study was to elucidate the mechanisms that mediate the antiproliferative effects of T44Bf over different models of human acute leukemia (KG1a, HL60, U937 and Jurkat cell lines). T44Bf treatment of the cell lines led to a significant reduction in cell growth in a dose/time

dependent manner (IC50=5μM). To evaluate whether this inhibition was due to the induction of apoptosis, we analyzed by western blot, caspase 3 and poly (ADPribose) polymerase (PARP) cleavage. The results obtained in the apoptosis studies correlates in time and concentration with the observed inhibition of proliferation for the four cell lines. Similar results were obtained when we measure caspase-3 activity by a colorimetric assay and phosphatidylserine extrusion by Anexine V staining. On the other hand, peripheral blood monocytes and lymphocytes isolated from healthy blood donors were treated with T44Bf up to 20 μM. Interestingly, T44Bf did not promote death of normal cells indicating selectivity of the compound at the working concentrations.

To elucidate the action mechanism involved in the pro-apoptotic activity of T44Bf we studied by Western blot, the phosphorylation state of MAPKs: ERK, Akt, p38 and JNK, using phospho-specific antibodies. T44Bf treatment increased phosphorylation in a time dependent manner of ERK1/2, while p-Akt, p-p38 and p-JNK levels remained unchanged. To evaluate whether the observed ERK1/2 modulation was involved in the pro-apoptotic activity of T44Bf, we measured caspase 3 and PARP clevage in cells treated with T44Bf in presence of the MEK inhibitor U0126. Inhibition of ERK phosphorylation by U0126 blocked T44Bf induced apoptosis, indicating that phosphorylation of ERK is a necessary step to achieve apoptosis by T44Bf.

Our results shed new light in the mechanism of action of thiosemicarbazones as anticancer agents and postulated T44Bf as a promising compound for the development of novel and selective antileukemic drugs.

No conflict of interest.

819 POSTER

The novel peroxisome proliferator-activated receptor gamma (PPARg) agonists CB11 and CB11d, induced apoptosis through DNA damage by reactive oxygen species (ROS) in a human non-small cell lung cancer (NSCLC) cell line H460

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Background: PPARγ agonists have been shown to induce apoptosis a variety of cancer cells. Lung cancer is the most common cause of cancer death in the world and the second most common cancer in the Republic of Korea. PPARγ is known to be highly expressed in human lung cancer cell lines. In this study we investigated effects of novel PPARγ agonists CB11 (8-(2-aminophenyl)-3-butyl-1,6,7-trimethyl-1H-imidazo[2,1-f]purine-2,4(3H,8H)-di-
one) and its derivative, CB11d1(8-(2-aminophenyl)-3-butyl-1,7-dimethyl-1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione) on a human lung cancer cell line H460.

Material and Methods: CB11 was selected using chemical library (ChemBridge, USA) screening and its derivative CB11d1 was purchased from the same company. These chemicals as PPARγ agonists were confirmed by Oil Red O staining or effect of specific antagonist GW9662 in differentiated 3T3L1 cells. Cell viability was measured by WST assay. The subdiploid cellular DNA fraction, mitochondrial membrane potential (MMP) collapse and ROS were analyzed by flow cytometry. Activation of the caspase pathway by PPARγ agonist treatment was detected by Western blot analysis and caspase activity assay.

Results: Both CB11 and CB11d1 dose-dependently inhibited cell growth and induced apoptosis in H460 cells. Pretreatment of H460 cells with GW9662, a PPARγ specific antagonist did not recover cell viability showing that both CB11 and CB11d1 induced apoptosis in a PPARγ-independent pathway. These agonists increased activation of caspase −3, −8 and −9 as well as cleavage of poly (ADP-ribose) polymerase (PARP). Moreover, MMP collapse was increased by these agonists and blocked by pretreatment of cell with cyclosporin A, MMP inhibitor, suggesting that these agonists induced apoptosis via a mitochondria-dependent pathway. Concomitantly, both CB11 and CB11d1 increased ROS generation and DNA damage in H460 cells. ROS inhibitors such as N-acetyl-cystein (NAC) suppressed ROS generation, apoptosis and DNA damage suggesting that apoptosis by CB11 and CB11d1 is involved in ROS generation.

Conclusions: Our results suggest that novel PPARγ agonists, CB11 and CB11d1, may be used for the treatment of NSCLC.

No conflict of interest.

820 POSTER

Niclosamide radiosensitizes non-small cell lung cancer cells through activation of c-Jun

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Background: Radiotherapy is one of the effective modalities in the clinical treatment of cancers, and currently has been tried to combine with chemotherapy to improve therapeutic efficacy. Therefore, we aimed to develop small molecules that enhance the cytotoxic effects of radiotherapy. In this study, we provide evidence that Niclosamide can be an effective radiosensitizer in non-small cell lung cancer cells.

Material and Methods: To identify small molecules that increase cell death following radiotherapy in H1299 lung cancer cells, we screened a chemical library (a US-Drug Collection) containing 1,040 compounds using a cell viability assay system with Cell Counting Kit-8 in a 96-well plate format. A potent radiosensitizer among hits was validated by clonogenic survival assay, annexin V/PI staining and immunoblotting.

Results: Using a cell-based high-throughput screening with the 1,040 compounds in combination with irradiation, we found Niclosamide, an FDA-approved antihelminthic agent, exhibited radiosensitizing effect on H1299 human lung cancer cells. Combination treatment with Niclosamide and IR significantly reduced clonogenic survival of H1299 lung cancer cells in a dose-dependent manner and induced more apoptotic cell death than IR or Niclosamide alone, determined by increased level of PARP cleavages via caspase-3 activation and annexin V-positive cells. Given that IR induced apoptosis through generation of reactive oxygen species (ROS), we next examined ROS-induced molecular target signaling by the combination treatment of Niclosamide with IR. The combined treatment significantly induced phosphorylation of c-Jun in H1299 cells. Moreover, Niclosamide combined with H_2O_2 which was employed another ROS generator also induced c-Jun and its phosphorylation, leading to more increased apoptosis. N-acetyl-L-cysteine (NAC) treatment abolished c-Jun activation as well as apoptosis. Inhibition of c-Jun by siRNA also decreased PARP cleavages and attenuated clonogenic cell death of H1299 cells.

Conclusions: Our findings suggest that Niclosamide could be a promising radiosensitizer in lung cancer patients through activation of c-Jun which plays a pivotal role in ROS-induced apoptosis.

No conflict of interest.

821 POSTER

NFkB subcellular modulation and gene expression profile of chronic myeloid leukemia cell lines after treatment with the new compound LQB-118

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Background: Development of drugs more capable of overcoming tyrosine kinase inhibitors (TKIs) resistance, observed in about 30 % of chronic myeloid leukemia patients (CML), is of great importance nowadays. It was recently demonstrated by our group that treatment of CML cell lines (K562 and Lucena) with the new compound pterocarpanquinone LQB118 reduced the cellular viability and induced high levels of apoptosis. It was also observed a reduction in protein levels of glycoprotein-P (Pgp), and the inhibitor of apoptosis proteins (IAPs), XIAP and survivin. The NFkB transcription factor, which is capable of activating the transcription of Pgp, survivin and XIAP, is related to oncogenesis since regulates the expression of a variety of genes related to apoptosis, cellular proliferation and differentiation. The aim of the study was to evaluate the mechanism of LQB118 in inducing apoptosis and the gene expression profile of CML cell lines K562 and Lucena after treatment with LQB118.

Material and Methods: After treatment with LQB118 the levels of caspase-8 and IkBa, NFkB endogenous inhibitor, were analyzed by Western blotting. For NFkB subcellular localization, an immunofluorescence assay was performed. Proteasome activity was measure using Proteasome-Glo™. DNA microarray was performed to evaluate the differential gene expression profile of the CML cell lines treated with LQB118.

Results: LQB118 induced the activation of caspase-8 in both cell lines and maintained or induced IkBa levels, suggesting that NFkB was inactive in the cytoplasm. These results were confirmed by immunofluorescence, once NFkB was predominantly observed in the cytoplasm of the cell lines treated with LQB118. Further analysis also demonstrated that LQB118 was able to inhibit proteasome activity in both cell lines. DNA microarray results showed that LQB118 altered the expression of 109 and 75 genes, in K562 and Lucena, respectively. *TOB2, TAP2, NCF1* and *IKZF5* genes were differentially expressed after treatment with LQB118.

Conclusions: Our results suggest that LQB118 induced high levels of apoptosis in both CML cell lines through the extrinsec pathway of apoptosis and seems to be able to modulate NFkB activity, preventing its translocation to the nucleus, probably by avoiding IkB degradation by proteasome and, therefore, preventing XIAP and survivin expression. The study of the gene expression profile allowed us evaluate the putative genes involved in LQB118 mechanism.

No conflict of interest.

822 POSTER

Evaluation of tolerability and anti-tumor activity of GDC-0032, a PI3K inhibitor with enhanced activity against PIK3CA mutant tumors, administered to patients with advanced solid tumors

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Background: GDC-0032 is an orally bioavailable, potent, and selective inhibitor of Class I PI3K alpha, delta, and gamma isoforms, with 30-fold less inhibition of the PI3K beta isoform relative to the PI3K alpha isoform. Preclinical data show that GDC-0032 has enhanced activity against PI3K alpha isoform (PIK3CA) mutant cancer cell lines.
Material and Methods: A Phase I dose escalation study was conducted with evaluation of GDC-0032 doses ranging from 3−16 mg QD in a modified 3+3 design. Dose expansion cohorts at 9 mg QD were conducted in patients with solid tumors or with HER2-positive breast cancer. Safety and tolerability of GDC-0032 was assessed, as well as pharmacokinetics (PK), pharmacodynamic (PD) assessment of PI3K pathway inhibition by paired tumor biopsies and by FDG-PET, and anti-tumor activity by RECIST.
Results: Enrollment onto the dose escalation stage has been completed (n = 34). Two DLTs (G4 hyperglycemia and G3 fatigue) were observed at the 16 mg cohort. As of Nov 30, 2012, adverse events (AEs) assessed by the investigator as related to GDC-0032 in ⩾10% of patients, were diarrhea, fatigue, hyperglycemia, decreased appetite, nausea, rash, stomatitis, and vomiting. GDC-0032 has dose-proportional PK and a mean half-life of 40 hours, from 3−16 mg QD. PD inhibition of the PI3K pathway was observed via paired tumor biopsies as assessed by reverse phase protein array. Metabolic partial responses via FDG-PET (⩾20% decrease in $mSUV_{max}$) were observed in 12 out of 17 patients assessed (71%). Confirmed partial responses (PRs) have been observed in 5 patients who were treated at doses ranging from 3−12 mg QD. Of the 6 patients with PIK3CA mutant breast cancer (RECIST −30 to −70%), there have been 4 confirmed PRs observed. One confirmed PR has been observed in a patient with PIK3CA mutant NSCLC. Enrollment onto the solid tumor cohort (n = 13) and the HER2-positive breast cancer cohort (n = 10) has been completed. Preliminary PK and safety data from these expansion cohorts are consistent with those observed in the dose-escalation portion of this study. Updated data on clinical outcomes and biomarker correlates will be presented.
Conclusions: GDC-0032 is a next-generation PI3K inhibitor with promising anti-tumor activity observed in patients with PIK3CA mutant tumors. GDC-0032 is being investigated in combination with endocrine therapies such as letrozole and fulvestrant for patients with hormone receptor-positive breast cancer.
Conflict of interest: *Ownership: Richard Graham, Tim Wilson, Jerry Hsu (Genentech). Advisory board: Ian Krop, Jose Baselga (Genentech)*

823 POSTER

Reactivation of apoptosis in cancer cells with new class of fusion proteins

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Introduction: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) was going to be promising, natural therapeutic that selectively targets tumor cells while omitting normal cells. However it occurs that many cancer cell lines have developed a number of resistance mechanisms to TRAIL. Among recently discovered, overexpression of X-linked IAP (XIAP) molecules is considered as the main cause of TRAIL resistance.
There are many strategies, regarding chemo- and radio-sensitization, enhancing TRAIL efficacy, however combining TRAIL with chemotherapy may also sensitize normal cells to TRAIL induced apoptosis.
Here we present a recombinant variant of TRAIL fused with the peptide derived from Smac/DIABLO protein. The peptide we used is responsible for the interaction with BIR domains of IAP molecules. The fusion protein contains membrane transduction motif followed by sequences recognized by tumor-specific proteases (MMPs). General mechanism of action of this protein is considered as specific targeting the tumor by TRAIL, followed by activation (release) and transduction of pro-apoptotic peptide into cancer cells. Delivery of the Smac/DIABLO peptide blocks X-linked IAP (XIAP) proteins activity and reactivates apoptosis in cancer cell.
Methods: TRAIL/APO2L-SMAC/DIABLO fusion protein was expressed in *E.coli* and purified by IEC chromatography. Obtained protein was tested regarding *in vivo* distribution, apoptosis induction, protease cleavage and MTT cell cytotoxicity assays. For *in vivo* potential we examined the efficacy of fusion protein on mice xenograft model of colorectal adenocarcinoma (Colo205) and uterine sarcoma (MES-SA/Dx5) cells in comparison to reference − active TRAIL.
Results: We had obtained molecule and verified its mechanism of action. Our Smac/DIABLO-TRAIL fusion variant showed *in vitro* specific cytotoxic effect on various cancer cell lines at the level of IC50 below 0.1 ng/ml. In contrast to IC50 values obtained for cancer cell lines the fusion molecule showed no or very low activity on normal cells. The fusion protein showed specific targeting into the xenograft tumor and superior effect displaying significant tumor volume regression *in vivo* when compared with TRAIL.
All those results confirm that we had developed very promising molecule with high potential of pro-apoptotic activity that could be considered as a novel anticancer therapeutic agent showing clear synergistic effect of TRAIL and pro-apoptotic peptide.
No conflict of interest.

824 POSTER

A phase 1 study of oral rucaparib in combination with carboplatin

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Background: Targeting poly (ADP-ribose) polymerase (PARP), an enzyme involved in DNA damage repair, may increase efficacy of DNA-damaging agents. This study evaluated the tolerability of oral rucaparib, a potent and selective PARP1/2 inhibitor, in combination with carboplatin (CP).
Methods: Patients (pts) aged ⩾18 with advanced solid tumors were included. Pts received lead-in doses of IV and oral rucaparib on Days −10 and −5, respectively, followed by CP on Day 1 and oral rucaparib on Days 1−14 q21 days. Treatment continued until disease progression. Pts with benefit could continue rucaparib monotherapy once CP dosing was completed. Dose escalation was based on toxicities observed in Cycle 1 in cohorts of n = 3−6. PK was assessed during Cycle 1.
Results: 26 pts (median age 60.5 yrs [range 20−76]; 19 female; 10 ECOG PS=0; 7 ovarian/peritoneal (OC), 6 breast (BC), 2 NSCLC, 11 other tumor) were enrolled. Rucaparib doses of 80, 120, 180, 240, and 360 mg were administered with AUC3 CP, followed by 360 mg rucaparib with AUC4 and AUC5 CP. Two of 5 pts treated with AUC5 CP/360 mg rucaparib experienced dose-limiting toxicity (Gr 3/4 neutropenia & thrombocytopenia). Evaluation of AUC5 CP and 240 mg rucaparib is being completed. Treatment-related adverse events in ⩾5 pts, all grades, include fatigue (n = 11), anemia (n = 10), neutropenia (n = 8), thrombocytopenia (n = 8), nausea (n = 7), lethargy (n = 6), and constipation (n = 5). One pt (OC, $BRCA^{wt}$, AUC3 CP/180 mg rucaparib) had a PR of 5.1 mo duration and 1 pt (BC; $BRCA2^{mut}$, AUC5 CP/360 mg rucaparib) is ongoing in week 10 with confirmation of PR pending. Three pts (1 CRC, 2 OC; 2 $BRCA^{unk}$, 1 $BRCA^{wt}$) discontinued CP (after 4 or 8 cycles) and continued on rucaparib (additional 2, 5, or 10+ cycles). An additional 3 pts (mesothelioma, NSCLC, pseudomyxoma peritoneii; all $BRCA^{unk}$) had stable disease (SD) >12 wks. Overall disease control rate (CR+PR+SD>12 wks) in evaluable OC pts across all dose levels was 50% (3/6). Dose-proportional increase in rucaparib exposure was observed with steady state achieved by Day 14 and mean $t_{1/2}$ of 15 h. Oral bioavailability was 38% and dose-independent. Rucaparib exposure was not changed by CP co-administration.
Conclusions: The combination of oral rucaparib and CP exhibits activity at clinically relevant doses of each agent. Further studies in platinum-sensitive and homologous recombination repair deficient populations are warranted.
Conflict of interest: *Ownership: Heidi Giordano, Dayna Simpson and Sarah Jaw-Tsai are employees/stock holder of Clovis Oncology, Inc. Advisory board: Prof Ruth Plummer is an advisory board member for Clovis Oncology, Inc. Corporate-sponsored research: Institutions for Drs. Roxburgh, Molife, Gupta, Wilson, Evans, and Cresti received research funding for trial from Clovis Oncology, Inc.*

825 POSTER

Magnetic nanoparticle-entrapping liposomes for localized hyperthermia and controlled drug release in solid tumours

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Background: Hyperthermia is a powerful tool to trigger content release from thermosensitive liposomes (TSL) encapsulating chemotherapeutic drugs. A major challenge of nowadays' hyperthermia and TSL-mediated chemotherapy treatment, is the precise heat delivery to only trigger drug release in the tumour area, preventing healthy tissue toxicity. Nanometre scale magnetic iron oxide nanoparticles (MNPs) can, when exposed to an alternating magnetic field (AMF), generate heat and can also be imaged by magnetic resonance imaging (MRI). When MNPs are co-encapsulated with chemotherapeutics into TSL, a drug carrier is obtained, which under imaging-guidance can be heated to release its contents at any desired moment using a non-invasive AMF impulse. The aim of this study is to develop such MNP entrapping thermosensitive liposomes.
Material and Methods: We have applied various liposome preparation methods, to optimize incorporation of hydrophobic MNPs into liposome bilayers or hydrophillic MNPs in the aqueous core. Magnetoliposomes were analysed by dynamic light scattering for size, polydispersity index and zeta potential, followed by transmission electron microscopy. Magnetic properties of these samples were determined by T1, T2 measurements and NMRD profiling.
Results: When using film hydration, hydrophllic MNPs were loaded in aqueous core of 100 nm liposomes. For hydrophobic MNPs, more delicate approaches were required for incorporation into liposomal bilayers. The magnetic properties of MNPs after liposome incorporation remained unaltered.
Conclusions: Hydrophilic and hydrophobic MNP were incorporated in TSL. Current studies focus on optimising MNP loading efficacy and further characterization. After this stage hyperthermia potential and heat triggered drug release will be studied in vitro and in vivo.
No conflict of interest.

826 POSTER

Dual inhibitors of NFkappaB and Akt activation: Synthesis, in vitro anticancer activity and molecular docking studies

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Background: The C-2 arylidene analogs of pentacyclic triterpenoid Lantadene A **(1)** and B **(2)** were synthesized as dual inhibitors of NF-kappaB (NF-κB) and Akt. The compounds were further evaluated for *in vitro* anticancer activity against four human cancer cell lines (HL-60, MCF-7, A549 and HCT-116).
Material and Methods: The cytotoxicity was evaluated by using MTT assay, the NF-κB and Akt inhibition was evaluated by NF-κB luciferase assay and Akt kinase inhibition assay respectively. Automated molecular docking was performed to find out molecular interaction and optimized geometry by using docking software AutoDock 4.2.
Results: Analogs **3, 4, 7** and **8** showed enhanced inhibitory activity as compared with parent compounds **1** and **2**. These analogs were found more active than standard drug cisplatin with selective toxicity towards cancer cells and were inactive against normal cells (VERO). Furthermore, the mechanistic studies to investigate the effects of the new compounds on Akt protein in lung cancer cell line A549 and the NF-κB signalling pathway suggested that the compounds may exert their inhibitory activity on cancer cells through inhibition of both Akt and NF-κB activation. The docking studies of most potent analog **(7)** with 3D crystal structure of the nuclear factor kappa-B (NF-κB) P50 homodimer (PDB ID: 1NFK) revealed that carbonyl group of ester side chain and C-28 carboxylic acid groups were mainly involved in hydrogen bonding interaction. The oleanane frameworks was involved in strong hydrophobic interaction with amino acid phenylalanine and structure of lead compound have the potential to be developed as potent NF-κB inhibitor and anticancer agent.
Conclusions: Novel dual inhibitors of NF-κB and Akt activation inhibitors were synthesized and found to have potent selective anticancer activities in low micro molar range.
No conflict of interest.

827 POSTER

A phase I of study of Everolimus (EVE) in combination with LBH589B (LBH) [HDAC inhibitor] in advanced malignancies with enrichment for EBV driven tumors

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Background: Pre-clinical evidence suggests that combination of HDAC and m-TOR inhibition abrogates multiple EBV-driven oncogenic pathways by increasing expression of EBV lytic genes coupled with immune-modulatory and anti-angiogenic effects.
Material and Methods: Patients with advanced malignancies enriched for EBV-driven cancers were enrolled to determine the safety, tolerability, maximum tolerated dose (MTD), pharmacokinetics, pharmacodynamics and preliminary anti-tumor activities in a 3+3 dose escalation design. LBH was administered 7 days prior to initiation of combination treatment. NPC patients received either acyclovir or val-acyclovir prophylaxis. Serum EBV-DNA levels were measured weekly and plasma cytokines profiled using a 31-plex luminex panel.
Results: 20 patients have been treated [male:female 15:5, median age 52.5 (37−63, 11 nasopharyngeal carcinoma (NPC) and 9 non-NPC] at 4 dose levels − LBH (3x/week)-EVE (daily):10 mg-2.5 mg (3pts), 10 mg-5 mg (6pts), 15 mg-2.5 mg (exploratory) (6pts) and 15 mg-5 mg (5pts). 73 cycles of treatment were administered in total. Two dose limiting toxicities of G4 (grade) thrombocytopenia were observed at LBH 15 mg-EVE 5 mg. Significant adverse events (AE) (G⩾3) were dysphagia (1),diarrhoea (1), epistaxis (1) and thrombocytopenia (3). Common AEs (G1/2) included mucositis (70%), fatigue (65%), anorexia (50%), fever (40%), cough (30%) and diarrhoea (30%). One patient experienced a partial response (lymphoma) and 6 patients (3 NPC, 2 breast and 1 renal cell carcinoma) had prolonged stable disease (>16 weeks). Modulation of EBV DNA titres was seen only in NPC patients, with median fold-change from baseline of 9 (0.9–174). In a limited patient subset (n = 9, 30 timepoints), plasma cytokine profiles were consistent with a T- cell response, specifically, elevated levels of FLT3L, IFN-gamma, IL-13 and IL-17. Target engagement was also observed with increased histone-3 acetylation as early as 4 hours after administration of LBH. Results of the PK studies would be presented during the meeting.
Conclusion: Combination of LBH and EVE resulted in induction of EBV DNA titres with concomitant T-cell host response. The recommended phase 2 dose is LBH 10 mg 3x/week and EVE 5 mg daily in an Asian population comprising of predominantly NPC patients.
No conflict of interest.

828 POSTER

p21Cip1/Waf1-mimetic peptide bound to elastin-like polypeptide carrier enhances bortezomib cytotoxicity in androgen-independent prostate cancer cell lines

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Background: Bortezomib is a proteasomal inhibitor approved for the clinical use in hematological tumors. However, due to a narrow therapeutic window it has a limited potential to be utilized as a treatment for solid tumors. In this work we have investigated the effect that externally delivered $p21^{Cip1/Waf1}$-mimetic peptide (aa 139–164) has on bortezomib cytotoxicity in DU-145 and PC-3 androgen-independent prostate cancer cell lines.
Materials and Methods: In this research elastin-like polypeptide (ELP) was used for the intracellular delivery of the p21-mimetic peptide. ELP is a genetically engineered, thermally responsive macromolecular carrier previously shown to be able to improve the stability of therapeutic peptides as well as to mediate their targeted delivery in response to mild hyperthermia. The effect that ELP-bound p21 peptide has on bortezomib cytotoxicity was measured using MTT test. Flow cytometry was used to quantify ELP-p21 peptide cellular uptake, as well as changes in the cell cycle distribution, apoptosis and autophagy induction after the combination treatment with bortezomib and ELP-p21 peptide. Western blot technique was used to monitor the change in the expression of various proteins involved in cell cycle regulation, while the senescence-associated beta-galactosidase assay was used to assess changes in the senescence activation.

Results: We demonstrated that co-treatment with bortezomib and ELP-bound p21-mimetic peptide carrier enhanced bortezomib cytotoxicity in both androgen-independent prostate cancer cell lines. In our research the ELP-p21 polypeptide led to a 1.5-fold decrease in the bortezomib IC_{50} value in the two cell lines tested. The combination treatment affected the cell cycle distribution and caused an intra-S phase cell cycle arrest. Additionally, the combination treatment augmented autophagy and apoptosis induction. On the protein level, we detected a different pattern in the cyclin D1, E and B1 expression, which we believe is due to the different status of the Rb protein in the two cell lines tested. Moreover, in contrast to PC-3 cell line that possesses wild type Rb protein, DU-145 cells, that contain non-functional Rb protein, did not display increase in the senescence induction upon the combination treatment.
Conclusion: In summary, our results suggest that ELP-bound p21-mimetic peptide may prove to be useful tool in combination therapy with proteasomal inhibitors.
Conflict of interest: *Other substantive relationships: D. Raucher is the president of Thermally Targeted Therapeutics, Inc., Jackson, MS, USA*

829 POSTER

Immunoliposomes with single-domain antibodies targeted against mucin 1 (MUC1)

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In response to the lack of specificity in anti-cancer drugs, we want to use anti-MUC1 antibodies to improve targeting of drug-loaded liposomes to cancer cells. Liposomes can be used as carrier for various molecules, including targeting moieties and chemotherapeutics, to increase specificity or reduce toxicity. After extravasation the drug needs to be targeted to the tumor cells to be most effective. We use MUC1 as target, since it is overexpressed in pancreatic cancer, which is difficult to treat and has a 5-year survival rate of 6%. In this project we will use liposomes targeted with human single domain antibodies, i.e. antibodies containing only the variable domain of the antibody's heavy chain. Due to their small size (10−15 kDa) they are likely to be much less immunogenic than whole antibodies coupled to liposomes, but with all complementary determining regions intact, they can still show high affinity for their targets.
A transgenic mouse has been developed, which enables the production of heavy-chain-only antibodies of human origin. After immunization with MUC1, antibodies have been isolated and expressed to create a clone library. These clones have been screened for affinity on FortéBio Octet and have been used for subcloning of heavy chain variable domains with an additional cysteine. This cysteine will be used for thiol-maleimide conjugation to the polyethylene glycol (PEG) chains of liposomes. Affinity measurements of the single-domain antibodies and the antibody-liposome complex will be performed on MUC1 positive cell lines, such as CFPAC-1, by flowcytometry and immunostaining.
After immunization of several mice, and selection steps against MUC1, twelve related heavy-chain-only-antibodies have been found with dissociation constants (Kd) between 1E-9 to 1E-10 M. These twelve will be tested on MUC1 positive cell lines and a selection will be made into a single-domain format, which can be used for liposome conjugation and further affinity measurements. Liposomal conjugation with other antibodies has been performed, where antibodies are conjugated to micelles, which are post-inserted into preformed liposomes. These liposomes will be optically labeled to enable *in vivo* biodistribution studies.
Research on liposomes equipped with single-domain antibodies will help to identify novel targeted liposomal chemotherapy formulations that combine the liposomal drug encapsulation together with improved cell targeting and intracellular drug delivery.
No conflict of interest.

830 POSTER

Paclitaxel resistance is associated with drug accumulation in intracellular compartments and paclitaxel-binding proteins in human lung cancer cell lines

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Background: Several mechanisms have been suggested for paclitaxel resistance in cancer cells, including overexpression of the multidrug transporter gene, ATP-binding cassette, sub-family B, member 1 (*ABCB1*), and the presence of a point mutation in the β-tubulin gene at the paclitaxel-binding site. However, the mechanisms underlying resistance to this agent have not yet been completely elucidated.
Material and Methods: Three human lung cancer cell lines, II18, A549, and RERF-LC-KJ, were analysed; their 50% inhibitory concentrations of paclitaxel were −8.33, −7.69, and −4.51 logM, respectively. The cell lines did not have any β-tubulin mutation. We evaluated the expression levels of *ABCB1*, intracellular accumulation of paclitaxel, paclitaxel-induced stabilisation of microtubules, and intracellular localisation of Oregon Green® 488-conjugated paclitaxel in these cell lines. Moreover, we prepared paclitaxel conjugated ferriteglycidyl metacrylate (FG) beads to purify paclitaxel-binding proteins from whole cell lysates of these cells.
Results: The *ABCB1* expression level was strongly correlated to intracellular [^{3}H]-paclitaxel accumulation (r^2 = −0.804) but was not related with paclitaxel resistance. The changes in the quantities of polymerized tubulin and acetylated tubulin after paclitaxel exposure were not related to paclitaxel resistance. Differences were observed between the intracellular localisation of paclitaxel in RERF-LC-KJ, the most resistant cell line, and in the other 2 cell lines. The use of Oregon Green® 488-conjugated paclitaxel enabled visualization of not only the normal microtubule formation in the partial cells but also the aggregated vesicle formation in RERF-LC-KJ cells; aggregated vesicle formation was not remarkable in the other cell lines. Affinity purification by paclitaxel immobilised beads revealed several specific bands in RERF-LC-KJ; these bands were not revealed in the other cell lines.
Conclusions: We propose that paclitaxel resistance is associated with intracellular compartments in which paclitaxel accumulates and paclitaxel-binding proteins expressed specifically in resistant cell line.
No conflict of interest.

831 POSTER

Naturally occurring isothiocyanates potentiate doxorubicin cytotoxicity in doxorubicin-resistant colon cancer cells

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Background: Doxorubicin and other anthracyclines are well-known, widely used and very efficient cancer therapeutics agents. Unfortunately, prolonged administration very often causes severe toxic effects and leads to resistance, thereby limiting the usability in chemotherapy. One of potential strategies to lower the toxicity and overcome resistance is to use an additional compound as a sensitizing agent making cancer cells more susceptible to cytostatics. Naturally occurring isothiocyanates (ITC), due to their low toxicity and multi-targeted mechanism of action, have been recently introduces as a potentially effective agents in combined therapy.
Material and Methods: Isothiocyanates, including benzyl, 3,4-dimetoxybenzyl and 6-hydroxyhexyl isothiocyanate were tested in combined therapy with doxorubicin in two colon cancer cell lines − drug-sensitive LoVo and its doxorubicin-resistant subline LoVoDX. Several different schedules of treatment were checked and their outcome was analyzed using MTT assay. Further studies on the combination mechanism of action involved determination of: ROS production, doxorubicin accumulation and caspase-3/7 activity, as well as cell cycle and glutathione level analysis.
Results: Short, 1 hour pretreatment with isothiocyanates (concentration in the range of 10−2.5μM) followed by medium removal and doxorubicin treatment for next 48 hours led to the most pronounced synergistic effect (Combination index CI, calculated using Chou-Talalay method 0.28–0.45). Isothiocyanate concentration proved to be an important factor affecting observed effect − concentrations lower than 2μM used in above mentioned schedule gave additive or even an antagonistic effect. In LoVoDX cell line 3,4-dimetoxybenzyl isothiocyanates almost completely abrogated doxorubicin resistance (resistance index RI calculated as a LoVoDX/LoVo IC_{50} ratio − 9 without isothiocyanate, and 1.6 for combination). Doxorubicin accumulation remained at the same level after isothiocyanates pretreatment, thereby increased drug concentration appears to be not involved in increased cytotoxicity. Further studies showed that glutathione depletion caused by isothiocyanates (30%-45% decrease in glutathione content after 1 hour) led to increased ROS-production in cells treated with combinations. Moreover, isothiocyanates pretreatment induced cell cycle arrest in G2/M phase which might be also associated with increased doxorubicin cytotoxicity. The final outcome of combined treatment was apoptotic cell death indicated by caspase-3/7 increased activity.
Conclusions: Naturally occurring isothiocyanates proved to be a potent sensitizing agents when used along with the doxorubicin in properly designed schedule. Their main mechanism of action in combined treatment appears to be based on the modulation of cell cycle and redox status.
No conflict of interest.

832 POSTER

Gene resilencing following decitabine therapy is initiated by nucleosome reoccupancy and is related to CpG island shore methylation

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Decitabine is a front line therapy for myelogenous leukaemias and is in clinical trials for various solid tumour types. This DNA demethylating agent reactivates genes silenced by promoter hypermethylation in cancer. However upon drug withdrawal reactivated genes undergo resilencing which may underlie drug resistance. While resistance is associated with resilencing of genes, the mechanism underlying this resilencing is unknown. We aimed to decode the ordered sequence of epigenetic events associated with resilencing in order to improve the clinical effectiveness of Decitabine. Using biallelically methylated MLH1 as a model gene, we profiled epigenetic changes at the MLH1 promoter associated with reexpression and resilencing in a colorectal cancer cell line before, during and after Decitabine treatment. In contrast to the closed chromatin structure observed before treatment, Decitabine induced increased MLH1 expression and 54% decreased promoter methylation. Using single molecule analysis at multiple time points, we show that gene resilencing, which occurs 6−8 days following removal of therapy, was initiated by nucleosome reassembly on demethylated DNA and only then was followed by remethylation and stable silencing. Furthermore, long-term monitoring of cells following treatment showed MLH1 resilencing never reverts to pretreatment levels with low-level MLH1 expression and demethylated MLH1 promoter alleles persisting up to 118 days after withdrawal of Decitabine. Genome-wide methylation profiling was used to categorise promoter CpG Islands (CGI) based on their degree of demethylation after treatment. This revealed that CGIs with persistence of demethylated promoter alleles after long-term recovery ($n = 108$) showed significantly lower levels of CGI shore methylation ($p = 0.0231$). Our findings suggest a role for shore methylation in susceptibility to CGI remethylation. The data also establishes the importance of nucleosome positioning in mediating resilencing of drug-induced gene reactivation and suggest a role for therapeutic targeting of nucleosome assembly as a mechanism to overcome drug resistance.

No conflict of interest.

833 POSTER

New pharmacological approaches against human epidermal growth factor receptor 2 (HER2+) resistant breast cancer

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Background: Therapeutic acquired resistance of HER2 positive breast cancer represents a major health care problem given the lack of curative interventions for a large subset of patients bearing this disease. Many efforts are being devoted to find new pharmacological strategies for patients unresponsive to existing treatments. Our group has shown that HER2+ tumors also overexpress fatty acid synthase (FASN+).

The purpose of this study is to test drugs inhibiting different molecular targets to assess the effects in HER2+ breast cancer, even in those resistant to anti-HER2 drugs.

Materials and Methods: We have developed long term HER2+ breast cancer cell lines (SKBr3) resistant to the HER2-monoclonal antibody (trastuzumab, herceptin®) (SK**TR**), the EGFR/HER2-tyrosine kinase inhibitor (lapatinib, tykerb®) (SK**LR**) or both (SK**LTR**). Once established, we have characterized these cells by studying a panel of EGF receptors signaling proteins with western blot analysis, changes in adherence to extracellular matrix proteins and invasion capacity with colorimetric assays. Using MTT assay, we have assessed the effect of a HER2 dimeritzation-inhibitor (pertuzumab, perjeta®), an mTOR-inhibitor (temsirolimus, torisel®) and a new FASN inhibitor developed in our laboratory (G28UCM) on the viability of parental and resistant cells. Currently, we are establishing orthotopic xenograft mice models to test the anti-cancer effect of these drugs, alone or in combination.

Results: Resistant cells maintained downstream HER2 pathway activation by stimulating the expression/activation of alternative EGF family receptors and/or those specific ligands. Moreover, these cells increased adherence to extracellular matrix proteins and invasion capacity.

The HER2-dimerization inhibitor (perjeta®, 50 μg/ml) produced a decrease of approximately 20−40% in cell viability, even in resistant cells non-responding to trastuzumab and lapatinib. Inhibition of mTOR had a potent effect in parental and resistant breast cancer cells, producing a drop of 30% in cell viability with 4.8−6 μM of temsirolimus. The inhibition of 30% in cell proliferation was obtained with 5.5–11.6 μM of G28UCM. Moreover G28UCM improved the cytotoxic effects of other known FASN inhibitors (C75 and EGCG).

Conclusions: FASN inhibition, alone or in combination with other targets (such as mTOR or EGF family receptors) could be a new pharmacological strategy to fight HER2+ breast cancer resistant to trastuzumab and lapatinib. New studies in ortoxenopatients are needed to strengthen this study.

Conflict of interest: *Other substantive relationships: Puigner V. is employed by Roche Farma SA, which kindly yielded us pertuzumab, perjeta™. Viqueira A, Bolos MV are employed by Pfizer, which kindly yielded us temsirolimus, torisel™.*

834 POSTER

Epithelial–mesenchymal transition confers resistance to FGFR inhibitors in gastric cancer cell line

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Background: Targeted therapies based on kinase inhibitors may bring encouraging results but they frequently elicit resistance that makes the therapy ineffective and is often accompanied with cross-resistance to other drugs. Understanding and anticipation of the resistance mechanism for novel targeted drugs provides new approaches to use alternative or combine therapy which would improve patients' chances for recovery.

Fibroblast growth factor receptor (FGFR) comprises a promising target for anticancer therapy as it's amplification, mutation or overexpression is considered to be an oncogenic driver in various types of human neoplasms. Several clinical trials are currently carried out with the use of FGFR inhibitors but there still lacks the information about possible mechanisms of resistance to therapy in treated patients. The aim of the study was to define the mechanisms of acquired resistance to FGFR inhibitors: AZD-4547, BGJ 398 and PD173074 in selected in vitro models.

Material and Methods: To explore the mechanism of acquired resistance to FGFR inhibitors we have applied SNU16 human gastric cancer cell line with FGFR2 amplification. The cells were cultured with increasing concentrations of each inhibitor: AZD-4547, BGJ398 or PD173074 until reaching a concentration exceeding the IC50 value ten times. The mechanism of resistance was verified using immunoblotting techniques.

Results: In the following study we have established three gastric cancer cell lines SNU16R AZD, SNU16R BGJ, SNU16R PD, resistant to selective FGFR inhibitors AZD-4547, BGJ398 or PD173074, respectively. Since we found the loss of FGFR phosphorylation in all three lines we concluded that the resistance results from activation of alternative signaling pathways and is not evoked by mutation in FGFR kinase gene. We found however loss of several cell surface growth factor receptors like cMet or EGFR in these lines. Concurrently, the protein profile of established cell lines indicated epithelial–mesenchymal transition (EMT). We found the decrease in E-cadherin level and an increase of vimentin which are markers of EMT.

Conclusion: Our results reveal that one of the mechanisms of acquired resistance to FGFR selective inhibitors can be epithelial–mesenchymal transition. To our best knowledge it is the first time to show EMT as mechanism of resistance to the therapy targeting at FGFR. This finding indicates that EMT could emerge in patients treated with FGFR inhibitors.

No conflict of interest.

835 POSTER

Molecular mechanisms of resistance to protein kinase B inhibitors of the alkylphosphocholine family

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Background: Resistance of solid tumors to standard chemotherapy and radiotherapy remains challenging to successful treatment. But new hypothesis based on current studies reveal that tumor cells also show resistance to small molecule signal transduction inhibitors, including the membrane-targeted apoptosis modulators of the alkyllysophospholipid drug family (van der Luit A, Biochem J 2007). Drugs of the ALP or alkylphosphocholine (APC) drug families potently induce apoptotic cell death in various cancer cells and enhance radiation-induced cell death as well as radiation induced eradication of clonogenic tumor cells.

Aim of the proposed project was to mimic clinically relevant long-term treatment with the intravenously applicable APC erucylphosphocholine (ErPC) to select for ErPC resistance and to use a proteomics approach

to identify proteins and signaling networks that promote acquired drug resistance in these cells.
Material and Methods: Non-small cell lung cancer cells (A549) were treated in 20 cycles with increasing concentrations of ErPC (10 to 50μM). Drug resistance was confirmed by using standard short term (proliferation, apoptosis) and long-term (clonogenic survival) assays. Potential cross-resistance to treatment with ionizing radiation (IR) was determined accordingly. Proteome analysis was performed by 2D differential gel electrophoresis (DIGE) and subsequent mass spectrometry of altered protein spots. Changes in protein expression ratio were calculated from related spot intensities.
Results: Chronic treatment with rising ErPC concentrations resulted in the selection of A549 with increased resistance to ErPC. Surprisingly, the drug-resistant cells displayed increased susceptibility to IR compared to the non-selected controls. Fluorescence microscope pictures showed morphological changes featuring multiple vesicle-like structures with high auto-fluorescence. Proteome analysis via 2D differential gel electrophoresis and mass spectrometry evidenced changes in expression level of structural proteins and of proteins involved in stress defense.
Conclusions: The role of specific deregulated proteins for cellular sensitivity to ErPC and IR is subject to current investigations. Understanding the mechanisms underlying drug resistance will allow the design of combination strategies that exploit predicted adaptive changes to prevent or overcome drug resistance and improve treatment outcome.
No conflict of interest.

836 POSTER

Curcumin-mediated oxaliplatin resistance reversal in CRC cell lines through modulation of NF-kB, STAT3 and CDK5 signaling pathways

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Background: Oxaliplatin (OXA) is a chemotherapeutic drug widely used in the treatment of colorectal cancer (CRC). Unfortunately, acquired resistance results in a major obstacle for effective treatment. In previous work we observed an up-regulation of Cyclin-dependent kinase 5 (CDK5), pS727-STAT3 and p-P65 (NF-κβ) in the HT29-derived OXA-resistant cell line, HTOXAR3. Curcumin (diferuloylmethane), the major active ingredient of turmeric (*Curcuma longa*), without discernable toxicity, has been shown to inhibit the growth of transformed cells and colon carcinogenesis in rodent models. Curcumin has also been shown to suppress activation of transcription factors NF-κβ and STAT3. The aim of this work was to demonstrate whether the combined treatment of curcumin and OXA could revert the acquired resistance to the latter in HTOXAR3 cells.
Material and Methods: Curcumin IC50 determination and the effect of curcumin and OXA treatment on the proliferation of HT29 and HTOXAR3 cells was determined by a MTT assay and data were analysed by Chou and Talalay method. Curcumin time-dependent phosphorylation status of CDK5 (tyr-15), STAT3 (S727) and P65 (Ser536) was studied by Western Blotting at different times after curcumin treatment.
Results: Curcumin IC50 (μM) was approximately the same for both cell lines (HT29 IC50: 10.2±0.85, HTOXA IC50: 11.2±0.43). HT29 and HTOXAR3 cells were treated with 10μM curcumin for 4, 8, 24 and 48 h. As compared with untreated cells, maximum effect was observed after 48 h. pSTAT3 inhibition was 40% in both HT29 ($p = 0.0036$) and HTOXAR3 ($p = 0.0016$); pCDK5 inhibition was 60% in both HT29 ($p = 0.0006$) and HTOXAR3 ($p = 0.0073$) and pP65 inhibition was 30% in HT29 ($p = 0.0067$) and 40% in HTOXAR3 ($p = 0.0011$). We investigated the effect of sequential and concomitant treatment of OXA and curcumin. Preliminary results indicate that the highest effect is obtained in the concomitant schedule for 24 h as compared with sequential treatments (24 h-treatment with OXA or curcumin followed by an additional 24 h-treatment with OXA or curcumin). Cell viability was 27% (curcumin + OXA), 9% (OXA + curcumin) and 2% (concomitant treatment) as compared with individual exposures.
Conclusions: We demonstrated that curcumin suppressed activation not only of NF-κβ, STAT3 but also CDK5 in HT29 and HTOXAR3 cell lines, providing evidence that curcumin can be used in therapeutic regimes directed against CRC and suggesting that in combination with OXA, it could revert the resistant phenotype to this drug. Further experiments are ongoing in order to elucidate the best combination schedule.
No conflict of interest.

837 POSTER

A novel 3D cell culture system for in vitro evaluation of anticancer compounds

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Background: Traditional method for evaluating chemo-therapeutic drug has generally employed single layer of cells grown on plastic surface (2-dimentional cell (2D) cultures), which is physiologically different from the natural environment of the cells. Screening compounds by using the method therefore has the potential to result in less effective anticancer drugs than anticipated when tested in clinical trial. Recently, a number of approaches have been developed to generate 3D cell culture models for cancer study; e.g. scaffolds, microcarriers, and spheroids. However, many challenges remain, such as applying them into high throughput screening systems and improving the efficiency of anticancer drug discovery.
Material and Methods: In this study, we screened large-molecule compounds for the ability to suspend cancer cells homogeneously in liquid culture medium with keeping the medium low-viscosity, and identified FP001 as the most potent compound. We also established a novel method for the generation of cancer cell spheroids in suspension by using FP001 in ultra-low attachment multiwell plates. The cultured spheroids of cancer cells were characterized in terms of cell growth, apoptosis, cell cycle, and susceptibility to anticancer drugs to demonstrate the competency of the method.
Results: We cultured A549, HCT116, and HeLa cells for 5 days in medium containing FP001 and observed their cellular appearance and growth. The spheroids formed in the 3D culture medium were appropriate in size and suspended homogeneously, which led to easy handling for various assay. The cultures with FP001 exhibited a >10-fold increase in the number of living cells and contained decreased numbers of $BrdU^+$ cells and increased numbers of Annexin V^+ cells as compared to those with vehicle. Furthermore, the cells cultured in the medium were more sensitive to Mitomycin C, and the inhibition concentration obtained by the method was closer to actual blood level than that by 2D cultures. These data suggest that FP001 promotes the proliferation of spheroid forming-cancer cells which allow practical sensitivity to anticancer compounds. We are now investigating the feasibility of the method for development of automated and miniaturised screening systems.
Conclusions: We have identified FP001 as a novel substrate for the 3D culture of cancer cells. The approach using FP001 would facilitate the development of novel models for in vitro evaluation of anticancer compounds.
No conflict of interest.

838 POSTER

High-throughput assay for screening cancer metastasis inhibitors in human cancer cells using adenoviral knock-down

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The de-differentiation from a normal non-invasive epithelial cell into an immortalized, metastatic cell requires deregulation of multiple cellular processes. Our aim was to establish a high-throughput assay in human cancer cell lines to identify novel drug targets that reverse this deregulation by inducing Mesenchymal-to-Epithelial transition (MET).
Invasive mesenchymal cells express vimentin whereas non-invasive epithelial cells express E-cadherin, and the ratio between these markers reflects the identity of the cancer cells. We established an image-based functional high throughput assay measuring these markers in different cancer cell lines. Images were acquired on an INCell2000 Analyzer and an in-house written algorithm was used to calculate vimentin and E-cadherin expression. Using this assay, we screened an adenoviral shRNA knock-down library directed against human drugable genes for their potential to induce MET.
We successfully developed an automated high-throughput MET assay in multiple cancer cell lines which can be used for discovery and validation of novel targets for their potential to inhibit metastasis. Using this approach, we identified new targets as well as known players involved in MET. Knock-down of the transcription factor Snail, known to control epithelial–mesenchymal transitions, induced MET, similar to knock-down of members of the Wnt-signaling pathway.
Discovery of novel targets in metastasis may lead to the development of small molecule compounds or antibody therapeutics in cancer therapy.
No conflict of interest.

839 POSTER

A novel cell assay to screen for wnt pathway inhibiting drugs

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The wnt pathway is one of the most crucial pathways in colon cancerogenesis: In most colorectal cancers (CRC) wnt correlated genes are mutated. Since screening assays for drugs modifying this important pathway are still rare we generated a stable cell line transfected with a new fluorescence-based reporter.
Therefore, we designed a reporter gene construct based on the well-established TOPFLASH reporters coupled with eGFP instead of luciferase and then stably transfected into SW480 cells. As the wnt-state in this cell line is known to be active the new cell line named SW480-SuperTopEGFP can easily be used as a screening tool for new inhibitors the pathway.
In preliminary experiments the known wnt inhibitory compounds acetylsalicylic acid, XAV939 (tankyrase inhibitor; Hollande et al., 2010), PKF118–310 (Tcf4/β-catenin complex inhibitor; Wei et al., 2010) or FH535 (β-catenin inhibitor, Handeli et al., 2008), bleached the fluorescence of the tested well and measured the fluorescence recovery 24 hours after treatment compared to the untreated control. This resulted in a lower fluorescence signal in treated cells than in untreated cells. Following this treatment we used a self-made set-up to bleach cells seeded in a 96-well plate simultaneously. For quantification the fluorescence recovery time can be measured after treatment to have a dimension for the effectiveness for specific wnt inhibitors.
In conclusion we established an assay-set-up capable for use in automated image screening assays. In comparison to already established assays this new approach is much more cost-effective and easy to perform. Therefore this is a further step towards in specific compound research targeting the wnt pathway.
No conflict of interest.

840 POSTER

XenoBase enabled growth rate analysis improves xenograft study design

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Xenograft models are widely used in the oncology drug discovery and development process. However, the lack of available information regarding the Xenograft models, such as growth curves, standard of care treatment results, IHC analysis of biomarkers, as well as target gene expression, mutation and amplification, hampers the selection of suitable models for accurate evaluation of therapeutic molecules. We have created XenoBase™ that combines public cell line profiling data with our own data on >180 xenograft models. A searching engine is also built in the database to search for models based on gene mutation, expression, amplification, as well as SOC information, types (orthotopic, subcutaneous, systemic) of the xenograft models. The XenoBase™ will enable informed decision in selecting the most relevant models for the development of targeted therapeutics.
Armed with hundreds of xenograft studies available in the XenoBase™, we analyzed the tumor growth rate of each study in order to compare the results with traditional T/C analysis. The T/C analysis is the current standard, and a T/C value less than 0.42 is widely accepted as an indication of efficacy in evaluating a test article. However, this T/C analysis is limited in using only the data points on one day, overlooking the fact that tumor growth curves are generated over a long period of time (months) and with many days of data collection. To take advantage of the tumor growth curves, we analyzed tumor growth rate utilizing every data points, and derived growth rate based on slopes. Our analysis with the XenoBase™ data indicated that the growth rate based approach is more powerful in evaluating test articles for efficacy. If adopted by the industry, this approach may save hundreds of millions of dollars spent in excess number of animals and prolonged observations that may not be necessary.
No conflict of interest.

841 POSTER

High-throughput 3D screening reveals differences in drug sensitivities between culture models of JIMT1 breast cancer cells

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Background: Many cellular features are impaired in the traditional 2D cell culture conditions and big alterations in gene expression in comparison to tumors have been reported. Three-dimensional (3D) cell culture models are suggested to be better models than 2D monolayers due to improved cell-to-cell contacts and structures that resemble in vivo architecture.
Materials and Methods: The aim of this study was to develop a simple high-throughput 3D drug screening method and to compare drug responses in JIMT1 breast cancer cells when grown in 2D, in polyHEMA induced anchorage independent 3D models and in Matrigel 3D cell culture models. We screened 102 compounds with multiple concentrations and biological replicates for their effects on cell proliferation. Gene expression patterns of cells in the different culture models were also compared to xenografts.
Results: Big variations in drug responses were observed between the models. We show that, in general, JIMT1 cells grown on Matrigel were significantly more sensitive to drugs than cells grown in 2D cultures, while responses of cells grown in polyHEMA resembled those of 2D. Furthermore, comparison of gene expression profiles of the cell culture models to xenograft tumors indicated that cells cultured in Matrigel and as xenografts most closely resembled each other.
Conclusions: 3D cultures can provide a platform for systematic experimentation of larger compound collections in a high-throughput mode and can be used as alternatives to traditional 2D screens towards better comparability to in vivo state.
No conflict of interest.

842 POSTER

5-FU Monitoring in clinical practice: Pharmacokinetic variability

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Background: Current dosing of 5-Fluorouracil (5-FU) is based on body surface area (BSA). However BSA dosing has been associated with clinically significant pharmacokinetic (PK) variability. Dosing based on BSA has been seen to result in low exposure and loss of efficacy, or high exposure and severe toxicity. The reported target area under the concentration vs. time curve (AUC) for 5-FU is 20−30 mg.h.l^{-1}. 5-FU therapy which achieves this AUC has been shown to improve response rates and minimize toxicity. The aim of this study was to evaluate the practicality of sampling 5-FU for dose management and evaluate the PK variability resulting from BSA dosing in commonly used continuous infusion (CI) regimens for colorectal cancer (CRC).
Material and Methods: Blood samples were obtained from 24 patients with colorectal cancer receiving 5-FU CI: 9 patients received FOLFOX 4, 7 received FOLFIRI, 7 received De Gramont and 1 5FU/LV. The mean dose of 5-FU administered was 1151 mg/m^2 (range 440–1270 mg/m^2). In total 57 EDTA samples were drawn a minimum of 1 hr before the end of the 5-FU CI at 5-FU steady state concentration. The 5-FU concentrations were subsequently quantified on a Roche Cobas® 6000 using a homogeneous immunoassay (MyCARE™ 5-FU, Saladax Biomedical, Inc.). The 5-FU AUC was calculated from the reported plasma concentrations and one result was discarded as it was clearly an outlier.
Results: The 24 patients analyzed demonstrated a wide range of AUCs: ranging from 2.7 to 37 mg.h.l^{-1} with a mean of 12.2 mg.h.l^{-1} and a standard deviation (SD) of 6.19 mg.h.l^{-1}. There was no significant correlation observed between 5-FU dose and AUC for any of the regimens − overall R^2=0.0312; p = 0.576.

Regimen	Patients/ Samples	AUC, mg·h·l^{-1}		Number of patients below range
		Mean	SD	
Folfox 4	9/24	13.7	7.0	8 (89%)
Folfiri	7/18	8.4	2.7	7 (100%)
DeGramont	7/13	14.4	6.2	6 (86%)
5-FU/LV	1/1	15.3	−	1 (100%)

Conclusions: These data support the previous reports that standard BSA dosing of 5-FU leads to a high PK variability. Using the optimal AUC range of 20−30 mg.h.l^{-1}, out of the 24 patients, 22 (92%) were under the target level with only 2 out of the 24 receiving an initial dose resulting in an AUC within the target range. Exposure appeared independent of regimen. Based on the results of this small study it appears that sampling and measuring concentrations of 5-FU during CI to adjust the dose to reach optimal exposure is practical and may be a rational approach to delivering effective treatment.
No conflict of interest.

843 POSTER

Nanostructured silica functionalized with an organotin compound induces differentiation of B16 melanoma cells

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Background: Cancer nanotechnology is a promising area of research in science and medicine that finds application for molecular imaging, molecular diagnosis, and targeted therapy. Using targeted nanoparticles with anti-cancer drugs offers the possibility of destroying tumors with minimal damage to healthy tissue and organs using significantly lower doses of toxic chemotherapy. Nanostructured silica-based materials proved to be an excellent candidates as drug carrier for cancer therapy. Here we evaluated potential of silica nanoparticles SBA-15p grafted with an organotin(IV) compound [$SnPh_3\{(CH_2)_8OH\}$] against B16 melanoma cells.
Material and Methods: Cell viability was estimated by MTT and CV tests. Flow cytometric analysis was done on cells stained by Propidium Iodide, Annexin V-FITC/PI, Apostat, DAF-FM, DHR or CFSE dye. Differentiation of melanoma cells was evaluated by measuring intracellular amount of melanin and activity of key enzyme involved in melanin synthesis − tyrosinase and microscopic analysis of cells stained with hematoxylin dye.
Results: SBA-15pSn as well as [$SnPh_3\{(CH_2)_8OH\}$] strongly suppressed the viability of B16 cells. The amount of [$SnPh_3\{(CH_2)_8OH\}$] in MC_{50} concentration of SBA-15pSn is approximately 100 times lower than IC_{50} dose of free [$SnPh_3\{(CH_2)_8OH\}$]. Carrier alone had no influence on cell viability. Reduced cell viability was the consequence of inhibited cell proliferation. In parallel, small percentage of cells died by caspase dependent apoptosis. The rest of the cells were transformed into large, granulated cells. Observed granules were in fact melanosomes since it was determined that SBA-15pSn strongly enhanced tyrosinase activity and melanin quantity. On the other hand free compound slightly increased tyrosinase activity and production of melanin. Amount of reactive oxygen and nitrogen species was not significantly changed in the presence of SBA-15pSn suggesting that antitumor activity was not associated with their production. Free compound reduced the production of ROS which can be explained as a defense mechanism against toxic stimuli.
Conclusion: Overall results show that packaging of toxic metal-based drug in nanoparticles achieved a stronger antitumor effect with less toxicity.
No conflict of interest.

844 POSTER

Physical and chemical compatibility of fosaprepitant dimeglumine for injection with concomitantly dosed medications

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Background: Fosaprepitant (EMEND IV®, IVEMEND® and PROEMEND®) is a key anti-emetic therapy for highly emetogenic chemotherapies (MASCC/ESMO Antiemetic Guideline 2011). As fosaprepitant is frequently co-administered with other agents, it is important to understand which combinations of drugs will not adversely impact the chemical or physical stability of Fosaprepitant. Therefore, we explored the impact of concomitantly dosed medicines on the physical and chemical stability of fosaprepitant (and only fosaprepitant). Mixtures included combinations of other anti-emetics, corticosteroids, anti-histamines, H2 blockers, anti-spasmodics, B vitamins, Vitamin K, diuretics and infusion solutions (including saline, glucose solution and multiple electrolyte solutions).
Materials and Methods: 172 different combinations of fosaprepitant with up to four other agents were prepared together to simulate simultaneous administration from a single IV bag. The impact on fosaprepitant stability was determined by visual observation and HPLC analysis after storage under ambient conditions for 24 hours.
Results: Fosaprepitant was found to be compatible with many of the admixtures. Degradate growth was determined to the most sensitive predictor of stability. The extent of degradation of fosaprepitant was strongly dependent on the solution pH. These results are consistent with the known chemistry of fosaprepitant. The only degradate observed was the active pharmaceutical ingredient aprepitant, which readily forms via hydrolysis from the prodrug fosaprepitant.
Conclusions: Acceptable in-vitro physical and chemical stability of fosaprepitant was demonstrated with many of the concomitantly dosed medicines. The pH of the admixture was shown to have a significant impact on the stability of fosaprepitant.
Conflict of interest: *Other substantive relationships: BD and MDS are employees of Merck and may own stock/stock ownership in the company.JS and HK are employees of Ono Pharmaceuticals Co., Ltd.*

846 POSTER

Safety results from a Phase I study with a new tablet formulation of olaparib (O) in combination with carboplatin (C) and paclitaxel (Pa)

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Background: This Phase I study evaluated safety/tolerability and preliminary antitumour activity of the PARP inhibitor O with C and Pa in patients (pts) with advanced solid tumours refractory to standard therapies (NCT00516724; sponsor, AstraZeneca). Previously, we reported data from cohorts 1−15 that evaluated the capsule form of O and identified a tolerable schedule with CPa: O 200 mg bid (d1−10) plus CPa AUC4/175 mg/m^2 q 3 weeks (van der Noll *et al* ASCO 2013). Here, we report data from cohorts evaluating a new tablet form of O with higher bioavailability and improved pt convenience vs the capsule.
Materials and Methods: O tablets were introduced at 200 mg bid (days 1−10 per 21-day cycle) with CPa AUC4/175 mg/m^2 q 3 weeks (both on day 1 per cycle); as this proved non-tolerable, other regimens were explored. AEs were graded by CTCAE v3.0. Objective tumour response was evaluated by RECIST.
Results: 102 pts were enrolled in 3 centres. The table shows dose schedules for tablet cohorts.

Cohort (pts, n)	O, mg bid (days)	C, AUC
16 (8)	200 (1−10)	4
17 (6)	100 (1−10)	4
17b (15)	100 (1−10)	4
18 (6)	100 (1−10)*	4
19 (6)	100 (1−5)	4
20 (6)	100 (3−12)	4
21 (13)	50 (1−5)	5
22 (6)	200 (1−2)	5
23 (6)	100 (1−2)	6
24 (6)	100 (1−5)	5
25 (6)	100 (1−2)	5
26 (6)	50 (1−2)	6
27 (6)	50 (1−2)	5
28 (6)	50 od (1−5)	5

Pa dose: 175 mg/m^2 in all cohorts. *100 mg od on day 1 (6 h post-C).

Most common tumour types were breast (53%) and ovarian (33%); 90 (88%) pts had received prior chemotherapy. 40 (39%) pts were known to have a *BRCA1/2* mutation. Common haematological AEs included neutropenia (53%), thrombocytopenia (41%) and anaemia (20%). Other common AEs included fatigue (82%) and nausea (73%). Neutropenia leading to delays in chemotherapy cycles was the main dose-limiting toxicity. Toxicities in tablet cohorts were generally consistent with those in capsule cohorts, but lower O tablet doses and/or treatment durations were needed to manage increased haematological toxicity (neutropenia) and treatment delays. Cohort 19 was tolerable but, due to the low C dose (AUC4), further dosing schedules were assessed leading to identification of a second tolerable schedule with C AUC5 (cohort 27). Preliminary antitumour activity was encouraging with 5 (5%) pts achieving a complete response and 39 (38%) a partial response.
Conclusions: When combined with CPa, a lower dose of the tablet formulation of O (vs capsule) is required to optimize tolerability. O 50 mg

bid plus CPa AUC5/175 mg/m^2 was considered tolerable, but with limited duration of O use (2 days per cycle).
Conflict of interest: *Ownership: I. Tchakov & K. Bowen are employees of AstraZeneca and own AstraZeneca stock*

847 POSTER

Discovery of naturally occurring toll-like receptor-4 signalling inhibitors: Their anticancer effects and mechanisms of action

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Background: Toll-like receptor 4 (TLR-4) plays a key role in pathogen recognition and activation of innate immunity. Very recent studies have suggested aberrant TLR-4 activation by tumour cells promotes their proliferation and survival. To the best of our knowledge, there are no studies on the potential use of TLR-4 signalling inhibitors in cancer treatment. Therefore with the aim of exploring the potential of TLR-4 signalling inhibitors in cancer treatment.
Materials and Methods: We have employed the target- and ligand-based *in silico* screening to discover novel natural products as TLR-4 signalling inhibitors. The best drug-like compounds were selected for *in vitro* studies to measure their efficacy in inhibiting the actions of lipopolysaccharide (LPSEc) in TLR-4/MD-2/CD-14 transfected HEK-293 cells. Their IC_{50} value was determined using dose-response curves. The IC_{50} value is defined as the concentration of these compounds inhibit the 50% of LPSEc's activity. The anticancer effects of these compounds were evaluated in 12 human cancer cells using MTT assay. We also evaluated the apoptotic induction effects of these compounds using fluorescence microscope and enzyme linked immunosorbent assay (ELISA).
Results: The majority of the selected compounds were shown significant inhibition of LPSEc induced TLR-4 activity. The experimental results are in good agreement with virtual screening results suggesting the constructed *in silico* model is a good model and can be used in discovering novel TLR-4 signalling inhibitors. The results suggested the TLR-4 signalling inhibitors are effective in inhibiting cancer progression. The cytotoxic effects exhibited by these compounds were also confirmed to be more selective towards cancer cells rather than non-cancerous cells. Our results suggested the TLR-4 signalling inhibitors induced apoptosis in cancer cells rather than necrosis.
In conclusion, we have discovered a natural product that inhibits TLR-4 signalling and have proven that TLR-4 signalling inhibitors play a a role in preventing cancer progression.
No conflict of interest.

848 POSTER

Targeting Immuno-liposomes using TCR-like antibodies directed against melanoma antigens

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Chemotherapeutic treatment of solid tumors is greatly hampered by factors like toxicity, rapid drug clearance and poor perfusion of the drug to tumor areas. Nanocarriers such as liposomes make an attractive alternative to free cytotoxic drug by reducing toxicity and promoting drug accumulation in tumors. When coupled to a ligand they can be internalized selectively by target tumor cells. Here, we aim to improve liposomal chemotherapy by developing drug-loaded liposomes that specifically target cell surface-expressed peptide-MHC complexes which constitute the natural targets for T-cells and are uniquely or overexpressed on melanoma cells.
Single chain Fv's G8 and Hyb3 against Melanoma Antigen 1 presented by human leukocyte antigen class 1 (MA1/A1) were derived from a phage-display library. scFv for liposome conjugation are cloned in a modified vector pABC4 which holds an additional cysteine at the C′ terminus of scFv during the production. These scFv are produced in periplasmic fractions and extracted by immobilized metal ion affinity chromatography. Purified scFv are validated by flow cytometry on APD cells pulsed with MA1/A1. Liposomes are prepared by film hydration and extrusion method and characterized by size, polydispersity and lipid concentration and then coupled to scFv via a thio-ether bond. Various analytical approaches are applied to validate this immunoconjugate.
scFv with C-terminal cysteins were produced and purified in considerable yield. Purified scFv were tested for target specificity toward MA1/A1 complex and demonstrated desired affinity for the ultimate molecular target. G 8 and Hyb 3 differ with respect to their ligand-binding affinity towards wild type MA1/A1, expressed by native tumor cells: KD's of 250 and 14 nM, respectively. The difference in ligand-binding affinity will allow us to compare the importance of this parameter for multivalent nanoparticles. The process of coupling scFv to liposomes is being optimized with regard to efficiency and antibody density. Produced immunoliposomes demonstrated cell specific targeting using flow cytometry, in vitro. Various cell assays will be done using fluorometry and confocal microscopy to confirm binding and internalization.
TCR like antibodies have been produced in a scFv format ready for coupling to liposomal nanocarriers. Preliminary data suggest cell specific binding of the newly produced immunoliposomes. Final aim is to evaluate these immuno-conjugates in vivo in relevant tumor models.
No conflict of interest.

849 POSTER

First in human phase I study of PankoMab-GEXTM: a novel glyco-optimized anti-TA-MUC1 monoclonal antibody

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Background: PankoMab-GEX is a potent humanized and glyco-optimized IgG1 recognizing the novel carbohydrate-induced conformational TA-MUC1 epitope expressed on the majority of tumor cells in a wide variety of cancers. Its epitope comprises a tumor specific carbohydrate antigen (TF or Tn) together with the immunodominant peptide region of MUC1, is human-specific and virtually only expressed on malignant cells. PankoMab-GEX promotes potent tumor cell killing via ADCC, phagocytosis, apoptosis induction and proliferation inhibition.
Methods: Eligible patients with TA-MUC1 positive (IHC reactivity score $\geqslant$3 on a 12 grade scale) advanced solid tumors, progressing after standard treatments, were enrolled into this first in humans phase I trial. Primary endpoints were safety and tolerability. Pharmacokinetics (PK), immunogenicity and anti-tumor activity were also assessed.
Results: Of the 74 pts included, 73 were treated per protocol: 52 (51) q3w (13 dose levels (DL) from 1–2200 mg), 18 q1w (5 DL from 300–700 mg), 4 q2w (1200 mg + 900 mg start dose one week prior). No MTD was reached. Infusion-related reactions (IRRs), mostly of º1/2 during and after cycles 1 or 2 occurred in ~50 % of the pts starting at the DL of 300 mg q1w. IRR consisted of dyspnea, rash, erythema and flushing, but no cytokine release, and no increases of factor C3a or eosinophilic cationic protein and hence no allergic reactions were observed. Premedication reduced IRRs at 1st inf. from 86% (6/7pts) to 53% (19/36pts) at DL $\geqslant$600 mg. PK was linear and dose-independent with a mean t1/2 =184 h (q3w). In pts with at least one post-baseline CT (62) overall confirmed clinical benefit rate (CBR) was 32% (20/62 pts) across all DL and schedules and 50% (17/34) at DL $\geqslant$600 mg including 1 CR with normalization of CA125 in an ovarian cancer case for 483 days at 1100 mg q3w, and 1 PR for 295 d in a NSCLC case at 600 mg q1w. Confirmed CBR in pts with OvCa was 45% (9/20) over all DL, and for DL $\geqslant$600 mg 60% (9/15). All 5 pts sensitive to their last platinum based therapy experienced CB. Maximum CB duration was in a pseudomyxoma peritonei pt (−21% SLD ongoing for >700 d at 900 mg q3w).
Conclusions: PankoMab-GEX is safe and demonstrated clinical activity in heavily pre-treated pts. The q1w, q2w and q3w administration schedules were feasible and associated with CB.
No conflict of interest.

850 POSTER

S100A4 neutralizing mAbs for the treatment of cancer

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Background: S100A4 belongs to the S100 family of small Ca-binding proteins. Secreted by tumor and stromal activated cells, plays an important role in tumor progression, angiogenesis and metastasis and therefore it turns out into a very compelling target for cancer therapy. S100A4 has gained increasing attention over the last two decades because of its metastasis-promoting properties and its over-expression has been reported to be associated with poor prognosis of cancer correlates. Although extracellular roles of S100A4 are closely associated with tumor invasion and metastasis, the mechanism by which it promotes an invasive phenotype is not fully understood. This study aimed to determine the role of S100A4 in tumor growth, angiogenesis and metastasis and we

addressed the use of a neutralizing monoclonal antibody (5C3) targeting the extracellular activity of S100A4, to inhibit tumor progression.
Materials and Methods: Endothelial cell-based studies were used pointing to the involvement of S100A4 in at least two steps in the angiogenic cascade: remodeling of the extracellular matrix and cell migration.
We have developed the 5C3 neutralizing monoclonal antibody against S100A4 protein. To examine the inhibitory effect of our antibody a panel of *in vitro* and *in vivo* experiments were run. We used several subcutaneous, orthotopic and intrasplenic mouse tumor models to test the efficacy in tumor development.
Results: Boyden chamber migration assay showed that S100A4 synergizes with VEGF on HUVECs migration and the combination of 5C3 mAb with Bevacizumab is more effective than the two drugs separately. In addition, S100A4 induces the secretion of active forms of MMP-9 and 5C3 mAb blocks this effect.
We observed that treatment with 5C3 reduced in vivo angiogenesis on MiaPACA-2, M21 and HT29 subcutaneous tumor model thereby affecting tumor growth ($p < 0.05$).
Treatment with 5C3 revealed a potent decrease in lung and liver metastasis using the 4T1 orthotopic model and the KM12L4luc intrasplenic model respectively.
Finally, we have also observed that extracellular S100A4 induces metastasis formation by building the pre-metastatic niche on the CT26 intrasplenic model.
Conclusions: Our results highlight the relevance of extracellular S100A4 in tumor development and prove it as a novel therapeutic target in cancer.
No conflict of interest.

851 POSTER

Assessing the potential of S100A7 as target for tumor therapy using neutralizing monoclonal antibodies

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Background: S100A7, a member of the S100 calcium-binding protein family, is a low molecular weight protein whose expression have been reported in several cancer types such as breast, lung, bladder, skin, esophageal, gastric and head and neck, correlating in most of them with a poor prognosis. It has been described that extracellular S100A7 can act as a pro-inflammatory, pro-angiogenic and pro-metastatic factor, but its mechanism of action remains poorly understood. In this study, we have developed neutralizing monoclonal antibodies against S100A7 and tested its functionality in different in vitro and in vivo models as therapeutic and diagnostic tools.
Materials and Methods: Cell migration and MTT assays were performed to test the capacity of the obtained specific monoclonal antibodies to neutralize extracellular S100A7 activity. We also studied the downstream signaling pathways and the secretion of pro-inflammatory factors in response to extracellular S100A7 and its blockade by our antibodies. Finally, we assessed the usefulness of the monoclonal antibodies as diagnostic tools for the detection of S100A7 in biofluids and in tumor samples by ELISA and immunohistochemistry respectively.
Results: Extracellular S100A7 induced an increase on MDA-MB-231 breast cancer cell migration and secretion of pro-inflammatory factors possibly by the activation of the MAPK pathway. Furthermore, S100A7 promoted the proliferation of HT1080 fibrosarcoma cells. Our anti-S100A7 specific monoclonal antibodies were effective in neutralizing the *in vitro* activity of S100A7 protein. Finally, we have demonstrated that our antibodies can be used in diagnosis and prognosis by determining the presence of S100A7 in tissue samples and in plasma from tumor bearing mice. A positive correlation between the S100A7 plasma levels and the tumor presence and tumor burden was found.
Conclusions: We have elucidated the extracellular action of S100A7 in tumor cells and obtained useful tools for cancer diagnosis and therapy by the blockade of this protein as a therapeutic target.
No conflict of interest.

852 POSTER

Targeting extracellular S100P: therapeutic potential of monoclonal antibodies

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Background: S100P has gained increasing attention over the last two decades because of its cancer-promoting properties, via its specific roles in survival, cell proliferation, angiogenesis, metastasis and drug resistance. Furthermore, high S100P expression has been identified as a significant marker for poor prognosis in several cancer types (such as pancreatic, breast, colon, prostate and lung) when compared with their matched normal tissues and associated to an increased incidence of metastasis. S100P have also shown to be a potential marker with diagnosis and prognosis value for several cancers. The purpose of this study was to validate S100P as a promising target for therapeutic and diagnostic applications by using neutralizing monoclonal antibodies.
Materials and Methods: Specific monoclonal antibodies able to neutralize the extracellular activity of S100P were obtained.
To better understand the role of S100P in tumor cell proliferation and survival, we used two cell lines; one expressing S100P (human pancreatic BxPC3) and one no-expressing (fibrosarcoma HT1080). Proliferation, drug resistance and migration properties were studied *in vitro*. *In vivo* tumor models (subcutaneous, orthotopic and intrasplenic) were developed to assess the efficacy of anti-S100P mAbs on tumor growth and metastasis.
Results: Here we have clearly demonstrated the inhibitory effect of our mAbs on S100P-induced tumor cell proliferation. Additionally, S100P protected BxPC3 and HT1080 cell lines to the cytotoxic effect induced by chemotherapeutic drugs by increasing their survival while anti-S100P mAbs reversed this effect.
Moreover, anti-S100P mAbs blocked tumor growth *in vivo* on a BxPC3 subcutaneous model either by i.t or i.p. route. Moreover, a clear reduction on liver metastasis formation and in the final staging of the disease was observed in an orthotopic and an intrasplenic tumor model when treated with anti-S100P mAbs.
Finally, we have demonstrated a positive correlation between S100P plasma levels and tumor incidence.
Conclusion: These results have shown the importance of S100P in tumor progression and aggressiveness. Therefore, blocking S100P is a valid therapeutic approach and it might improve the response to other therapeutic treatments as well as to decrease the metastatic capacity of the tumor.
No conflict of interest.

853 POSTER

Immunogenicity assessment of PF-05280014, a potential biosimilar to trastuzumab, in healthy subjects (REFLECTIONS B327−01)

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Background: PF-05280014, a proposed biosimilar to trastuzumab, was evaluated for immunogenicity in a phase 1 pharmacokinetic (PK) similarity study.
Methods: In this double-blind trial (NCT01603264), 105 healthy male subjects with no known prior biologics exposure, were randomized to receive a single 6 mg/kg IV dose of PF-05280014, or trastuzumab sourced from the US (trastuzumab-US) or the EU (trastuzumab-EU) and followed for 10 weeks for PK, safety, and immunogenicity assessments. Serum samples for detecting anti-drug antibodies (ADA) and neutralizing antibodies (Nab) were collected at 0, 336, 672, 1008, and 1680 hours post-dosing. ADA was detected using two validated electrochemiluminescent immunoassays, one each to detect antibodies against PF-05280014 and reference drugs. Samples were first tested for antibodies against the dosed product. Confirmed positive samples were further tested for Nab using a validated semi-quantitative competitive ligand binding assay, and for ADA cross-reactivity. All subjects provided informed consent.
Results: Samples for immunogenicity assessment were collected from all 105 subjects. Only 2 samples tested positive; the rest (99.6%) tested negative in the ADA assay specific for the dosed product. One positive sample was collected at 1680 hours after dosing from a subject who received trastuzumab-EU (Subject 1). This sample had a low titer of ADA and tested negative against PF-05280014. There were no adverse events attributable to the ADA finding for this subject. The other sample testing positive was collected before dosing from a subject who subsequently received PF-05280014 (Subject 2). This low false-positive rate (1/105) of ADA at baseline was consistent with assay validation requirements to ensure high probability of identifying all subjects who develop ADA. The 2 ADA-positive samples were negative for Nab. The 3 study agents were well-tolerated and adverse events were similar. The PK profile of Subject 1 was similar to other subjects in the trastuzumab-EU group. The PK of the 3 study agents were shown to be similar based on C_{max}, AUC_t, and AUC_{inf} and the standard bioequivalence criteria of 80–125%.
Conclusions: Consistent with reported data for trastuzumab in patients with cancer, PF-05280014 appears to have low immunogenic potential

when given as a single IV infusion to healthy subjects. Overall, PF-05280014 demonstrated PK similarity and comparable safety and immunogenicity profiles to trastuzumab in healthy subjects.
Conflict of interest: *Ownership: Stock in Pfizer (DY, CTT, KBB, XM, DR, RL, ADR, CZ). Advisory board: None. Board of directors: None. Corporate-sponsored research: None. Other substantive relationships: All authors are current employees (DY, CHC, CZ, DR, KBB, RL, XM), former employees (ADR), or contract employees (CTT, SDR) of Pfizer Inc*

854 POSTER

Neoadjuvant-adjuvant treatment of breast cancer: A model for extrapolation of clinical data for trastuzumab biosimilar candidates

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Background: Trastuzumab (Herceptin®) is approved for HER2-positive EBC, MBC and MGC. Trastuzumab biosimilars raise questions about how best to conduct mandatory, head-to-head clinical equivalence studies, to show similarity in efficacy, safety and immunogenicity and mitigate risks associated with extrapolation to indications not investigated by the biosimilar mAb. EMA mAb biosimilar guidelines recommend sensitive and homogenous populations and sensitive endpoints for such studies. MBC patient (pt) populations are generally heterogenous, and pts may have a compromised immune response. Establishing clinical similarity in the neoadjuvant–adjuvant setting may be the better risk mitigation strategy for extrapolating clinical data to MBC and may be more acceptable to regulators than the reverse.
Materials and Methods: Data from NOAH (neoadjuvant–adjuvant trastuzumab + CTx vs. CTx, N = 231) were used to retrospectively assess treatment effect size (difference between arms) and identify a sensitive, homogenous population, as per the EMA guidelines. tpCR treatment effect differences were evaluated as a sensitive endpoint for investigation of clinical benefit. This can then allow assessment of equivalence only based on tpCR endpoint.
Results: tpCR treatment effect size was 19%. Based on the study results, a 15−20% difference in tpCR rate may translate to a clinically meaningful difference in disease-free survival. If biosimilars were evaluated using an equivalence design and margins of 10% based on the tpCR endpoint (40% response), the sample size would be 500 pts per arm (80% power, 18% drop-out rate). In trials of this sort, similarity in immunogenicity could be assessed when pts are given trastuzumab monotherapy excluding bias or immunosuppressive effects due to concomitant CTx.

	tpCR %
Trastuzumab + CTx (n = 115)	40
CTx alone (n = 116)	21
Effect size	**19**

Conclusions: Sensitive populations as defined by EMA regulations are those that are homogenous, often the most responsive to therapy and are treatment naive. If equivalence in efficacy, safety and immunogenicity of trastuzumab (reference product) and a trastuzumab biosimilar candidate is demonstrated in the neoadjuvant–adjuvant setting, this may provide a reasonable basis for extrapolation to other indications not specifically studied during biosimilar development. This may be a more sensitive approach than one based on ORR in MBC, as ORR is less sensitive to treatment differences and only weakly correlated with clinical endpoints.
Conflict of interest: *Other substantive relationships: All authors are employees of Genentech or F. Hoffmann-La Roche Ltd*

855 POSTER

Anti-FGFR1 humanized monoclonal antibody OM-RCA-01 inhibits FGF-induced angiogenesis

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Background: The growth of new blood vessels is regulated by several proangiogenic factors. We believe that the angiogenesis induced by basic fibroblast growth factor (bFGF) is resistant to anti-VEGF/R therapy and can be targeted by anti-FGFR1 monoclonal antibody.
Methods: In our in vivo study, angiogenesis was measured with subcutaneously implanted Matrigel plugs containing: 1) bFGF; 2) VEGF; 3) bFGF+bevacizumab; 4) VEGF+bevacizumab; 5) bFGF+anti-FGFR1 monoclonal antibody OM-RCA-01 (Tsimafeyeu et al. 2012 ASCO meeting); 6) VEGF+OM-RCA-01. Control group was without stimulation or treatment. Doses of bFGF, VEGF-A (R&D Systems), bevacizumab (Roche), OM-RCA-01 (OncoMax) were 100 ng, 200 ng, 10 mg/kg, and 10 mg/kg per animal, respectively. Number of endothelial cells/vessels was calculated.
Results: There was no neovascularization in bFGF negative, VEGF negative group (mean, 0). bFGF and VEGF strongly induced angiogenesis ($P<0.001$). There were no vessels and endothelial cells in anti-FGFR1 antibody FGF-stimulated group (mean, 0). In bFGF-induced angiogenesis, bevacizumab did not impact on neovascularization in comparison with bFGF positive control ($P = 0.5$). The angiogenic effect of VEGF was significantly inhibited by bevacizumab in comparison with VEGF positive control ($P<0.0001$). OM-RCA-01 not significantly inhibited growth of vessels in VEGF positive group ($P = 0.064$).
Conclusion: Anti-FGFR1 monoclonal antibody OM-RCA-01 inhibited FGF-induced angiogenesis. Targeting VEGF(R) by bevacizumab significantly impacted on VEGF-induced angiogenesis and not on bFGF-induced neovascularization.
Conflict of interest: *Other substantive relationships: N. Golub, E. Zaveleva are employers of OncoMax LLC*

856 POSTER

Eudesmol isomers induce caspase-mediated apoptosis in human hepatocellular carcinoma HepG2 cells

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Background: Eudesmols are naturally occurring sesquiterpenoid alcohols that present cytotoxic effect to cancer cells. In this work, we studied the mechanisms of cytotoxic action of eudesmol isomers (α-, β- and γ-eudesmol) in human hepatocellular carcinoma HepG2 cells.
Material and Methods: In first, Three tumour cell lines (HepG2, K562 and B16-F10) were treated with increasing concentrations of eudesmol isomers for 72 h and analysed by methyl-[^{3}H]-thymidine incorporation assay. The pro-apoptotic affect of these compounds was assessed in HepG2 cells by morphological analysis (using hematoxylin/eosin staining and acridine orange/ethidium bromide staining), flow cytometry (cell membrane integrity, mitochondrial transmembrane potential, cell cycle distribution and internucleosomal DNA fragmentation analysis) and caspase-3 activation assay after 24 h incubation.
Results: All eudesmol isomers displayed cytotoxicity to different tumour cell lines. α-Eudesmol showed IC_{50} values ranging from 5.38 to 10.60 μg/mL for B16-F10 and K562 cell lines, β-eudesmol showed IC_{50} values ranging from 16.51 to 24.57 μg/mL for B16-F10 and HepG2 cell lines and γ-eudesmol showed IC_{50} values ranging from 8.86 to 15.15 μg/mL for B16-F10 and K562 cell lines, respectively. After 24 h incubation, HepG2 cells treated with eudesmol isomers presented typical hallmarks of apoptosis, as observed by morphological analysis in cells stained with hematoxylin–eosin and acridine orange/ethidium bromide. Significant increases in internucleosomal DNA fragmentation without affecting membrane integrity were also found. In addition, eudesmol isomers induced loss of mitochondrial membrane potential and an increase of caspase-3 activation in HepG2 cells, suggesting that this apoptotic cell death was caspase-dependent.
Conclusions: In conclusion, the eudesmol isomers herein investigated are able to reduce cell proliferation and to induce tumour cell death through apoptotic pathways.
No conflict of interest.

857 POSTER

Eusynstyelamide B: a novel topoisomerase II inhibitor isolated from an ascidian from the Great Barrier Reef

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Researchers have shown that marine ascidians are an excellent source of new anticancer compounds that can exhibit new mechanisms of action. The Great Barrier Reef is a rich environment harbouring a prolific ascidian biodiversity, and many of the species have never been explored for bioactive natural products. The aim of this project was to isolate and characterize

anticancer compounds from ascidians belonging to the family Didemnidae. We tested a total of 143 ascidian specimens from the Eskitis Biota Library for anti-proliferative effects in the prostate cancer cell line LNCaP using the real-time cell analyser xCELLigence System. Twenty-one hit extracts were identified, and the most interesting extracts were selected for bioassay-guided fractionation in order to purify the bioactive compound(s). From these studies we identified a previously reported modified tryptophan-arginine dipeptide dimer, named eusynstyelamide B (EB). We found that EB inhibited proliferation of LNCaP cells with an IC_{50} of 5 μM. Cell cycle studies and analysis of histone H3 phosphorylation showed that EB arrested LNCaP cells in the G2 phase. RNA expression profiling by micro array and qRT-PCR suggested that the EB-induced G2 arrest was caused through DNA damage pathways. Western blotting experiments confirmed phosphorylation of ChK2, but not of p53 or ChK1, and down-regulation of CDC2 protein expression. EB-induced DNA damage was confirmed by neutral comet assay and the formation of γH2AX foci. Intercalation displacement assays and melting curve analysis demonstrated that EB did not interact with DNA. Importantly, when incubated with kDNA and topoisomerase II EB strongly inhibited the decatenation of kDNA. These data indicate that EB is a novel topoisomerase II inhibitor which causes DNA damage in LNCaP cells. Studies are ongoing to further characterize the potential anti-cancer properties of this compound and validate the findings in additional cancer cell lines.
No conflict of interest.

858 POSTER

Allylbenzenes as potential chemosensitizers and P-glycoprotein inhibitors

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Background: Allylbenzenes are a family of compounds found essentially in spices. Some of them can induce apoptosis in tumour cells. In our previous studies we determined that eugenol can inhibit topoisomerase II and induce apoptosis in mammalian cells, while myristicin can also induce apoptosis in human leukaemia K562 cells. Eugenol was genotoxic while myristicin was non-genotoxic. The apoptotic activity of the allylbenzenes raised the possibility of them potentiating the activity of standard chemotherapeutic drugs, particularly in cases of multidrug resistance, a major cause of failure of cancer chemotherapy. Multidrug resistance can also occur as the result of drug extrusion due to up-regulation of membrane efflux pumps (EP) such as P-glycoprotein (P-gp, ABCB1). Much research has been performed to identify EP inhibitors, and a large number of natural compounds have shown promise in inhibiting EP.
Material and Methods: We analysed five allylbenzenes for their ability to inhibit the function of P-gp by using a Semi-automated Fluorimetric Method that monitors ethidium bromide (EB) uptake and extrusion, on a real-time basis by L5178 mouse T-cell lymphoma cells expressing the human ABCB1 (P-gp) gene. Monitoring of uptake and extrusion of EB was assessed using the Rotor-gene™ 3000 (Corbett Research). MDA-MB-231 breast metastatic cancer cells were used to analyse synergy with doxorubicin using the MTT assay.
Results: We observed that eugenol, α and β-asarone potentiated the cytotoxicity mediated by doxorubicin in MDA-MB-231 breast metastatic cancer cells. Extrusion of EB took place readily in control cells expressing ABCB1. In cells exposed to known inhibitors of efflux pumps (verapamil and cyclosporine A) there was marked EB accumulation. The allylbenzenes α and β-asarone strongly inhibited EB efflux, followed by myristicin and eugenol. The allylbenzene trans-anethole did not present significant inhibitory activity.
Conclusions: In conclusion, α and β-asarone can potentiate the cytotoxicity of doxorubicin and also inhibit P-gp efflux pumps and can be regarded as promising candidates, in co-treatments, to increase cell death and overcome resistance to known chemotherapeutics.
No conflict of interest.

859 POSTER

Population pharmacokinetics (PPK) of eribulin in cancer patients

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Background: Eribulin mesylate (Halaven®) is a non-taxane microtubule dynamics inhibitor, approved for the treatment of certain patients with breast cancer. A combined population PK analysis was conducted using data from seven Phase 1 studies, one Phase 2 study and one Phase 3 study in cancer patients to characterise eribulin PK profile and identify covariates that affect eribulin exposure. Eribulin mesylate was administered intravenously at doses between of 0.25 and 2 mg/m^2.
Materials and Methods: Data from 69 males and 444 females (389 with breast cancer), aged 27 to 81 years and weighing 39 to 161 kg were available for PPK analysis. The pooled dataset comprised of 4093 eribulin concentrations. PPK was conducted using NONMEM with first-order conditional estimation method with interaction (FOCEI). Stepwise covariate building was performed and the final model was evaluated using bootstrap analysis and predictive check simulations. Covariates tested were age, weight (WGT), hepatic function markers (albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate trasnferase (AST), bilirubin (BILI)), renal function (creatinine clearance (CLCr)), gender, race, ECOG status and tumor type (breast vs. other).
Results: Eribulin PK was characterised by a three-compartment model where both volume (V_1, V_2, V_3) and clearance (CL, Q_2, Q_3) parameters were proportional to WGT according to an allometric model. Other covariates were related to hepatic function with effects of ALB, ALP and BILI levels significantly affecting CL. Population estimates were: CL = 3.11 L/h, $V_{1=}$ 4.06 L, Q_2 = 2.64 L/h, $V_{1=}$ 4.06 L, V_2 = 2.42 L, Q_2 = 6.60 L/h and V_2 = 121 L. Eribulin CL increased proportionally with ALB levels (exponent: 0.946) and decreased proportionally with ALP (exponent: −0.209) and BILI levels (exponent: −0.180). Inter-individual variability was moderate ranging between 37 % for V3 and 52 % for CL. Proportional residual variability in eribulin concentrations was moderate (24 %). Evaluation from bootstrap and predictive checks suggested robustness of the final model for all patients. Eribulin PK was unaffected by age, renal function, gender, race, ECOG status and tumor type.
Conclusions: The population model adequately described eribulin PK in breast and other cancer patients. WGT, ALB, ALP and BILI were significant predictors of eribulin CL. The current model can be utilised to characterise eribulin PK and predict eribulin exposure in cancer patients.
Conflict of interest: *Corporate-sponsored research: All authors are employees of Eisai Europe Ltd, or Eisai Inc.*

860 POSTER

Lurbinectedin (PM01183) in combination with doxorubicin (DOX): Preliminary results of a phase Ib study

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Background: PM01183 has wide antitumour activity through minor groove DNA-binding. Synergism with DOX was observed preclinically. PM01183 monotherapy is undergoing clinical evaluation in pancreatic, ovarian and breast cancer patients (pts). Reversible neutropenia and high emetogenic potential are its main side effects.
Methods: Pts were included in successive cohorts aiming to define the recommended dose (RD) of PM01183 combined with 50 mg/m^2 of DOX (fixed dose) q3wk, with or without colony-stimulating factors (CSF) prophylaxis. Less than 1/3 of at least 9 pts had to have dose-limiting toxicities (DLTs) in Cycle 1, at the RD. Pharmacokinetics (PK) were assessed. Consenting adults ⩽75 years, ECOG PS 0−1, adequate organ function and up to 2 prior chemotherapy lines were included. Prior adjuvant DOX was allowed. DOX had to be withdrawn before exceeding 450 mg/m^2 of cumulative dose. PM01183 could be continued alone if clinical benefit.
Results: As of March 2013, 43 pts were treated: 53.5% were males, median age was 61 years (r: 22−78). SCLC (30%), STS (21%) bladder (12%), and gynaecological, stomach and NET (9%, each) were the most frequent locations. DLTs occurring at the MTD, 5.0 mg flat dose (FD) of PM01183, with or without CSF, were febrile neutropenia (FN=3), sepsis, grade 4 thrombocytopenia and grade 3 diarrhoea (1 each). PM01183 at 4.0 mg FD combined with DOX, 50 mg/m^2 without CSF is the RD; 1 pts out of 9 had a DLT (FN). Toxicities in ⩾10% of pts, in addition to myelosuppression, were generally mild, including anaemia, ALT/AST increases, fatigue, alopecia, mucositis, nausea/vomiting, diarrhoea, anorexia and constipation. One pt had >10% asymptomatic decrease in left ventricular ejection fraction (LVEF). Out of 41 evaluable pts, 14 responded, for an ORR = 34% (95% CI: 20−51%), including 3 radiological CRs (7%). Of note, 5 out of 12 evaluable SCLC pts had PRs (42%). Over 1/3 of pts received ⩾6 cycles. There are ongoing pts at cut-off. DOX and PM01183 clearance (CL) were not affected, whereas doxol CL decreased with PM01183 dose.
Conclusions: The RD is PM01183 4.0 mg + DOX 50 mg/m^2. Toxicity is manageable and predictable. CSF prophylaxis is not required. This combination showed impressive response rates, including CRs

in relapsed/refractory solid tumour pts. This activity warrants further investigation in specific disease-settings, including SCLC.
Conflict of interest: *Advisory board: PharmaMar. Corporate-sponsored research: PharmaMar*

861 POSTER

Ruta chalpensis: a promising phytotherapeutic candidate against multiple forms of cancer

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Background: Studies on medicinal plants are mostly based on information provided by traditional healers in ethno botanical surveys. The treatment of cancer is mainly based on the use of Doxorubicin as chemotherapeutic agent, despite its potential to elicit serious cardiotoxicity often leading to degenerative cardio-myopathy/heart failure. The proposed mechanism of DOX-toxicity is complex and involves increased oxidative/nitrosative stress. Thus, inflammatory responses could be in the top of induction of cancer and hence treatment target. Based on Tunisian traditional medicine, *Ruta chalpensis* was explored for its anti-cancer activities and modulation of immune responses.
Material and Methods: The activity of *Ruta chalpensis* methanolic extract was tested against different cancerous cells; human carcinoma of bladder (RT112), of laryngeal (Hep2) and myelogenous leukemia (K562). Cellular viability was evaluated by MTT assay and microscopic count of nucleus upon extract treatment. In addition, annexinV staining and [^{3}H]Thymidine incorporation assays were used to control the viability within K562 cells. Furthermore, the release of free radicals was analyzed within the lipid peroxidation by AAPH assay and iNOS mRNA and NO production.
Results: In this study, biological activities of *Ruta chalpensis* methanolic extract related probably to its high tenor in phenolic compounds were investigated. This extract showed high antioxidant activity, and inhibited the production of NO, in murine macrophages, *via* transcriptional regulation indicating appreciable anti-inflammatory activities. Cytotoxicity assay results indicate a specific anticancer therapeutic property while there was no effect on healthy PBMC.
The observed decrease of viability was not due to cellular death but to an anti-proliferative effect of *Ruta chalpensis* methanolic extracts with ERK-dependent growth inhibition.
Conclusion: The use of plant compounds of *Ruta chalpensis* may be a novel approach for specifically inhibiting cancer cells' growth, which will lead to the development of new anti-cancer agents. Interestingly, our findings show, in opposite to the doxorubicin treatment, that *Ruta chalpensis* inhibits nitrosative stress. These results demonstrate the remarkable potential of traditional medicinal plants as valuable source of anti-oxidants exhibiting anti-inflammatory and anticancer properties.
No conflict of interest.

863 POSTER

Phase I study of U3-1565, a fully human anti-HB-EGF monoclonal antibody, in Japanese patients with advanced solid tumors

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Background: Human heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a member of an EGF family and a ligand that binds to and activates the EGF receptor and human epidermal growth factor receptor 4 (HER4). U3-1565 is a fully human monoclonal antibody directed against HB-EGF that has demonstrated anti-tumor activity in preclinical models. This phase I study explored safety, tolerability, pharmacokinetics (PK) and potential anti-tumor activity of U3-1565 in Japanese patients with solid tumors.
Material and Methods: This study was conducted using a 3+3 design and tested U3-1565 at 2, 8, 16, and 24 mg/kg once every two weeks (with the second dose given three weeks after the first), and at 24 mg/kg weekly.
Results: Fifteen patients (6 females) were enrolled, 3 in each dose level cohort, and median age of 62 (range 52−73) years. Tumor types were colorectal (10), ovarian (2) and others (3). No dose-limiting toxicities and no treatment-related serious adverse events were observed; the MTD was not reached. PK data indicated serum U3-1565 concentration was dose-proportional, similar to US phase I study in parallel, though slightly lower exposure was observed. The highest administered dose of 24 mg/kg weekly generated Ctrough above the predetermined target concentration resulting in 90% tumor growth inhibition in preclinical study. Drug related AEs included malaise(20.0%), dermatitis acneiform(13.3%), and decreased appetite(13.3%), which were G1 or G2. Of 15 patients enrolled, 3 patients(20.0%) had stable disease and 12 patients had progressive disease as best response based on RECIST, and the median duration of stable disease was 15 (range 13−20) weeks with no decrease in tumor volume.
Conclusions: U3-1565 was well tolerated without DLT up to the dose level of 24 mg/kg weekly in Japanese patients. The dose of 24 mg/kg weekly was considered to be appropriate for the following phase. Biomarker evaluation in serum and tumor biopsy specimens and exploratory anti-tumor activity are ongoing in the additional study at the dose level of 24 mg/kg weekly as dose expansion part of this phase I study. (Trial Registry Number: JapicCTI-111484.)
Conflict of interest: *Ownership: This study was funded by DAIICHI SANKYO CO.,LTD.(JapicCTI-111484). Advisory board: A.Ohtsu: DAI-ICHI SANKYON.Fuse: DAIICHI SANKYO. Corporate-sponsored research: T.Yoshino: DAIICHI SANKYON.Fuse: DAIICHI SANKYO. Other substantive relationships: A.Ohtsu: Honoraria: DAIICHI SANKYOS.Fujitani, Y.Aramaki: Employed by DAIICHI SANKYO CO.,LTD.All other authors have declared no conflicts of interest.*

864 POSTER

Phase I study of LOR-253, a novel inducer of Kruppel-like factor 4, in patients with advanced solid tumors

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Background: LOR-253 is a novel small molecule inducer of tumor suppressor Kruppel-like factor 4 (KLF4) that has shown potent antitumor activity in NSCLC and colon tumor xenograft models. Objectives are to determine the maximum tolerable dose (MTD) or target-appropriate dose (TAD), and to characterize safety.
Materials and Methods: Patients with advanced solid tumors who progressed on standard therapies received LOR-253 by i.v. infusion on Days 1, 2, 15, and 16 of each 28 day cycle. The study design consisted of a brief run-in (Stage I) with 100% dose escalation until 2 patients with grade 2 toxicity or 1 patient with grade 3 toxicity, followed by a standard 3+3 design with escalating doses (Stage II). Dose limiting toxicity (DLT) was defined as $\geqslant$ grade3 other than reversible electrolyte abnormalities. RECIST 1.1 assessments were performed every 2 cycles (8 weeks). Serum samples for PK were collected in Cycle 1 pre-treatment, 0, 0.5, 1, 2, 4 and 7 hours (hr.) after end of infusion on Day 1 & on Day 2 omitting the 7 hr. sample and adding 24, 48 and 144 hr. samples.
Results: Twenty seven patients have been enrolled, with a mean age of 59 (range, 39−75), 67% male, and a mean of 4 (range, 1−7) prior regimens. Primary tumors included 16 colorectal, 3 appendiceal, 2 non small cell lung, 2 esophageal and, 4 others. Of 24 patients dosed only 1 patient experienced a $\geqslant$ grade 3 toxicity at least possibly related to drug (grade 3 hypophosphatemia). The most frequent grade 2 toxicity was hypersensitivity (2 patients) preventable by pre-treatment. Dosing was at 20, 40, and 80 mg/m^2 in Stage I until a DLT of grade3 hypophosphatemia, and at 80, 104, 135, 176, and 229 mg/m^2 in Stage 2. Of 17 pts evaluable for RECIST assessment, 7 (41%) had stable disease as best response. Stable disease of $\geqslant$4 cycles (4 Pts; mean 154 days) was seen exclusively at the higher dose levels from 176 to 229 mg/m^2 which corresponds to a preclinically efficacious KLF4 inducing dose. PK elimination appeared biphasic with mean $T\frac{1}{2}$ at doses $\geqslant$80 mg/m^2 ranging from 48−61 hr. AUC(0-t) was dose proportional with a median accumulation ratio of 4 on Day 2 vs. Day 1.

Conclusions: LOR-253, a first-in-class molecule, is well tolerated to a TAD of 229 mg/m^2 without significant toxicity. A biomarker investigation has therefore been initiated with continued evaluation of PK and expansion for pre- and post-dose biopsies and correlative tissue analyses.
Conflict of interest: *Other substantive relationships: P. Murray, S. Zhou and Y. Lee work are employed by Lorus Therapeutics, the manufacturer of the drug LOR-253.*

865 POSTER

Evaluation of the interaction of copper(II) and ruthenium(II) compounds with fibronectin and tubulin proteins: two potential chemotherapeutic targets

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Most current research efforts with respect to the quest for novel metal-based drugs are devoted to investigating their interaction with the DNA double helix, considering the accepted mechanism of action for the well-established anticancer drug cisplatin. However, this approach neglects the fact that other cellular components may be targeted by metal complexes. In the present study, fibronectin and tubulin, two proteins involved in fundamental cellular processes that are cell division and cell migration, have been chosen as (cytotoxic) targets for copper(II) and a ruthenium(II) compounds.
The potential interaction of a series of metal-based molecules with these two proteins was first assessed by circular dichroism (CD) and atomic-force microscopy (AFM). MTT assays were subsequently used to determine the cell-growth inhibitory activities (IC_{50}) of the compounds in HeLa and HL60 cell lines. Immunofluorescence assays were then carried out with the two most cytotoxic metal complexes, with HeLa cells, using anti-α-, anti-β-tubulin and anti-fibronectin antibodies to investigate their effect on microtubules and the extracellular matrix. The microtubule-depolymerizing agent Nocodazole was used as positive control. Cell cycle analyses by flow cytometry and annexin V-FITC + PI apoptosis assays (with cisplatin as positive control) were performed with both cell lines to better understand the mechanisms of action of the two compounds.
The AFM and CD experiments clearly evidenced the interaction of the Cu(II) and Ru(II) compounds with both proteins, the most efficient being the copper molecule. IC_{50} values lower than those of the reference compound cisplatin® were obtained for both the Ru(II) (13.01 μM HeLa; 2.48 μM HL60) and the Cu(II) (3.63 μM HeLa; 14.50 μM HL60) complexes. The immunofluorescence assays revealed a microtubule-depolymerizing behaviour for the copper molecule and the formation of apoptotic nuclei with both compounds. The cell cycle tests did not show an arrest at the G2 phase, which would have indicated microtubule stabilization (that prevents cell division), therefore suggesting that the cells had died by apoptosis, as confirmed by the annexin assays.
In summary, fibronectin and tubulin are legitimate target proteins for potential metal-based anticancer drugs such as the two compounds evaluated herein, which showed apoptotic cytotoxic activities.
No conflict of interest.

866 POSTER

Factors predisposing to development of hyperglycaemia in phase 1 studies involving PI3K, mTOR, AKT and mTORC1 and mTORC2 inhibitors

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Background: Dysregulation of the PI3K/AKT/mTOR pathway is implicated in human cancer growth and progression. Agents targeting this pathway can be associated with on target effects of hyperglycaemia due to partial compensation of the insulin-glucose regulatory axis. Identifying those factors predictive of developing hyperglycaemia in patients (pt) treated with these agents may help direct future management.
Materials and Methods: Clinical characteristics and outcomes of pts treated consecutively with PI3K, AKT or mTOR inhibitors in the Drug Development Unit, The Royal Marsden between 2007 and 2012 were recorded. Baseline variables and their association with grade (G) 3 hyperglycaemia (CTCAE version 3.0) were analysed, using chi-square test and significant values were further analysed by multivariate regression analysis (MVA).
Results: 341 pts were identified and treated on 12 phase I trials of PI3K/AKT/mTOR inhibitors, during the study period. Clinical, laboratory and demographic data including personal and family history of diabetes, use of steroids, body mass index (BMI), and baseline blood sugar levels (BSL), biochemistry, treatment and outcomes of hyperglycaemia were recorded. 81.5% of pts developed hyperglycaemia during treatment. Majority had G1 (n = 217, 63.6%) and G2 hyperglycaemia (n = 61, 17.9%). Development of G $\geqslant$3 (n = 20, 5.9%) hyperglycaemia was associated with age <65 (p = 0.03), previous history of diabetes (p = 0.003) and treatment with AKT and mTOR inhibitors (p = 0.00). On MVA, maximum BSL at Cycle (C) 1 [inter quartile range (IQR) =6.3−8.5 mmol/l; odds ratio (OR) =2.4(1.66–3.46)] and fasting BSL at C2 [IQR=4.9−5.9 mmol/l; OR=2.07(1.09–3.95)] were associated with development of G $\geqslant$3 hyperglycaemia. BMI>30, history of steroid use, tumour type or histology, fasting BSL and HbA1C were not predictive of the risk of developing G3 hyperglycaemia. The majority of patients did not require intervention for hyperglycaemia [n = 316; 92.7%]; however, metformin [n = 20; 5.9%] and/or insulin [n = 2; 0.6%] were the most common pharmacological agents used, where required. One pt required a dose reduction and there were no permanent drug discontinuations.
Conclusion: Pts aged <65 y, with a history of diabetes, treated with AKT/mTOR inhibitors are more likely to develop significant hyperglycaemia on study. These predictive factors may warrant further validation in a prospective setting, and may help in the future management of pts treated with this important class of agents.
No conflict of interest.

867 POSTER

Fatty acid synthase inhibition as a potential therapeutic target in triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) are defined by the lack of detectable expression of estrogen receptor, progesterone receptor and do not have amplification of HER2. There is no clinically validated molecularly targeted therapy for TNBC and patients have a poor prognosis due in part to the high probability of relapse after chemotherapy. Thus, the identification of novel targeted therapies for TNBC patients is needed.
The expression levels of FASN, a multi-enzyme protein that catalyses de novo synthesis of fatty acids, are low or undetectable in normal tissues. In contrast, high levels of FASN expression have been detected in several human carcinomas. Some reports highlight that FASN over expression correlates with progression, aggressiveness and metastatic potential of the disease.
The aim of our study is determine the FASN tumor expression levels in TNBC patients and in parallel evaluate the therapeutic effect of FASN inhibition (alone or in combination with current treatments) in a panel of TNBC cells sensitive and resistant to conventional therapies (such as doxorubicin and paclitaxel).
Methods: FASN and EGFR expression was retrospectively evaluated in 29 paraffin-embedded core-biopsies of patients with TNBC by Immunohistochemistry (IHC).
TNBC cell lines were long-exposed to increasing doses of doxorubicin (DR), paclitaxel (PR) or both (DPR) to establish long-term chemoresistant cells. Western-Blot (WB) analysis were performed to evaluate the main signaling pathways in both sensible and resistance cells. The cytotoxic effect of the anti-FASN compounds were determined by an MTT assay. Quantitative Real-Time PCR (qRT-PCR) was used to analyze the expression of basal and mesenchymal markers to determine the molecular subtypes and for the study of cell population changes under chemotherapy.
Results: FASN staining was positive in all 29 TNBC tumor samples, with low (69%) and moderate (31%) levels. EGFR were positive in 76% of the tumors respectively. Analysis by WB and qRT-PCR showed higher levels of FASN in TN CK5/6$^+$ and EGFR$^+$ cells than in TN VIM$^+$ cells. FASN pharmacological inhibition (alone and in combination) was cytotoxic and induced apoptosis in all TNBC cells treated.
Conclusions: FASN is expressed in TNBC tumors and *in vitro* FASN inhibition (alone and in combination) induces apoptosis in TN cells. The absence of target therapies for this breast cancer subtype and its poor prognosis lead to the exploration of FASN as a therapeutic target for TNBC patients.
No conflict of interest.

868 POSTER

Phase 1 dose escalation study of the investigational drug TAK-960, an oral polo-like kinase 1 (PLK1) inhibitor, in patients (pts) with advanced solid tumors

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Background: PLK1 is involved in mitosis and DNA integrity and is over-expressed in various tumor types, presenting a target for cancer therapy. This first-in-human study evaluated TAK-960 in pts with advanced solid tumors to determine safety, tolerability, MTD/RP2D, pharmacokinetics (PK), anti-tumor activity and pharmacodynamics (PD) (NCT01179399; sponsored by Millennium: The Takeda Oncology Company).
Materials and Methods: Key eligibility: ⩾18 y with metastatic solid tumor unresponsive to current treatment, ECOG status of 0−1. TAK-960 PO QD in a 3+3 design for 21-d in a 28-d cycle. Samples for plasma PK assessment were taken pre-dose and at time points post-dose on d 1 and 21 of cycle 1. 25 pts provided skin biopsies at screening, pre-dose and 6 hr post-dose on day 7 of cycle 1. Average mitotic index and phosphorylated keratin (pKeratin) intensity were determined by immunohistochemistry.
Results: 32 pts (median age 65 y [range 47−84]) received 1−28 mg TAK-960 (median 2 cycles, range 1−12; 7 pts ⩾6 cycles). Safety population was 32 pts, 1 dose limiting toxicity reported (grade 4 neutropenia). 20 pts (63%) had a drug-related AE, fatigue (34%), decreased appetite (25%), nausea (19%). 6 pts (19%) had a grade ⩾3 drug-related AE; 1 pt (3%) had a drug-related serious AE and discontinued (grade 4 neutropenia); 3 (9%) unrelated on-study deaths. The study was terminated due to business reasons before the MTD or RP2D were established, a further 21-d cycle was considered (7-d QD then 14-d rest period). In 31 PK evaluable pts, TAK-960 was characterized by a median T_{max} of 6 hr, low fluctuation at steady-state (overall mean peak-to-trough ratio of 1.4), and moderately long mean $t_{1/2}$ of 48 hr. Overall mean accumulation ratio was 3.8-fold following repeated QD dosing for 21 d. Steady-state exposures (d 21 AUC, Time 0 to end of dosing interval $AUC_{(0-tau)}$,) increased in an approximately dose proportional manner over 1 to 28 mg. Average mitotic indices were <1 at screening, with indices >2 in 2 pts (1, 20 mg; 1, 28 mg) on day 7 of cycle 1. No trends in pKeratin intensity were observed. Best response was stable disease (14 pts, 3 pts >3 months, 3 pts >6 months: pancreas, CRC, breast, ovarian, H and N x2, squamous, bladder, thyroid, colon x2, prostate, rectal, unknown). Progressive disease observed in 17 pts.
Conclusions: TAK-960 was generally well tolerated, exhibiting linear PK and characteristics that support QD dosing. Stable disease in 14 pts with a range of tumor types.
Conflict of interest: *Corporate-sponsored research: Research funding: Emiliano Calvo, Kyriakos P Papadopoulos, Antonio Cubillo, Drew Rasco (Millennium: The Takeda Oncology Company). Sunil Sharma (Millennium: The Takeda Oncology Company Beta Cat Pharmaceuticals Salarius ConverGene). Other substantive relationships: Employment: Hongliang Shi, Stephanie Faucette, Xiaofei Zhou, Keisuke Kuida, Cristina Oliva (Millennium: The Takeda Oncology Company).*

869 POSTER

Rapid validation of a novel kinase target FAM20C through integration of large scale genomic databases and matched patient derived tumor models

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Background: Target discovery and validation in oncology has largely relied on molecular and functional studies performed in cell lines. Recent advances in genomics have now created large databases based on well-characterized tumor tissue, which has enabled direct investigation of patient tumors for novel targets.
Material and Methods: We have developed a target validation platform based on a large scale genomic database matched to patient-derived tumor models. The platform relies on Molecular Response's proprietary bank of more than 144,000 patient derived tumor cells, of which nearly 400 tumors have been genomically characterized and databased for target discovery studies. The database is growing, but currently features the following cancer indications: colon carcinoma, NSCLC, melanoma, ovarian carcinoma, prostate cancer and Non-Hodgkins Lymphoma. Upon discovery of a novel target, tumors of interest are immediately implanted into mice to perform functional studies in direct patient derived models–either in vivo or ex vivo.
Results: Through use of this platform, we have identified the novel kinase target FAM20C for therapeutic development. We investigated prevalence of target overexpression across 7 cancer indications, and identified melanoma as a clinical indication of high interest. We examined growth characteristics from patient tumors featuring high kinase gene expression vs. low expression to help characterize the role of this target in oncology disease progression. Finally, we performed functional knockdown studies in patient derived models to further validate this novel kinase as a druggable target of pharmaceutical interest. Studies are ongoing to develop small molecule and antibody-based therapeutics which will serve as drug candidates for further development.
Conclusions: FAM20C represents a novel molecular target for development of targeted therapeutics in the treatment of melanoma, and potentially other cancers.
No conflict of interest.

870 POSTER

Novel receptor-mediated transport of the anticancer agent Dp44mT

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Background: Defective iron homeostasis in cancer cells, owing to the perturbed expression of iron-related proteins, may confer a survival advantage to neoplastic cells and poorer patient prognosis (*Cancer Res* 2011;71:1511–1514). Iron chelators have emerged as anti-tumour agents that disrupt vital iron and copper trafficking of cancer cells. Several *in vitro* and *in vivo* studies have demonstrated the potent anti-cancer and anti-metastatic activity of the chelator, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT; *PNAS* 2006;103:14901−6; *EMBO Mol Med* 2012;4:93–108). Recent investigations have shown that Dp44mT accumulates within the lysosome, disrupting lysosomal membrane integrity and resulting in apoptosis (*Cancer Res* 2011;71(17):5871−80). However, the mechanism by which this drug is transported into cells to induce cytotoxicity is unknown.
Materials and Methods: ^{14}C-Dp44mT was employed to assess membrane transport mechanisms using 3 tumour cell lines, namely: SK-N-MC neuroepithelioma, SK-Mel-28 melanoma and DMS-53 lung carcinoma and mortal MRC-5 fibroblasts.
Results: The cellular uptake of ^{14}C-Dp44mT as a function of concentration was saturable in SK-N-MC cells (B_{max} 4.28 x 10^7 molecules of chelator/cell and K_d 2.45 μM), suggesting it enters cells *via* a receptor-mediated process. Saturable uptake was also observed in DMS-53 and SK-Mel-28 cells as well as MRC-5 fibroblasts. The uptake of ^{14}C-Dp44mT was examined in the presence of its unlabelled precursors, namely dipyridyl ketone (Dp) and 4,4-dimethyl-3-thiosemicarbazide (44mT), in order to decipher the stereospecificity of the transport mechanism involved. ^{14}C-Dp44mT uptake was significantly (p<0.01) decreased in the presence of unlabelled Dp44mT, while its unlabelled precursors, Dp or 44mT, had no significant effect (p>0.05) on ^{14}C-Dp44mT transport in SK-N-MC cells. A range of structurally similar and diverse thiosemicarbazones were also screened to determine the specificity of the receptor, including the DpT series (DpT, Dp4mT, Dp4eT, Dp4aT, Dp4pT and DpC); the BpT series (BpT, Bp4mT, Bp44mT, Bp4eT and Bp4aT); the ApT series (ApT, Ap4mT, Ap44mT, Ap4eT and Ap4pT); as well as the well know chelators, Triapine® and PIH. All unlabelled ligands of the DpT series and unlabelled Bp44mT, Bp4mT and Ap44mT significantly (p<0.01) inhibited the uptake of ^{14}C-Dp44mT in comparison to the control (^{14}C-Dp44mT alone). The other members of the BpT and ApT series as well as Triapine® and PIH had no significant (p>0.05) effect on ^{14}C-Dp44mT uptake. As may be expected, unlabelled Dp44mT appeared to most markedly (p<0.001) decrease the cellular uptake of ^{14}C-Dp44mT.
Conclusion: The receptor involved in the uptake of Dp44mT by cells shows high affinity for Dp44mT. Our studies highlight the importance of the saturated N4 structural moiety in the receptor-mediated transport of our ligands. The uptake of Dp44mT through a receptor-mediated system may reveal a potential mechanism to selectively target chemotherapeutics to malignant cells. These findings have great clinical implication for the extent of uptake and bioavailability of the drug within the circulation.
No conflict of interest.

871 POSTER

Syntactic analysis for single agent phase I trials in Japan, from National Cancer Center Hospital (NCCH) experience

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Background: In Japan, to develop investigational new drug, most phase I trials, especially 'first in human' trials are starting from about 50% of MTDs of dosage to the MTD levels based on the initial phase I studies and results in the West, from where the trials are usually started. However, little has been well recognized about actual status in phase I trials in Japan. This study is aimed to evaluate the toxicity profiles including MTD levels as compared with those in the West, anti-tumor response, and survival of patients enrolled in phase I trials in Japan.
Patients and Methods: Between July 1995 and December 2012, we retrospectively analyzed the data of patients enrolled in single agent phase I trials at NCCH. The doses with anti-tumor response observed, DLT profiles, and MTDs were compared with those in the West. Cox proportional hazards model was examined to assess potential prognostic factors for survival.
Results: A total of 777 patients were enrolled in 44 phase I trials including 5 first in human trials. The median age was 57 (range, 18−76) years and 57.9% were male. ECOG performance status (PS) of 0 and 1 were 38.6 and 61.1%, respectively. The common cancer types were lung cancer (31.9%), colorectal cancer (21.0%), sarcoma (13.8%), esophagus cancer (4.6%). DLTs were observed in 12.1% of the patients. The dose levels in which DLTs observed were as follows: 5.3% were in +1 dose level as compared with those in the West, 31.9% were in the same dose level, 28.7% in −1 dose level, 14.9% in −2 dose level, and 3.2% in −3 dose level. Tumor shrinkage was observed in 20.8% of the patients and the response rate was 6.3 (95% CI: 4.6−8.0)%. Almost all (93.9%) responding patients were in and around the MTD levels (34.7, 32.7 and 26.5% were in the MTD, −1, and −2 dose levels, respectively). Median OS was 11.5 (95% CI: 10.5–12.4) months. Male, PS of 1, body weight loss (⩾5% within 3 months), liver metastasis, elevation of AST and LDH (⩾ upper limit of normal), and hypoalbuminemia were independent poor prognostic factors for survival.
Conclusions: This is the first report of syntactic analysis for single agent phase I trials in Japan. Dose levels with DLTs and observed responders were almost similar to those in the West, and these were around the MTD levels (from −2 to +1 dose levels from MTD). Prognostic factors were similar to those in previous reports in the West. Phase I studies in Japan are practically carried out on the same time lines and study qualities with those in the West.
No conflict of interest.

872 POSTER

Cumulative safety experience of telotristat etiprate in clinical trials supports advancement to phase 3

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Background: Telotristat etiprate (TE) (or LX1606) is a novel, oral inhibitor of serotonin synthesis being developed for treatment of carcinoid syndrome (CS). Telotristat etiprate reduced CS symptoms such as diarrhea in Phase 2 studies. In preparation for Phase 3, we conducted a systematic review of safety data up to 19 January 2013 from 3 completed studies of healthy subjects and 2 ongoing clinical studies of patients (pts) with CS.
Material and Methods: To detect potential signals, cumulative safety databases were pooled and examined using analyses of: 1) 77 special MedDRA queries covering 22 disease areas, 2) adverse event (AE) accumulations by study day, 3) System-Organ class (SOC) and preferred term, and 4) laboratory testing. Serious adverse events (SAEs) and other cases of interest were reviewed for potential relationship to study drug.
Results: Of the 121 subjects in this analysis, 88 were healthy subjects who received single- or multiple-dose levels of up to 1500 mg/day for up to 14 days; 33 were pts with CS exposed to at least one dose of TE, with dose levels up to 1500 mg/day for up to 12 weeks of initial treatment, with an option to continue into an open-label phase for a total of 124 weeks. Of the 33 pts with CS, 16 were on TE for ⩾6 months, 11 were on TE for ⩾12 months, and 6 patients were on TE for ⩾24 months. Across the studies, there was no common theme in AEs. All SAEs reported by subjects receiving TE were assessed as unrelated to study drug with the exception of 1 case of severe nausea and vomiting, which resolved in 10 days. Most AEs were assessed as of mild to moderate intensity, and most resolved spontaneously while continuing study drug. In Phase 1 studies of healthy subjects, mild increases in hepatic transaminase levels (mostly <2 x ULN) were noted, with 1 subject discontinuing from therapy at the 500 mg bid dose level. In Phase 2 studies, no signal for transaminase abnormalities has been observed to date. In both active drug and placebo, the most common SOC in which AEs were reported was Gastrointestinal (GI) Disorders. Nausea and vomiting accounted for a large proportion of events in this SOC, and occurred relatively early in treatment, usually without recurrence.
Conclusions: This safety review supports advancement of telotristat etiprate to Phase 3 for treatment of CS. Treatment-related SAEs and discontinuations due to AEs have been rare. The majority of events identified in studies thus far are GI symptoms, consistent with the underlying disease.
Conflict of interest: *Ownership: Stock ownership (Lexicon Pharmaceuticals, Inc.) − DF, GLY, JJ, SJ, PL. Other substantive relationships: employment (Lexicon Pharmaceuticals, Inc.) − DF, GLY, JJ, SJ, PL contract employment − DM*

873 POSTER

Clinical outcomes of patients treated within early phase cancer trials: An audit of the NIHR/Wellcome UCLH clinical research facility

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Background: A number of centres have devised prognostic scores to predict the outcome of patients treated within Phase I trials using baseline characteristics. The Royal Marsden Hospital (RMH) score has demonstrated significantly increased risk with the presence of 2 or 3 of the following factors; low albumin (<35 g/L), elevated lactate dehydrogenase (>upper limit normal) or more than 2 sites of metastases. We assessed the clinical outcomes of patients treated within our early phase trial unit and analysed the relevance of the RMH score within this patient population.
Methods: We retrospectively analysed baseline characteristics and clinical outcomes including best response, progression free (PFS) and overall survival (OS) in 165 sequential patients treated between April 2010 and January 2013. Survival distributions were estimated and differences in survival according to RMH score were analysed using the log-rank test.
Results: Of 165 patients treated in 24 trials, 107 were treated within Phase 1 trials and 58 within Phase II. Median age was 60 years (range 21−83). Best overall response was; partial response in 14%, stable disease in 51% and disease progression in 21%. Ninety-day mortality rate for patients treated in a Phase I trial was 11.9% and 30-day mortality was 3.8%. The median OS for all patients (Phase I and II) was 12.8 months and median PFS was 4.8 months. OS was significantly longer in patients treated within phase II trials than those treated in a phase I trial (median 14 vs 12.5 months, p = 0.043). The RMH score could be applied in 81 patients of which 71% of patients had a 'good' prognostic score of 0−1, and 29% a 'poor' score of 2−3 (no patients scored 3). OS in the 0−1 group was not significantly better compared to the 2−3 group (0−1 group HR = 1.0, 2−3 group HR = 1.02, p = 0.95).
Discussion: In our series of patients, the RMH score was not prognostic however no patients had a high risk score of 3. This may reflect the increasingly stringent eligibility criteria required for current trials. Overall survival and 90-day mortality of our Phase I patient population compares favourably with data reported by other centres. This study demonstrates the possible clinical benefit gained by patients recruited into early phase trials and is in keeping with recently published data.
No conflict of interest.

874 POSTER

Improving patient selection and outcomes in phase I trials: validating the Royal Marsden Hospital prognostic score

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Background: Phase I clinical trials remain an essential step in the development of novel anti-cancer agents. Patient selection is pivotal to maximising patient benefit and avoiding premature study withdrawal of unfit patients and the need for additional recruitment. Performance status (PS) and a life expectancy ⩾3 months are often used to define patients' fitness and trial eligibility. However these methods are subjective and it is reported that up to 20% of patients die within 90 days of starting a phase I trial. The Royal Marsden Hospital (RMH) prognostic score, based on three variables: Lactate dehydrogenase (LDH) > upper limit of normal, albumin <35 g/l and >2 sites of metastases has been shown to predict patient survival. We sought to validate this score in our phase I patient population.

Materials and Methods: Retrospective review of patient referrals for phase I trials to the Sir Bobby Robson Cancer Trials Research Centre, Northern Centre for Cancer Care, Newcastle, UK between January 2009 and December 2011. Baseline characteristics: age, sex, tumour type, WHO PS, number of metastatic sites and baseline blood parameters including haemoglobin, albumin and LDH collected. Clinical outcome assessed by: ⩽90 day mortality rates, best response by RECIST and overall survival defined by date of death or last follow-up. Reasons why individual patients failed to enter phase I trials were recorded.
Results: Data for all 287 patient referrals reviewed. In our centre volume of referrals exceeded trial availability and many patients deteriorated waiting for a trial. 80 (28%) of these patients with a range of advanced solid malignancies entered a total of 21 phase 1 trials (48% combination studies of a novel agent + cytotoxic and 52% single agent). The commonest reason for not taking part was declining PS (59%) and 39% of patients failed screening (52% due to impaired liver function). Median age of trial participants was 63 years (range 23−79) with 23% aged ⩾70 years. Baseline PS was 0 (23%), 1 (70%), 2 (3%). Best response was: partial response 5%, complete response 1% with 50% of patients achieving stable disease. The median survival of all 80 patients was 221 days and the ⩽90 day mortality rate was 9%. Patients with a good RMH score (0−1) at baseline had a longer median survival than patients with poor prognostic scores (2−3); 225 days vs. 207 days; P <0.05. In multivariate analysis the RMH score was an independent variable that predicted survival.
Conclusions: The poor RMH prognostic score predicted a shorter survival in our patients, validating further its use in phase I patient selection.
No conflict of interest.

875 POSTER

Phase I clinical trial investigating maximum tolerated dose, safety and pharmacokinetics of volasertib in Japanese patients with advanced solid tumours

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Background: Polo-like kinase 1 (Plk1) is a key regulator of mitosis and is a promising therapeutic target in cancer. Volasertib (BI 6727; an investigational agent) is a selective and potent Plk inhibitor that induces mitotic arrest and apoptosis. The maximum tolerated dose (MTD) for volasertib was established and volasertib had a manageable safety profile and favourable pharmacokinetics (PK) in Caucasian patients (Phase I studies). Here, for the first time the MTD, safety, PK and clinical benefit of volasertib were investigated in Japanese patients (NCT01348347; sponsored by Boehringer Ingelheim), and the results were compared with those of Caucasian patients.
Material and Methods: In this ongoing, open-label, dose-escalation Phase I study, Japanese patients with refractory advanced solid tumours were treated with escalating doses of volasertib (200, 300 and 350 mg). The primary endpoint of this study was the MTD of volasertib. Secondary endpoints included safety, PK and clinical benefit.
Results: Fifteen patients with advanced solid tumours were treated. Dose-limiting toxicities (DLTs; Common Terminology Criteria for Adverse Events [CTCAE] grade 4 neutropenia for ⩾7 days and CTCAE grade 4 thrombocytopenia) were experienced by 2/6 patients in the 350 mg cohort. The MTD of volasertib in Japanese patients was 300 mg; this is consistent with the recommended Phase II dose (300 mg) and comparable to the MTD (400 mg) in the Caucasian Phase I study. In this study, the most common (⩾3 patients) drug-related non-haematological adverse events included fatigue, decreased appetite and nausea. Exposures of volasertib and its metabolite increased with increasing doses and were comparable with those of Caucasian patients (Phase I). Partial response (n = 1; gastric cancer) and stable disease (n = 11, including 3 patients for >12 weeks prior to disease progression) were observed.
Conclusions: In Japanese patients, volasertib had a manageable safety profile up to the MTD determined as 300 mg. Reversible myelosuppression (neutropenia and thrombocytopenia) constituted DLTs as expected from the mode of action. The results on safety and PK of volasertib in Japanese cancer patients are comparable with those previously obtained in Caucasian patients and support enrolment of Japanese patients in global clinical trials without dose modification.
Conflict of interest: *Other substantive relationships: Taube,T is employed by Boehringer Ingelheim Pharma GmbH & Co KG. Takeuchi, Y is employed by Nippon Boehringer Ingelheim Co Ltd*

876 POSTER

Exploratory study of health-related quality of life in a phase I trial studying Idarubicin-loaded beads for chimioembolization of hepatocellular carcinoma

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Background: Phase I trials aim to identify the recommended phase II dose: the maximum-tolerated dose (MTD) that will be investigated in further trials. In this context, the added value of health-related quality of life (QoL) of patients to complement the usual toxicity assessment should be questioned. The objective was to investigate QoL in a phase I trial.
Methods: A phase I dose-escalation trial of transarterial chemoembolization (TACE) with idarubicin-loaded beads was performed in cirrhotic patients with hepatocellular carcinoma. Idarubicin dose was escalated according to a modified continuous reassessment method. MTD was defined as dose level closest to that causing dose limiting toxicity (DLT) in 20% of patients.
QoL was evaluated using the EORTC QLQ-C30 at baseline and at days 15, 30 and 60 after TACE. Changes in QoL scores were described using mean difference in scores with baseline as reference and scores at each follow-up, for all patients and according to the idarubicin dose level. The time to QoL score deterioration (TTD) was investigated as a modality of longitudinal analysis. TTD was defined as the time from randomization to a first QoL score deterioration with a 5-point MCID as compared to the baseline score or death. Univariate Cox analyses were performed to identify factors influencing TTD.
Results: Between March 2010 and March 2012, 21 patients were included: 9, 6, and 6 patients were treated at idarubicin dose levels of 5-, 10-, and 15-mg, respectively. Calculated MTD of idarubicin was 10 mg. The median TTD was 0.76 months [95% CI 0.62-NA] for Global Health Status, 0.69 months [0.59-NA] for fatigue and 2.50 months [0.76-NA] for pain. At 10-mg idarubicin dose level, patients presented a longer TTD than at 5-mg dose level for Global Health Status (HR 0.87 [95% CI 0.14–5.36]), physical functioning (HR 0.67 [0.11–4.13]), fatigue (HR 0.77 [0.13–4.71]) and pain (HR 0.52 [0.09–3.16]). Women presented a shorter time to pain deterioration than men (HR 14.7 [1.31–164.7]).
Conclusions: These results show the importance to study QoL in phase I trials. These results are consistent to the idarubicin dose level of 10 mg retained. Nevertheless, if we had integrated TTD in DLT, we could retain idarubicin dose level of 15 mg as the MTD. Moreover, it raises the issue of a specific questionnaire for phase I trial which would be more focused on toxicities.
No conflict of interest.

877 POSTER

Adverse and expected drug reactions to therapy with mistletoe extracts (Viscum album L.) in cancer patients

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Background: Mistletoe (*Viscum album L.)* is one of the most frequently prescribed complementary treatments for cancer in Europe. As an immunomodulating substance it can induce local and systemic immune responses. According to product information, dose-finding is related to local reactions (3−5 cm) and temperature increase (up to 38ºC). Formally, these parameters are defined as adverse drug reactions (ADRs). In terms of safety, therefore, it is necessary to distinguish between adequate immune reactions (expected reactions like local erythema, induration or pain and increased body temperature) and other ADRs. The present study investigates ADRs that occurred in cancer patients treated with mistletoe in an integrative oncological setting.
Material and Methods: The Network Oncology is a conjoint clinical registry of German hospitals and out-patient practitioners that systematically records cancer diagnoses, therapies, ADRs and disease progress. This database was analysed for mistletoe related ADRs, which were classified as MedDRA terms (15.0) and rated on severity. Logistic regression analyses were performed.
Results: A total of 1657 cancer patients (1194 females [72%]; 463 males [28%]) were treated with mistletoe extracts by subcutaneous application. Of these, 378 patients (23%) reported a total of 692 formal ADRs. The majority of formal ADRs (64%) were rated as grade I (mild) in severity, while 10 cases (1%) were rated as grade II (moderate) and 9 cases (1%) as

grade III (severe). No cases were judged as grade IV (life threatening). More than 99% of all formal ADRs were expected (increased temperature [49%], erythema [29%], induration [4%] and pain [3%]). Only 11 patients had other ADRs (chills, headache, nausea and vomiting), collectively making up less than 1% of formal ADRs. Based on logistic regression analysis, females were more likely to experience a formal ADR (OR = 1.5; CI = 1.07, 2.12; p = 0.02), while older age (OR = 0.98 per year; CI = 0.97, 0.99; p < 0.001) and UICC stage IV (OR = 0.53; CI = 0.31, 0.92; p < 0.02) were associated with a lower risk.

Conclusions: Less than a quarter of cancer patients treated with mistletoe reported a formal ADR. These were almost exclusively expected reactions, such as increased body temperature and injection site erythema indicating stimulation of the immune system. With increasing age and tumour stage immunoreactivity decreased. Based on these results, treatment with mistletoe extracts is safe.

Conflict of interest: *Advisory board: Weleda AG*

878 POSTER

Chymase inhibitor, TY-51469, attenuates monocrotaline-induced sinusoidal obstruction syndrome in hamsters

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Background: The recent chemotherapy regimens of adding oxaliplatin improve the survival of patients with metastatic colorectal cancer. However, treatment with oxaliplatin induces hepatic sinusoidal dilation and haemorrhage, and sinusoidal obstruction syndrome (SOS) is observed in the most of hepatectomy specimens from these patients as well. To date, however, any useful strategy for prevention of SOS has not been established. Previous reports have demonstrated a significance of matrix metalloproteinase (MMP)-9, which is formed from a precursor proMMP-9 by chymase stored in mast cells, in the progression of SOS. In this study, we investigated the preventive effect of a chymase inhibitor, TY-51469, on monocrotaline (MCT)-induced SOS in hamsters.

Material and Methods: Hamsters were orally administrated with a single dose of MCT (120 mg/kg) to induce SOS. Treatment with TY-51469 (1 mg/kg per day) or placebo was started 3 days before the MCT administration. Blood samples and liver tissue were examined two days after the MCT administration. Furthermore, to determine the survival rate, either TY-51469 or placebo was administered from 3 days before up to 14 days after MCT administration.

Result: Two days after the MCT administration, significant increases of aspartate aminotransferase, alanine aminotransferase and total bilirubin and a significant reduction of albumin were observed in plasma, but their changes were significantly attenuated by treatment with TY-51469. The numerous hepatic necrosis areas were observed in the placebo-treated group, but the ratio of necrotic area to total area in liver was significantly reduced by treatment with TY-51469. Chymase activity and the levels of MMP-9 and tumour necrosis factor (TNF)-α in the liver were significantly augmented in the placebo-treated group. However, these were significantly attenuated in the TY-51469-treated group. Furthermore, both gene expressions of chymase and MMP-9 were significantly augmented in the placebo-treated group, which were significantly attenuated in the TY-51469-treated group. Until 14 days after MCT administration, survival rates in the placebo- and TY-51469-treated groups were 25% and 70%, respectively, and there was a significant difference between the two groups.

Conclusion: Chymase inhibition by TY-51469 may prevent the accelerating of severity in MCT-induced SOS in hamsters.

No conflict of interest.

879 POSTER

MPDL3280A (anti-PDL1): Clinical activity, safety and biomarkers of an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors

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Background: PD-L1 and PD-L2 have been reported to regulate Th1 and Th2 immune responses. Tumor-expressed PD-L1, when bound to PD-1 or B7.1 on activated T cells, can mediate cancer immune evasion. Inhibiting the binding of PD-L1 to its receptors represents an attractive strategy to restore tumor-specific T-cell immunity. However, PD-L2 expressed in the tumor microenvironment may also bind PD-1-expressing T cells, dampening their function. MPDL3280A (anti-PDL1), a human monoclonal antibody containing an engineered Fc-domain designed to promote a Th1-driven response to optimize efficacy and safety, is described here along with Phase I results.

Materials and Methods: A study was conducted with MPDL3280A administered IV q3w in pts with locally advanced or metastatic solid tumors, including 3+3 dose-escalation and expansion cohorts. ORR was assessed by RECIST v1.1 and includes u/cCR and u/cPR. PD-L1 was measured by IHC (pos vs neg), and PD-L2 was measured by qPCR (high vs low) in archival tumor specimens.

Results: As of Feb 1, 2013, 171 pts were evaluable for safety. Administered doses include $\leqslant$1 (n = 9), 3 (n = 3), 10 (n = 35), 15 (n = 57) and 20 mg/kg (n = 67). Pts in the dose-escalation cohorts did not experience DLTs. No MTD was identified. Pts had received MPDL3280A for a median duration of 147 days (range 1–450). 41% of pts reported G3/4 AEs, regardless of attribution. No acute pneumonitis was observed. 122 pts enrolled prior to Jul 1, 2012 were evaluable for efficacy. RECIST responses were observed in multiple tumor types including NSCLC (9/37), RCC (5/39), melanoma (9/35), CRC (1/4) and gastric cancer (1/1). An ORR of 21% (25/122) was observed in nonselected solid tumors with a duration of response range of 1+ to 253+ days. Other pts had delayed responses after apparent radiographic progression (not included in the ORR). The 24-week PFS was 42%. 94 pts had tumors evaluable for PD-L1 status, and 81 pts had tumors evaluable for PD-L2. Median PD-L2 expression was $\approx$2x higher in PD-L1-pos tumors versus PD-L1-neg tumors. The ORR was 39% (13/33) for pts with PD-L1-pos tumors versus 13% (8/61) for pts with PD-L1-neg tumors. Pts with PD-L2^{High} tumors showed an ORR of 27% (11/41), versus 13% (5/40) for pts with PD-L2^{Low} tumors. Updated data will be presented.

Conclusions: MPDL3280A was well tolerated, with no pneumonitis-related deaths. Durable responses were observed in a variety of tumors. PD-L1 and PD-L2 tumor status appears to correlate with responses to MPDL3280A.

Conflict of interest: *Advisory board: Amgen, Boehringer, Bristol-Myers Squibb, Genentech, Imclone, Lilly, Merck KGaA, Millennium, Novartis, Onyx, Pfizer, Roche, Sanofi, Celgene, Genentech. Corporate-sponsored research: Genentech, Roche, BMS, Novartis. Other substantive relationships: Amgen, Merck KGaA, Novartis, Roche, Sanofi, PharmaMar*

880 POSTER

Survival and long-term safety in patients (pts) with advanced solid tumors receiving nivolumab (anti-PD-1; BMS-936558; ONO-4538)

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Background: Blockade of programmed death-1 (PD-1), a co-inhibitory receptor expressed by activated T cells, can overcome immune resistance and mediate tumor regression (Topalian et al, NEJM 2012). We present survival and long-term safety results from a clinical study of nivolumab, a PD-1 receptor blocking monoclonal antibody, in pts with advanced solid tumors.
Methods: Pts received nivolumab (0.1−10 mg/kg IV Q2W) in an outpatient setting during dose escalation and/or cohort expansion. Tumors were assessed by RECIST 1.0 after each 4-dose cycle. Pts received ⩽12 cycles or until discontinuation criteria were met.
Results: 306 pts with non-small cell lung cancer (NSCLC; n = 129; squamous and non-squamous), melanoma (MEL; n = 107), renal cell (RCC; n = 34), colorectal (n = 19) or prostate cancer (n = 17) were treated. Objective responses (OR) were observed in NSCLC, MEL and RCC (Table). Additional pts with NSCLC, MEL and RCC manifested stable disease (SD) for ⩾24 weeks (Table). In these heavily pretreated pts (47% with 3−5 prior systemic therapies), median OS in NSCLC, MEL, and RCC was 9.6, 16.8 and >22 months, respectively. Drug-related adverse events (AEs; any grade) occurred in 75% (230/306) of pts, the most common being fatigue (28%), rash (15%), diarrhea (13%), and pruritus (11%). Grade 3−4 drug-related AEs occurred in 17% (52/306) of pts. Drug-related pneumonitis (any grade) occurred in 4% (12/306) of pts, with grade 3−4 drug-related pneumonitis occurring in 1% (4/306) of pts and associated with 3 deaths in the trial. Exploratory data correlating PD-L1 expression to outcomes using an automated assay with the 28−8 anti-PD-L1 antibody will be presented.
Conclusions: Nivolumab produced durable tumor regression, and was associated with OS and landmark 1−2 year survival values which are unexpected in such heavily pre-treated pts with advanced NSCLC, MEL and RCC. We observed an acceptable safety profile with long-term drug administration in the outpatient setting. These findings support the ongoing clinical development of nivolumab in phase 3 trials with survival endpoints.

Tumor type	Dose, mg/kg	OR Rate, n/N (%)	SD ⩾24 wk, n/N (%)	Median OS, mo (95% CI)	OS Rate, % (95% CI); pts at risk, n 1 y	2 y
NSCLC	1−10	22/129 (17)	13/129 (10)	9.6 (7.8−12.4)	42 (33−51); 43	14 (4−24); 5
MEL	0.1−10	33/107 (31)	7/107 (7)	16.8 (12.5−31.6)	62 (53−72); 55	43 (32−53); 26
RCC	1 or 10	10/34 (29)	9/34 (27)	>22[a] (13.6−NE)	70 (55−86); 23	50 (31−70); 8

[a]Median OS was not reached at 22 mo, the longest time to death so far.
NE, not estimable.

Conflict of interest: *Ownership: JM Wigginton: employee stock ownership, BMS. Advisory board: JR Brahmer: BMS, uncompensated. DF McDermott: BMS. S Gettinger: Nivolumab (April 2013), BMS. JM Taube: BMS. M Sznol, BMS. Corporate-sponsored research: FS Hodi: BMS, institute received clinical trial support. SL Topalian: BMS. DC Smith: BMS, OncoMed, Celgene, MedImmune, Millennium, AstraZeneca, Atterocor, Debiopharm. JM Taube: BMS. Other substantive relationships: FS Hodi: non-paid consultant to BMS. SL Topalian: uncompensated consulting for BMS consulting for Jounce Therapeutics spouse consulting for Amplimmune, Inc spouse royalties through institution from BMS and Amplimmune. JM Wigginton: employee, BMS.*

881 POSTER

A phase I dose-escalation and pk study of continuous oral rucaparib in patients with advanced solid tumors

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Background: Rucaparib, a potent, oral small molecule inhibitor of poly (ADP-ribose) polymerase (PARP) 1 and −2, is being developed for treatment of homologous recombination repair deficient (HRD) ovarian cancer. This study evaluated rucaparib as monotherapy. Primary objectives were to define the maximum tolerated dose (MTD), recommended Phase 2 dose (RP2D), and PK of continuous oral rucaparib.
Materials and Methods: A standard 3+3 dose escalation design was used. Intra-patient dose escalation was allowed. Patients (pts) aged ⩾18 with advanced solid tumor that progressed on standard treatments were recruited. Measurable disease was not required. Rucaparib was taken orally qd or bid continuously until disease progression. Plasma PK assessments included full profile, trough levels, and food effect.
Results: 33 pts (median age 49 yrs [range 21−71]; 30 female; 17 ECOG PS=0; 18 breast cancer (BC), 10 ovarian/peritoneal cancer (OC), 5 other tumor) were enrolled in 7 dose cohorts (40, 80, 160, 300 and 500 mg qd, and 240, 360 mg bid). One pt at 360 mg bid experienced DLT of CTCAE grade 3 nausea. No pts discontinued treatment due to toxicity. Treatment-related adverse events (primarily grade 1−2) reported in ⩾10% of pts include fatigue (n = 8), nausea (n = 5), anorexia (n = 4), vomiting (n = 4), and diarrhea (n = 3). Grade 3/4 toxicities have been minimal and no myelosuppression has been observed. To date, two pts (1 BC, 1 OC; both $BRCA1^{mut}$; 300 mg qd) achieved a PR (duration 14 and 21+ wks, respectively). An additional 10 pts (5 OC, 4 BC, 1 CRC; 7 $BRCA^{mut}$, 2 $BRCA^{unk}$, 1 $BRCA^{wt}$) achieved a best response of stable disease (SD) >12 wks thus far. Three pts (all $BRCA^{mut}$ OC) are ongoing in wks 26, 27, and 40. Five recently enrolled pts are also ongoing. Overall disease control rate (CR+PR+SD>12 wks) to date in all evaluable OC pts across all dose levels is 86% (6/7). Dose proportional PK was observed up to 500 mg qd with mean $t_{1/2}$ of 15 h (range 4.3−29 h). Following qd dosing, steady state was achieved by Day 8. As expected, bid dosing increased trough levels above 2 μM target with low interpatient variability.
Conclusion: Continuous oral rucaparib is well tolerated, with encouraging clinical activity, including objective responses and durable SD, observed during dose-escalation. Once confirmed, the RP2D will be evaluated in platinum-sensitive OC pts with a gBRCA mutation.
Conflict of interest: *Ownership: Heidi Giordano, Jennifer Borrow and Sarah Jaw-Tsai are employees/stock holders of Clovis Oncology, Inc. Advisory board: Rebecca Kristeleit is an advisory board member for Clovis Oncology, Inc. Corporate-sponsored research: The institutions for Drs. Flynn, Shapiro, LoRusso, Kristeleit, Patel, Infante and Burris receive trial funding from Clovis Oncology, Inc.*

882 POSTER

A phase I dose-escalation study of buparlisib (BKM120), an oral pan-PI3K inhibitor, in Chinese patients with advanced solid tumors

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Background: Activation of the PI3K/Akt/mTOR signaling pathway promotes tumor growth. Buparlisib is an oral pan-class I (α, β, γ, δ) PI3K inhibitor that has demonstrated clinical antitumor activity in a range of cancer types. In a first-in-man Ph I study (NCT01068483) conducted in Western patients (pts) with advanced solid tumors (aST), the maximum tolerated dose (MTD) of single-agent buparlisib was declared as 100 mg/d. Here, we present interim results of a Ph Ib trial of single-agent buparlisib in Chinese pts with aST (NCT01626209).
Material and Methods: The primary objective was to determine the MTD or recommended Phase II dose (RP2D) of single-agent buparlisib in Chinese pts, based on clinical safety profile, and supported by PK results. Pts (age ⩾18 y, ECOG PS⩽2) with advanced breast cancer or

other cancers with squamous cell (SC) histology, who progressed on standard therapy, or for whom no standard anticancer therapy exists, received once-daily oral buparlisib. Other key eligibility criteria included availability of archival/fresh tumor biopsy to determine PI3K pathway activation status and measurable/non-measurable disease (RECIST v1.1). A Bayesian logistic regression model guided dose escalation.
Results: As of March 11, 2013, 14 pts (median age 45.5 y [range 24−75]; 43% male; primary cancer site: breast (5), SC lung (6), head and neck (3); 43% ⩾4 prior antineoplastic therapy lines) had received buparlisib at 80 mg/d (n = 6) or 100 mg/d (n = 8). Median exposure duration was 47 d [range 9–105]. DLT only occurred in 1 pt at 80 mg (Grade [G] 3 depression). The MTD was declared as 100 mg/d. Other drug-related G3/4 AEs (CTCAE v4.03) occurred in 2 pts at 80 mg/d (anemia and depression) and 3 pts at 100 mg/d (increased alanine and aspartate aminotransferases [1 pt], decreased platelet count [1 pt], and hyperglycemia [1 pt]). Primary reasons for treatment discontinuation were progressive disease (n = 8), AEs (n = 2 [G3 depression and G3 pneumonia]), patient/guardian decision (n = 2), and death (n = 1 [terminal lung cancer]). Preliminary PK analysis revealed no major difference in PK parameters between Chinese and Western pts.
Conclusions: In these Chinese pts with aST, buparlisib had a favorable safety profile and a similar PK profile to that seen in Western pts. The MTD for single-agent buparlisib in Chinese pts was declared as 100 mg/d. Short duration on treatment may reflect heavily pretreated disease. Antitumor activity is under evaluation in expansion cohorts and will be presented at the meeting.
Conflict of interest: *Ownership: Katharine Hazell owns shares in Novartis. Corporate-sponsored research: Li Zhang, Bhinge Xu, and Yi-Long Wu are investigators on Novartis-sponsored clinical trials. Other substantive relationships: Katharine Hazell, Anil Gaur, Junfang Xu, and Lucia Trandafir are employed by Novartis.*

883 POSTER

A multiple ascending dose phase I clinical, pharmacokinetic, and pharmacodynamic study of CG200745, a histone deacetylase (HDAC) inhibitor, in patients with advanced solid tumors

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Background: The aim of this study was to assess the safety, maximum tolerated dose (MTD), pharmacokinetics, pharmacodynamics, and efficacy of multiple dose of intravenous CG200745, a novel histone deacetylase (HDAC) inhibitor, in patients with advanced solid malignancies.
Materials and Methods: Two to six patients received intravenous CG200745 weekly for 3 weeks, then 1 week off according to the '2+4' dose-escalating method. Pharmacokinetic sampling and pharmacodynamic sampling of acetylated histone H4 (Acetyl-H4) in peripheral blood mononuclear cells (PBMCs) were performed on day 1 and 15 of the 1st cycle. Pre- and post-biopsy for acetyl-H4 in tumor tissue was performed in accessible patients.
Results: Eighteen patients were treated at one of nine doses (24.0–250 mg/m^2) and received 1.5 (1−11) cycles of CG200745 (median, range). No dose-limiting toxic effects or QTc prolongations were noted. Dose proportionality was observed for both Cmax and AUC. The elimination half-life and mean residual time was 5.67±2.69(mean±SD) and 3.97±1.63 hrs. An increase in PBMC acetyl-H4 correlated with dose and Cmax up to 51 mg/m^2 and plateaued in higher dose levels. At 24 hrs post administration, acetyl-H4 values higher than two times of baseline values in tumor tissue were observed in 50% (4/8) of measured patients. Stable disease was seen in half of the patients (9/18).
Conclusions: CG200745 can be safely administered at the effective dose levels that inhibit HDAC in PBMCs and tumor tissue. Although MTD was not reached, further escalation was not performed as the acetyl-H4 plateaued at dose levels higher than to 51 mg/m^2. Further phase II trials are recommended at 250 mg/m^2 due to the tolerability.
No conflict of interest.

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Hyperglycemia in patients treated with the pan-PI3K inhibitor buparlisib (BKM120): characterization, management, and assessment for pharmacodynamics

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Background: PI3K serves a central role in glucose homeostasis. Here, we characterize hyperglycemia (HG) observed in two clinical trials of the pan-class I PI3K inhibitor buparlisib (BKM120), assess its relevance as a pharmacodynamic marker, and evaluate the relationship of HG and insulin release (assessed by C-peptide [C-pep]) with clinical response.
Methods: Pts were treated with single-agent buparlisib in two completed studies in pts with advanced solid tumors: S1 − a Ph I dose-escalation (DE) study (CBKM120X2101; 12.5–150 mg/d [N = 83]); and S2 − a Ph I DE study in Japanese pts (CBKM120X1101; 25–100 mg/d [N = 15]). Here, we report observations for pts treated with 80 mg/d (n = 11) and 100 mg/d (n = 55) in S1, and 100 mg/d (n = 9) in S2, unless otherwise stated. C-pep was measured throughout the studies. HG was assessed according to CTCAE v3. Clinical response was assessed by best % change from baseline in sum of longest diameters (SLDs) and best overall response as per RECIST v1.0 and v1.1 in S1 and S2, respectively.
Results: In S1, mean max % change in C-pep at baseline and at C_{max} on C1D1 was −10, 13, 58, 41, 70, and 72% at 12.5, 25, 50, 80, 100, and 150 mg/d, respectively. There was a slight inverse correlation between max post-baseline C-pep value in C1 and best % change in SLDs (Pearson's r = −0.17) in pts treated at 80 or 100 mg in S1. All-grade (G) HG was noted in 25 of 75 (33%) pts at 80/100 mg in S1/S2; although most cases were mild (G1/2) and transient, G3/4 HG was noted in 6 (8%) pts. HG was managed with glucose-lowering medications, such as metformin and insulin when needed, and with buparlisib interruption/dose reduction. Only 1 pt permanently discontinued buparlisib due to HG (treated at 100 mg in S1). Among pts reporting no HG (n = 50), 2% had PR, 44% had SD, and 46% had PD; among those with HG G1−4 (n = 25), 36% had SD and 48% had PD.
Conclusions: HG is observed in buparlisib clinical trials and is well managed with glucose-lowering agents. Buparlisib exhibited a dose-dependent effect on C-pep levels and is a potential pharmacodynamic marker. There was a slight inverse correlation between C-pep and tumor shrinkage in this preliminary analysis, indicating a potential relationship between PI3K pathway inhibition and tumor response. No clear relationship between HG grade and clinical response was observed. Further analyses investigating the relationship between HG/change in C-pep and tumor shrinkage in additional single-agent buparlisib studies will be presented at the meeting.
Conflict of interest: *Ownership: D Mills owns shares in Novartis. Advisory board: J Rodon serves as a consultant for Novartis, Lilly, Servier, and Lipopharma. Corporate-sponsored research: A Azaro, J Rodon, JF Vansteenkiste, Y Ando, T Doi, and RW Naumann are investigators on Novartis-sponsored clinical trials. Other substantive relationships: D Mills, C Sarr, E di Tomaso, and C Massacesi are employed by Novartis. Y Ando is a speaker for Novartis, receiving honoraria.*

885 POSTER

Phase 1b study of albumin-binding doxorubicin (aldoxorubicin) plus free doxorubicin

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Background: Preclinical studies of aldoxorubicin, a doxorubicin conjugate that binds covalently to circulating albumin, plus free doxorubicin, have demonstrated complete and prolonged remissions of pancreatic and ovarian cancer xenografts when administered at 50% of their MTD, and with less toxicity than each drug administered at their MTD. The safety

and activity of a fixed dose of free doxorubicin and escalating doses of aldoxorubicin were evaluated in patients with advanced solid tumors who had no other accepted therapeutic options.
Methods: Open label dose-escalation study of aldoxorubicin administered at either 175, 240 or 320 mg/m^2 (130, 180 or 240 mg/m^2 doxorubicin equivalents) iv + 35 mg/m^2 doxorubicin iv, both on Day 1 of 21 day cycles, up to 8 cycles. MTD is the dose level immediately below where 2/6 patients experience a dose limiting toxicity (DLT), or the maximum dose of 320 mg/m^2 of aldoxorubicin.
Results: 10 subjects have been treated as of March 31, 2013. 7 subjects received 230 mg/m^2 aldoxorubicin and 3 patients received 320 mg/m^2 aldoxorubicin. No DLT was observed. The MTD thus was 320 mg/m^2 aldoxorubicin + 35 mg/m^2 doxorubicin administered as above. Patients were able to receive 4.5 cycles (median). 4 subjects were terminated either due to progressive disease or death. No subjects stopped treatment due to an adverse event. Grade 3/4 neutropenia occurred in 8/10 patients, grade 3/4 thrombocytopenia in 6 patients and grade 3/4 anemia in 4 patients. Febrile neiutropenia was observed in 3 patients. Grade 3/4 liver enzyme elevations or fatigue occurred in 1 and 2 patients, respectively. An objective partial tumor response was documented in one patient with advanced soft tissue sarcoma that had not responded to previous doxorubicin as well as a patient with advanced breast cancer. 7 patients had stable disease.
Conclusion: The combination of aldoxorubicin at 320 mg/m^2 and doxorubicin at 35 mg/m^2 can be safely administered to patients with solid tumors and shows anti-tumor activity. Since neutropenia is very common, prophylactic use of G-CSF is rexcommended. The dose of aldoxorubicin is 90% of the MTD of aldoxorubicin. Thus, doxorubicin does not appear to add to the toxicity of this combination.
Conflict of interest: *Ownership: CytRx Corporation. Corporate-sponsored research: CytRx Corporation. Other substantive relationships: Employment, CytRx Corporation*

886 POSTER

Pharmacokinetic study of aldoxorubicin, a novel albumin-binding drug, in solid tumor patients

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Background: Aldoxorubicin consists of doxorubicin conjugated to a pH sensitive linker that binds covalently to the cycteine 34 position in circulating serum albumin. Previous studies demonstrated that aldoxorubicin can be administered at doses up to 350 mg/m^2 (260 mg/m^2 doxorubicin equivalents) every 21 days for up to 8 cycles. We have investigated aldoxorubicin pharmacokinetics, including albumin-bound and free doxorubicin, and doxorubicinol after administration of 2 dose levels of aldoxorubicin in patients with advanced solid tumors.
Methods: Patients with solid tumors and no standard therapy were eligible. Other entry criteria: ECOG PS 0−2; LVEF>45% of predicted normal; ANC. 2000/mm^3; platelets >100,000/mm^3; Hct >25% (male), 28% (female). Patients were administered either 230 mg/m^2 aldoxorubicin (165 mg/m^2 doxorubicin equivalents) or 350 mg/m^2 aldoxorubicin (260 mg/m^2 doxorubicin equivalents) iv over 30 minutes on day 1 of each cycle. Blood samples were taken prior to administration and at multiple time points up to 72 hr post administration during cycles 1 and 3. Serum concentrations of albumin-bound doxorubicin, unbound doxorubicin and doxorubicinol were analyzed.
Results: As of March 31, 2013, 10 subjects have been entered in the study. 7 subjects have received 230 mg/m^2 aldoxorubicin and 3 patients 350 mg/m^2 aldoxorubicin. No serious adverse events have been reported. Grade 3 and 4 adverse events include neutropenia, thrombocytopenia and anemia. A partial response has been documented in 1 patient with small cell lung cancer that had received 3 prior chemotherpy regimens, and ongoing stable disease in another patient with previously-treated small cell lung cancer, both at the 230 mg/m^2 dose. Results for the 230 mg/m^2 cohort are complete and show for the albumin-bound doxorubicin during cycle 1: C_{max}=64 μg/mL; t_{max}= 0.25 hr; $t_{1/2}$= 19.7 hr; $AUC_{t-\infty}$=1500 h*μg/mL; CL_{pred}= 0.153 L/h/m^2; V_{sspred}= 3.91 L/m^2. Results are similar for cycle 3. Free doxorubicin accounted for less than 0.8% of total doxorubicin at each time point, and doxorubicinol for less than 0.0007% of total drug. Results from the 350 mg/m^2 cohort are pending.
Conclusion: Aldoxorubin binds rapidly and almost completly to albumin, has a narrow V_d and is cleared slowly from circulation, which distinguishes it from doxorubicin. Very little free doxorubicin is released into the circulation, potentially mitigating some of the drug's toxicity.
Conflict of interest: *Ownership: CytRx Corporation. Corporate-sponsored research: CytRx Corporation. Other substantive relationships: Employment, CytRx Corporation*

887 POSTER

Description and impact of topotecan dosing in ovarian cancer and SCLC

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Background: Topotecan (topo) is a treatment (tx) for advanced ovarian and small cell lung cancer (SCLC). Weekly (WK) dosing vs. conventional (daily x 5, Q21D) (CO) has shown similar outcomes in both diseases with less severe neutropenia. We describe patterns of use in the community, measure tx duration, and capture WBC growth factor (GF) utilization during topo.
Methods: Eligible pts: in US Oncology's (USO) iKnowMed^{TM}(iKM) EHR; SCLC or ovarian cancer diagnosis; tx with single agent topo. Pts were classified as WK or CO dosing cohorts. Percent of pts crossed over from CO to WK was captured. Age, sex, KPS, line of therapy (LOT), tx duration, and GF use were collected. Chi squared tests assessed the association of clinical factors with CO vs. WK dosing. Wilcoxon rank sum tests assessed continuous variables.
Results: From 1/1/2007 to 10/31/12, 2,534 pts were included (1071 ovarian; 1463 SCLC). CO: WK dosing for ovarian and SCLC were 168: 903 and 624: 839, respectively. Characteristics for SCLC pt were similar for both cohorts. In the ovarian population, a higher percent of pts age >65 yo received WK ($p = 0.005$). Less than 1% of pts crossed over from CO to WK. Pts receiving CO topo received more administrations in ovarian and SCLC (mean: 15 vs. 6, $p < 0.001$ SCLC and 18 vs. 10, $p < 0.001$ ovarian), but overall treatment duration was not different for CO dosing. Pegfilgrastim (PEG) was used less frequently with WK tx in ovarian and SCLC (OR: 0.1, $p < 0.01$) and average number of PEG admin was greater in CO tx (SCLC: 2 vs. 1, $p < 0.01$; ovarian: 3 vs. 2, $p = 0.014$).
Conclusions: Published data support topo WK as an alternative to CO dosing with less toxicity. This report shows more use of WK topo in a community oncology setting in ovarian and SCLC; more PEG use in CO topo dosing and similar tx duration for CO and WK dosing.
No conflict of interest.

	Cohort	Mean	Median	Standard deviation	P-value
SCLC N: CO=624; WK=839					
# Topo admin	CO	15.5	10.0	12.0	<0.001
	WK	6.1	5.0	4.6	
# peg admin	CO	3.1	2.0	2.3	<0.001
	WK	2.1	1.0	1.6	
# filgrastim admin	CO	4.9	3.0	7.4	NS
	WK	5.0	3.0	6.3	
Topo tx (mths)	CO	67.5	46.0	79.3	NS
	WK	52.9	42.0	63.7	
Ovarian N: CO=168; WK=903					
# Topo admin	CO	18.1	15.0	12.4	<0.001
	WK	9.6	7.0	8.5	
# peg admin	CO	3.7	3.0	2.5	0.014
	WK	3.4	2.0	3.4	
# filgrastim admin	CO	6.5	3.0	9.0	0.50
	WK	7.2	4.0	7.9	
Topo tx (mths)	CO	84.4	67.0	73.1	0.93
	WK	89.8	70.0	95.3	

888 POSTER

Phase I, open-label, dose-escalating clinical and pharmacokinetic study of the novel antimicrotubulin agent PM060184 administered over 10 Minutes on day 1 and 8 every three weeks to patients with advanced malignant solid tumors

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Background: PM060184 is a new chemical entity that inhibits tubulin polymerisation, causing microtubular fragmentation and mitotic arrest. *In vitro*, it has activity against solid tumours, especially breast, colon, renal and ovarian tumour cell lines. *In vivo,* evaluation of PM060184 demonstrated significant antitumour activity in patient-derived xenograft models (AVATAR) of gastric, NSCLC and pancreatic ductal adenocarcinoma.
Materials and Methods: Patients (pts) with advanced solid tumours were enrolled in a phase I, open-label, accelerated dose-escalating clinical and pharmacokinetic (PK) study of intravenous (i.v.) PM060184 given over 10 min on Day 1 and 8 every 3 weeks.
Results: 30 pts were distributed in 9 dose levels (DLs). Median age was 60 y (range, 36−78 y), 19 pts (63%) were males. ECOG PS was 1 in 67% of pts (range, 0−2). Most common tumours were colorectal (33%), GIST, NSCLC and breast cancer (13% each). Most pts were heavily pretreated, with a median of 4 (range, 1−13) lines. Pts received a median of 2 (range, 1−23) PM060184 cycles. Starting DL was 1.3 mg/m^2. DLTs occurred at 10.4 mg/m^2 [1 pt G3 peripheral neuropathy (PN)], 11.6 mg/m^2 (1 pt G3 PN), 14.5 mg/m^2 (2 pts G3 PN; and G3 vomiting and G4 neutropenia), and 12.0 mg/m^2 (3 pts G3 abdominal pain; symptomatic G3 hyponatremia; and G3 myalgia and G3 arthralgia). The MTD was 12.0 mg/m^2 and the RD was 9.3 mg/m^2. Most toxicities and abnormalities were mild. G3 AEs were: peripheral neuropathy (n = 3 pts), vomiting, fatigue, abdominal pain, intestinal obstruction, arthromyalgia, hyperkalaemia and hyponatraemia (n = 1 each). Haematological abnormalities were G3 thrombocytopenia (n = 2), G3 neutropenia (n = 2) and G4 neutropenia (n = 1). Preliminary PK data show a short half-life (4.4 h), wide distribution (251 L) and moderate inter-patient variability. Evidence of activity was observed in 2 breast carcinoma pts with tumour shrinkage around 20% and one partial response as per Choi criteria in one pt with GIST (resistant to 2 prior lines of standard therapy) who is still on treatment after 23 cycles. Overall, stabilisation for >3 mo has been observed in 4 pts.
Conclusions: The RD of PM060184 for further development was 9.3 mg/m^2 and has shown an acceptable safety profile; early non-cumulative PN was the main DLT. The antitumour activity observed in heavily pretreated pts warrants further study.
Conflict of interest: *Corporate-sponsored research: Funding to STAT for conduct of clinical trial*

889 POSTER

First-in-man phase I, open-label, dose-escalating clinical and pharmacokinetic study of the novel antimicrotubulin agent PM060184 administered over 10 minutes on days 1, 8, and 15 every four weeks to patients with advanced malignant solid tumours

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Background: The new chemical entity PM060184 inhibits tubulin polymerization, causing microtubular fragmentation and mitotic arrest. It has activity *in vitro* against several solid tumours (especially breast, colon, renal and ovarian tumour cell lines). *In vivo,* evaluation of PM060184 demonstrated significant antitumour activity in patient-derived xenograft models of gastric, NSCLC and pancreatic ductal adenocarcinoma.
Materials and Methods: Patients (pts) with advanced solid tumours were enrolled in a phase I, open-label, accelerated dose-escalating clinical and pharmacokinetic (PK) study of PM060184 given i.v. over 10 min on Days 1, 8 and 15 every 4 weeks.
Results: 22 pts were distributed in 8 dose levels (DLs) during escalation. Median age was 58 y (range, 22−72 y), 15 pts (68%) were males. ECOG PS was 1 in 36% of pts (range, 0−1). Most common tumours were colorectal (50%) and pancreas (14%). Most patients were heavily pretreated, with a median of 4 (range, 1−9) lines. Pts received a median of 2 (range, 1−7) PM060184 cycles. The starting DL (DL1) was 1.3 mg/m^2. The highest DL (DL7) 14.5 mg/m^2 was the MTD, as 2 pts (both pretreated with oxaliplatin) had DLT (G3 peripheral sensory neuropathy). One DLT (G3 peripheral sensory neuropathy) occurred at (DL8) 12.0 mg/m^2 in the expansion cohort, which is still ongoing. Common toxicities were fatigue, alopecia, peripheral sensory neuropathy, nausea, vomiting, musculoskeletal pain and diarrhoea. Most were mild (G1−2); G3 toxicities were peripheral sensory neuropathy (3 pts), fatigue and abdominal pain (1 pt each). Myelosuppression was mild, 5 pts had G2 neutropenia and 3 pts had G3 anaemia. G3 biochemical abnormalities comprised transient AST increase (2 pts) and AP increase (2 pts). Preliminary PK data show a short half-life (5 h), a wide distribution (286 L) and moderate inter-patient variability. Remarkable tumour shrinkage (~20%) occurred in 2 pts: 1 with head and neck carcinoma and 1 with breast carcinoma. Stabilization >3 mo also occurred in 3 pts with colorectal carcinoma.
Conclusions: PM060184 has acceptable safety and tolerability profile; non cumulative peripheral neuropathy is the only DLT observed. Evidence of activity has been found. Recruitment into the expansion cohort is ongoing; updated data will be presented at the meeting.
Conflict of interest: *Advisory board: PharmaMar*

890 POSTER

A phase I study in patients with advanced solid tumors for the human monoclonal antibody vantictumab (OMP-18R5; anti-Frizzled) targeting the WNT pathway

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Background: The WNT pathway is a key oncologic pathway in numerous tumor types and implicated in cancer stem cell (CSC) function. Vantictumab is a first-in-class anti-CSC antibody that interacts with the extracellular domain of 5 Frizzled receptors (Fzd 1, 2, 5, 7, 8) and blocks canonical Wnt signaling. In patient-derived xenograft models, vantictumab inhibits growth of many tumor types, reduces CSC frequency, promotes differentiation of tumor cells, and synergizes with many chemotherapy agents (*PNAS 109, 11717*).
Methods: Using a 3+3 design, vantictumab was given intravenously, first every 1 week (q1w) or q2w, and, ultimately, q3w. Objectives were to determine maximum tolerated dose, safety, pharmacokinetics (PK), pharmacodynamics (PD), and efficacy.
Results: 23 patients have been treated in 7 dose-escalation cohorts (0.5 & 1 mg/kg q1w; 0.5 mg/kg q2w; 1, 2.5, 5 and 10 mg/kg q3w). Most common related Grade 1 and 2 adverse events (AEs) included fatigue, vomiting, abdominal pain, constipation, diarrhea and nausea. Only related Grade $\geqslant$3 AEs were dose-limiting toxicities of Grade 3 diarrhea and vomiting in one patient at 1 mg/kg q1w. Vantictumab clearance was dose-dependent, consistent with target-mediated drug disposition, with the half-life ranging from 1.5 (0.5 mg/kg) to 3.3 days (5 mg/kg). Exposure at current dose levels correlates with efficacy in nonclinical tumor models. PD biomarkers indicate inhibition of WNT pathway in patient tumors and surrogate tissue. One patient at 0.5 mg/kg q1w had a bone fracture on Day 110 and a ~4-fold increase by Day 28 of β-C-terminal telopeptide (β-CTX), a marker of increased bone turnover. A revised safety plan, including Vitamin D_3 and $CaCO_3$ prophylaxis, and q3w dosing enabled further dose escalation. Upon β-CTX doubling, 2 patients received zoledronic acid, and β-CTX returned to baseline. Three patients with neuroendocrine tumors (NETs) with investigator-confirmed progressive disease on prior therapy had stable disease (SD) for ~4, 9+ and 12+ months; 2-, 0.8- and 8.4-fold length, respectively, of prior therapy. One patient with pancreatic NET (12+ months) had tumor shrinkage of −21%.
Conclusions: Vantictumab is well tolerated at current dose levels. An increase in bone turnover can be managed with increased monitoring and intervention with zoledronic acid. Prolonged SD in 3 NET patients, with a minor response in a pancreatic NET patient, may represent single-agent activity. The cohort for 10 mg/kg q3w continues to enroll.
Conflict of interest: *Corporate-sponsored research: KP Papadopoulos, AW Tolcher: Oncomed support for conduct of clinical trials to START. LS Rosen: research funding to institution from OncoMed. R Chugh: research funding from Novartis, Mabvax and Infinity. D Smith: Bristol-Myers Squibb, OncoMed, Celgene, MedImmune, Millennium, AstraZeneca, ImClone Debiopharm.*

891 POSTER

Properties of pyrrol derivate as potential anticancer compound

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Introduction: Gastrointestinal organs are the first to be affected by drugs including anticancer ones and provide major alterations because gut epithelium sensitivity through its high proliferative activity and liver vulnerability through xenobiotics detoxification. Targeted inhibitors of proliferative activity such as protein kinase inhibitors are known as high-efficiency and low-toxic anticancer agents, but only few ones have received US Food and Drug Administration approval as colorectal cancer treatments. Therefore, evaluation of 'small molecule' protein kinase inhibitor pyrrol derivate (D1, 5-amyno-4-(1,3-benzothyazol-2-yl)-1-(3-methoxyphenyl)-1,2-dihydro-3H-pyrrol-3-one) as anticancer agent, based on assessment of its anticancer efficacy and gut toxicity in comparison with common therapeutic 5-fluorouracil (5FU) ones was aimed.
Methods: Macroscopic analysis of colon internal surface, histological (light microscopy and morphometry of stomach, liver, small and large intestine sections), biochemical (serum liver enzymes activities, urinary 7,8-dihydro-8-oxoguanine (8-oxoG) concentration as a marker of DNA oxidative damage).
Results: D1 ingested for 10, 50 and 190 days (2.3 mg/kg daily) to normal rats didn't alter gut mucosa unlike 5FU, inhibited colon mucosa cell proliferation by 27% only after 190 days action (as opposed to 40−50% inhibition by 5FU after all investigated terms), caused no inflammation, had practically no effect on submucosa vascular bed, caused no hepatotoxicity. 1,2-Dimethylhydrazine(DMH)-induced rat colon cancer model was used to evaluate D1 antitumor activity. Total tumor lesions area decrease by 41−46% caused by D1 when acts concomitantly or following DMH (by 43% caused by 5FU) was revealed. Concomitant action of D1 and 5FU following DMH increased this rate to 54%.
Protective effect of D1 against DMH alterations in 'normal' gut mucosa and liver, manifested by recovery of gut mucosa and liver morpho-functional state and vascular bed, decrease of inflammation in stomach and intestine mucosa, normalization of serum aspartate aminotranspherase and alkaline phosphatase, diminution of elevated (by 5 times) urinary 8-oxoG, was found. On the contrary 5FU caused aggravation of DMH alterations in gut organs, urinary 8-oxoG further enhancing. Partially neutralization of 5FU toxic effects, caused by D1 under concomitant action, was also detected.
Conclusions: Low toxicity of pyrrol derivate and its efficacy against experimental colorectal cancer was concluded, so further D1 preclinical investigations are suggested.
No conflict of interest.

892 POSTER

A phase I trial assessing the safety and pharmacokinetics of afatinib and weekly vinorelbine in patients with advanced solid tumours

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Background: Afatinib (A) is an irreversible ErbB Family Blocker. Final safety and pharmacokinetic (PK) data from a phase I trial of A + vinorelbine (V) intravenous (iv) or oral (po) are presented. PK data focus on V po, as data for V iv were previously shown.
Methods: Eligible patients (pts) were ⩾18 years old with refractory advanced, non-resectable and/or metastatic tumours known to overexpress EGFR and/or HER2, had an ECOG performance status (PS) 0−1, and adequate organ and bone marrow function. In a 3+3 dose-escalation design, pts received escalating doses of daily A po (20/40/50 mg) continuously + V iv (25 mg/m^2; Part A) or V po (60 mg/m^2 for 3 weeks, escalated to 80 mg/m^2 thereafter; Part B) on Days 1, 8, 15 and 22 of a 4-week course. Primary endpoint was maximum tolerated dose (MTD) of A + V iv or po (the dose at which ⩽1/6 pts had a dose-limiting toxicity [DLT] during Course 1). A and V PK parameters were analyzed by intra-individual comparison to assess possible drug–drug interactions.
Results: 55 pts were treated (24 male/31 female) with A + V iv (n = 28) or A + V po (n = 27); median age 54 years, ECOG PS 0/1 36%/64%. MTD for both combinations was A 40 mg daily based on 1/6 DLTs in the MTD cohort (Table). Febrile neutropenia (8/55 pts) and diarrhoea (7/55 pts) were the most frequently reported DLTs during Course 1. Three pts had a confirmed partial response (PR) at A 40 mg + V po (RECISTv1.0); median duration of response was 114 days. Stable disease was seen in 16 pts in the A + V iv arm (4 of which had an unconfirmed PR at A 40 mg/50 mg [1/3]) and in 11 pts in the A + V po arm. In PK expansion cohorts for both combinations at the MTD, geometric mean $C_{max,ss}$ and $AUC_{\tau,ss}$ of A with/without V iv/po were similar, as were C_{max} and AUC_{0-24} of V iv/po with/without A. Intra-individual comparisons did not show any systematic trend with higher/lower exposure of A in the presence of V iv/po or *vice versa*.

	DLTs in Course 1					
	A + V iv − Part A			A + V po − Part B		
	20 mg A	40 mg A	50 mg A	20 mg A	40 mg A	50 mg A
MTD cohort	0/3	1/6	4/6	0/4	1/6	3/5
PK expansion cohort	NE	7/13	NE	NE	2/12	NE

NE = not evaluated.

Conclusions: The recommended Phase II/III dose of A was determined to be 40 mg daily in combination with iv or po V weekly. Final PK analyses suggest no drug–drug interactions between A and V iv or po. Both combinations had a manageable safety profile and showed signs of clinical activity in pretreated pts with solid tumours.
Conflict of interest: *Other substantive relationships: Martina Uttenreuther-Fischer and David Schnell are employed by Boehringer Ingelheim GmbH & Co. KG.H*I*ne de Mont-Serrat and Inga Tschoepe are employed by Boehringer Ingelheim France S.A.S.Antoine Hollebecque, Rastislav Bahleda, Yann Berg*, Christophe Massard, Jean-Charles Soria and Jean-Pierre Delord have nothing to disclose.*

893 POSTER

Design and development of active and selective FGFR kinase inhibitor CPL-043 as potential anticancer targeted therapy

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Background: Fibroblast growth factor receptors (FGFRs) family of receptors with tyrosine kinase activity comprises a group of extensively studied targets for small molecule inhibitors development. Alterations in gene copy number of different FGFRs and their point mutations have been correlated with many types of cancer, making FGFR kinases an interesting target for novel anticancer therapy. There are several FGFR inhibitors in clinical development but there is still a niche for the drug with properly balanced selectivity profile.
Material and Methods: Using sophisticated drug design methods we have designed CPL-043 − a small molecule FGFR kinase inhibitor with high potency in vitro. To establish the activity and the selectivity of the compound we have used kinase activity assay based on recombinant kinases and cell proliferation assay, using the cell lines dependent on FGFR signaling − SNU-16 and UM UC-14. To confirm biological activity of the inhibitor we performed immunoblot assay detecting the level of FGFR pathway related proteins.
Results: Our results indicate that CPL-043 inhibits FGFR1, 2 and 3 activity in vitro in low nanomolar concentrations. Concurrently the IC50 for the most common FGFR off-targets − KDR and PDGFR is over ten times higher. CPL-043 inhibits proliferation of FGFR-dependent cell lines including SNU-16 and UM-UC-14. Treatment of cells with CPL-043 for 1 h evokes dramatic decrease in the level of pFGFR, pFRS and pErk proteins in a dose dependent manner. Moreover the inhibitor has no effect on the lines with low FGFR activity like HCT-116 or H1703, suggesting that the compound is not cytotoxic.
Conclusion: We have designed a very potent and selective FGFR inhibitor, which displays biological activity in selected cellular models without evoking cytotoxic effects on the FGFR-independent cell lines. The compound has proper ADME predicted profile as is currently under investigation in the in vivo study.
No conflict of interest.

894 POSTER

First-in-human phase I trial investigating the oral selective c-Met inhibitor MSC2156119J (EMD 1214063) in patients (pts) with advanced solid tumors

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Background: MSC2156119J is a highly selective, reversible, ATP-competitive inhibitor of c-Met, a frequently deregulated oncoprotein. In preclinical models, MSC2156119J inhibited tumor growth and induced regression of hepatocyte growth factor-dependent and -independent tumors.
Methods: This phase I dose-escalation trial (3+3 design) included pts with advanced solid tumors not amenable to standard therapy (NCT01014936; ongoing; sponsored by Merck KGaA). Primary objective was to assess the MTD of MSC2156119J; secondary endpoints included safety, antitumor activity, pharmacokinetics (PK), and pharmacodynamics (Pd). Pts were treated once/d with oral MSC2156119J: d1−14 followed by 7d rest (regimen [R]1), continuously 3 times/wk (R2), or continuously d1−21 (R3), all 21-d cycles. In Aug 2011, an optimized formulation (OF) was introduced.
Results: Until Dec 04, 2012, 100 pts were treated (R1=42; R2=41; R3=17). Doses were escalated from 30–230 mg/d in R1 and 30–115 mg/d in R2 with the initial formulation, and from 30–400 mg/d in R1, 60–175 mg/d in R2, and 300–500 mg/d in R3 with the OF. Bioavailability was higher with the OF; AUC and C_{max} increased with dose. DLTs were reported in 4 pts: G4 lipase and G3 amylase increase (R1; 115 mg/d), G3 lipase increase (R2; 60 and 100 mg/d OF), and G3 nausea and vomiting (R2; 130 mg/d OF). One pt experienced drug-related G3 peripheral edema (R3; 300 mg/d OF). G2 drug-related AEs (R1−3) included fatigue (n = 8), lipase increase (n = 3), nausea (n = 2), decreased appetite (n = 2), vomiting (n = 2), and neutropenia (n = 2). Most pts (80%) had no drug-related AE >G1. Paired tumor biopsies (pre-/on-therapy) revealed phospho–c-Met inhibition in 13/15 evaluable pts. Two pts (NPC and NSCLC) experienced unconfirmed partial responses. Fifteen pts had SD >4 mo, including 1 pt with SD >32 mo. This pt (sarcomatoid bladder cancer) had multiple MET copies due to Chr 7 polysomy. In line with preclinical PK/Pd models, 500 mg was considered biologically active and sufficient for target inhibition. In the 500-mg cohort, no DLTs were observed in 12 evaluable pts. 500 mg once/d was confirmed as the recommended phase 2 dose (RP2D). Since the cutoff date in Dec, doses were further escalated to 700 and 1000 mg to explore effects of MSC2156119J above the RP2D. At 1000 mg, one DLT was observed (G3 AST/ALT elevation). This cohort is currently expanded to 6 pts.
Conclusions: MSC2156119J was well tolerated and showed antitumor activity. 500 mg was defined as the RP2D.
Conflict of interest: *Advisory board: GS Falchook has an advisory relationship with EMD Serono to disclose. K Kohler has a consultancy/advisory relationship with Merck KGaA to disclose. Corporate-sponsored research: GS Falchook, HM Amin, and R Kurzrock have received a research grant from EMD Serono. Other substantive relationships: MB Klevesath and A Johne are Merck KGaA employees.GS Falchook has received honoraria from EMD Serono.*

895 POSTER

Epidermal growth factor receptor (EGFR)-mediated adverse events (AEs) in patients (pts) with EGFR mutation positive (EGFR M+) non-small cell lung cancer treated with afatinib

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Background: Afatinib (A) is an oral, irreversible ErbB Family Blocker showing superior efficacy to first-line pemetrexed/cisplatin (PC) in EGFR M+ pts. In the LUX-Lung 3 trial, median progression-free survival was 11.1 months for A and 6.9 months for PC (hazard ratio=0.58; p = 0.0004). A similar Phase III trial, LUX-Lung 6, comparing A with gemcitabine/cisplatin in Asian pts was recently reported (ASCO 2013). Here, we present the data on common EGFR-mediated AEs from both Phase III trials.
Methods: 345 (LUX-Lung 3) and 364 (LUX-Lung 6) EGFR M+ pts were randomized (2:1) to receive A or chemotherapy. A was administered until progression or intolerable AEs. The A starting dose of 40 mg could be escalated to 50 mg daily or reduced to 30 mg or 20 mg daily based on study criteria. On-treatment AEs were summarized by preferred and grouped terms. No AE diaries were supplied; pts recalled start and stop dates for each AE during clinic visit. AEs were graded using NCI-CTCAE version 3.0.
Results: In LUX-Lung 3, 229 pts received A and median exposure was 336 days (range 7–827 days). All pts reported at least one AE and the commonly observed EGFR-mediated AEs in LUX-Lung 3 are included in the table.

	Diarrhoea	Rash*	Stomatitis*	Paronychia
All grades (%)	96.1	90.0	73.4	56.8
Grade 3 (G3) (%)	14.8	16.2	8.7†	11.4
First G3 before/after 6 weeks (%)	11.8/3.1	3.9/12.2	6.6/2.2	0.4/10.9
Median duration of G3 (days)	5.0	10.0	8.0†	14.0
G3 recurred after dose reduction (%)	2.6	1.3	0.4	1.3
Serious AE (%)	6.6	0.4	1.3	0.0
Led to dose reduction (%)	19.7	19.2	10.0	13.1
Led to treatment discontinuation (%)	1.3	0.0	0.0	0.9

*Group term; † Includes one pt with G4 AE.

Two-thirds of pts who reported diarrhoea experienced two episodes (25%) or less (46%). The majority of pts with G3 diarrhoea, rash, stomatitis or paronychia had a single occurrence. Other drug-related EGFR-mediated AEs included dry skin (29.3%), cheilitis (12.2%), conjunctivitis (8.3%) and dry eyes (4.8%). Related interstitial lung disease-like events occurred in three pts (one G1, one G3 and one G5). Additional information on the AE profile of A and data from LUX-Lung 6 will be presented.
Conclusions: The most common AEs observed with A were characteristic of EGFR-inhibiting agents. G3 AEs were short-lived, and responded to dose interruptions/reductions with little recurrence at a lower dose of A. Treatment discontinuation due to EGFR-related AEs was low, which indicates that A has a manageable safety profile and is suitable for the long-term treatment of EGFR M+ lung cancer pts.
Conflict of interest: *Other substantive relationships: James Yang has held compensated consultant or advisory roles for Roche, Astrazeneca, Genetech, Pfizer, Novartis, Takeda, Clovis, Innopharma. He has held uncompensated consultant or advisory roles for Eli Lilly, Boehringer Ingelheim. He has held honoraria for Bayer, Astrazeneca, Roche, Merck, Pharmengine. Lecia Sequist has held uncompensated consultant or advisory roles for Boehringer Ingelheim, Merrimack, Clovis, AstraZeneca, GlaxoSmithKline. Kenneth John O'Byrne has held consultant or advisory roles for Boehringer Ingelheim, and has received honoraria from Boehringer Ingelheim, and has received other renumeration from Boehringer Ingelheim. Martin Schuler has received research funding from Boehringer Ingelheim and has received travel support from Eli Lilly. Tony Mok has held consultant or advisory roles for Astrazeneca, Boehringer Ingelheim, Roche, Eli Lilly, Beigene, Pfizer, Merck Serono, Bristol-Myers Squibb, Jannsen, Taiho. He has held honoraria from Astrazeneca, Boehringer Ingelheim, Roche, Eli Lilly, Pfizer, Merck Serono, Taiho. Dan Massey and Victoria Zazulina are employed by Boehringer Ingelheim Limited, Bracknell, UK. Dennis O'Brien is employed by Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA. Yi-Long Wu has received honoraria from Roche, Eli Lilly, AstraZeneca, Pfizer, Sanofi, and research funding from Roche, Eli Lilly, AstraZeneca, Pfizer, Sanofi. Sarayut L Geater has nothing to disclose.*

896 POSTER

Pharmacokinetic analysis of Asian patients in a phase 2 study of dovitinib (TKI258) in metastatic renal cell carcinoma

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Background: Dovitinib potently inhibits FGFR (IC_{50} = 8−40 nM) as well as VEGFR and PDGFR (IC_{50} <40 nM), drivers of tumor growth, angiogenesis, and anti-angiogenic escape in renal cell carcinoma (RCC) and other solid tumors. In a phase 2 study of dovitinib in patients with RCC, tolerability was within the known safety profile of dovitinib (Angevin E, et al. ASCO 2011, abstract 4551). Here, we provide the pharmacokinetic (PK) results from this study in Asian and non-Asian patients.
Materials and Methods: Patients with advanced or metastatic RCC with predominant clear cell histology who failed both VEGF and mTOR therapy were eligible, as were small subsets of patients who were refractory to standard treatment or treated with agents that were not VEGFR or mTOR inhibitors. Patients of Asian ethnicity who failed standard treatment or for whom no standard treatment existed were also included. Patients were treated with dovitinib 500 mg/day on a 5-days-on/2-days-off schedule. PK parameters were determined using a noncompartmental method for area under the curve (AUC), maximum concentration (C_{max}) and time to maximum concentration (T_{max}).
Results: PK results for Asian (n = 12) and non-Asian (n = 53) patients are summarized in the Table. Asian patients had a higher coefficient of variation (as high as 68%) due to a smaller number of patients than that of non-Asian patients. Both day 1 AUC and C_{max} for Asian patients were similar to those for non-Asian patients. For both Asian and non-Asian patients, the day 15 AUC and C_{max} were 16%-33% lower than those of day 1.
Conclusions: PK parameters were similar between Asian and non-Asian patients. Day 15 PK was lower than that of Day 1 in both Asian and non-Asian patients due to auto-induction of CYP1A2 as shown in other in vitro and in vivo studies.

PK Parameter	Asian patients n = 12 (day 1) n = 11 (day 15)	Non-Asian patients n = 53 (day 1) n = 45 (day 15)
Geometric mean AUC_{0-last}, h·ng/mL (coefficient of variation [CV%])		
Day 1	5381 (68)	5221 (29)
Day 15	4428 (50)	3501 (34)
Geometric mean C_{max}, ng/mL (CV%)		
Day 1	333 (62)	300 (31)
Day 15	281 (41)	239 (36)
Median T_{max}, h		
Day 1	7.1	6.1
Day 15	6.0	6.0

Conflict of interest: *Ownership: M. Shi (Novartis). Advisory board: C.C. Lin (Novartis) Y.H. Chang (Pfizer, Novartis, GlaxoSmithKline, Bayer, Millennium-Takeda, Janssen, IPSEN) V. Grunwald (Novartis, Pfizer, GlaxoSmithKline, Roche) B. Escudier (Novartis, Pfizer, Bayer, GlaxoSmithKline, Aveo). Corporate-sponsored research: V. Grunwald (Pfizer). Other substantive relationships: C.C. Lin, honoraria (Novartis) V. Grunwald, honoraria (Novartis, Pfizer, GlaxoSmithKline, Roche) J. Chang, employment (Novartis) E. Tan, employment (Novartis) N. Pirotta, employment (Novartis) M. Shi, employment (Novartis)*

897 POSTER

A phase I/II study of cancer peptide vaccine S-288310 in patients with advanced urothelial carcinoma

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Background: S-288310 is a cancer peptide vaccine composed of two kinds of HLA-A*24:02-restricted peptides, S-288301 and S-288302, that were developed from two oncoproteins, DEP domain containing 1 (DEPDC1) and M-phase phosphoprotein 1 (MPHOSPH1), highly expressed in urothelial carcinoma (UC).
Purpose: The objective of this study was to examine the safety, tolerability and immune response (induction of cytotoxic T lymphocytes, CTLs) specific to S-288301 and S-288302 in patients with advanced UC.
Patients and Methods: HLA-A*24:02-positive patients with histologically confirmed UC after documented progression or intolerance to prior platinum-based chemotherapy(ies) were eligible. 1 or 2 mg of each peptide emulsified with Montanide ISA51VG was administered s.c. once a week in the axillary or inguinal region.
Results: Three patients each were treated with S-288310 at 1 or 2 mg/each peptide in the phase I study. S-288310 was well-tolerated and no DLTs were observed. In the phase II study, 32 patients were treated with either 1 or 2 mg/shot in a random manner. The protein expression of DEPDC1 and MPHOSPH1 was confirmed by immunohistochemical analysis in 36 (97.3%) and 35 (94.6%) of the 37 tissues examined, respectively. The CTL responses to S-288301 and S-288302 were detected in 22 (66.7%) and 24 (72.7%) of the 33 patients so far examined, respectively, and 87.9% of the patients responded to at least one peptide. No significant difference in CTL induction or safety was observed between the 1 and 2 mg groups. In the phase II study, 2 of the 32 cases (6.3%) revealed irPR, and 16 of the 32 (50.0%) were judged to be irSD assessed by the immune-related response criteria. Seven of the 16 patients with irSD showed tumor necrosis or regression although they did not meet the criteria of partial response. The median progression-free survival and overall survival (OS) was 1.9 months (90% confidential interval [CI] of 1.2−2.2 months) and 9.4 months (90% CI of 4.2–12.2 months). The OS of cases, in which CTL induction to both peptides was observed, tended to be improved, compared with those that showed the response to one peptide. The most frequently observed AE was the injection site reaction.
Conclusion: S-288310 was well-tolerated and induced antigen-specific CTLs in 87.9% of the patients and revealed clinical responses. Our results suggested S-288310 is a promising drug for the treatment of UC.
Registration: JapicCTI-090980
No conflict of interest.

Poster Session (Sat, 28 Sep)
Regulatory/Trial Methodology/Pharmacy

900 POSTER

Defining dose limiting toxicity (DLT) for phase I testing molecularly targeted agents: results of an international survey

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Background: Using traditional definitions of dose limiting toxicity (DLT) in phase I trials of molecularly targeted agents (MTA) is challenged by their specific toxicity profiles and often continuous administration. For instance we recently reported that 50% of patients receiving MTAs have their worst toxicity after cycle 1 (Postel-Vinay, JCO 2011). An international survey to

collect expertise on defining DLT for MTA phase 1 trials was initiated by the EORTC-led research group.
Material and Methods: A 15-question electronic survey was sent to corresponding authors of phase I reports identified in EJC, JCO, Annals of Oncology, Lancet as well as to phase I experts identified by the co-authors. Questions included: DLT assessment period duration, incorporation of specific grade 1 (G1) or G2 adverse events (AEs), and their minimum duration to qualify as DLT, exclusion of specific G3 AEs, inclusion of dose modification/delay, and relative worsening of AEs. The potential impact of schedule, both oral and IV dosing was considered.
Results: Among the 400 investigators contacted, 119 replied; 67 questionnaires were 100% complete. In the last 5 years, 30% participated in more than 10 trials, 35% were principal investigators of 5 or more. 11% (8/67) opted for a DLT assessment period of 1 cycle, 33% for 2 cycles and 54% requested all cycles to be assessed, with the proviso not to delay patient accrual. Suggestion was made to define maximum tolerated dose on cycle 1 data only, to reanalyze all accumulated data before the expansion cohort and to recommend the phase II dose based on the toxicity data from multiple cycles. 90% evaluated pre-existing symptoms to qualify a DLT. 92% suggested including dose modification and temporary interruption in the DLT definition when dose intensity drops down to 50−60% (13% of the responders) and to 70−80% (50%).
Moderate toxicity was deemed relevant by 70% (IV treatment) and 80% (oral). Selected G1/2 varied: visual disorders and confusion (30%); QTc prolongation, dyspnoea, some GI disorders (diarrhoea, pancreatitis) (20%). For G2 events, >1 week duration was relevant by 40 to 58% depending on AEs. Responses for IV and oral routes were consistent in 70%.
Conclusions: The majority of experts favoured a longer assessment period as well as the incorporation of specific G2 AE. However, no clear agreement on a re-definition of DLT was reached. A large international data warehouse is being evaluated to develop guidelines.
No conflict of interest.

901 POSTER

A population-based analysis of outcomes in cancer patients who do not satisfy clinical trial eligibility criteria (CTEC)

W. Cheung[1], H. Kennecke[1], H. Lim[1], D. Renouf[1], S. Gill[1], C. Speers[1], O. Goktepe[1], K. Javaheri[1]. *[1]British Columbia Cancer Agency, Medical Oncology − GI, Vancouver BC, Canada*

Background: Trials have stringent inclusion and exclusion criteria in order to maintain internal validity. However, study findings are subsequently applied to patients in routine practice who frequently do not meet CTEC. Our aim was to characterise the outcomes and magnitude of treatment benefit, if any, in these patients.
Material and Methods: Patients diagnosed with stage 3 colon cancer from 2006 and 2008, referred to any 1 of 5 regional cancer centers in British Columbia, and assessed for adjuvant chemotherapy (AC) within 12 weeks of surgery were analysed. Patients were considered trial-eligible (TE) if aged 18 to 79 years, ECOG 0/1, CEA <10, did not receive prior chemotherapy or radiation, and had adequate blood counts and normal cardiac, liver and kidney function. All other patients were deemed trial-ineligible (TI).
Results: A total of 820 patients were identified: median age was 69 years (range 60−76), 423 (52%) were men, 365 (45%) were ECOG 0/1 and 592 (72%) received AC. Among patients treated with AC, 370 (63%) were TE and 222 (37%) were TI. Compared to TI patients, those who were TE were younger (63 vs 70 years, $p < 0.01$) and more likely to receive combination rather than single agent AC (56 vs 33%, $p < 0.01$). Outcomes were significantly different among patients who were TE, TI, and those who did not receive AC (Table). In multivariate analyses that adjusted for confounders such as age, ECOG and T and N stages, both TI patients and those not treated with AC had worse prognoses than TE patients (HR for colon cancer deaths 1.32, 95% CI 0.86–2.02 and 2.77, 95% CI 1.92–3.99, respectively, p trend <0.01; HR for all deaths 1.24, 95% CI 0.85–1.80 and 2.95, 95% CI 2.17–4.00, respectively, p trend <0.01).

Group	5 year CSS rate	p-value	5 year OS rate	p-value
TE and received AC	82%		74%	
TI and received AC	75%	<0.001	65%	<0.001
No AC	57%		35%	

CSS=cancer-specific survival; OS=overall survival.

Conclusions: In this population-based cohort, patients who did not fit CTEC were frequently treated with AC. Outcomes in this TI group were inferior to those in the TE group, but they were better than the subset that did not receive AC. Trials specifically designed for patients traditionally deemed TI are needed.
No conflict of interest.

902 POSTER

Adherence to CONSORT adverse event reporting guidelines in medical oncology clinical trials, a systematic review

J. Peron[1], D. Maillet[2], H.K. Gan[3], E.X. Chen[4], B. You[5]. *[1]Hospices Civils de Lyon − Centre Léon Bérard, Lyon, France; [2]Hospices Civils de Lyon, Medical Oncology, Lyon, France; [3]Austin Hospital, Joint Austin-Ludwig Oncology Unit, Melbourne, Australia; [4]Princess Margaret Hospital University Health Network, Department of Medical Oncology and Hematology, Toronto, Canada; [5]Hospices Civils de Lyon, Department of Medical Oncology, Lyon, France*

Background: The Consolidated Standards of Reporting Trials (CONSORT) guidance was extended in 2004 to provide a set of 10 specific and comprehensive guidelines regarding adverse event (AE) reporting in randomized controlled trials (RCTs). There is little data though regarding adherence to these guidelines by published oncology RCTs.
Material and Methods: All phase III RCTs published between 2007 and 2011 were reviewed using a 16-point adverse event reporting quality score (AERQS) based on the 2004 CONSORT extension. Multivariable linear regression was used to identify features associated with improved reporting quality. All statistical tests were two-sided.
Results: A total of 325 RCTs were reviewed. The mean AERQS was 10.1 on a 0-to-16 scale. The most common items that were poorly reported were the way adverse event data were collected (adequately reported only in 10% of studies), the description of AEs' characteristics leading to withdrawals (15%) and the attribution of AEs to trial interventions (38%). Even when reported, the methods of AE data collection and analysis were highly heterogeneous. The multivariable regression model revealed that industry funding, intercontinental trials and trials in the metastatic setting were predictors of higher AERQS. The quality of AEs reporting did not improve over time and was not better among manuscripts published in high-impact-factor journal.
Conclusion: In conclusion, our findings show that methodological aspects of AEs collection and analysis were poorly reported. Given the potential impact of poorly reported trials, oncology journals should emphasize the importance of conformity to the 2004 CONSORT guidelines regarding adverse event reporting.
No conflict of interest.

903 POSTER

Area-based measures for assessing survival benefit in Kaplan–Meier's curves

E.J. Alegre-Del Rey[1], J. Diaz-Navarro[1], S. Fenix-Caballero[1], J.F. Lopez-Vallejo[1], C. Martinez-Diaz[1], E. Rios-Sanchez[1], M.A. Blanco-Castaño[1], M.J. Gandara-LadronDeGuevara[1], C. Palomo-Palomo[1], J.M. Borrero-Rubio[1]. *[1]Puerto Real Universitary Hospital, Pharmacy Department, Puerto Real (Cádiz), Spain*

Background: Medians comparison in survival curves is erratic and doesn't sometimes provide good assessment of survival benefit.
Objectives: The aim is to test a new area-based method for assessing survival in a set of Kaplan–Meier's curves. The primary outcome is to analyze the correlation between survival increments by the area method and excess risk calculated by hazard ratio (HR). A second outcome is to compare area-based vs. median-based results.
Methods: A 30 curves sample for R 0.7, α 0.05 and β 0.8 was calculated, and a Pubmed search for articles with survival analysis in colon, lung and breast cancer was conducted. Articles with an overall survival figure showing patients at risk, both curves reaching the median and $p < 0.05$ for HR, were included.
Three lines were defined for each figure: V: vertical cut line intersects abscissa at the longest time (t) with at least 10 patients at risk in each group or 30 in total. H: horizontal cut line crosses the intersection of V with the upper survival curve. T: Horizontal top line intersects ordinates in its maximum value (100%). Area under curve (AUC) was defined among the ordinates axis, the curve and H. Reference area (RA) was defined as the rectangle among ordinates axis, V, H and T. It represents the survival time in case no patient died (t). Survival was calculated as AUC/RA*t. SISA, R-code and Photoshop CS6 were used.
Pearson's correlations were calculated between excess risk in A vs. B group (HR-1)% and survival increment in B vs. A (B survival time/A survival time −1)%, assuming longer survival for B group. Survival increments were calculated by medians and area methods. Steiger's z-test was used to compare which method (area or medians) correlated better with HR-calculated excess risk. A Bland-Altman's concordance analysis was performed.
Results: The search identified 485 articles, 41 of them had survival curves that met inclusion criteria. By excluding tails with few patients at risk, area-based measures included survival data from 71% patients.
Concordance analysis showed a standard deviation of 85%, and a mean difference in survival (area minus median) of −1.9 months.

Method	Correlation survival increment – excess risk (HR-based)	
	R	p
Medians	0.854	<0.01
Area	0.922	<0.01
p(Medians vs Area)	0.036	

Conclusions: Area- and medians-based results show discrepancies between them. Area method benefits more from curve information, correlates better with HR-based excess risk and provides a measure of survival time.
No conflict of interest.

904 POSTER

Quality of reporting of phase II trials in oncology in highly ranked oncology journals

T. Grellety[1], A. Petit-Monéger[1], A. Diallo[1], S. Mathoulin-Pelissier[1], A. Italiano[1]. [1]*Institut Bergonié, Bordeaux, France*

Background: Phase II trials represent an essential step in the development of anti-cancer drugs. The aim of this study was to assess the quality of their reporting in highly-ranked oncology journals, to investigate predictive factors of quality and to develop better reporting guidelines for authors.
Material and Methods: We reviewed the tables of contents of 8 peer-reviewed oncology journals published between January 2011 and December 2011 and with a 2011 impact factor >4: Ann Oncol, Br J Cancer, Clin Cancer Res, Eur J Cancer, J Clin Oncol, J Natl Cancer Inst, Lancet Oncol and Oncologist. Two reviewers assessed the quality of each report by using a 44-point overall quality score (OQS; range, 0 to 44 points) inspired from the revised Consolidated Standards of Reporting Trials statement. Primary endpoint definition, justification of sample size and definition of the evaluable population for each endpoint, were assessed separately because of their crucial methodological importance using a 3-point key methodological score (KMS; range, 0 to 3). Exploratory analyses were used to identify predictive factors associated with the different scores.
Results: 156 articles were included. Agreement between the reviewers for each item was good (kappa coefficient range: 0.62−1). The median OQS was 28 (range: 9−35). OQS sub-score analysis showed that reporting of statistical methods was particularly low with a mean of 2.5 (6 items). The median KMS was 2 (range 0−3). Primary endpoint definition, justification of sample size and definition of the evaluable population were reported only in 107 (68.6%), 121 (77.6%), and 52 (33.3%) cases, respectively. On multivariate analysis, reporting of clinicaltrials.gov registration was associated with improved OQS, OR = 3.2 (95CI, 1.5 to 7.1). No predictive factor for KMS were identified.
Conclusions: Phases II trials reporting is still poor even in journals with strict editorial policies. This may lead to biased interpretation of phase II trial results. We have developed a checklist for use by authors, reviewers, and editors to improve reporting of these studies. As well as using a checklist during the preparation of their manuscript, we recommend that authors provide reviewers and readers with the last version of the study's protocol.
No conflict of interest.

905 POSTER

Quality of reporting of chemotherapy compliance in randomized controlled trials of breast cancer treatment

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Background: The adoption of any therapy in clinical practice requires a detailed knowledge of treatment administration and expected compliance as reported in the literature. The Consolidated Standards of Reporting Trials (CONSORT) statement requires detailed reporting of interventions in publications of randomized controlled trials (RCTs). We hypothesized that there was variable reporting of chemotherapy compliance in published randomized controlled trials in breast cancer and surveyed the literature to assess the quality of reporting chemotherapy compliance and to determine the study characteristics associated with a reporting quality.
Materials and Methods: MEDLINE, EMBASE, and CENTRAL were searched systematically for published original articles (Jan 2005 through Dec 2011; English language) of Phase III RCTs evaluating chemotherapy in breast cancer. Selected articles were scored 1 point for reporting each of 4 measures − number of chemotherapy cycles, dose modification, early treatment discontinuation and relative dose intensity (RDI). Logistic regression was performed to identify study characteristics associated with higher reporting quality score of $\geqslant 2$.
Results: Key study characteristics of the eligible 115 RCTs were: published in high impact journals, 79 (69%); published 2008 onward, 66 (57%); advanced-stage disease, 43 (37%); industry sponsorship, 37 (32%). RDI, number of cycles received, dose modification, and early treatment discontinuation rates were mentioned in 70 (61%), 53 (46%), 65 (57%) and 81 (70%) of the articles, respectively. Only 25 (22%) articles mentioned all 4 compliance measures. Quality score was $\geqslant 2$ for 82 (71%) articles. Study characteristics associated with a significantly higher quality of reporting chemotherapy compliance were articles published 2008 onward ($P = 0.035$) and advanced-stage disease ($P < 0.001$).
Conclusions: Our study demonstrates that there is variable reporting of chemotherapy compliance in published RCTS, although a modest improvement is seen in recent years. Incorporating standards for reporting chemotherapy compliance in scientific guidelines or the journal peer review process may decrease the variability and improve the quality of reporting.
No conflict of interest.

906 POSTER

Poor quality of adverse event reporting in oncology phase III trials: A systematic review

D. Maillet[1], B. You[1], J. Peron[1]. [1]*Hôpital Lyon Sud, Pierre Bénite, France*

Background: In the present study, we assessed the quality of toxicity reporting (description and impacts of AEs) in oncology phase III trials.
Material and Methods: This is a systematic review of all medical oncology phase III studies published in 10 major oncology journals (NEJM, JCO,

Table 1 (abstract 906). Reporting of the consequences of AEs on trial or patient outcomes ($n = 325$)

Adverse event reporting	RCTs	
	n	%
AEs leading to patient death: lethal adverse events		
Existence of lethal adverse events		
Not reported: unknown	88	27
Reported	237	73
• No lethal AE (n = 0)	60	18
• One or more lethal AEs (n⩾1)	177	54
• Including those with reporting of type of specific adverse events leading to death	130	73% of RCT with n⩾1 lethal AEs reported
Adverse events leading to drug discontinuation		
Existence of AEs leading to trial discontinuation		
Not reported: unknown	85	26
Reported	240	74
• No AE leading to drug discontinuation (n = 0)	4	1
• One or more AEs leading to discontinuation (n⩾1)	236	73
• Including those with type of adverse events reported	49	21% of RCT with n⩾1 AEs leading to drug discontinuation reported
Adverse events leading to dose modification		
Existence of AEs leading to dose modification		
Not reported	197	61
Reported	128	39
Type of AEs reported	32	10

Lancet, Lancet Oncol, JNCI, Ann Onc, EJC, BJC, BCRT, Cancer) between 2007 and 2011 using PubMed. For each publication, we analyzed the trial characteristics; the presentation of AEs (including the description clarity) and the reporting of AE consequences on trial or on patient outcomes (i.e. AEs leading to patient death or to trial discontinuation/dose modification).
Results: Total of 325 published randomized control trials (RCT) were analyzed. Results are presented in Table 1. The potential existence of lethal AEs was reported in only 73% of them. Among 177 RCT publications where one or more lethal AEs were mentioned, the specific type of AE leading to patient death was mentioned in 73%. The existence of AEs leading to trial discontinuation was mentioned in 74% RCT publications. Among them, the types of AEs responsible of discontinuation were reported in only 21%. Only 39% of publications reported the existence of AEs leading to dose modifications. Regarding descriptions of AEs, aggregations of AEs were used in 29% of studies. For example, the most commonly reported aggregated outcomes were reported as 'dermatologic AEs' (45%); 'cardiologic AEs' (33%), and 'neurologic AEs' (26%) without any additional definitions. Although these aggregations were considered, only 25% were clearly described. Sources of confusion in definition of AEs were found in 10% reports.
Conclusions: Our findings suggest the quality of AEs reporting in oncology RCTs published in 10 major oncology journals is insufficient. In particular, the proper reporting of potential lethal AEs, as well as the types of AEs leading to death, to trial discontinuation or dose alteration is found in 50% of the trials only. Moreover, although unclear in essence, aggregated AE outcomes are too frequently used in publications.
No conflict of interest.

907 POSTER

Pneumatic conveying systems and physical stability of monoclonal antibodies: example of cetuximab

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Background: Proteins such as monoclonal antibodies (mAb) are sensitive products which could undergo complex degradation pathways during the various manipulation steps also during transport. Aggregation can be induced by mechanical stresses which can occur during manipulations and transport and could induce loss of efficacy and/or toxic effects such as immunogenicity. Currently pneumatic conveying systems are in place in some hospitals but are not currently used for transport of proteins. Previous studies with Rituximab showed that the use of these systems were possible on the condition of removing air of bags. The objective of this study was to confirm these results with another antibody, Cetuximab.
Material and Method: Various protein characterization methods: size exclusion chromatography (SEC), dynamic light scattering (DLS) describing submicronic populations and corresponding mean diameters, turbidity (350 nm) and infra-red spectroscopy (FTIR) were used to determine changes in physical properties of Cetuximab aggregation mechanically induced. Several conditions were tested: presence of residual air in bags, travel time, number of travel cycles (1 to 3). One concentration was tested (2 mg/ml). All experiments were performed in the same day.
Results and Discussion: Considering the results obtained with Rituximab, we have limited our experiments to 3 travel cycles. Up to 3 travel cycles and without head space or bubbles into the bags, no modification was noticed in comparison with the control (no run). Indeed, we observed only one peak by SEC with a retention time of 18, 42$\pm$0.01 min, a monodisperse population (polydispersity index $\leqslant$ to 0.1) with a mean diameter of 12.56$\pm$0.121 nm by DLS, a slightly increased of optical densities (OD) at 350 nm (0.00123 up to 0.00216) and no modification of the FT-IR spectra (similarity coefficients were close to one). In the opposite, in presence of air, significant modifications were found after 1 cycle since OD reached to 0.00264 and 2 populations were found by DLS with a polydispersity index of about 0.22. Moreover, modifications of FTIR spectra were also observed (similarity coefficient <1) suggested alteration of the secondary structure.
Conclusion: As shown for Rituximab, aggregation of monoclonal antibodies during the pneumatic conveying is strongly dependant on the presence of air/liquid interfaces.
No conflict of interest.

Poster Session (Sat, 28 Sep)
Diagnostics/Biomarkers

950 POSTER

Randomised phase II trial comparing therapy based on tumour molecular profiling versus conventional therapy in patients with refractory cancer: results of the feasibility part of the SHIVA trial

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Background: Two recent studies suggest that a histology-independent approach consisting in selecting molecularly targeted agents based on the molecular profile of patients' tumours, whatever the tumour location and histology are, improves patients' outcome [Von Hoff et al., 2010; Tsimberidou et al., 2012]. However, the lack of randomisation versus standard of care in these studies did not allow drawing robust conclusions.
Material and Methods: The SHIVA trial is a multicentric randomized proof-of-concept phase II trial comparing molecularly targeted therapy based on tumor molecular profiling versus conventional therapy in patients with any type of refractory cancer. The primary endpoint is progression-free survival (PFS). The molecular profile performed on a mandatory biopsy includes the assessment of 1) hot spots mutations using the AmpliSeq cancer panel on Ion Torrent/PGM (Life Technologies), 2) gene copy number alterations using Cytoscan HD/Affymetrix, and 3) expression of oestrogen, progesterone and androgen receptors by immunohistochemistry on formalin-fixed sample. The algorithm used by a Molecular Biology Board (MBB) to guide treatment in the experimental arm is presented in the Table. The efficacy analysis will be performed on 200 randomized patients. A cross-over is proposed at disease progression. Feasibility included the first 100 patients.
Results: Between 10/2012 and 04/2013, 143 patients have been included in the study. Results of the feasibility part are available for the 53 first patients at the time of the abstract submission. Full feasibility results will be presented at the meeting. Biopsy was performed in 50 out of 53 patients (94%). Complications occurred in 1 patient (2%). Median time between the biopsy and the MBB was 26 days [range: 14−42]. Mutations, gene copy number alterations and IHC profile were obtained in 32 (64%), 34 (68%) and 45 (90%) patients, respectively. A molecular abnormality leading to randomisation was present in 21 patients (42%).
Conclusions: The establishment of a comprehensive tumour molecular profile is safe, feasible and compatible with clinical practice. A molecular abnormality matching with the approved drugs available in the trial was present in 42% of patients.
No conflict of interest.

Molecular abnormalities	Type of molecular abnormality	Molecularly targeted agents
KIT, ABL, RET	Activating mutation or amplification	Imatinib
PI3KCA, AKT1	Activating mutation or amplification	Everolimus
AKT2,3, mTOR, RAPTOR, RICTOR	Amplification	Everolimus
PTEN	Inactivating mutation and LOH	Everolimus
STK11	Inactivating mutation and LOH	Everolimus
BRAF	Activating mutation or amplification	Vemurafenib
PDGFRA/B, FLT-3	Activating mutation or amplification	Sorafenib
EGFR	Activating mutation or amplification	Erlotinib
HER-2	Activating mutation or amplification	Lapatinib + Trastuzumab
SRC	Activating mutation or amplification	Dasatinib
EPHA2, LCK, YES	Amplification	Dasatinib
ER, PR	Protein expression >10%	Tamoxifen (or letrozole if contra-indication)
AR	Protein expression >10%	Abiraterone

ER = Estrogen receptor; PR = Progesterone receptor; AR = Androgen receptor; LOH = Loss of heterozygosity

951 POSTER

Evaluation of HER2 expression status using multiple chromosome 17 reference probes

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Background: Targeted therapies to HER2(+) breast cancer have improved outcomes in this aggressive phenotype. A recent unconfirmed study suggested that HER2 gene status may be misidentified in a small group of patients due to co-amplification of HER2 and CEP 17, leading to low HER2/CEP17 ratio with high HER2 gene copy number. We used alternate probes to chromosome 17 to reinterpret HER2 status in a group of patients in a single center to attempt to confirm these findings.
Methods: We identified 60 breast cancer biopsies done at the University of Pittsburgh Medical Center in 2012 with equivocal HER2 protein expression (2+ on IHC) that underwent FISH, had an increased HER2 copy number (⩾4), but were categorized as either equivocal or negative due to an elevated CEP17 (>2.6) copy number. We used Abbott Laboratory probes to SMS (17p11.2) and RARA (17q21.2) to reclassify cases based on HER2:SMS and HER2:RARA ratios. Equivocal was defined if either ratio was 1.8−2.2; and positive if either ratio was >2.2.
Results: For 60 cases, the average HER2 copies/cell ranged from 4 to 7.83. Eight cases had >6 HER2 copies/cell. Of these 8 cases, 7 (88%) became unequivocally positive on re-interpretation using HER2:SMS or HER2:RARA ratio. Fifty-two cases had 4−6 HER2 copies/cell. Of these 52 cases, 18 (34%) became unequivocally positive on re-interpretation using HER2:SMS or HER2:RARA ratio. The status remained negative on 30 cases (58%), 2 (4%) changed from negative to equivocal and 2 (4%) changed from equivocal to negative. Overall, the HER2 status changed in 29 of 60 cases (48%) using an alternate probe; however, the alternate probe that resulted in change of status was SMS in 23 cases (79%), both SMS and RARA in 5 cases (17%) and RARA alone in 1 case (4%).
Conclusions: Tumors with a HER2 gene copy number >6/cell may be assumed to be HER2(+) as they generally have ratios >2.2 using alternate reference probes. The reference probe implicated in changing amplification status in majority of cases is SMS, which is located on 17p while RARA is located on 17q (similar to HER2). Elevated CEP17 and RARA mean copy numbers may skew the ability for these probes to accurately assess HER2 gene status, especially when average HER2 copies/cell range between 4−8 copies.
No conflict of interest.

952 POSTER

Feasibility and safety of image-guided biopsy procedures for personalized cancer treatment

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Background: For implementing tumor DNA guided personalized cancer treatment in daily clinical practice it is important to determine the feasibility and safety of real-time image-guided fresh frozen tumor biopsies.
Material and Methods: An umbrella protocol (CPCT-02; NL35781.041.11) has been developed by the Dutch Center for Personalized Cancer Treatment (www.CPCT.nl), a collaboration between three Dutch cancer centers, to prospectively acquire fresh frozen tumor tissue as well as radiological response data from patients with advanced solid tumors. Image-guided biopsy procedures were performed before initiation of systemic anti-cancer treatment. The protocol allowed 2−4 specimens per time point with a maximum of four time points. DNA isolation of macrodissected tumor rich areas was performed if tumor cellularity exceeded 20%. Another pre-set criterium for performing Next Generation Sequencing (NGS) was a DNA yield of >50 ng for limited (but deep) sequencing of common somatic mutations in approximately 50 genes. A tumor cellularity of at least 50% and DNA yield >500 ng was required for performing extended sequencing of nearly 2000 cancer related genes using sequence enrichment technology. All clinical and genetic data was stored in the CPCT database allowing us to identify biomarkers for response. CPCT-02 (sponsored by the UMC Utrecht) is still recruiting.
Results: Currently, we report on 189 consecutive biopsy procedures performed in 182 patients, the majority being ultrasound-guided liver biopsies (40%). There were over 30% superficial tissue biopsies and in 9% CT-guided lung biopsy was performed. Biopsies were generally well tolerated. No pneumothorax ⩾ grade 2 occurred and no bleeding or other major complications were observed. In 1.1% local pain at the biopsy site (grade 3) occurred. In almost 50% of samples tumor cellularity exceeded 70% and in general more than sufficient quantities of DNA were isolated (85% over 1000 ng). Given the pre-set criteria limited sequencing could be performed on 86% and extended sequencing on 65% of samples.

Feasibility of image-guided tumor biopsies for personalized oncology

	Feasibility (%)
Tumor percentage	
<20%	12
20−50%	20
>50%	68
DNA yield	
<500 ng	7
500–1000 ng	8
>1000 ng	85
Sequencing	
No	14
Limited	86
Extended	64

Conclusions: In daily clinical practice, image-guided tumor biopsies are feasible yielding sufficient material to perform NGS in 86% of patients and can be safely (99%) performed and implemented for personalized cancer treatment.
Conflict of interest: *Ownership: PIFA Therapeutics B.V., Naarden, the Netherlands. Advisory board: InteRNA Technologies, Nijmegen, the Netherlands*

953 POSTER

Genomic testing in cancer (GTC): Patient knowledge, attitudes and expectations

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Background: Genomic testing in cancer (GTC) characterizes oncogenic genes within a patient's cancer. This form of DNA testing is currently being studied for its ability to guide cancer therapy. The objective of this study was to describe patients' knowledge, attitudes and expectations towards GTC.
Materials and Methods: The 42-item self administered GTC questionnaire was developed by a multidisciplinary group (n = 9) and pilot patient pretesting (n = 8). The questionnaire was distributed to advanced stage cancer patients referred to The Drug Development Program at Princess Margaret Cancer Centre, Toronto, Canada, for a phase I clinical trial or for GTC testing as part of an ongoing research study.
Results: Results are reported from 93 surveyed patients with a response rate of 75% (n = 70). Patient characteristics: female 52 (74%); median age 57 years (range 22−77), prior chemotherapy 65 (93%), prior targeted therapy 35 (50%), current or prior clinical trial enrollment 49 (70%), high school diploma 16 (23%), university degree 30 (43%). A total of 73% of patients were interested in learning more about GTC and 60% reported that GTC would significantly improve their cancer care. The mean score of a 12-item questionnaire to assess knowledge of cancer genomics = 8/12 (67%) (SE: 0.25; 95% CI: 7.57–8.55). Patients' knowledge scores significantly correlated with their education level ($p < 0.0001$) and desire for further genetic counseling ($p = 0.004$). A needle or surgical biopsy for GTC if required would be acceptable to 66% and 37% of patients respectively. The primary reason for testing reported by 69% of patients was to help guide their ongoing treatment. The risk of a serious biopsy complication and potential for a treatment delay were listed as the most important reasons to avoid GTC. More than 78% of patients requested disclosure of incidental test results that might impact their own health or increase their family's risk of developing cancer. A reported 87% of patients agreed with biobanking their GTC results and tissue samples for future scientific research. Only

42% of patients reported having sufficient knowledge to make an informed decision to pursue GTC while 36% of patients indicated a need for formal genetic counseling prior to GTC.
Conclusions: Advanced cancer patients are motivated to participate in GTC. Educational tools and counseling programs need to be developed to support understanding and decision-making among patients interested in pursuing GTC.
No conflict of interest.

954 POSTER

Integrated microRNA and mRNA signature associated with the transition from the locally confined to the metastasized renal cell carcinoma

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Background: MicroRNAs (miRNAs) are endogenous small non-coding RNAs that regulate gene expression by interfering translation or stability of target transcripts. One miRNA can interact with several hundred mRNAs, while one mRNA can be regulated by several miRNAs. This interplay between miRNA and their mRNA has been proposed as an important process in cancer development and progression. We have investigated molecular networks impacted by predicted mRNA targets of differentially expressed miRNAs in patients with clear cell renal cell carcinoma (ccRCC) diagnosed with or without metastasis.
Material and Methods: miRNA and mRNA microarray expression profiles derived from primary clear cell renal cell carcinomas from patients with (in total 16 samples) or without diagnosed metastasis (in total 22 samples) were used to identify anti-correlated miRNA-mRNA interaction in ccRCC. For this purpose, Ingenuity pathway analysis microRNA Target Filter, which enables prioritization of experimentally validated and predicted mRNA targets was used. By applying an expression pairing tool, the analysis was focused on targets exhibiting altered expression in our analysis, finding miRNAs and their target genes with opposite or same expression. The resulting identified interactions were revalidated by RT-qPCR in another cohort of RCC patients. The predicted miRNA-mRNA interactions were also tested by functional analyses using miRNA knock-down and over expression experiments in renal cancer cell lines.
Results: Among the significantly differentially expressed miRNAs, we have identified 3 miRNAs (miR-146a, miR-128a and miR-17-5p) that were upregulated in primary tumors from patients without metastasis and down regulated in primary tumors from patients with metastasis. We have further identified the mRNA targets which expression were inversely correlated to these 3 miRNAs, and have been previously experimentally demonstrated in cancer setting in humans. Specifically we showed that BRAC1, MCM10, CDKN3, UHRF1, IL8 were downregulated and targeted by miR-146a-5p. The relation between these identifies target genes and miRNA-146a was validated in cell culture experiments.
Conclusions: We identified novel target genes of dysregulated miRNA which are involved in the transition from primary RCC without metastases into tumors generating distant metastasis.
No conflict of interest.

955 POSTER

Clinical application of molecular profiling in treatment selection for rare and advanced refractory solid tumours: An Australian experience

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Background: Patients with advanced refractory solid tumours including rare cancer types present significant treatment challenges. In many cases, patients have either exhausted standard of care options or, for the more rare cancer types, there are limited treatment options known to be effective. The correlation of biomarkers to associated clinical treatments in a number of cancer types, has allowed for a more targeted and personalised approach to cancer management. The aim of this study was to explore molecular profiling (MP) as a tool for guiding treatment selection in these difficult-to-treat patients within a clinical practice setting.
Materials and Methods: Tissue samples from rare cancer patients (n = 10) and heavily pre-treated ($\geqslant$2 lines of prior therapy) advanced cancer patients (n = 30) with an ECOG performance score $\leqslant$2 underwent MP based on immunohistochemistry, microarray, q/RT-PCR, Sanger and Next-Generation Sequencing (NGS) analyses (Caris Molecular Intelligence®; Caris Life Sciences, Irving, USA).
The rare cancer cohort included: ethmoid sinus, adrenal cortex adenocarcinoma, anaplastic thyroid, fibro sarcoma, astroblastoma, cervical carcinosarcoma, pseudopapillary mucinous neoplasm, endometrial stromal sarcoma, and medullary thyroid. The heavily-pretreated cohort included: cervical, gall bladder, skin (Merckel), colorectal, lung, pancreas, gastric, breast, ovarian, melanoma, oesophagus, cholangiocarcinoma and mesothelioma. MP-guided therapy was considered to have clinical benefit if the patient showed complete response, partial response or stable disease.
Results: In the rare cohort, two patients showed progression and two had non-evaluable disease. Six of the eight (75%) evaluable patients with rare tumours were shown to have clinical benefit following MP-guided treatment. In the heavily pre-treated cohort, 17 of the 30 evaluable patients (56.7%) have demonstrated clinical benefit, while 13 (43.3%) progressed following MP-guided therapy. Of the 23 patients with clinical benefit, eight have sustained clinical responses ranging from 140 days (breast cancer $ER^{-}HER2^{+}$patient) to 365 days (lung pleomorphic patient).
Conclusion: MP-guided treatment selection resulted in clinical benefit in over half of the patients in this study. While requiring further clinical validation, these data lend support to the use of MP in identifying therapeutic interventions for advanced refractory and rare solid tumours who have limited treatment options and poor prognosis.
No conflict of interest.

956 POSTER

DNA damage response deficiency signature predicts response to platinum-based therapy in ovarian cancer

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Background: Ovarian cancer is the leading cause of death from gynaecological malignancies among women. The standard first line therapy is a combination of carboplatin and paclitaxel. Although this treatment regimen confers a response rate in the region of 70%-80%, the majority of women subsequently relapse. Acquired resistance to further chemotherapy is generally responsible for treatment failure, resulting in an overall 5-year survival rate of only 10−30% for late-stage ovarian cancer. At present, empiric-based treatment strategies are used and result in a significant number of patients with chemotherapy-resistant disease, receiving multiple cycles of toxic therapy before the lack of efficacy is identified.
Materials and Methods: We have developed and validated a DNA damage response deficiency (DDRD) signature from microarray data, which predicts response to DNA damaging agents in breast cancer. This assay is based on a molecular subgroup defined by loss of the DNA damage response FA/BRCA pathway, which results in extreme sensitivity to DNA damaging chemotherapeutic agents. We have investigated the utility of the DDRD signature in its ability to predict response to platinum based therapy in 1078 ovarian cancer samples profiled on the Affymetrix microarray platform. Samples were tested with the signature and using the 70th percentile cut-off value, 30% of samples were classified as DDRD-positive and 70% as DDRD-negative.
Results: For overall survival, the DDRD signature yielded HR = 0.56 (95% confidence interval [CI] = 0.46 to 0.68) with a 5-year overall survival rate of 0.42 and 0.24 for patients in the gene signature DDRD-positive and DDRD-negative groups respectively. For relapse free survival, the DDRD signature yielded HR = 0.57 (95% CI = 0.48 to 0.69). The 5-year relapse free survival rate was 0.17 and 0.10 for patients in the DDRD-positive and DDRD-negative groups respectively.
Conclusions: This analysis demonstrates that the DDRD assay, identifying the DDRD molecular subgroup, enriches for patients with an improved overall and relapse free survival following DNA damaging carboplatin-based chemotherapy. We propose that the DDRD assay could be used as a patient stratification tool for existing chemotherapy or as a clinical trial enrichment tool for DNA-damaging or repair targeted drugs in development for use in ovarian cancer.
Conflict of interest: *Other substantive relationships: Almac Diagnostics*

957 POSTER

Soluble CXCL16 in urine as biomarker for bladder cancer diagnostics

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Background: The non-invasive thus patient-friendly detection of bladder cancer by determination of protein biomarkers in urine remains a challenge in cancer diagnostics. Here we present the identification of soluble CXCL16

in urine as a sensitive and specific biomarker for diagnosis of bladder cancer.
Material and Methods: Urine samples from patients with primary bladder cancer and healthy control volunteers were collected. Differential protein expression analysis in urine was carried out using antibody arrays. The results were reproduced in an additional and independent set of urine samples of 31 tumour patients and 31 healthy controls using immunoassays. In addition, urine samples from 21 patients with acute inflammation of the bladder were analysed in order to further verify the diagnostic specificity of CXCL16. Finally, we evaluated expression and distribution of CXCL16 and its receptor, CXCR6, in formalin-fixed patient tissue by immunohistochemistry.
Results: Urinary CXCL16 was found to be significantly higher in patients with primary bladder cancer (median 332.7pg/mg creatinine) in comparison to healthy controls (median 150.1pg/mg creatinine; $p = 0.0026$). In addition, soluble CXCL16 in bladder cancer patients was significantly increased compared those with bladder inflammation only (median 132.7 pg/mg creatinine; $p = 0.0002$). Results did not differ between men and women, smokers and non-smokers, or patients with and without leukocytes in urine. In contrast to the results in urine, most prominent expression of transmembraneous CXCL16 was detected in tissue samples from patients with urocystitis, whereas cancer cells from tumour patients showed only weak or no immunoreactivity.
Conclusions: We were able to identify and successfully confirm soluble CXCL16 as a promising target protein for the diagnosis of bladder cancer. Thereby, CXCL16 is capable of distinguishing tumour patients from patients with urocystitis rather than tumour patients and healthy controls only. Thus, analysis of CXCL16 offers a significant step forward in non-invasive bladder cancer diagnosis.
No conflict of interest.

958 POSTER

Osteomodulin expression profile in human breast cancer

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Background: Osteomodulin (OMD) a cell-binding keratin sulphate proteoglycan, also referred to as osteoadherin, was originally isolated from bovine bone. OMD belongs to the family of leucine rich repeat proteins found in the mineralised matrix of bone and is primarily expressed by mature osteoblasts, whilst also been shown to bind other osteoblasts via the $\alpha_5\beta_3$ integrin. Breast cancer preferentially metastasises to the bone primarily forming osteolytic lesions characterised by loss of bone density. OMD has been proposed to be involved in the regulation of cell proliferation and migration, and therefore this may of particular interest in breast cancer progression to bone metastasis.
Materials and Methods: The expression profile of OMD transcript was examined in a cohort of human normal breast (n = 22) and breast cancer specimens (n = 122) using quantitative polymerase chain reaction (qPCR). This was subsequently used in conjunction with clinical and pathological data, as well as clinical outcome after 10 years follow up, to explore the importance of this molecule in breast cancer progression.
Results: In patients with a good prognosis and survival OMD transcript levels were shown to be high. OMD transcript levels were significantly lower in patients with a Nottingham Prognostic Index of $\geqslant 5.4$ ($p = 0.032$) compared to those who have an index of $\leqslant 3.4$. When comparing clinical outcomes, patients who were disease free at the time of 10 year follow up had significantly higher levels of OMD compared to patients with metastasis ($p = 0.0009$), local recurrence ($p = 0.0079$) and those who had died of the disease ($p = 0.0025$). This trend continued when comparing patients who had developed bone metastasis ($p = 0.009$).
Discussion: In our cohort high OMD expression is associated with a better prognosis and clinical outcome. Reduced levels were associated with metastasis, local recurrence and of particular interest in this study bone metastasis. OMD might therefore provide a potentially novel new biomarker for breast cancer progression and clinical outcome.
No conflict of interest.

959 POSTER

A microarray-based gene expression analysis identified diagnostic biomarkers for unknown primary cancer

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Background: Patients with cancer of unknown primary (CUP) present with metastatic disease for which the primary site cannot be found, despite extensive standard investigation. Clinically, CUPs exhibit common characteristics, such as rapid progression and early dissemination, with a silent primary tumor that may either have a slow growth pattern or may become involuted and undetectable. Existence of such common properties prompts us to hypothesize that there may be potential biological markers that elucidate CUP as a whole.
Methods: We are presently involved in a multicenter clinical study to predict the primary site of CUP based on the analysis of gene expression patterns. Tumor mRNA samples from 60 patients with CUP were measured for the expression of ~22,000 genes using the Affymetrix U133A Plus 2.0 GeneChip® and analyzed by applying normalization and classification algorithms to the gene expression data. The similarity of each tumor specimen's gene expression pattern is then compared to the gene-expression profiles specific to non-CUP groups (containing tumors from 24 known primary sites) that were constructed using publicly available raw microarray datasets. The t-tests were performed to compare the CUP with non-CUP groups and the top 59 CUP specific genes with the highest fold change were selected (p-value <0.001).
Results: This study enabled the identification of genes that exhibited a unique expression pattern in CUP. As a high metastasis potential and vulnerability to apoptosis would explain the properties of CUP well, we first searched for genes related to metastasis and apoptosis and found 14 genes among 44 that were up-regulated by more than 2.5-fold in the CUP samples. Some of these genes were associated with the epithelial-to-mesenchymal transition (EMT), a function that has been increasingly recognized as a key step in cancer metastasis. We also identified 6 genes for ribosomal proteins among 44 up-regulated genes, two of which (*RPS7* and *RPL11*) were known to be involved in the Mdm2 − p53 pathway. Of 15 genes that were down-regulated by more than 2.5 fold in the cup samples, we focused on *CD24*, *KRAS* and *DICER1*.
Conclusions: The protein products of the up-regulated and down-regulated genes identified in this study suggest a biological attribute of CUP and, therefore, may become clinically useful biomarkers for CUP.
No conflict of interest.

960 POSTER

Serological detection of specific protein fingerprints of collagen and laminin degradation can differentiate cancer patients from healthy controls

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Background: In normal tissue extracellular matrix (ECM) remodeling and composition is tightly controlled to ensure homeostasis. In the tumor microenvironment, however, stromal cells deposit increased amounts of ECM components and the proteolytic activity is altered with matrix metalloproteinase (MMPs) as key actors in ECM protein degradation. As the ECM is an important and dynamic part of the tumor microenvironment due to the mutual interaction between cells and the ECM, many cancer hallmarks are promoted by an abnormal ECM composition. Thus, the alterations in the ECM remodeling and composition associated with cancer has a significant impact on the development and progression of the disease. The aim of this study was to investigate whether specific MMP generated ECM protein fragments (neoepitopes) of the ECM components collagen type I, III, IV and laminin may differentiate cancer patients from healthy controls when measured in serum.
Material and Methods: Using well-characterized and validated competitive ELISAs the levels of MMP degraded collagen type I (C1M), III (C3M), IV (C4M, C4M12) and laminin (LAM) were assessed in serum from patients with gastric cancer (n = 11), non-small cell lung cancer (NSCLC) (n = 10), pancreatic cancer (n = 10) and healthy controls (n = 9).
Results: In all three groups of cancer patients analyzed, serum biomarkers reflecting MMP-9 degraded collagen type IV (C4M, $p < 0.001$) and laminin (LAM, $p < 0.05$) were elevated compared to healthy controls. Biomarkers of

MMP-12 degraded collagen type IV (C4M12) were elevated only NSCLC ($p < 0.05$) and gastric cancer ($p < 0.01$) and MMP degraded collagen type III (C3M, $p = 0.05$) only in NSCLC ($p < 0.05$). No significant differences were observed with the biomarkers reflecting MMP degraded collagen type I (C1M).
Conclusion: Serum biomarkers reflecting specific MMP generated ECM-protein neoepitope fragments of collagen and laminin are able to differentiate cancer patients from healthy controls. Although validation in larger clinical settings is needed, this small study emphasizes that highly specific biomarkers for assessing alterations in the ECM remodeling and composition may prove valuable for diagnosing cancer.
No conflict of interest.

	Gastric cancer (n = 11)		NSCLC (n = 10)		Pancreatic cancer (n = 10)		Normal (n = 9)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Age	*56*	*49–63*	*57*	*51–63*	*64*	*59–66*	*70*	*65–76*
C1M	95	53–137	118	54–182	85	47–123	57	40–73
C3M	30	23–36	33	24–42	30	22–39	23	17–29
C4M	83	66–100	98	82–115	91	69–113	46	36–57
C4M12	191	139–244	212	143–282	176	117–236	124	88–159
LAM	12	8–17	11	7–14	9	6–11	5	4–7

961 POSTER

Expression profile of amphyphysin I in normal and breast cancer specimens

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Introduction: Breast cancer is currently the second leading cause of cancer-related death among women. Amphyphysin, a neuronal protein encoded by the *AMPH I* gene, is not only the auto-antigen of Stiff-Man Syndrome (patients are positive for auto-antibodies against this protein) but is also associated with breast cancer. Currently there is little data on the role of AMPH I in the biology and involvement in breast cancer progression therefore our study looked at the expression profile of *AMPH I* gene in a cohort of breast cancer patients.
Methods: The expression profile of Amphyphysin I was examined in a cohort of human normal (n = 20) and breast cancer specimens (n = 91) with 10 year follow-up. Gene transcript expression in the samples was analysed using Real Time RT-PCR and compared to patient data. Additionally, immunohistochemistry technique was used to visualise amphyphysin protein in normal and cancerous tissue sections.
Results: Immunohistochemistry results show expression of the amphyphysin protein to be higher in normal tissues when compared to tumour tissues. Normal breast epithelial cells stain mostly within the cytoplasm with some degree of staining in the nuclei regions. These results seem to correlate with gradually lowered expression levels of *AMPH I* observed throughout breast cancer grading: the highest expression for low grade 1 (mean = 633) and the lowest for the high grade 3 (0.2) breast cancer specimens.
Especially significant are the *AMPH I* expression differences between samples from patients classified as disease free (expression level mean=299) and patients with cancer metastasis (mean=8.8) and local recurrence of breast cancer (mean=1.25) with *p values* 0.037 and 0.032, respectively. The slight increase in expression of *AMPH I* in the samples from patients who died of breast cancer was revealed (mean=28) but it was still significantly lower (*p value* = 0.055) than in cancer-free samples.
Elevated *AMPH I* gene expression seems to correlate with Nottingham prognosis index staging. Its expression levels means were increased from 93.2 in samples from patients with good prognosis to 278 in moderate stage with the highest expression level mean of 526 being observed for patients with the poorer prognosis.
Conclusion: Together the data suggests that amphyphysin I expression may be a useful molecule to identify poorer prognostic breast cancers in patients.
No conflict of interest.

962 POSTER

PRAME gene and protein expression in bone marrow of patients with acute myeloid and lymphoid leukemia

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PRAME is a cancer-testis antigen highly expressed in a number of solid and leukemia tumors. PRAME protein has brought attention due to its ability to activate T-cell cytotoxic PRAME-specific antitumor response. It is used as a diagnostic marker of MDR in leukemia. While RT PCR is used for PRAME expression evaluation, PRAME-based immunophenotyping has not yet been involved into leukemia diagnosis due to the lack of appropriate anti-PRAME antibodies. We have developed recently two hybridoma cell lines producing anti-PRAME moAbs highly specific to detect PRAME protein both in Western blotting and inimmunophenotyping tests.
Aims: To evaluate PRAME expression and cellular localization of this protein in bone marrow (BM)cells of leukemia pts by means of anti-PRAME moAbs and to compare these results with the data of RT PCR analysis. To stain PRAME protein in BM cells we have used two moAbs produced by hybridoma cell lines 5D3F2 and 6H8F12. In order to generate fluorescent signal we have performed additional treatment by secondary fluorescent Ab (Invitrogen) giving green signal. Images were analyzed by Karl Zeiss Axiovert 40CL and AxioVision software to detect the exact cellular PRAME localization.
Results: BM of AML (N = 8) and ALL (N = 2) leukemia pts has been investigated. According to the level of PRAME gene expression all pts subdivided into two groups with relatively high PRAME expression (0.587−6.3% PRAME/Abl) and with low level of PRAME expression (0–5.87×10^{-3}%). Using PRAME staining on the fixed cells we have obtained the following results. In the group of high PRAME expression (AML M7,M5) we have observed PRAME protein both inside the cells (in the nuclei − 33.3%, in the cytoplasm − 23.3%) and on the cell surface (43.3%). In the group of low PRAME expression (AML M7, M3, ALL) there have been no PRAME signal in nuclei, in the cytoplasm it has been observed in 26.6% cases, in most cases it has been on the cell surface (46.2%), some cases (27.2%) have been PRAME-protein negative. When we have performed PRAME staining on the alive none-fixed cells, fluorescent signals have been found in 13.5−14% on the cell surface of both group. Our study suggests that in leukemia pts PRAME protein is very frequently localized on the cell surface. In the cases with low PRAME expression it is nether found in thenucleus. When PRAME expression in leukemia BM cells is increased, it is localized not only on the cell surface and within the cytoplasm but also in the nucleus.
No conflict of interest.

963 POSTER

Oncomodulin is a novel early marker of urinary bladder carcinogenesis in F344 rats

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Background: Short-term carcinogenicity assay using molecular biomarkers that can predict the results of long-term rodent cancer bioassays is greatly desired. The purpose of the present studies is to identify early markers of bladder carcinogenesis in F344 rats.
Material and Methods: Microarray analyses were conducted on 12 *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)-induced rat bladder cancers, 11 dimethylarsinic acid (DMA)-induced rat bladder cancers and 4 normal bladder urothelium using Affymetrix GeneChip Rat Genome 230 Array. mRNA expression analysis was perform using Taqman real-time PCR.
Results: Microarray analyses of BBN and DMA-induced bladder cancers revealed that 85 genes were commonly overexpressed in all cancers compared to control bladder urothelium. To select the candidate markers capable of predicting carcinogenicity of chemicals at the early stage of bladder carcinogenesis, mRNA expression levels of 20 of above genes which were selected based on the overexpression levels and the results of ingenuity pathway analysis, were examined in bladder urothelium of rats treated with BBN for 2, 4 and 8 weeks, respectively. Twelve of the above 20 genes were found to be consistently overexpressed from the week 2, and therefore were selected as candidate early marker genes and their mRNA expression levels were examined in bladder urothelium treated with 7 genotoxic and nongenotoxic bladder carcinogens ((DMA, 2-acetylaminofluorene, sodium o-phenylphenol, phenethylisothiocyanate, benzyl isothiocyanate, uracil and BBN)), bladder carcinogens and 3

nonbladder carcinogens (liver carcinogen: diethylnitrosamine; kidney carcinogen: N-ethyl-N-hydroxyethylnitrosamine; colon carcinogen: 1.2-dimethyhydrazine) and 3 nonbladder carcinogens(liver carcinogen: diethyl-nitrosamine; kidney carcinogen: N-ethyl-N-hydroxyethylnitrosamine; colon carcinogen: 1.2-dimethyhydrazine) respectively, for 4 weeks. Oncomodulin was consistently significantly overexpressed in all urothelium treated with various bladder carcinogens regardless of the degree of histopathologic changes, but not in the urothelium treated with any of nonbladder carcinogens. The functional analysis of oncomodulin is in progress.
Conclusion: Oncomodulin is a novel early marker of bladder carcinogenesis in rats. The 4-week oncomodulin-based bioassay is useful to predict bladder carcinogenicity of chemicals in rats.
No conflict of interest.

965 POSTER

Is HPV-16 integration a predictor biomarker of cervical lesions?

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Background: Persistent infection with oncogenic types of *human papillomavirus* (HPV) has been established as the main etiological factor for the development of cervical lesions and invasive carcinoma. The integration of HPV genome into hosts' genome is considered the hallmark of HPV-associated carcinogenesis, however, the significance of HPV physical status detection remains unclear. The aim of this study was to characterize the physical status of HPV-16 in samples with different histological classifications.
Material and Methods: We have selected 53 cervical specimens from women with different histological classification that have been identified with HPV-16 infection (45 single infection and 8 co-infections). The physical status of HPV16 was analyzed using a multiplex Real-time PCR that allows simultaneous amplification of the E2 and E6 regions. HPV-16 status classification was based on the principle that, when integration occurs, the E2 gene is partially or totally disrupted while the E6 gene remains intact.
Results: In this study, the prevalence of HPV16 integration was of 26.4% (14/53, 13 mixed forms and 1 integrated only). Results showed no significant differences when comparing HPV-16 integration within single vs co-infections ($p = 0.647$). The prevalence of HPV-16 integration among different cervical lesions was 28.6% (2/7) in samples without cytological lesion (normal), 13.3% (2/15) in atypical squamous cells of unknown significance (ASC-US), 33.3% (4/12) in low-grade squamous intraepithelial lesions (LSIL), 33.3% (5/15) in HSIL high-grade squamous intraepithelial lesions and 25.0% (1/4) in invasive cervical cancers (ICC). Additionally, we no found statistical significant differences among the histological specimens ($p = 0.735$).
Conclusion: Our study revealed that HPV16 integration is not exclusive event of high-grade lesions/ICC. Moreover, it was not possible to detect integrated forms in all cases of HSIL/ICC. This study shows that HPV16 integration occurs early during HPV-associated carcinogenesis and therefore there is an emergent requirement to reconsider the role of viral genome integration in HPV-associated carcinogenesis.
No conflict of interest.

966 POSTER

An innovative approach for in-vivo isolation of circulating tumor cells (CTCs)

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Background: Currently, circulating tumor cells (CTCs) are isolated *in vitro* from a small volume of blood samples. Furthermore, CTC results for different kinds of cancer as a prognostic and stratification biomarker are scarce. The aim was to assess a medical device (CellCollector) for *in vivo* isolation of CTCs directly from the blood of non-small cell lung cancer (NSCLC), breast cancer (BC), prostate cancer (PC) and colorectal cancer (CRC) patients prior surgery/ therapy start and partially in the course of therapy. Additionally, CTC enumerations were compared to the Cell Search® method.
Material and Methods: The device was inserted in a cubital vein through a standard cannula for thirty minutes. The interaction of target CTCs with the CellCollector was mediated by an antibody directed against the epithelial cell adhesion molecule (EpCAM). To confirm the CTCs binding to the wire, the immunohistochemical staining against EpCAM and/or Cytokeratin as well as CD45 for negative cell selection was performed. There were more than 380 applications of the wire in NSCLC, BC, PC and CRC patients and over 45 applications in control subjects. Enumeration data was available for 159 cancer patients and 37 control subjects. For 98 cancer patients and 22 control subjects, samples were also tested in the CellSearch® system.
Results: The device was well tolerated in more than 380 applications without side effects. We obtained in vivo isolation of CTCs in 126 of 159 cancer patients (79.2%) with a median (range) of 3 (0–515) CTCs and a mean of 17 CTCs. The sensitivity was similar for early and late stage in cancer patients. In the control groups, only in 6 of 37 subjects CTCs were detected (84% specificity). The sensitivity and specificity for CTC detection by the CellSearch® method was 16.7% and 79.5%, respectively. With exception of three samples, in all 78 paired samples the number of CTCs detected with the CellCollector was higher or equal to CellSearch®, regardless of the disease stage.
Conclusions: Whilst well tolerated without side effects, the CTC detection rate of the CellCollector in NSCLC, BC, PC and CRC patients was 79.2%. In contrast, 16.7% detection was obtained using the CellSearch® analysis. A specificity of the medical device of 84% could be reached. This innovative method may have important clinical implications, as the implementation of the device into clinical practice may improve early detection, prognosis and therapy monitoring of NSCLC, BC, PC and CRC patients. The method may also allow the molecular analysis of the CTCs, with the possibility of establishing more personalized treatment regiments.
No conflict of interest.

967 POSTER

Early technology foresight for the development of biomarkers for prostate cancer screening: Prospective Health Technology Assessment (ProHTA)

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Background: Currently, screening for prostate cancer is mostly performed using the digital rectal examination (DRE) and the measurement of the prostate-specific-antigen (PSA). Although PSA-testing is very sensitive, it lacks in specificity. Therefore an additional biomarker with high specificity could lead to a significant reduction of the biopsies performed unnecessarily.
Foresight methods such as modeling and simulation can help to assess the impact of future health technologies. 'Prospective Health Technology Assessment' (ProHTA) aims to develop a platform targeting health care manufacturers and decision makers that facilitates the assessment of innovative health technologies prior to their launch.
Methods: The objective of this work is to reduce the number of biopsies by introducing a novel and fictive biomarker additional to PSA-measurement. The potential impact of this biomarker is investigated in a simulation. Using clinical pathways, a model for the diagnostic process of prostate cancer is designed. This model serves as the basis for 'hybrid simulation' that consists of system dynamics models for macro-simulation and discrete event models for micro-simulation.
Results: The simulation shows that the use of a fictive biomarker in addition to DRE and PSA measurement will reduce the biopsies performed. The extent of the reduction depends on the sensitivity and specificity of PSA and DRE testing, as well as on the parameters determined for the biomarker. For example, if the estimated sensitivity of the biomarker is 100% and the specificity is 80%, then 25% of all biopsies can be avoided.
Conclusions: The ProHTA simulation approach is innovative and shows with the example of the fictive prostate cancer biomarker the potential to predict if an innovation and research in this field will be successful. Thus, ProHTA represents a useful decision-making tool for foresight and adds value to existing methodologies for pre-assessing health technology at a very early stage of technology research and development. It offers a valuable approach with an emphasis on strategic planning and therefore helps to improve the efficiency of health care delivery in different care settings.
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No conflict of interest.

968 POSTER

Opportunities and pitfalls in developing imaging biomarkers (IB) in oncology clinical trials

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Background: The assessment of tumor progression is fundamental to cancer clinical trials. Progression-free survival or disease-free survival tend to substitute overall survival as trial endpoint whenever feasible and meaningful. Those endpoints are based on imaging evaluation of tumor size. However, new technologies have been developed that provide additional information according tumor location or treatment administered, e.g. metabolic assessment provided by FDG-PET. Those advanced techniques have been widely used in routine clinic for staging or evolution of the disease. Up to date, they also play an increasingly important role in response assessment. Nevertheless, the assessment by functional imaging is different from that by anatomic imaging, where tumor size is a reproducible measure. Functional imaging has the additional requirement that an observed change of the parameter in response to treatments must be greater than the intrinsic and extrinsic variability of the parameter in the absence of treatment.
Methods: In order to use IB in clinical practice, robust criteria taking into account all the sources of variability of the measurement must be developed. Four steps are needed in such development: 1) evaluation of the different source of variability (multicentric test-retest) 2) development of imaging guidelines (standardization, quality assurance, central review) 3) evaluation of the treatment effect on the imaging measurement (correlation with pathological assessment) 4) validation studies (correlation with long term endpoints).
Results: We will detail the process and trial designs needed to fully develop robust IB and will illustrate how to do this in an imaging protocol that fits with the constraints of the primary clinical trial to which it is attached. When an IB is developed through a clinical trial as an (optional) translational research project, the imaging protocol has to be adapted to not negatively impact on the main protocol as the assessment of the tumor with the new techniques will not be blinded to the investigator. The sample size of the imaging protocol will also be constrained by that of the main clinical trial.
Conclusion: Appropriately used, IB could offer interesting treatment options and help fasten and target treatment development in clinical trials. Therefore we propose guidelines to help researchers to develop add-on IB protocols to therapeutic trials.
No conflict of interest.

969 POSTER

NGS panel V1.1 for the routine deep sequencing-based diagnostic of somatic hotspot theranostic mutations on FFPE tumours: A prospective study of 500 cancer samples

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In cancer, targeted therapies development induces an increasing number of mutations with theranostic values. Improving of patient care now relies on tumour genetic profiling that will guide treatment strategy. This implies the ability of parallel genotyping of a growing number of mutations. By providing an increase in sequencing throughput, NGS technology is suitable for routine implementation of such diagnostic in the near future. To address this challenge, we tested whether NGS sequencing detects somatic alterations in formalin-fixed, paraffin-embedded tumour samples. We aimed at determining the sensitivity and specificity of NGS compared to pyrosequencing (PS) and allele-specific PCR (ARMS) methods on 500 patients. A panel of 48 amplicons covering a 5kb region was designed to detect hotspot mutations in 17 key cancer genes. Low amount of input FFPE DNA was used to generate libraries on 48.48 Fluidigm access arrays for targeted sequence library. Libraries were sequenced using 150bp paired-end reads on an Illumina MiSeq. Trimmed reads were aligned to the hg19 genome using BWA in single end mode. Local indel realignment was performed with GATK. Calling algorithms were used to detect mutations even at a low frequency (SNVmix, lofreq, samtools, GATK and freebayes). NGS data was obtained on 500 patients (240 NSCLC, 230 colorectal cancers and 30 melanomas) and compared with reference technics (PS and ARMS) used in the routine molecular diagnostic. Deep sequencing of our custom gene panel results in a minimum of 3000X coverage for all amplicons with a mapping quality greater than 30. NGS detects 219 mutations in the 500 patients, including single nucleotide substitutions, small deletions (*EGFR* exon 19) and insertions (*ERBB2* exon 20). Among theses, 193 were also found with the two reference methods. NGS lead to 10 false negative mutations due to poor DNA quality. Sixteen false positive mutations were detected very likely due to the increased sensibility of NGS. Considering pyrosequencing and ARMS as references, deep sequencing provides a 95% sensitivity and a 98% specificity. We demonstrate that our deep sequencing approach can be reliably implemented as a diagnostic test for routine detection of somatic mutations. Our strategy allows the simultaneous analysis of more than 100 somatic hotspot mutations in 17 key cancer genes for 96 samples in a single assay and thus improves the molecular diagnosis of tumours in a cost and time efficient manner.
No conflict of interest.

970 POSTER

A comprehensive low-cost germline genetic test for over 260 conditions, including 50 affecting cancer diagnosis and treatment

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Background: Historically it has only been feasible to sequence 1 or 2 genes to test for hereditary cancer in a patient. Hence, cancer-related genetic conditions such as Bloom syndrome and Fanconi anemia can go undiagnosed. Also, conditions not directly related to a patient's cancer often go untested, although they can affect treatment and outcome, such as bleeding syndromes (i.e. surgery) and cardiomyopathies (i.e. chemotherapy).
Materials and Methods: A single assay was developed to test for 264 germline genetic conditions, including 50 hereditary cancer conditions. This assay covers nearly all published clinically-relevant variants (both coding and non-coding) in the associated genes and also detects novel likely-pathogenic variants, such as previously unseen loss-of-function mutations in tumor suppressors, in the full gene sequence. Extensive scientific review was conducted to curate these clinical conditions, storing the validated genes, variants and risk models in a database that informs our targeted next-generation sequencing platform. Custom variant interpretation software describes known and novel substitutions, insertions and deletions, and copy number variants, including those in traditionally hard to assay sites such pseudogenes and homopolymer stretches. Clinical reports are automatically generated and variants reported according to ACMG guidelines, with an additional category for variants observed in patients but which have uncertain pathogenicity. This test requires less than 2 weeks from arrival of blood sample to clinical report delivery.
Results: Analytical validation was performed on a panel of reference samples with >11,000 known variant sites and ~2.1 million non-variant sites. For coding sequence substitutions, we demonstrated 99.7% sensitivity and 99.998% specificity. For insertions/deletions in coding sequence, we demonstrated 98.3% sensitivity and 99.994% specificity. In direct comparisons against established diagnostic laboratories using traditional Sanger sequencing, we saw 100% concordance with those results, both in terms of analytical concordance and clinical interpretation.
Conclusions: We report progress in aggregating many genetic tests into one comprehensive assay at low cost, including a comprehensive inherited cancer test. This curation approach enables scalable, automatable, and reproducible diagnostic reporting, using accurate and scalable DNA sequencing methods.
Conflict of interest: *Ownership: employees of InVitae and stock ownership in InVitae*

971 POSTER

The diagnostic value of one step nucleic acid amplification (OSNA) for sentinel lymph nodes in colon cancer

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Background: Colorectal carcinoma (CRC) is the second most commonly diagnosed malignancy in the Netherlands. Determining the lymph node (LN) status is a good prognostic factor in CRC and is critical in the staging of these tumors. Earlier studies have shown that 20−30% of the Dukes stage I−II patients will still develop metastases within 5 years. Literature has also shown that with the use of the ex vivo sentinel lymph node

mapping procedure (SLNM) and the immunohistochemical Keratin Pan staining at 4 levels an upstaging of 20% can be achieved in these patients. The examination of SLN in breast cancer with One Step Nucleic acid Amplification (OSNA) has been proved to be valuable. The aim of this study was to determine the value of the sentinel node mapping procedure and the diagnostic value of OSNA in colorectal cancer.
Materials and Methods: In this study 313 SLNs of 122 patients from the Jeroen Bosch Hospital in 's-Hertogenbosch and the Leiden University Medical Center were investigated by the routine examination (H&E), fine examination (H&E and Keratin Pan immunohistochemical straining) and OSNA. The SLNs were harvested by the ex vivo sentinel lymph node mapping procedure, using Patent blue or Indocyanine green. Half of the SLN was used for the routine and fine examination and the other half for the OSNA assay. OSNA uses the reverse-transcription loop-mediated isothermal amplification (RT-LAMP) for mRNA amplification.
Results: The diagnostic value of OSNA, determined on all 122 patients was 81% and 100% for the fine examination and combined method (OSNA and fine examination together). An upstaging rate of 21% was obtained with the use of OSNA and 37% with the use of fine examination. An upstaging rate of 48% was obtained by combining these two methods together.
Conclusion: SLNM proved to be of value in CRC. OSNA and Fine examination both showed a good diagnostic value. The combination of OSNA and Fine examination was associated with an increased diagnostic value and also showed more upstaging of patients. Future research needs to be done to show whether routine examinations should be replaced by the investigation of sentinel lymph nodes using the OSNA assay and the fine examination together and the amount of SLNs to be investigated.
No conflict of interest.

972 POSTER

High resolution in vivo imaging for cancer detection and evaluation of tumor heterogeneity

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Background: Early tumor diagnostics and imaging of solid tumors are critical areas of cancer research. Imaging of tumor heterogeneity gives insights into proliferation, metastases and response to therapies. The majority of imaging methods does not demonstrate competitive resolution, penetration depth or cannot utilize fluorescent imaging agents widely used in preclinical research. In this work, we analyze *in vivo* spatial distribution of nanoparticle-based vascular and targeted contrast agents using novel imaging methods: hybrid fluorescent molecular tomography-X-Ray computer tomography (FMT-XCT) and high-resolution multispectral optoacoustic microscanner (microMSOT) in different animal models: transgenic model for Non-Small Cell Lung Cancer (NSCLC) and tumor xenograft/allograft one.
Material and Methods: The animals of transgenic K-ras model for NSCLC were injected with different targeted and activatable contrast agents and imaged *in vivo* with FMT-XCT imaging system. Foxn athymic nude mice were used for inoculation of subcutaneous (s.c.) 4T1 murine breast cancer allografts or HT29 human colorectal xenografts. The tumors were grown up to the size of 7−8 mm. Then the animals were injected with nanoparticles or vascular contrast agents and imaged with microscanner MSOT. The results of *in vivo* imaging were validated and analyzed with cryo-slicing epifluorescence imaging system and immunofluorescence.
Results: FMT-XCT *in vivo* imaging demonstrated the potential of $\alpha v\beta 3$-integrin targeting for detection of early lesions in K-ras mouse model of NSCLC. $\alpha v\beta 3$-integrin targeted fluorescence could selectively resolve pulmonary lesions in mice from 2 weeks of age vs. wild type. FMT-XCT imaging showed tumors and heterogenic profiles for $\alpha v\beta 3$-integrin spatial distribution in the lung tumors of 4-week-old animals. *In vivo* studies were verified versus immunofluorescence.
Heterogenic profiles of s.c. tumors were studied by microMSOT visualizing development of tumor vasculature with high resolution. Distribution profiles of nanoparticles, oxy- and deoxygenated hemoglobin were detected *in vivo* through entire tumor. *In vivo* optoacoustic signal correlated with *ex vivo* cryo-slicing and immunofluorescence profiles.
Conclusions: Our results demonstrated potential of FMT-XCT imaging of targeted contrast agents for early detection of lung lesions and *in vivo* study of heterogenic tumors.
MicroMSOT could offer high resolution imaging of intrinsic contrast and extrinsically applied probes for *in vivo* investigation of tumor vascular properties with impact on cancer research.
No conflict of interest.

973 POSTER

Development of a next generation sequencing assay for the identification and quantitation of fms-like tyrosine kinase internal tandem duplication sequences, and mutation analysis

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Background: Activating mutation in fms-like tyrosine kinase (FLT3) gene aberrations, internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations, play an important role in pathogenesis of acute myeloid leukemia. FLT3 Internal Tandem Duplications (ITD) are in frame duplications occurring most frequently in the juxtamembrane and less frequently in the tyrosine kinase domains. FLT3 ITDs cause ligand independent dimerization and activation of FLT3 leading to uncontrolled cellular proliferation. About 30% of AML patients are FLT3 ITD+ and demonstrate a worse prognosis and response to chemo therapy. Treatment of FLT3 ITD+ patients with the appropriate tyrosine kinase inhibitor represents a viable option for these patients. Selection of the appropriate tyrosine kinase inhibitor is dependent on patient FLT3 genotype and the presence of any resistance conferring mutations vs. the candidate treatment. To this end, we developed and characterized a next generation sequencing assay for the simultaneous detection, identification, and quantitation of FLT3 ITDs and TKD point mutations.
Methods: DNA fragment libraries prepared from 100 or 1000 ng of test sample gDNA are hybridized to enrichment oligos specific for FLT3 exonic sequences. Captured libraries are amplified and sequenced via Ion Torrent. TMAP aligned data were realigned for larger INDELs and filtered for the identification/quantitation of ITD sequences. Additionally, point mutations were called by VarScan and further characterized by SIFT/PolyPhen2/COSMIC for their damaging effects.
Results: Using model cell lines (MV4−11, MOLM-13), the assay demonstrates a sensitivity (LOD) of 10% for the detection of ITD sequences and 5% for point mutations. Further assay validation in AML patient samples demonstrates detection of ITDs between 30–178 bp at frequencies as low as 10% in addition to identifying resistance conferring point mutations within the tyrosine kinase domain.
Conclusions: The developed FLT3 genomic assessment assay simultaneously determines FLT3 ITD status, monitors ITD clonal populations for the presence of specific ITD's and TKD point mutations, and may be useful in determining patient response to tyrosine kinase inhibitors.
No conflict of interest.

974 POSTER

Automated large-volume extraction of circulating, cell-free DNA to improve the sensitivity of tumor biomarker detection

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Background: Because of its low concentration and high degree of fragmentation, the extraction and detection of tumor-derived circulating cell-free DNA (ccfDNA) is technically challenging. An optimized ccfDNA extraction method was developed and evaluated by comparison to existing manual and automated reference methods.
Material and Methods: ccfDNA was bound from 5.8 ml EDTA plasma of healthy donors (with IRB approval) to novel magnetic particles and recovered in 150 µl. The QIAamp® Circulating Nucleic Acid (CNA) Kit was used as a reference and for the purification of any residual ccfDNA from the plasma supernatant after binding. Alternatively, the 'Virus cell-free 1000 protocol' using the QIAsymphony® DSP Virus/Pathogen Midi Kit was modified for the processing of higher sample volumes. ccfDNA was extracted from 4 ml plasma and eluted in 90 µl. ccfDNA yield was quantified by qPCR (66bp within the 18S rDNA). To determine the DNA fragment size-dependent recovery, targets from 67bp to 475bp within the APP gene were quantified by qPCR.
Results: The mean ccfDNA recovery (18S 66bp target; compared to the QIAamp® CNA Kit) was 7% (N = 12; +/−4.1%) for the supernatant and 95% (N = 12; +/−19.2%) for the eluate. For the APP assay, ratios between the copy numbers of different target sizes were calculated. The mean ratios were: 67/476bp = 11 (N = 12; +/−6.2), 180/476bp = 8.1 (N = 12; +/−3.6) and 67/180bp = 1.4 (N = 12; +/−0.3). The modified Virus cell-free 1000 protocol led to a mean ccfDNA recovery of 140% (N = 24; +/−48.4) compared to the QIAamp® CNA Kit.
Conclusions: The automated protocol versions led to an overall similar ccfDNA recovery compared to the QIAamp® CNA Kit (complete ccfDNA binding to magnetic particles). Using the new extraction chemistry an improved recovery of tumor-derived ccfDNA is possible and allows for a higher sensitivity of tumor DNA detection, which is, besides a high specificity, critically important for the application of tumor ccfDNA biomarkers as a tool in cancer diagnosis and prognosis. The presented

applications are for research use only. Not for use in diagnostic procedures. This work has received funding from the European Union FP7 Programme under grant agreement no. 222916, SPIDIA project (www.spidia.eu).
Conflict of interest: *Other substantive relationships: All authors are employed at QIAGEN GmbH.*

975 POSTER

Is there a place for the UCA1 test in bladder cancer detection?

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Background: Bladder cancer detection and surveillance are traditionally performed by cystoscopy and urine cytology. Recently, *Urothelial Carcinoma Associated 1 (UCA1)* was identified as a very sensitive and specific urinary marker of bladder cancer. This study aimed to compare the clinical value of the *UCA1* test with routine diagnostic methods.
Material and Methods: Between October 2009 and December 2011, 564 *UCA1* tests were performed on urinary samples collected from 467 patients. Histological diagnoses were available in 108 cases. Patients were divided into screening and follow-up groups based on the absence or presence of prior urothelial carcinoma. The test performance was evaluated in each group and compared to cystoscopy, urine cytology and routine follow-up results.
Results: The overall sensitivity, specificity and positive and negative predictive values for the *UCA1* test were 69, 67, 86 and 42%, respectively. We observed no difference in performance for tumors of higher grade or stage, but sensitivity was increased in the screening population (84%) compared to patients under follow-up (58%). The *UCA1* test successfully detected 8/8 cases of *carcinoma in situ* and 2/4 cases of urothelial dysplasia. The positive predictive value of the test was comparable to cystoscopy.
Conclusion: The *UCA1* test can be used as a complementary method to cystoscopy and cytology for the detection of bladder cancer. While we found no evidence supporting the use of the *UCA1* test as a replacement for cystoscopy, it does seem beneficial for detection of *carcinoma in situ* lesions and, in some cases, for urothelial dysplasia.
No conflict of interest.

976 POSTER

Prevalence of KRAS-LCS6 polymorphism (rs61764370) within three different tumour types (breast, colorectal and non small cell lung cancer, NSCLC): A study of Czech population

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Background: KRAS is one of the most frequently mutated oncogenes in human cancer, with almost a quarter of different tumor types showing altered functions of KRAS. The let-7 family of microRNAs were found to regulate KRAS activity by binding to the 3'UTR of human KRAS gene. A germline SNP (rs61764370) is located in a let-7 complementary site (LCS6) in the 3'UTR of KRAS oncogene. The LCS6 SNP consists of a T-to-G base change and it was found to alter the binding capability of the mature let-7 miRNA to the KRAS mRNA. In several studies G-allele of rs61764370 was found to be associated with higher risk of breast cancer, colorectal cancer, melanoma, oral cancer and ovarian cancer. It is potential biomarker of poor response to targeted therapies in colon cancer too. Controversially, other authors did not find significant association with cancer risk. Thus, the relevance of rs61764370 in cancer predisposition is still debated and deserves further investigations.
Material and Methods: DNA of tumours tissues was isolated from fixed, fresh-frozen, cytology specimens and formaline fixed paraffin embedded tissue from 262 mCRC, 158 breast and 117 NSCLC patients. DNA from 387 healthy controls was isolated from peripheral blood. Analysis of SNP rs61764370 (KRAS-LCS6) was performed by PCR and RFLP method.
Results: The KRAS-LCS6 G-allele (T/G genotype) was detected in 9.9% (26/262) of mCRC cases; 15.2% (24/158) of breast cancer cases and 14.5% (17/117) of NSCLC patients. T/G genotype of the SNP (rs61764370) was identified in 15.8% (61/387) of healthy controls. In our study we did not find G-allele carriers in homozygous state (G/G genotype) among the cancer patients and healthy controls.
Conclusions: The frequency of the LCS6 G-allele varies across geographic populations, with European populations exhibiting the variant allele most frequently 5−10%. In our study, in healthy Czech population (controls), frequency the LCS6 G- allele was 15.8%. Prevalence of the KRAS-LCS6 G-allele was not significantly different among stadied tumours (NSCLC, breast cancer) and the healthy controls. The acquisition of new data from different populations could have a paramount importance to establish the real contribution of each polymorphism to the cancer risk. The study of polymorphisms, affecting miRNA-dependent pathways and involved in cancer susceptibility is rapidly growing and is becoming increasingly important on the fast growing field of the personalized medicine.
No conflict of interest.

977 POSTER

microRNA expression in BRCA 1/2 hereditary breast cancer: Exploratory analysis

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Background and Objectives: microRNAs (*mirs*) are small non-coding regulatory RNAs involved in gene expression regulation at posttranscriptional level. *mirs* expression is deregulated in breast cancer, but there is very limited data about their expression in hereditary breast cancer (HBC). The objectives of this study are: 1- to analize if there is any difference in the expression of a *mirs* pannel (*mir*21, *mir*155, *mir*195, *Let*7a and *mir*16) between sporadic breast cancer (SBC) and *BRCA1/2* HBC; and 2- to analize the change of expression of the mirs pannel, prospectively, in *BRCA1/2* HBC.
Methods: RNA was extracted from whole peripheral blood, reverse transcribed and quantified by real-time quantitative polymerase chain reaction analysis for *mir* 21, *mir* 155, *mir* 195, *Let* 7a and *mir* 16 (endogenous control). We have 3 groups of patients (pts): (A) 20 with *BRCA1/2* HBC, (B) 13 with SBC (*BRCA1/2* wildtype) and (C) 15 healthy individuals. Five of 20 *BRCA1/2* HBC started this study prospectively, at breast cancer diagnosis, and *mir* expression was evaluated before surgery, 2 weeks after surgery and after adjuvant treatment. All other pts in group A were breast cancer survivors. All pts in group B were breast cancer survivors.
Results: Group A- 18 female, 2 male; BRCA1: 3 pts, BRCA2: 17 pts; median age: 58 years. Group B- 12 female, 1 male; median age: 45 years. Group C- 11 female, 4 male; median age: 45 years. For *mir*21, *mir*155 and *mir*195 *BRCA1/2* HBC and SBC cancer survivors had significant different levels of *mir* 195 expression. In the exploratory analysis of the first 4 prospective pts a trend for decreasing levels of *mirs* 21, 155 and 195 after surgery was observed (but not significant after log conversion). The only case already measured after adjuvant treatment had more pronounced descent of *mirs* 21 and 155 than after surgery. Analysis for *Let*7a is not yet complete.
Conclusions: This exploratory analysis, suggests two study hypotheses: 1- *mirs* expression may not be similar in HBC and SBC and 2- change in *mirs* expression before and after cancer treatment of HBC may be more significant after completion of adjuvant treatment than after surgery. These are very rare pts but enrollment in the prospective study continues.
No conflict of interest.

978 POSTER

Sensitive detection of EGFR mutations using mutant-enriched PCR and reverse-hybridization teststrips

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Background: Activating mutations in the epidermal growth factor receptor gene (EGFR) allow the therapeutic use of tyrosine kinase inhibitors (TKI), such as erlotinib or gefitinib in non-small cell lung cancer (NSCLC) therapies. In contrast, the presence of the T790M mutation in tumor tissue predicts resistance to TKI.
Materials and Methods: We have developed a reverse-hybridization StripAssay for the detection of three mutations in exon eighteen, twenty-four deletions and complex mutations in exon nineteen, the T790M mutation in exon twenty and two mutations in exon twenty-one of the EGFR gene. The test is based on mutant-enriched PCR in the presence of EGFR wild-type suppressors, followed by hybridization of biotinylated PCR products to teststrips presenting a parallel array of allele-specific oligonucleotide probes. The hybridization and detection steps can be carried out fully automated using commercially available instrumentation.
Results: StripAssay performance was evaluated using genomic DNA obtained from cultured cell lines and formalin-fixed paraffine-embedded

(FFPE) tumor tissue. Plasmid clones harbouring the respective EGFR mutations served as reference templates to control for test specificity. By using normal DNA spiked with serial dilutions of DNA from EGFR-mutant tumor cell lines all mutations were shown to be detectable at a level of 1%.
Conclusions: The simultaneous detection of EGFR mutations with a sensitivity of 1% makes the StripAssay a very useful tool for the assessment of the EGFR mutation status of cancer patients.
No conflict of interest.

979 POSTER

Relationship between insulin resistance and tumor burden in cancer patients

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Background: Insulin resistance (IR) is a risk factor for various cancers in many epidemiologic and preclinical studies. Prevalence of IR remains unknown in cancer patients.
Methods: We measured homeostasis model assessment (HOMA) defined as fasting glycaemia (mmol/l) x fasting insulinemia (mU/l)/22.5 and recorded criteria of metabolic syndrome (waist circumference, hypertension, triglyceridemia, HDL cholesterol level and fasting glycaemia) and C Reactive Protein (CRP) in a cohort of cancer patients. Patients with diabetes mellitus and corticosteroid treatment were excluded.
Results: We included 101 patients, 54 males, with a median age of 59 years (range 20−89), and with a WHO Performance Status $\geqslant$2 in 21.8%. Median HOMA was 2.1 [95% CI 2.148–2.719] and 23% of patients had a metabolic syndrome. HOMA was not related to waist circumference, BMI or CRP. The most frequent primary tumors were sarcoma, genito-urinary and gastro-intestinal (21.8% each). HOMA was found in the highest quartile (HOMA>3.15) in 8 out of 22 (36%) patients with sarcoma. Cancer status was long-term remission (n = 11), adjuvant setting (n = 10) and macroscopic disease (n = 80). HOMA was significantly lower in long-term remission patients versus adjuvant setting and macroscopic disease (p = 0.0149 and p = 0.0011 respectively). Among the 25 patients with HOMA>3.15, median age was 55 years (range 24−82) and all had macroscopic disease (p = 0.003 by Chi2 test). Twenty of these patients (80%) had no metabolic syndrome.
Conclusion: We could identify insulin resistant cancer patients. These results should guide the selection of the patients for inclusion in metformin or IGF-R inhibitors clinical trials.
No conflict of interest.

980 POSTER

Neutrophil gelatinase-associated lipocalin in platin induced renal injury

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Background: Acute renal injury (ARI) is an important issue in chemotherapy receiving patients. Neutrophil gelatinase-associated lipocalin (NGAL) is a novel marker used in early detection of ARI. In this study we aimed to assess the role of urine NGAL levels in patients receiving platin compounds.
Material and Method: Patients who had treated with cisplatin or carboplatin or oxaliplatin containing regimens at Cumhuriyet University Medical Faculty Cancer Center, included in this study. Patient's baseline and postchemotherapy serum urea, creatinine, urine NGAL and urine creatinine level had determined. To avoid false urine NGAL levels due to hydration during chemotherapy infusion urine NGAL/urine creatinin ratio had used to determine ARI. To examine the relationship between pre and post chemotherapy urine NGAL change, Wilcoxon Signed Ranks Test had used. A p-value of <0.05 had considered significant. The analysis had performed by SPSS version 14.0 software (SPSS Inc., Chicago, USA).
Results: A total of 42 patients, receiving platin compounds, included in this study. Fourteen of them (33.3%) received cisplatin containing regimes, 14 patients (33.3%) received carboplatin and 14 patients (33.3%) received oxaliplatin. The median age was 60 (37−76) years. Sixteen of the patients (38.1%) were lung cancer, 15 were (35.7%) colorectal cancer and 11 were (26.2%) other cancers. The median pre and post chemotherapy urine NGAL/urine creatinin ratio in cisplatin group was 15.6 ng/mg and 35.8 ng/mg (p = 0.016), in carboplatin group was 32.5 ng/mg and 86.3 ng/mg (p = 0.019) and in oxaliplatin group was 40.9 ng/mg and 62.3 ng/mg (p = 0.3).
Conclusion: Nephrotoxicity is a serious side effect of chemotherapeutic agents. Although not statistically significant, oxaliplatin may also have nephrotoxic effect. So all platin compounds must be used carefully and urine NGAL measurement seems to be promising in detecting ARI earlier then creatinin.
No conflict of interest.

981 POSTER

HER2-based treatment decisions in breast cancer (BC): Test accuracy and its clinical, economic and social impact

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Background: Treatment choice for patients with BC is guided in part by HER2 status. Although FDA-approved *in vitro* diagnostic (IVD) tests are available, nonapproved IVDs are used in many laboratories (approx 30% in Nordic region). Incorrect HER2 results can impact greatly on patient outcomes and healthcare budgets. We analysed the accuracy of HER2 testing approaches using pooled data from the Nordic Immunohistochemical Quality Control (NordiQC) real-world testing programme and used the results in an economic BC treatment model.
Methods: Data were obtained from the NordiQC HER2 BC test programme run from 2008–2012 (www.nordiqc.org). False-negative (FN; a 3+/2+ amplified tumour but stained as 0 or 1+) and false-positive (FP; a 0/1+/2+ unamplified tumour but stained as 3+) rates were calculated for approved vs nonapproved IVDs and used to calculate economic costs of inaccurate results, loss of survival, productivity benefit and QALYs. Costs were extrapolated to numbers of US patients with BC (early BC [EBC] n = 209 737; metastatic BC [MBC] n = 20 743) using a 1-y time horizon. US costs and population sizes were used because of the homogeneity of the US healthcare system pricing framework.
Results: 1703 tests were performed (1145 [67%] approved IVDs; 558 [33%] nonapproved IVDs). Pooled FN rates were 11% and 25%, respectively; FP rates were 0% and 5%, respectively. Incorrect results were largely due to misclassified 2+ samples. The total direct cost/patient of inaccurate tests in the model was $69 for approved and $263 for nonapproved IVDs. Extrapolation to the US EBC population gave a potential $41 million cost saving for approved vs nonapproved IVDs. Costs and benefits are shown below. Results were similar for MBC although cost savings were reduced due to the lower incidence of MBC.

Outcome	EBC		
	Approved IVD	Nonapproved IVD	Difference
Total direct cost of inaccurate test, $	14,378,394	55,112,205	40,733,810
Missed QALYs	158	361	203
Lost productivity, $	3,285,434	7,510,050	4,224,615
Missed survival benefit, y	187	426	239

Conclusions: This analysis suggests that incorrect HER2 test results have far-reaching clinical, social and economic consequences that should be considered when requesting a test. Oncologists and pathologists need to be aware that differences between available tests impact patient outcome in terms of missed survival benefit and exposure to unnecessary toxicity, as well as costs. Adhering to testing guidelines ensures accurate results and correct treatment for patients with BC.
Conflict of interest: *Ownership: None. Advisory board: None. Board of directors: None. Corporate-sponsored research: None. Other substantive relationships: Ventana employees * Ranger-Moore, Sheppard, Walk; Hoffmann-La Roche employees * Gartemann, Teichgr*ber, Rohr.*

982 POSTER

A new imaging biomarker to predict anti-angiogenic therapy efficacy in phase 1 trials: AUC changes with dynamic contrast enhanced-ultrasonography (DCE-US)

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Background: Antiangiogenics compounds are widely prescribed but lack a predictive biomarker of efficacy. The objective of this analysis was to confirm the decrease of AUC in DCE-US at day 15 and day 30 as a tool able to predict time to progression (TTP) in phase 1 trials enrolling patients exposed to anti-angiogenic compounds.
Material and Methods: All patients (pts) included in phase 1 trials with anti-angiogenic compounds, between 2005 and 2013 in our institution and having underwent standardized DCE-US evaluation at baseline, D15 and D30 were retrospectively reviewed. DCE-US methodology: a bolus injection of contrast medium, 3 minutes of recorded raw linear data with 4 frames per second and the quantification using ESFUMB guidelines. Pts were identified from a prospective database. TTP and OS were calculated from the initiation of the new agents, according to clinical data and using RECIST v1.1 criteria. A decrease of more than 40% of AUC at D15 and one month was assumed to be predictive of TTP.
Results: Two hundred and seven patients were analyzed from September 2005 to March 2013. Seventy pts were excluded: no anti-angiogenic agents (n = 31), screen failure (n = 27), discontinuation of study participation before day 30 (n = 2), no US-evaluation at 1 month (n = 7), no US-target (n = 3). A total of 137 pts from 7 trials were included: 32 pts with vascular disrupting agent (VDA), 93 pts with a TKI, 2 pts with monoclonal antibodies (mAb), 10 pts with both VDA and mAb. The median overall survival was 15.5 months, with 110 deaths at the time of statistical analysis. In the training cohort (n = 26), pts with a decrease of more than 40% of AUC at one month had a median TTP of 5.5 months, versus a median TTP less than 2 months for the other patients. The decrease of AUC at one month was correlated to TTP (p = 0.004). The 111 patients from the validation cohort are under radiological review (349 CT scans) for TTP.
Conclusion: The decrease of more than 40% of AUC with DCE-US at one month is a potential predictive biomarker of response for anti-angiogenic treatments in metastatic patients. The final results will be presented on 137 patients.
No conflict of interest.

983 POSTER

Tumor–stroma ratio as a predictor for response to neoadjuvant chemotherapy (TAC) in breast cancer (BC): A Dutch Breast Cancer Trialists' Group (BOOG) side-study

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Background: Intra-tumoral stroma has profound influences on tumor behavior. The tumor–stroma ratio (TSR) has previously been shown to be of prognostic value in breast cancer and other types of solid tumors. However, the role of this parameter regarding the prediction of pathological complete responses (pCR) after neoadjuvant chemotherapy is unknown.
Methods: 250 patients were included in the NEOZOTAC trial: a national, multicenter, randomized study comparing the efficacy of TAC (docetaxel, adriamycin and cyclophosphamide i.v. day 1) chemotherapy followed by G-CSF on day 2 with or without zoledronic acid 4 mg i.v. 3 weeks in patients (pts) with stage II/III, measurable, HER2-negative BC. The percentage of intra-tumoral stroma was visually estimated on diagnostic sections from primary tumor tissue by two observers. A third independent observer was consulted in case of outcome discordances. The cut-off point between stroma-rich and stroma-poor tumors was set to 50% (as determined in previous investigations). Tumor–stroma ratio was related to centrally revised pCR data. Reproducibility of results will be further investigated by comparing CNB and resection specimens.
Results: 194 specimens were evaluated. Cohen's kappa coefficient for inter-observer agreement showed a substantial agreement in classification (k=0.64, 82% concordance). 37% of the specimens were classified as stroma-rich. Stroma-rich tumors were significantly associated with T-stage (P = 0.08) and ER-status (P = 0.004). In univariate analysis, TSR predicted for pathological complete response (P = 0.03) with greater pCR rates observed in tumors with a a low percentage of intratumoral stroma (22.7% vs. 10.3%). After multivariate analysis, this effect did not persist (OR 1.58, 95% C.I. 0.63–3.93), presumably because of a current low number of patients across groups. Final results will be presented at the ECCO.
Conclusions: These results suggest that the TSR is a reproducible marker for response to treatment with neoadjuvant chemotherapy. Considering the simplicity and low cost of TSR assessment, it should be considered as a candidate for further investigation and eventually for implementation in pathology reports.
No conflict of interest.

984 POSTER

Evaluation of a chemoresponse assay as both a prognostic and predictive marker in the treatment of persistent or recurrent ovarian cancer

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Background: A recent study demonstrated significant improvement in clinical outcome in recurrent epithelial ovarian cancer (EOC) patients treated with therapies identified as sensitive (S) based on an in vitro chemoresponse assay. Together with previous studies, these results support that the assay provides prognostic value. The assay's ability to function as a predictive biomarker of patient outcome is investigated in this analysis.
Material and Methods: Women with persistent or recurrent EOC (n = 262) were treated with one of 15 therapies based on the medical judgment of the treating physician, blinded to assay results. Each patient's tumor was assayed for response to the 15 therapies in vitro. Three approaches were used for estimating the assay's predictive value. First, the assay's relative ability to predict progression-free survival (PFS) for patients treated with a therapy for which they were considered to be S vs. resistant (R) (match) was compared to the average prognostic value of S vs. R to a randomly selected treatment (mismatch) based on repeated resampling of the patient population. Next, patients were classified into groups based on assay results and clinical treatment: S to all tested therapies and treated with a S therapy (SA), S to some therapies and treated with a S therapy (SP), R to all therapies and treated with a R therapy (RA), and R to some therapies and treated with a R therapy (RP). Clinical outcome was compared among groups whose response was primarily dependent on tumor biology (SA, RA; prognostic) and groups whose assay results were heterogeneous and may benefit from specific treatment choice(s) (SP, RP; predictive). Third, the percentage of S therapies was included in multivariate analysis.
Results: The assay result for 'match' was significantly associated with PFS (HR = 0.67, 95% CI=0.50–0.91, p = 0.009). Based on 1000 simulations, the mean HR[mismatch] was 0.81 (95% range = 0.67–0.91), suggesting that HR[match] was predictive of response to a specific treatment. The improvement in median PFS for both SA vs. RA (HR = 0.72, 95% CI=0.44–1.18, p = 0.191) and SP vs. RP (HR = 0.70, 95% CI=0.46–1.05, p = 0.081) was consistent. The association between assay result for administered therapy and PFS remained statistically significant in multivariate analysis (HR = 0.61, 95% CI=0.41–0.89, p = 0.010), independent of the percentage of S therapies, indicating the value of the assay for predicting outcome was not due to tumor biology alone.
Conclusions: These results support that the chemoresponse assay is likely both predictive and prognostic of patient outcome. Further, these results suggest that recurrent EOC patients treated by assay-sensitive therapies may obtain improved clinical outcomes.
Conflict of interest: *Ownership: M Gabrin, S Brower, C Tian hold stock options from Precision Therapeutics, Inc. Other substantive relationships: D Sargent received consulting income from Precision Therapeutics, Inc.*

985 POSTER

Relationships of peripheral blood lymphocyte counts (PBLC) with antitumor activity of NGR-hTNF in combination with chemotherapy (CT)

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Background: NGR-hTNF, a tumor-targeted antivascular agent, produces antitumor effects at low dose by inducing an early vessel stabilization that greatly improves both intratumoral CT uptake and T-cell infiltration.

Synergism with CT has been shown in immunocompetent mice, but not in nude mice lacking functional T cells.
Methods: The associations of baseline PBLC with the antitumor activity of N (with or without CT) and CT alone was assessed by means of an individual patient pooled analysis of 427 patients (pts) from 7 phase II trials in 6 tumor types. NGR-hTNF was given at low dose (0.8 μg/m^2) in combination with CT in 183 pts. As control groups, 140 and 104 pts receiving N and CT alone, respectively, were also analyzed. CT consisted of doxorubicin or a platinum-based regimen. In all trials, response to treatment according to RECIST was evaluated every 6 weeks. Endpoints of interest were response rate (RR, complete plus partial response), disease control rate (DCR, RR plus stable disease), duration of response (DOR) and progression-free survival (PFS). Continuous PBLC data were categorized into high vs low levels using the median cutpoint (1.5/mL; 95% CI, 1.4−1.6). In multivariate logistic and Cox regression models, age, sex, PS and tumor type were included as covariates.
Results: There were no statistically significant differences in treatment effect according to baseline PBLC in both N-alone and CT-alone groups. Conversely, in the N plus CT group, high PBLC were related to better outcomes, as compared with low PBLC. In this N plus CT group, high PBLC (vs low) were associated with higher RR (OR=2.3; 95% CI, 1.0−5.3; p = 0.04) and DCR (OR=2.7; 1.4−4.9; p = 0.002), and with longer DOR (HR = 0.31; 0.12–0.79; p = 0.01) and PFS (HR = 0.59; 0.43–0.81; p = 0.001). For high vs low PBLC, RR was 23% vs 11%, DCR 74% vs 51%, median DOR 8.7 vs 6.3 months, and median PFS 5.0 vs 3.0 months, respectively. On multivariate analyses, high PBLC remained an independent predictor of increased RR (OR=2.4; 1.0−5.5; p = 0.04) and DCR (OR=2.7; 1.4−5.2; p = 0.003), and improved DOR (HR = 0.24; 0.08−0−80; p = 0.02) and PFS (HR = 0.59; 0.43–0.82; p = 0.002).
Conclusions: Consistently with preclinical data, these results highlight the potential value of PBLC in predicting tumor response to NGR-hTNF in combination with CT, which merits further clinical investigation.
No conflict of interest.

986 POSTER

Identification of the activated form of the estrogen receptor (ER) in breast cancer (BC)

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Background: About 50% of ER positive (ERpos) BC are resistant to hormone treatment. In absence of ligand, ERs are evenly distributed in nuclei in normal tissue. Upon ligand binding, ERs dimerizes and form a discrete focal subnuclear distribution pattern (FDP), which are associated with transcriptional activation of ER. This ER FDP is observed in BC. We hypothesized that in BC that the presence/absence of ER in the FDP could predict antiestrogen activity. This study describes an immunohistochemistry (IHC) method, by which a biomarker could be developed to investigate this hypothesis.
Methods: 37 paraffin embedded, formalin fixed archived BC samples were processed using 4 ER-alpha antibodies (Ab) under different IHC conditions to develop and refine this biomarker, the ER nuclear morphology was analyzed with standard microscopy at x1000. Interpretation of the IHC slides was done by an experienced pathologist. Standard ER, progesterone receptor (PR), and Ki67 testing were done. Tumor grade was obtained from the patients' records. Analysis of the different Ab was with the kappa statistics. Comparison of the IHC technique to immunofluorescence (IHF) was done on 8 samples.
Results: Consistent with prior research observations, tumors had two ER nuclear morphologies: 1. Diffuse pattern (D) where the ER was distributed evenly in a fine granular pattern, 2. Aggregate pattern (A) where the ER is distributed in distinct clumps or aggregates.
This defined 3 tumor phenotypes: A cells only (A) 1% (4/37), D cells only (D) 65% (24/37), and a heterogenous mix of A + D cells (AD) 18% (7/37), ER Neg 14% (5/37). IHC and IHF analysis for ER nuclear morphology was concordant. The ER activation status (A/AD or D) was not correlated with staining ER intensity, ERpos %, PRpos%, but was correlated with % Ki67 staining (p = 0.09) and tumor grade (0.035).
Conclusions: ERpos early BC biopsies can be grouped in two categories based on ER nuclear morphology: A group with diffuse and homogenous nuclear staining, and a different group with heterogeneous area of cells having a nuclear pattern consistent with a functional or activated ER. These observations warrant clinical pathological studies to determine if this biomarker is potentially predictive of clinical outcomes.
Conflict of interest: *Ownership: Invivis Pharmaceuticals, Arno Therapeutics*

987 POSTER

Pharmacogenetic assessment of toxicity in patients treated with taxane-based chemotherapy for solid tumors

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Background: Taxanes are active agents widely used to treat many solid tumors. However, the utility of taxane-based therapy could be limited by gastrointestinal toxicities, hematological toxicities, hypersensitivity and cumulative neurotoxicity. Taxanes are metabolized by CYP3A4 and CYP3A5 isoenzymes, and they are a substrate for the ATP binding cassette multidrug-transporters ABCB1. Metabolic pathways of these antitumor agents need a thorough evaluation to understand why some patients experience severe adverse effects. Aim of our study was to evaluate the association between taxane-related toxicities and their metabolism-related genetic polymorphisms in patients affected by solid cancers undergoing taxane-based chemotherapy regimens.
Material and Methods: We examined 182 adult patients, ECOG Performance Status ⩽1, affected by solid tumors who underwent treatment with taxane-based regimens in adjuvant or metastatic setting, planned for at least 3 courses of therapy. Through a peripheral venous blood sampling we genotyped them for selected polymorphisms and ABC-transporters that may influence cellular sensitivity to taxanes: CYP3A4*1B (A> G), CYP3A5*3 (G> A) and ABCB1 (1236 C> T; 3435 C> T). SNPs (Single Nucleotide Polymorphism) were characterized by pyrosequencing. Statistical analysis was conducted by MINITAB 16.2.3 software. A value of p < 0.05 was considered statistically significant.
Results: Toxicities and polymorphisms were evaluated in 182 patients (12 males and 170 females). Median age of patients was 59 (range 30−82). Patients who received taxanes were 95 in adjuvant setting and 87 in the metastatic one. We observed a significant association between normal homozygous genotype for ABCB1 polymorphism (3435 C> T) and lower toxicity during therapy with taxane-based regimens (p = 0.012). An association between mutant homozygous and normal homozygous genotypes with dose limiting toxicities was demonstrated, even though not statistically significant (p = 0.058). A larger cohort of patients must be investigated. The multivariate analysis results were independent from the different taxane-based regimens adopted, age and stage of disease.
Conclusions: ABCB1 3435 C>T polymorphism seems a toxicity predictive biomarker for taxanes. On the other hand, a larger cohort of patients must be investigated to define the role of CYP3A4, CYP3A5 and ABCB1 (1236 C>T) polymorphisms.
No conflict of interest.

988 POSTER

EGFR homodimers in tumor samples quantified by a new antibody based TR-FRET assay are associated with colorectal cancer progression

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Background: Following the development of targeted therapies against EGFR and HER2, two members of the human epidermal receptor (HER) family of receptor tyrosine kinases, much interest has been focused on their expression in tumors. EGFR is frequently expressed on colorectal cancer tumors and EGFR targeted therapies are currently used in the metastatic setting. Data on EGFR homodimerization, an index of receptor activation, are lacking.
The aim of the study was the description and clinical relevance of EGFR protein and dimer expression levels in colorectal tumors.
Material and Methods: Analysis of EGFR was assessed in a series of 60 frozen tumor samples from patients with colorectal cancer by using recently developed antibody-based time-resolved Förster resonance energy transfer (TR-FRET) assays. Quantification of EGFR protein expression levels was determined together with EGFR:EGFR homodimers. EGFR and EGFR-EGFR fluorescence signals were normalized for cell content in the tumor samples and correlated to clinicopathological parameters and outcome of patients.
Results: The median absolute EGFR protein expression levels was 7 400 (range 2 700−1 474 400) and EGFR:EGFR homodimers were detected in 19 (31.7%) tumors. Correlations with clinicopathological patients' characteristics demonstrate that EGFR:EGFR homodimer presence was

significantly associated with advanced pTNM stage (P = 0.003). The potential role of EGFR:EGFR dimers as predictive markers could be assessed in twenty chemotherapy-refractory metastatic patients that were treated with cetuximab. In this subgroup of patients no significant correlation was observed between EGFR activation observed through EGFR:EGFR homodimer presence and response to EGFR-targeted therapy.
Conclusion: Quantitative measurement of expression and dimerization of EGFR by the novel TR-FRET assays provides new information on colorectal cancer patients. Indeed, the signaling pathways triggered by EGFR:EGFR homodimers seems to play an important role in colon cancer dissemination. Validation is ongoing in an independent cohort of 134 patients with colorectal cancer and will be presented.
No conflict of interest.

990 POSTER

Fragmented peptides of prostate-specific antigen (PSA) as novel urinary biomarker candidates for diagnosis of prostate cancer

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Background: Prostate cancer (PCa) will affect one in five men and is now the second leading cause of cancer deaths among men in the Western countries. Biomarkers, such as prostate specific antigen (PSA) level in serum, play pivotal roles in the management of the cancer patients. However, currently used biomarkers for PCa are sub-optimal. Therefore, great emphasis has been placed on the need to discover novel biomarkers for PCa diagnosis.
Methods: We focused on urine samples voided after prostate massage (digital rectal examination [DRE]) and conducted peptidomic and proteomic analyses of the urine samples using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS^n). The urinary biomaterials were concentrated and desalted by a weak cation-exchange resin prior to MALDI-TOF/MS analyses.
Results: A high-resolution and high-sensitivity MALDI digital ion trap (DIT) TOF/MS^n was utilized in order to analyze the urinary peptides and protein-fragments in this study. Mass profiles of urine samples from healthy, BPH, and PCa subjects were compared. Several differences among these mass profiles were detected, especially between PCa and BPH. The most pronounced peak was detected around m/z 2,300. Other characteristic peaks in PCa were also detected around m/z 1,200, 1,300 and 4,700. Two peaks around m/z 1,200 and 1,300 became larger than those in the BPH mass profiles, whereas the peak around m/z 4,700 of the BPH became larger than that of PCa. The most pathognomonic peptide around m/z 2,300 in the DRE urine samples of PCa patients was confirmed as a C-terminal PSA fragment. It was verified that the C-terminal peptide was produced from PSA in prostate glands and was secreted into the urine by DRE. The two peptides around m/z 1,200 and 1,300 were also confirmed as the fragments of PSA near the center.
Conclusions: Our findings indicate: 1) the peptide fragments, especially the C-terminal fragment, of PSA in the DRE urine may become a novel pathognomonic biomarker candidate for PCa diagnoses; and 2) the fragmentation may be a pathognomonic bio-reaction in microenvironment of the PCa cells. Further examinations are underway to prove this possibility regarding the PSA fragmentation in the urine samples after prostate massage.
No conflict of interest.

991 POSTER

The predictors for micro-invasion of hepatocellular carcinoma ⩽2 cm

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Background and Aims: Hepatocellular carcinoma (HCC) of 2 cm diameter or less (⩽2 cm) is considered to have a low potential for malignancy based on the so-called stepwise progression hypothesis. But in fact, there have been cases with HCC⩽2 cm accompanied by micro-invasion (MI) and poor prognosis based on an alternative hypothesis of de novo development. The aim of present study was to identify independent predictors for MI of small HCC⩽2 cm.
Methods: A retrospective review was undertaken of 149 patients with primary solitary HCC⩽2 cm who underwent initial hepatic resection. The independent predictors of the MI such as portal venous, hepatic vein, or bile duct infiltration and/or intra-hepatic metastasis were indentified using multivariate analysis. Prognosis of patients with HCC⩽2 cm accompanied by MI was compared to that of patients with HCC⩽2 cm without MI.
Results: Forty-three patients with HCC⩽2 cm had MI (28.9%). Three independent predictors of the MI were revealed: invasive gross type (simple nodular type with extranodular growth or confluent multinodular type), des-γ-carboxy prothrombin (DCP) ⩾100 mAU/ml, and poorly differentiated. The sensitivity of DCP>100 mAU/ml for MI in HCC⩽2 cm of 53.5% (23/43 cases) was not very high, but its positive predictive value of 79.3% (23/29 cases) was relatively high. The sensitivity of preoperative imaging diagnosis for invasive gross type in HCC⩽2 cm was low 30.0% (6/20 cases), but the positive predictive value was relatively high 66.7% (6/9). Disease-free survival rates of patients with HCC⩽2 cm with MI were significantly worse than those for HCC⩽2 cm without MI (3-year; 44% vs. 72%). This disadvantage of disease-free survival rate of patients with HCC⩽2 cm with MI could be dissolved by hepatic resection with a wide tumor margin ⩾5 mm (p = 0.04).
Conclusion: Even in cases of HCC⩽2 cm, patients who are suspected of having invasive gross type tumors in preoperative imaging diagnosis or who have a high DCP level (⩾100 mAU/ml) are at risks for MI. Therefore, in such patients, hepatic resection with a wide tumor margin should be recommended.
No conflict of interest.

992 POSTER

Predictive value of plasma D-dimer for asymptomatic metastasis in cancer patients

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Background: Plasma D-dimer levels are high in patients with advanced tumors and can be used to predict the outcomes of cancer patients. At most advanced tumor stages, patients have asymptomatic metastasis, which contributes to early tumor recurrence after surgery. We hypothesize that plasma D-dimer can be used to identify patients with potential metastasis.
Methods: We first verified our hypothesis in different in vivo murine metastasis models (subcutaneous tumor, intraperitoneal metastasis and hematogenous metastasis), and we examined the plasma D-dimer levels using the enzyme-linked immunosorbent assay (ELISA) method. We then enrolled and examined plasma D-dimer levels by the latex-enhanced immunoturbidimetric assay (LEIA) method in 1268 primary cancer (1042 gastric cancer, 96 esophageal cancer, 50 lung cancer, 37 melanoma, 43 pancreatic cancer) patients in 3 cancer centers in northwestern China. We also followed 395 of 1042 gastric cancer patients at one cancer center to analyze the 2-year survival rate and early tumor recurrence.
Results: Among the three in vivo murine metastasis models, the plasma D-dimer level was extremely elevated in the hematogenous metastasis and intraperitoneal metastasis murine models but not in the subcutaneous tumor model and control group model. These results supported our previous hypothesis. In this large-scale clinical study, we found that plasma D-dimer levels were increased in distant metastasis, especially in patients with hematogenous metastasis. The cut-off value of the D-dimer levels was determined to be 1.5 mg/ml based on the ROC cure, and the sensitivity and specificity for predicting metastasis were 63.2% and 88.5%, respectively. In addition, patients with increased plasma D-dimer levels displayed early hematogenous-associated tumor recurrence and bad outcomes during the follow-up study.
Conclusion: Plasma D-dimer is a potential marker that is easy to measure at a low cost, and this marker can be considered for routine testing in cancer patients to predict asymptomatic metastasis.
No conflict of interest.

993 POSTER

Predictive role for pemetrexed sensitivity of thymidylate synthase expression in advanced cancer patients

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Background: High expression of thymidylate synthase (TS) in malignant cells has been proposed as a resistance mechanism to the antifolate pemetrexed. This study is aimed to evaluate the association of TS expression by a quantitative assessment in tumor cells with the efficacy of pemetrexed in patients with advanced non-small cell lung cancer, small cell lung cancer (SCLC) and mesothelioma.

Methods: 54 patients were studied: 40 stage IV NSCLC (26 adenocarcinomas, 11 large cell, and 3 squamous cell carcinoma), 3 SCLC and 11 mesothelioma. 21 patients received platins-pemetrexed as first line NSCLC, 20 pemetrexed in monotherapy as second and further lines and 3 carboplatin-pemetrexed fo extensive disease SCLC. RNA was obtained from FFPE tumor sections and the expression of TS was analyzed by RT-qPCR using appropriate mRNA specific primers and probes. TS levels was calibrated to expression in normal tissue.
Results: From 54 cases, TS expression was available in 32 cases, detecting overexpression in 23 (71.8%) and low expression in 9 (28.2%) patients. The response rate for patients with low TS expression was 0.63 compared with 0.15 in patients with overexpression ($p = 0.015$). A significant benefit in time to progression was observed in patients with low expression (median TTP 12 vs. 2 months respectively, $p = 0.002$), whereas did not impact on overall survival (median OS 20 vs. 19 months respectively, $p = 0.595$).
Conclusions: TS overexpression in tumor is associated with a reduced response to pemetrexed-containing chemotherapy and might be used as a predictive biomarker in advanced lung and mesothelioma cancer patients.
No conflict of interest.

994 POSTER

Predictive impact for bevacizumab of VEGF-A 165 family of isoforms in patients with non-squamous non-small cell lung cancer (NSCLC)

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Background: Bevacizumab is a recombinant monoclonal humanized antibody against vascular endothelial growth factor (VEGF) that improves Time to Progression (TTP) in patients with advanced non-squamous NSCLC, but currently no proven predictive markers exist. The VEGF-A 165 splice variant has been described as the most abundant and active isoform in cancer, where exon 8 modifications generates two family of isoforms with opposite *in vivo* effects, one pro-angiogenic (VEGF 165a) and other anti-angiogenic (VEGF 165b). The objective of this study is to explore the predictive role of $VEGF_{165}$a and $VEGF_{165}$b isoforms in patients with non-squamous NSCLC treated with a doublet of platin plus bevacizumab.
Methods: 22 patients were included (20 adenocarcinomas and 2 large cell carcinomas): 5 received carboplatin–taxol–bevacizumab, 14 carboplatin–taxotere–bevacizumab and 3 cisplatin–gemcitabine–bevacizumab. RNA was obtained from routine clinical samples and $VEGF_{165}$a and $VEGF_{165}$b expression was analyzed by RT-qPCR. Individual $VEGF_{165}$a and $VEGF_{165}$b family of isoforms expression was calibrated to normal tissue and the ratio between both isoforms was calculated.
Results: $VEGF_{165}$a overexpression was detected in 14 (63.6%) cases and $VEGF_{165}$b overexpression in 15 (68.2%) tumors. A predominant expression of the pro-angiogenic $VEGF_{165}$a in tumor was correlated with a significant benefit compared with cases with a predominant $VEGF_{165}$b expression (median TTP, 15 vs. 8 months respectively, $p = 0.005$). However, the expression of these isoforms did not impact on Individual overexpression of these isoforms was not associated with benefit to bevacizumab therapy ($p = 0.933$ and 0.166) or overall survival ($p = 0.477$).
Conclusion: The overexpression of $VEGF_{165}$a isoforms associated with a low expression of $VEGF_{165}$b was correlated with a clinical benefit to bevacizumab therapy in stage IV non-squamous NSCLC patients, supporting a potential use as predictive biomarkers.
No conflict of interest.

995 POSTER

Risk factors of thrombotic microangiopathy in patients treated by antiangiogenics in phase I trials

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Background: Antiangiogenics are effective treatments for several advanced cancers. Thrombotic microangiopathy (TMA) is a well known vascular and renal side effect of these drugs and such adverse event may limit its use. No risk factor of TMA has been described so far. Our objective was to assess the association of high blood pressure (HBP) and other prognostic factors with TMA.
Methods: Our retrospective study included data on all patients recruited from 2010 to 2012 at Gustave Roussy Institute in phase I trials evaluating antiangiogenesic drugs. The definition of TMA was clinical or histological. Patients with and without TMA were compared according to HBP defined as superior to 140 and/or 90 mmHg, sex, age, body mass index, history of antiangiogenic therapy, history of nephrectomy, various biological results, using a multivariable logistic regression model.
Results: Among the 56 patients exposed to antiangiogenic therapy, TMA occurred in 17 patients (30%). The diagnosis of TMA was histologically confirmed in 10 (59%) of 17 patients and schizocytes were present in one patient.
Proportion of women (59%), median age (57 years), history of partial or total nephrectomy (7%) and exposure to bevacizumab for more than 6 months (38%) did not differ in patients with or without TMA. Median level of fibrinogen in g/L was lower in patients with TMA (3.9) than in patients without TMA (5.1, $p = 0.0108$). In the multivariable analysis, TMA was associated with fibrinogen level (OR= 2.27 (95% CI=1.22; 4.35)) and with a clear trend was observed with history of treated hypertension (OR= 3.35(95% CI=0.73;15.34)). Analysis of data on sarcopenia and HBP characterization are on-going.
Conclusion: In our population antiangiogenic, TMA is only correlated with low level of fibrinogen at baseline. However, patients with a history of HBP exhibit a trend to present a higher risk of TMA. Further studies are warranted in order to better select the population eligible to antiangiogenic treatment.
No conflict of interest.

996 POSTER

RRM1 protein expression heterogeneity between diagnostic biopsies and resection specimens and changes in expression during carboplatin and paclitaxel treatment in non-small cell lung cancer

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Background: It is of interest if potentially predictive biomarkers such as RRM1 expression changes during chemotherapy if they are to be used for deciding treatment beyond first line.
Materials and Methods: RRM1 immunohistochemistry was performed on tumor samples from a total of 118 NSCLC patients T1−4N0−2M0. Samples from 65 patients, among which 53 had paired samples from before and after paclitaxel and carboplatin and 53 patients which had not been treated with chemotherapy, were included.
Results: No change in RRM1 expression was observed between paired samples of primary tumors in the NAC-group ($p = 0.524$) nor in the OP-group ($p = 0.171$). A mean H-score decreased of 0.2 in both groups ($p = 0.905$). RRM1 expression was higher after chemotherapy than before in N2-node metastases ($p = 0.010$). A discordant RRM1 expression (low vs. high) was observed in 32% paired diagnostic and subsequent resection specimen in the OP-group.
Conclusion: The substantial discordance between paired samples emphasizes the need of sufficient tumor tissue in biopsies when evaluating RRM1 expression. No change in RRM1 expression was observed in primary tumors but RRM1 was increased in N2-lymph node metastases following chemotherapy. Potential post-chemotherapy changes in RRM1 expression may be considered when basing treatment decisions beyond first line on RRM1 expression.
No conflict of interest.

997 POSTER

Significance of smudge cell on blood smear in chronic lymphocytic leukemia patients: A prospective single institutional study from India

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Background: Smudge cells are ruptured lymphocytes seen on routine blood smears of chronic lymphocytic leukemia (CLL) patients. We evaluated significance of smudge cells percentage on a blood smear in CLL patients.
Methods: We calculated smudge cell percentages (ratio of smudged to intact cells plus smudged lymphocyte) on blood smears of 175 consecutive untreated CLL patients registered at I.R.C.H, AIIMS, New Delhi over a period of 5 years (2006–2010).
Results: There were 130 males and 45 females. The median age was 59 years (30−88). Median absolute lymphocyte count was 40 $X10^9$/L. Clinical Rai stage distribution was: stage 0−5%, stage I − 25%, stage II − 40%, stage III −10 % and stage IV − 20%. The median smudge cells percentage was 28% (4% −76%). There was no correlation of proportion of smudge cells with age, sex, lymphocyte count, lymphocyte doubling time, beta 2 microglobulin, organomegaly, ZAP 70 + or CD 38 + CLL patients, but there was significant correlation with stage of disease. Median smudge cell

percentage in stage 0 & I −36% (12−76), stage II- 30% (12−61) and stage III&IV-20% (4−51) [p < 0.001]. Eighty five patients of early stage (0, I &II) patients required treatment during follow up [65% required treatment with smudge cell <30%, against 35% patients requiring treatment with smudge cells >30%, p = 0.01]. The percentage of smudge cells as a continuous variable correlated with OS [HR 0.96, p < 0.001]. The 5-year survival rate was 51% for patients with 30% or less smudge cells compared with 76% for patients with more than 30% of smudge cells. Median OS was 4.8 years with median follow up period of 3.6 years. Smudge cells percentage (<30% vs. >30%) had significant association with OS [HR 0.97, 95% CI (0.62–1.21), p = 0.001)].
Conclusions: Simple and inexpensive detection of smudge cells on blood smears on routine diagnostic test useful in predicting progression free and OS in CLL patients and may be beneficial in countries with limited recourses.
No conflict of interest.

998 POSTER

Circulating endothelial cells and dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) parameters as predictive biomarkers for survival benefit in patients with advanced non-small cell lung cancer (NSCLC) treated with sorafenib and metronomic oral vinorelbine

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Background: Biomarkers to predict benefit from anti-angiogenic therapy are still lacking. RECIST response criteria are inadequate for accurate evaluation of response. Sorafenib and metronomic oral vinorelbine combination was explored in this study and changes in blood and DCE-MRI parameters were investigated as potential predictive biomarkers of benefit.
Material and Methods: Eligible patients with advanced NSCLC who failed multiple prior lines of palliative chemotherapy were recruited to 3 successive cohorts. Each cohort was given a fixed metronomic (thrice a week) dose of oral vinorelbine at 60 mg/week, 90 mg/week, and 120 mg/week respectively. Each patient within each cohort received a starting dose of sorafenib at 200 mg bid for 4 weeks. In the absence of dose-limiting toxicities, the dose of sorafenib would be escalated to 400 mg bid for 4 weeks, 600 mg bid for 4 weeks and finally 800 mg bid. Biomarkers measured serially include DCE-MRI parameters, circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPs), and plasma thrombospondin-1 level (TSP-1). The following DCE parameters were analyzed: blood flow(F), permeability surface area product(PS), fractional intravascular blood volume (v1), and extracellular-extravascular volume (v2).
Results: 46 evaluable patients were analysed. There were 5 partial responders (10.9%) and 22 with stable disease lasting at least 4 months (47.8%). Evaluation for biomarker response was performed only for the first 2 cycles of treatment due to the subsequent high attrition rate. There was no significant change in the biomarker parameters between the 3 cohorts. Collective analyses of the 3 cohorts demonstrated a significant near-universal decline in CEP, TSP and PS, and a significant increase in V2 after 2 cycles of treatment. These parameters however, were not predictive of survival benefit. Using multivariate modeling, high baseline or rise in CEC and lower baseline V1 predicted for improved overall survival(OS), while a low baseline or a decline in F predicted for improved progression-free survival (PFS).
Conclusions: Sorafenib and metronomic oral vinorelbine is active in advanced NSCLC. Baseline levels and changes in DCE parameters (F and V1) and CEC may be useful predictive biomarkers for survival benefit with this anti-angiogenic regimen.
No conflict of interest.

999 POSTER

Class-III-β-tubulin expression levels remains unchanged during carboplatin and paclitaxel treatment in non-small-cell lung cancer

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Introduction: Class-III-beta-tubulin (TUBB3) is a tubulin isoform involved in microtubule formation during mitosis. Its expression may be a potential predictive factor for outcome in non-small cell lung cancer during microtuble interfering cytotoxic treatments such as vinca alcaloids and taxanes. We investigated for changes in TUBB3 expression during neoadjuvant chemotherapy including taxanes, which may be of interest if future choice of chemotherapy is to be based on TUBB3 expression. If the biomarker expression changes during chemotherapy, biopsies before initiation of chemotherapy beyond 1st line may be required. Thus, the aim was to explore on TUBB3 expression heterogeneity and changes during chemotherapy.
Materials and Methods: TUBB3 expression immunohistochemístry on diagnostic biopsies and on available subsequent resection specimens in 65 non-small cell lung cancer (NSCLC) patients stage T1−3N0−2 (NAC-group). These patients received preoperative carboplatin and paclitaxel. Another group of 53 NSCLC patients stage T1−4N0−1 were treated with surgery alone without preceding chemotherapy (OP-group). Paired samples of diagnostic and resection specimens were compared in order to evaluate for changes in TUBB3 expression.
Results: No statistically significant change in TUBB3 expression was observed between initial diagnostic biopsies and subsequent surgical resections of primary tumors in either the OP-group (p = 0.124) or the NAC-group (p = 0.414). When dichotomized into high and low TUBB3 expression, discordance between diagnostic biopsies and resection specimens of the primary tumors occured in 22% and 40% in the OP-group and NAC-group, respectively (p = 0.169). Changes in TUBB3 expression were not associated with prognosis but significantly more patients having low TUBB3 expression experienced down-staging during neoadjuvant chemotherapy compared to patients having high TUBB3 expression (p = 0.0220).
Conclusion: We observed a high degree of discordance of TUBB3 expression between paired serial tumor samples, which likely reflects intratumoral heterogeneity. This emphasizes a need for sufficient tumor tissue in order for stratification of patients based on TUBB3 expression. However, there were no significant changes in TUBB3 expression after neoadjuvant carboplatin and paclitaxel chemotherapy, suggesting no need for rebiopsy in case of need for second line chemotherapy with microtubule interfering cytotoxic treatments. Low TUBB3 expression predicts down-staging during neoadjuvant chemotherapy.
No conflict of interest.

1000 POSTER

Expression of progesterone receptor membrane component-1 in non-small cell lung cancer

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Background: The progesterone receptor membrane component-1 (Pgrmc1) protein is upregulated in cancer, and it is required for tumor cell proliferation, motility and tumor formation in vivo. Inhibition of Pgrmc1 suppressed the growth of lung, breast and cervical cancer cell lines. Recent studies in vitro have shown that one of the reasons of platinum resistance can be overexpression of the Pgrmc1. Taking in account that treatment of non-small cell lung cancer (NSCLC) is based on platinum included chemotherapy and its efficacy is not satisfactory because of tumor resistance, we have proposed this resistance mechanism dealing with Pgrmc1expressin in cancer patients. To answer the question we have studied level and frequency of Pgrmc1expression in NSCLC tissues.
Materials and Methods: NSCLC surgical biopsy specimens of smoking male patients with squamous cell carcinoma (40) and adenocarcinoma (20) were analyzed by flowcytometry. Single-cell suspensions obtained from the tumors were incubated with primary rabbit polyclonal IgG to Pgrmc1 antibodies (Abcam) overnight and with secondary FIT?-conjugated goat polyclonal to rabbit IgG antibodies (Abcam) for 1.5 h. Mean number of specifically stained cells were analyzed by WinMDI software and Kolmogorov-Smirnov statistical approach. Pgrmc1 expression was estimated as ratio of the specific parameter to the same isotype one. Two indexes of Pgrmc1 expression were used: high level − Pgrmc1was revealed in 20−50% of the cells, low − less than in 20%.
Results: 1. Pgrmc1 expression (specific fluorescence more than in 10% of the cells) was revealed in 70% of NSCLC patients. High level (29.9±8.1%) was in 36% of the tumors, 67% of which were adenocarcinomas. Low level (14.8±2.4%) was in 64% of the tumors, 81% of which were squamous cell carcinomas. 2. In squamous cell carcinomas mean Pgrmc1 level was 15%, but in adenocarcinomas − 1.7 times higher (26%, p = 0.013). 3. In squamous cell carcinoma patients low Pgrmc1 level was revealed in 81% of cases, but high − in 19% only. In contrast, in adenocarcinoma patients high Pgrmc1 level was revealed in 67%, but low − in 33% only.

Conclusion: Pgrmc1 expression was revealed in the majority of NSCLC patients. In adenocarcinomas mean Pgrmc1 level and frequency of high level of Pgrmc1 expression were about 2−3 times higher than in squamous cell carcinomas. Taking into account our results and clinical observations that squamous histologic features as compared with adenocarcinomas is factor associated with improved survival platinum included chemotherapy, we believe that Pgrmc1 could be predictive marker of platinum resistance in NSCLC patients. Supported by Russian Foundation for Basic Research (Grants 13−04–01004-a, 12−04–00028-a).
No conflict of interest.

1001 POSTER

Clinical and pathological correlation of the activated form of the progesterone receptor (PR) in breast cancer (BC)

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Background: Ligand binding of the PR in normal tissue causes the formation of discrete transcriptionally active subnuclear foci. The presence of these activated PRs (APR's) in post menopausal patients with BC and endometrial cancer suggests that the PR is activated by pathways requiring minimal or no PR ligand or is constitutively active. The goal of this study is to correlate the APR status in BC biopsies to any clinical and/or pathological relationships. APRpos was defined as any tumor with more than 5% APR cells.
Methods: 303 archived BC biopsies were analyzed for standard HES, ER, PR and Ki67. Clinical and pathology data was obtained from patient (pts) records. APR status (PR nuclear morphology) was determined with distinct antibodies for the PR A & B isoforms and the nuclear morphology patterns were analyzed at $\times$1000 magnification. The APR determination by PRA & PRB was combined as appropriate for the analysis.
Results: Average age was 58 (17−89). Histology: ductal 85%, lobular 13%, other 2%. 86% ERpos and 83% were either PRApos or PRBpos; 7% were only ERpos, 7% were only PRApos or PRBpos. All but 3 PRpos pts had received antiestrogens. Staging: I 50%, II 43%, III 6%, IV 1%. Tumor grade; I: 24%, II: 51%, III: 25%. Median follow up was 31 months. Local or distant progression (PD) was observed in 19% of the pts. APR status was positive in 22%, negative in 61%, and PRneg 17% of the biopsies. PD was not associated with PR status ($p = 0.68$). With DFS defined as time to PD or death with a 5 year cut off, PRpos was superior to PRneg ($HR = 0.3$, $p = 0.002$). DFS (5 year cut off) was superior for APRpos vs APRneg ($HR = 0.58$, $p = 0.48$). In univariate analyses, APRpos was associated with higher tumor grade ($p = 0.001$). No association was found between APR status and age, stage of disease, Ki67 or HER2 status.
Conclusions: Although there was an association with higher tumor grade, the APR status was not clearly linked or associated with other clinical characteristics, markers for proliferation, ER or PR positivity, disease stage; indicating that APR cannot be predicted by routine clinical data or pathological testing. Further work on the APR is warranted, as this biomarker may be able to determine which patients could benefit from antiprogestin treatment.
Conflict of interest: *Ownership: Invivis Pharmaceuticals, Arno Therapeutics*

1002 POSTER

Clinical and pathological correlation of the activated form of the progesterone receptor (APR) in endometrial cancer (EC)

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Background: Ligand binding of the progesterone receptor (PR) in normal tissue causes the formation of discrete subnuclear foci, which are known to be transcriptionally active (APR). The presence of APR's in menopausal EC and breast cancer suggest that the PR is constitutively active or activated by pathways requiring minimal or no PR ligand. The goal of this study is to determine the APR status in EC and to describe any clinical or pathological relationships with the ultimate goal to develop a companion diagnostic predicting the efficacy of anti-progestins in patients with EC.
Methods: 72 archived 1º ECs were processed with standard IHC for estrogen receptor (ER) and PR & proliferation (Ki67). APR was determined using antibodies specific to the A and B isoforms of the PR (PRA and PRB) and evaluating nuclear morphology. Pathology and clinical information was collected from patients' records.
Results: Histology; endometrioid 78%, clear cell 8%, serous/papillary 7%, other 7%. 2 PR nuclear distribution patterns were observed: an aggregated pattern (A) indicative of APR, and a diffuse or finely granular pattern (D), indicative of an inactivated PR. This resulted in three tumor phenotypes: A cells only, D cells only, and a mix of A + D cells (AD). For endometrioid cancers, 84% and 68%, respectively, were PRpos or ERpos. APR was present with PRA in 33% and PRB in 37%, and 48% either PRA or PRB. The mean age was 65 in both the APRpos/neg groups. APR status was not associated with Ki67 expression, tumor grade, lymphatic or vascular invasion or FIGO stage, progression of disease or death, but was associated with lower ER positivity. 8 progressions were observed and not associated with APR status. More deaths from any cause were observed in the PRB APRneg group.
Conclusions: Lack of association with clinical characteristics suggests APR status is distributed across clinical sub-groups of EC and cannot be predicted by routine clinical or pathological testing. The small number of progressions/deaths does not allow APR to be tested as a prognostic variable. Further work on a larger cohort is warranted, as this biomarker may be able to select patients who may have increased benefit/risk ratio of antiprogestin treatment.
Conflict of interest: *Advisory board: Arno Therapeutics*

1003 POSTER

Cholesterol and its esters as serum biomarkers in malignant obstructive jaundice: A single step 1H NMR metabonomic approach

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Introduction: In obstructive jaundice (OBJ), composition of bile and cholesterol metabolism changes drastically with growing severity towards malignancy. The timely diagnosis of underlying malignancy with OBJ is complex, as malignant tumors are often asymptomatic in their earlier course and thus preclude its curative resection. The complex regional anatomy of hepatopancreatobiliary system confounds the recognition of potentially resectable lesion and hinders the pre-operative histological confirmation of malignancy. Therefore, objectives of the present study include: (i) identification of the variations in low molecular weight metabolites of serum under the pathological state of benign OBJ and hepatopancreatobiliary malignancy induced obstructive jaundice from normal conditions, (ii) to explore the role of serum cholesterol (Chol) and cholesterol esters (CE) and their relative ratio estimation in sera of benign and malignant OBJ with the help of H NMR spectroscopy and, (iii) the evaluation of status of bile acids, cholesterol and choline containing compounds in bile and their contribution towards differentiating between malignant and benign OBJ.
Material and Methods: Serum and bile specimens from benign OBJ patients ($n = 28$), malignant OBJ patients ($n = 36$) and serum of healthy controls ($n = 57$) were analysed by H NMR spectroscopy. Relative- and semi-quantitation of serum metabolites viz. isobutyrate, lactate, alanine, acetone, glutamine, creatine, threonine and 1-methylhistidine, total cholesterol (tCho), cholesterol (Chol) and cholesterol ester (CE) were performed. In bile, total bile acids (BA), cholesterol, phosphatidylcholine (PC) and glycerophosphatidylcholine (GPC) were quantified. The effect of benign and malignant OBJ on small metabolites and lipids was analysed by non-parametric Mann-Whitney U test.
Results: Serum levels of isobutyrate, alanine, acetone, glutamine, threonine and 1-methylhistidine were significantly lower in both classes OBJ patients and glutamine levels were further lowered in malignant OBJ. Serum Chol and CE were significantly altered in healthy control, malignant and benign OBJ. Malignant OBJ had significantly decreased levels of tCho, Chol/CE and lipid content when compared with benign OBJ.
Conclusions: The single step estimation of alterations in serum Chol and CE may have potential for early and differential diagnosis of malignant and benign OBJ. This may augment the novel insights in local and systemic effects of OBJ patients.
No conflict of interest.

1004 POSTER

Circulating tumor cells counts in advanced and metastatic colorectal cancer by immunomagnetic labeling: Results reflect the reality?

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Background: According to Jemal et al. (2011), colorectal cancer (CRC) was the third most commonly diagnosed cancer in men in the world, and the second in women in 2008. The first strategy for treatment is complete resection of the lesion. However, some patients experience recurrence, believed to be due to residual micrometastases. Traditional diagnostic methods are unable to detect CTCs present in these sites and released into the circulation. Our objective was to count and correlate CTCs levels and MRP-5 (multidrug resistance associated protein 5) expression with progression free survival (PFS).
Materials and Methods: Prospective study made by blood collection of patients with metastatic or advanced CRC. Blood was collected before the beggining of chemotherapy and after 60 days, in accordance with image exams. The enrichment of CTCs was made by direct immunomagnectic labeling of positive cytokeratin (CK) cells. These cells were permeabilized and labeled with antibody against pan- CK conjugated to ficoeritrin to identify epithelial cells. Leucocytes were identified by anti-CD45 antibody. CTCs were analysed by immunofluorescence and by light microscope and quantified by 8 mL of blood. The protein expression of CTCs was analysed after blood filtration by ISET (Isolation by Size of Epithelial Tumor Cells). Blood was collected in EDTA tubes and diluted in buffer for filtration. Then, membranes were stored at −20°C until analysis. After incubation with antibodies (MRP-5), the membranes were counterstained with DAB. PFS curves were made by Kaplan Meier method and the difference between curves were analysed by log-rank.
Results: There were included 16 patients treated with FOLFOX or FOLFORI and bevacizumab. The median age was 63.5 years (30−81). The majority of patients was men (62.5%) and included at stage IV (68.7%). The PFS after the treatment was observed by image exams and showed a media of 6.14 months (0.79–8.55 months). The median CTCs numbers detected in these patients were 23.5 CTCs/8 mL at baseline. Patients with lowest levels of CTCs (above the median) showed worse PFS (4.15 months) in relation to those with higher levels of CTCs (7.78 months, $p = 0.037$). The same was true for the CTCs counts in the first follow-up (4.20 × 7.73 months, $p = 0.047$, respectively). Only 11 patients were analysed for MRP-5 protein expression and no correlation was observed between this expression and PFS.
Conclusion: Although our CTCs counts seems conflicting, the lowest counts found in patients with worst PFS can be explained by the inhibition of tumor angiogenesis by bevacizumab, which may lead to hypoxia, invasive cell behaviour and epithelial mesenchymal transition (EMT), as postulated by Gazzaniga et al. (2011). As the method used was based on epithelial markers, it is possible that these patients with poorest PFS were under EMT. The expression of EMT and endothelial cells markers in CTCs filtered on ISET are under investigation in our lab.
No conflict of interest.

1005 POSTER

Quantitative assessment of lymph vascular space invasion (LVSI) provides important prognostic information in node-negative breast cancer patients

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Background: The prognostic value of lymph vascular space invasion (LVSI) has been in investigated in multiple studies, some of which have demonstrated an association of this phenomenon with disease-free survival. Clear definitive criteria and optimal determination of this parameter remain unclear, especially whether some sort of quantification of LVSI is clinically relevant.
Materials and Methods: EORTC trial 10854 investigated the efficacy of perioperative chemotherapy in 2795 patients with T1-T3, N0−2 and M0 breast cancer. A subset of 427 node-negative breast carcinomas from premenopausal patients from this trial were selected and scored for LVSI. The number of LVSI foci were counted and the cell number was determined in the largest tumor embolus within the lymph vessels. These two parameters were multiplied in order to calculate the LVSI tumor burden (LVSI-TB). The optimal cut-off for this parameter was calculated in a discovery set and tested in a validation set. This parameter was also compared to simple quantitation of the number of LVSI foci regarding the sensitivity and specificity for identifying patients with disease relapse.
Results: Tumors with a single LVSI focus are not at increased risk for disease relapse (HR 1.423, 95% CI 0.762–2.656) which indicates that quantitation of LVSI is necessary. The LVSI-TB had the highest sensitivity and specificity for performing quantitative LVSI assessment compared to simple determination of the number of LVSI foci. This parameter was independently associated with disease-free survival in the validation set (HR 2.366, 95% CI 1.369–4.090, $P = 0.002$) in multivariate analysis and provided prognostic information in both the low- and high-risk node-negative breast cancer groups ($P<0.001$ and $P = 0.007$ respectively).
Conclusion: In order to optimally implement determination of LVSI into the standard clinical care for breast cancer patients, this parameter needs to be quantified. This study shows that the determination of the number of LVSI foci multiplied by the number of tumor cells in the largest tumor embolus provides the most reliable quantitative assessment of this parameter.
No conflict of interest.

1006 POSTER

Facilitates chromatin transcription (FACT) complex as a marker and target of aggressive poorly differentiated cancers

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Background: The Facilitates Chromatin Transcription (FACT) is involved in chromatin remodeling during transcription, replication and DNA repair and was considered to be aubiquitously expressed complex that had no known associations with any disease. However, we discovered that FACT is expressed in very limited number of cells of the adult mammalian organism, mostly presented by stem and undifferentiated cells (Garcia, 2011). Moreover a novel anti-cancer agents, Curaxins, entered recently Phase I clinical trials, exert tumor cell killing though inhibition of FACT function (Gasparian, 2011). The goal of this study was to elucidate what role FACT plays in cancer.
Material and Methods: We assessed expression of FACT in normal and cancerous tissues of different organs on mRNA (>20,000 samples) and protein (>800 samples) levels to evaluate the correlation between FACT expression and clinical features of different cancers. We also ran *in vitro* and *in vivo* experiments to evaluate how modulation of FACT levels affects tumorigenic transformation and tumor cell properties. Finally we obtained genome wide distribution of FACT using ChIP-seq to identify genes which transcription requires FACT assistance.
Results: FACT expression is significantly higher in tumors of patients with poor overall survival (all cancers, breast cancer (BC), NSCLC), higher incidence of metastasis (BC, NSCLC, RCC) and the presence of other markers associated with poor prognosis (BC, NSCLC, colon cancer). Ectopic expression of FACT in normal cells does not drive transformation, but increases efficiency of oncogene-driven transformation. Conversely a reduction of FACT level, using a RNAi approach, reduces transformation efficiency and interferes with tumor, but not normal cell growth.
Genome wide analysis revealed non-random FACT distribution in tumor cells with significant enrichment over the bodies of genes regulated by transcriptional factors associated with cancer (Myc, AP1-, ets-families, YY1), stress response (NF-kB, HSF1, HIF1a) and pluripotent cell state (Oct3/4, Myc, Hox family). This pattern suggests selective assistance of FACT to the transcription of genes involved in cancer and early development.
Conclusion: FACT is an attractive target and marker of poorly differentiated aggressive cancers based on its role as an accelerator of oncogenic transformation through selective chromatin remodeling of genes involved in cancer stress response and maintenance of pluripotent cell state.
No conflict of interest.

1007 POSTER

Combined use of estrogen receptor status and TP53 mutation status regulated gene expression data to predict risk of relapse in neoadjuvant chemotherapy treated breast cancer patients

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Background: Breast cancers (bc) carry a complex repertoire of nucleic acid variations including polymorphisms, germline and somatic mutations that can influence gene expression and the clinical behavior of the disease. In the present study we aimed to identify a gene signature driven by *TP53* mutations and to assess its clinical relevance in ER-positive and ER-negative bc.
Methods: Bc data containing *TP53* mutation status and gene expression (for 22,277 genes) derived from five independent datasets were used (n = 695; ER+ n = 511 and ER− n = 184). The gene expression data was obtained using Affymetrix HGU133A microarrays. The raw array data was normalized in the R environment using the affy Bioconductor package. In this discovery cohort, ROC analysis was performed for each gene separately and the genes were ranked by their achieved AUC values. Kaplan–Meier analysis was performed by employing an updated version of our online available KM-plotter using 635 neoadjuvant chemotherapy-treated patients − none of these patients was included in the discovery cohort. Statistical significance was set at $p < 0.01$. *In vitro* assay with specific inhibitors against selected kinases were performed.
Results: Single genes were highly capable to discriminate *TP53* mutant and wild type tumors as well as patients with high and low risk of relapse in ER positive patients. The combination of multiple genes did not significantly improve classification. Among the most significant genes were: TTK (AUC = 0.82), *CDC20* (0.804), *STC2* (0.780), *CDCA8* (0.780), *CCNB2* (0.800) and *MYBL2* (0.79). All of the top *TP53*-regulated genes correlated to survival as well. Identical analysis in ER negative tumors did not reveal any gene associated with survival. Notably, TTK inhibitor (SP600125) was used in 2 TP53 mutated bc cell lines, MDA-MB-231 (ER−) and T47D (ER+) with a significant reduction in cell proliferation and increase in cell death, particularly in the T47D.
Discussion: By using a new statistical approach, we identified *TP53*-mutation driven genes highly correlated to shorter survival in ER-positive chemotherapy-treated breast cancer patients. We identified an important kinase, TTK, regulated by mutant TP53. Inhibition of TTK caused a significant cell death in ER+/TP53 mutated bc cells.
No conflict of interest.

1008 POSTER

Prognostic role of neutrophil to lymphocyte ratio (nlr) in solid tumors: A systematic review and meta-analysis

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Background: Inflammation may play an important role in cancer progression, and a high NLR has been reported to be a poor prognostic indicator in several malignancies. However, the exact magnitude of the prognostic impact of this readily available and inexpensive potential biomarker remains unclear.
Methods: A systematic review of electronic databases was conducted to identify publications exploring the association of blood NLR and overall survival (OS) in all solid tumors. Data from studies reporting a hazard ratio (HR) and 95% confidence interval (CI) or a *P*-value were pooled in a meta-analysis. The pooled HRs for OS by disease group and by extent of disease (non-metastatic versus metastatic versus mixed) were computed using inverse-variance and random-effect modeling.
Results: 68 studies comprising 31,026 patients were included in the analysis. 50 (74%) of the studies were published in 2011 or later and 21 (31%) reported on metastatic disease alone. The median cut-off for high NLR was 4.0 (range 1.9−5.0). Overall, NLR above the cut-off was associated with a HR for OS of 1.68 (95% CI 1.57–1.80, $P<0.001$). The magnitude of effect on OS was non-significantly greater in lower gastrointestinal (GI) and genitourinary tumors compared to other cancer sites (*P* for subgroup difference = 0.10, table). Compared with patients with non-metastatic disease, high NLR was associated with a differentially worse survival in those with metastatic cancer (*P* for subgroup difference = 0.004, table).

	Studies (N)	Patients (N)	Median cut-off NLR	HR for OS	95% CI	*P*-value
Site						
Upper GI	21	5429	3.3	1.59	1.39–1.83	<0.001
Lower GI	17	4542	5.0	2.00	1.59–2.53	<0.001
Lung	10	1977	3.9	1.53	1.31–1.80	<0.001
Genitourinary	8	2379	3.0	2.15	1.71–2.69	<0.001
Gynecological	3	1488	2.6	1.48	0.98–2.23	0.06
Other	9	15211	4.0	1.67	1.45–1.93	<0.001
Stage						
Non-metastatic	19	3981	4.8	1.39	1.24–1.56	<0.001
Mixed	28	20744	4.0	1.68	1.52–1.87	<0.001
Metastatic	21	6301	3.5	1.83	1.64–2.04	<0.001

Conclusion: A high NLR is associated with an adverse OS in many solid tumors. The magnitude of association of high NLR with worse OS is greater for metastatic than for non-metastatic disease. The addition of NLR to established prognostic scores warrants further investigation.
No conflict of interest.

1009 POSTER

PD-1+ immune cell infiltration inversely correlates with survival of patients with operable breast cancer

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Background: Programmed death-1 (PD-1), a coinhibitory checkpoint receptor, is mainly expressed on functionally 'exhausted' $CD8^+$ T cells, dampening the host antitumour immune response. We evaluated the ratio between effective and regulatory T cells (Tregs), and PD-1 expression as prognostic factors for breast cancer patients.
Material and Methods: A series of 218 newly diagnosed invasive breast cancer patients who had undergone primary surgery at Ruijin Hospital between 2004 and 2008 were identified. The influence of $CD8^+$ cytotoxic T lymphocytes (CTLs), $FOXP3^+$ (Treg cell marker), and $PD\text{-}1^+$ immune cell counts on prognosis was analysed utilising immunohistochemistry.
Results: A total of 208 breast tumours were examined after exclusion of 10 patients with uninformative slides. No correlation between $CD8^+$ cells and clinicopathologic variables were demonstrated, while both $PD\text{-}1^+$ immune cells and $FOXP3^+$ Tregs counts were significantly associated with unfavourable prognostic factors. In univariate analysis, high tumour infiltrating $PD\text{-}1^+$ cell counts were correlated with significantly shorter overall survival (OS) ($p = 0.004$, log rank = 9.55), with a hazard ratio of 3.29 (95% CI, 1.48–7.32). However, multivariate analysis showed PD-1 expression not to be independently associated with OS (HR = 2.37; 95% CI, 0.99–5.65; $p = 0.051$).
Conclusions: Tumour progression was seen with higher PD-1 expression on previously functional immune cells and more aggressive cancers harboured increased Tregs. Our results suggest a prognostic value of the $PD\text{-}1^+$ immune cell population in breast cancer patients. Targeting the PD-1 pathway as well as depletion of Tregs may be a feasible approach to treat patients with breast cancer.
No conflict of interest.

1010 POSTER

Protein biomarker signature for risk classification of hormone receptor positive breast cancer identified by reverse phase protein array based tumor profiling

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Background: Around 70% of breast cancer cases belong to the luminal intrinsic molecular subtype, characterized by hormone receptor overexpression. This subtype can be further divided into luminal A and luminal B, which is commonly used as surrogate for good and bad prognosis, respectively. The classification is crucial for therapy decision as patients of the luminal B subtype are at higher risk of recurrence and require chemo-endocrine therapy, contrasting low risk patients who do not benefit from chemotherapy. However, accurate definition of low and high risk luminal breast cancer has remained a challenge so far. Thus, the objective of this study was the identification of a robust protein biomarker signature to facilitate the risk classification of luminal breast cancer.
Materials and Methods: Reverse phase protein arrays (RPPA) were applied to quantify over 120 breast cancer relevant target proteins of hormone receptor positive breast cancer tumor samples. Subsequently, we used a novel bioinformatics workflow combining a bootstrap approach with three different classification methods for biomarker selection. To validate our RPPA derived results we have applied Western blot, immunohistochemistry, and mRNA profiling.
Results: Our results confirm that markers for cell proliferation are prominent factors to distinguish between low and high risk tumors with Ki-67, TOP2A, and PCNA appearing among the top hits. However, NDKA, RPS6, and caveolin-1 were selected as prime candidates. Comparably to Ki-67, NDKA and RPS6 were expressed at an elevated level in high risk tumors whereas caveolin-1 was observed to be downregulated.
Conclusions: We have identified a protein biomarker signature (consisting of caveolin-1, NDKA, RPS6, and Ki-67) using RPPA based tumor profiling with the potential to facilitate the risk of recurrence classification in luminal breast cancer. In addition, we present RPPA as promising experimental platform for the identification of biomarkers in clinical samples.
No conflict of interest.

1011 POSTER

Generation of a potent uPAR-antagonist by forced-proximity engineering of the receptor binding domains of urokinase and vitronectin

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Background: Extensive in vitro, in vivo and clinical evidence suggest that urokinase-type plasminogen activator receptor (uPAR) plays important functions in wide range of pathological processes including tumor growth, invasion and metastasis, inflammatory diseases and viral infections. Drugs interfering effectively with uPAR-function may therefore provide novel therapeutical regimens in a variety of pathological conditions. We here describe the conception, construction and validation of a novel type of inhibitor of the uPAR.
Material and Methods: *a)* Inspection of the crystal structure of the ternary complex between uPAR, the aminoterminal fragment of urokinase-type plasminogen activator uPA (growth factor-like domain, GFD) and the somatomedin B domain (SMB) of vitronectin (VN) reveals that the peptide backbone of uPA and VN are closely located. In particular Lys48 in uPA and Pro42 in VN are only distant 18Å. Connecting residues 1−48 of uPA (i.e. GFD) and 1−42 of VN (i.e. SMB) to a common scaffold, via their C-termini, is thus predicted to generate a chimera. To join GFD and SMB onto a common scaffold the constant region (Fc) of human IgG was chosen to form stable dimers (named uPAR-lock).
b) To evaluate the activity of uPAR-lock in inhibiting uPAR-signalling in live cells we quantified cell adhesion to VN coated wells by impedance measurements using a real-time cell analyser.
Results: *a)* Potent uPAR inhibitor was generated by forced proximity engineering. The uPAR binding domains of uPA and VN are natural and specific inhibitors of uPA and VN binding to uPAR and resulted in uPAR-lock.
b) uPAR-lock is a potent inhibitor of uPAR function. Addition of uPA induced a rapid and strong increase in the adhesion of 293/uPAR cells. Importantly, the treatment with uPAR-lock completely abrogates the increase in 293/uPAR cell adhesion induced by uPA addition. These data clearly showing that uPAR-lock is a potent and specific inhibitor of uPA induced, uPAR mediated, cell adhesion to VN.
Conclusions: The generated inhibitor (uPAR-lock) is a hetero-bivalent uPAR-ligand containing the receptor binding domains of the extracellular protease uPA and the Extracellular matrix protein VN positioned in close proximity on a common scaffold. Binding of uPAR-lock to uPAR results in a complex where the binding sites for both uPA and VN are occupied contemporarily and efficiently, thus blocking both the proteolytic and signalling activities of the receptor.
No conflict of interest.

1012 POSTER

Increased body mass index shortens telomeres through elevated C-reactive protein

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Rationale: Obesity is associated with decreasing telomere length (TL). The cause of this association is unknown.
Objectives: By measuring leukocyte TL, body mass index (BMI) and C-reactive protein (CRP) in 45069 individuals, we tested the hypothesis that increased BMI causes TL shortening, and that low-grade inflammation contributes through elevated CRP. Using the three obesity-associated polymorphisms rs9939609, rs17782313, and rs6548238, and the CRP promoter polymorphism rs3091244 in instrumental variable analyses, we estimated the causal associations between BMI and TL and between CRP and TL.
Findings: In multivariable adjusted observational analyses, TL decreased with 5 base pairs (bp) (95% confidence interval −7; −3) per unit increase in BMI, and further adjustment for CRP attenuated this association to −2 bp (−4; 0.1). In accordance, instrumental variable analysis showed a causal non-significant TL shortening of 6 bp (−37; 25) per unit increase in BMI. Furthermore, in observational analyses TL decreased with 10 bp (−17; −3) for a doubling in CRP, supported by the instrumental variable analyses showing a causal decrease of 66 bp (−124; −7).
Conclusions: The association between increasing BMI and decreasing TL may be causal, and possibly mediated through elevated CRP: elevated CRP *per se* is a causal determinant of TL shortening.
No conflict of interest.

1013 POSTER

Pooled analysis of two phase III studies provides prognostic indicators for clinical out-come after catumaxomab treatment for malignant ascites

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Background: For identification of factors with potential impact on efficacy of catumaxomab (CATU) in patients (pts) with malignant ascites data from two phase III studies (IP-REM-AC-01 and CASIMAS) were used for a meta-analysis.
Materials and Methods: All randomized pts of both studies were included: 389 pts treated with CATU plus paracentesis and 88 control pts (paracentesis only). Efficacy parameters were overall survival (OS), puncture-free survival (PuFS) and time to first puncture (TTPu). Karnofsky Index (KI), total serum protein (protein), presence/absence of distant metastases (metastases) and number of prior chemotherapies (prior CTX) at inclusion were analysed as potential prognostic factors for the treatment effect of CATU.
Results: Pts treated with CATU showed a significant prolongation of PuFS (44 vs 11 d, $p < 0.0001$), TTPu (88 vs 13 d, $p < 0.0001$) and OS (88 vs 68 d, $p = 0.007$) compared to control.
CATU treated pts with a KI of 80–100 at inclusion had a significantly better OS and improved PuFS compared to those with a KI of 60−79 (120 vs 57 d, $p < 0.001$ and 55 vs 27 d, $p < 0.001$, respectively). TTPu was not improved (88 vs 96 d, $p = 0.186$). Linear Cox regression of KI as a co-variable showed a significantly positive impact of high KI on CATU efficacy for OS (HR 0.97, $p < 0.001$) and PuFS (HR 0.98, $p < 0.001$) but not for TTPu (HR 0.99, $p = 0.054$).
Cox regression analysis of the presence of distant metastases and low (< normal) total serum protein on CATU efficacy showed a negative impact on

OS (metastases: HR 1.43; p < 0.001; protein: HR 1.39, p = 0.002), PuFS (metastases: HR 1.37, p = 0.003; protein: HR 1.45, p < 0.001) and TTPu (metastases: HR 1.28, p = 0.069; protein: HR 1.41, p = 0.011).
Median number of prior CTX was 3. Pts with >3 prior CTX had a reduced treatment effect of CATU compared to those with 1 or 3 prior CTX. Overall, significant treatment effects on ascites (PuFS and TTPu) were observed in all subgroups for all factors; however, significant effects on OS were only found in pts with KI 80–100, with no distant metastases, with ⩾ normal total serum protein and with 3 prior CTX.
Conclusion: Karnofsky Index, distant metastases, total serum protein and number of prior CTX at screening were identified as factors having a significant impact on OS in CATU-treated pts and better prognostic pts compared to controls. In contrast, the effect of CATU on ascites control was observed in all subgroups irrespective of whether the pts were in the good or poor prognostic group.
Conflict of interest: *Advisory board: Fresenius Biotech GmbH. Corporate-sponsored research: Fresenius Biotech GmbH. Other substantive relationships: Employee Fresenius Biotech GmbH*

1014 POSTER

A new IDH1/2 PCR assay for one-step detection of 12 IDH1 and IDH2 mutations in glioma

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Background: Isocitrate dehydrogenase (IDH) mutation status is a strong diagnostic and prognostic marker in glioma which will probably be introduced in the WHO classification system. Current screening for IDH mutations is performed with an IHC assay specific for IDH1 R132H, the most frequent mutation; subsequent sequencing is recommended when IHC is negative or equivocal. A qPCR assay was designed to detect in one single step, IDH1R132H and 11 rare IDH1/2 mutations in FFPE samples. This study describes development, analytical performance and validation of this new assay.
Material and Methods: PCR Clamping was used to detect IDH1 R132H and 11 additional mutations (5 IDH1 R132, 1 IDH1 R100, 5 IDH2 R172). ARMS was combined to selectively identify IDH1 R132H/R132C and IDH2 R172K. Limit of detection (LOD) (minimum % mutant DNA detected in a WT DNA background) was determined using five low positive samples per mutation (n = 5x30 measurements/mutation). FFPE glioma samples (n = 170) were retrospectively collected from 3 independent sources. The assay was validated on samples meeting assay requirements (<10 yrs, ⩾50 mm^2, ⩾40% tumor cells) comparing PCR IDH status to IHC (mIDH1 R132H) and bidirectional sequencing. Additionally, to better document minor mutations, synthetic samples for the 11 minor mutations (30% and 45% mut DNA in WT DNA) were tested.
Results: Assay sensitivity varied across mutations with LOD <5% for 11/12 mutations (mean=3.3%). From the first 120 clinical samples analyzed, 103 were <10 yrs (48/103 glioblastomas). IDH status was successfully obtained by PCR for these 103 samples. All IHC positive cases were concordantly identified as R132H by PCR (n = 45). One R132H case detected by PCR was negative by IHC (negative concordance 98%). PCR additionally detected 9 rare mutations (8 IDH1, 1 IDH2). Moreover, the kit produced 100% correct results on the synthetic samples with rare mutations.
Conclusions: The one-step IDH1/2 PCR assay showed a 100% technical success rate and is more sensitive than published references for bidirectional sequencing. Positive concordance with IHC detection for IDH1 R132H was 100%. Complete validation results including sequencing data (to be presented at the meeting) will further document the value of this new assay to screen in one step for 12 IDH12 mutations.
Conflict of interest: *Ownership: No. Advisory board: No. Board of directors: No. Corporate-sponsored research: No. Other substantive relationships: HG, CP, FM, AC, and HPSP are full time employees of QIAGEN Marseille*

1015 POSTER

Plasma testosterone in the general population, cancer prognosis and cancer risk

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Background: Testosterone is an important anabolic hormone in humans and, i*n vitro*, testosterone stimulates growth of lung and colon cancer cells. We tested the hypothesis that increased plasma testosterone associates with increased risk of cancer and with increased risk of early death after cancer in the general population.
Material and Methods: Plasma testosterone was measured in 8771 20−94 year old men and women who participated in a prospective study of the general population. Participants were included in 1981−83 and followed for up to 30 years.
Results: During follow-up, 1140 men and 809 women developed cancer. For risk of early death after cancer and after adjustment for age at diagnosis, tumour stage at diagnosis, and time since blood-sampling, the hazard ratios in men were 1.30 (95% confidence interval 1.03–1.65) for the 2nd quintile, 1.31 (1.02–1.67) for the 3rd quintile, 1.52 (1.19–1.93) for the 4th quintile, and 1.52 (1.20–1.91) for the 5th quintile, versus the 1st quintile. In women, corresponding hazard ratios were 1.09 (0.81–1.46), 1.17 (0.86–1.59), 1.03 (0.76–1.39), and 1.80 (1.32–2.46). For risk of any cancer for a doubling in testosterone levels, multifactorially adjusted hazard ratios were 1.07 (0.98–1.18) in men and 1.06 (0.93–1.22) in women. For both men and women, a doubling of testosterone levels was not associated with risk of any individual cancer type.
Conclusions: In this prospective study of 8771 men and women from the general population followed for up to 30 years, increased testosterone levels were associated with a 30−80% increased risk of early death after cancer, but with unchanged risk of incident cancer.
No conflict of interest.

1016 POSTER

Expression of cancer stem cell (CSC) markers in primary tumors (PT) and matched lymph node metastases (LNM) in breast cancer patients

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Background: Breast cancer is characterized by high intra-tumor heterogeneity, with multiple cell populations differing in metastatic potential. CSCs, representing a minor subset of cells, play a critical role in breast cancer initiation, progression, metastasis and drug resistance. However, their status in LNM remains unclear. We evaluated in PT and in matched LNM protein levels of CSC markers: CD44 and ALDH1, and epithelial–mesenchymal transition (EMT) markers: E-cadherin and vimentin. The results were correlated with clinicopathological data, overall (OS) and disease-free survival (DFS).
Material and Methods: We examined formalin-fixed paraffin-embedded samples from PT and matched LNM from 42 stage II−III breast cancer patients. Protein expression was measured by immunohistochemistry on tissue microarrays. For CD44 and ALDH1 staining intensity (scored as: 0-negative, 1-weak, 2-intermediate, 3-strong) and percentage of positively stained cells (0–100%) were multiplied to count the expression score. For E-cadherin and vimentin ⩾10% of stained cells defined a positive result. Results were considered concordant if PT and LNM were both positive or both negative. Concordance was measured by Cohen's kappa coefficient (κ), with κ value equal 1 indicating perfect agreement. DFS and OS were compared using F-Cox test. Hazard ratios (HRs) with 95% confidence intervals (95% CI) were computed using Cox regression analysis.
Results: Median expression score of CD44 was significantly higher in LNM compared to PT (p = 0.04). Status of ALDH1 and CD44 between PT and LNM was discordant in 16/42 (38%) and 7/27 (26%) of cases, respectively (κ=0.24 and κ=0.5), with only negative-to-positive conversion for CD44. Status of E-cadherin was fully concordant (κ=1), and of vimentin has changed in 7% (3/40, κ=0.63). CD44 positive status correlated with vimentin expression (p = 0.03). Increased expression of CD44 in LNM was strongly associated with shorter OS (HR 8.4, 95% CI 1.04–67.6, p = 0.001) and DFS (HR 9.6, 95% CI 1.2–76.7, p = 0.0005), whereas its expression in PT had no prognostic impact. Status of ALDH1 and vimentin in both PT and LNM did not correlate with OS and DFS.
Conclusions: Compared to PT, LNM are enriched in cells with CSC markers, suggesting that phenotype of these aggressive cell subpopulations might easier be captured in LNM than in PT. High level of CD44 in LNM confers worse prognosis, confirming the correlation of CSC-phenotype with aggressive disease behavior.
No conflict of interest.

1017 POSTER

HER3 expression in primary colorectal cancer and corresponding lymph node metastases

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Background: To evaluate the prognostic value of the epidermal growth factor receptor HER3 and to elucidate heterogeneity of HER3 expression in primary colorectal cancer (CRC) and corresponding lymph node metastases.
Material and Methods: HER3 expression was analyzed immunohistochemically (IHC) in primary tumors and in corresponding lymph node metastases from 236 patients with stage II and III CRC. In 58 primary tumors, fluorescence in situ hybridization (FISH) detection was performed.
Results: HER3 was detected at high frequency in the cellmembrane. Seventy per cent of the primary tumors were categorized with high HER3 expression compared to 75% in the lymph nodes metastases. HER3 expression in the primary tumor was an independent prognostic factor for overall survival in the entire group of patients ($p = 0.026$) and in the subgroup of patients with colon cancer stage II ($p = 0.030$). A high HER3 expression in the primary tumor was associated with worse clinical outcome. The expression of HER3 was homogenous within the tumor ($p < 0.0001$) and correlated with the HER3 expression in corresponding lymph node metastases ($p < 0.0001$). No gene amplification with respect to HER3 was seen in primary tumors using FISH analysis.
Conclusion: High HER3 expression was found in about 70% of the primary CRC tumors and corresponding lymph node metastases. HER3 expression in the tumor was an independent prognostic factor where a high HER3 expression was associated with worse clinical outcome. There was correlation in HER3 expression between primary tumors and corresponding lymph node metastases.
No conflict of interest.

1018 POSTER

Can we accurately report PTEN status in advanced colorectal cancer?

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Background: The tumour suppressor gene PTEN is considered to have a potential role as a biomarker for anti-EGFR therapy in CRC, although results are inconsistent and there is debate as to the optimal method of reporting low PTEN expression/loss of function. Immunohistochemistry (IHC) is frequently used, although other methods have also been reported (Fluorescence in situ hybridisation and mutation status). PTEN status also appears to vary between primary and secondary tumour samples, further complicating interpretation of the data. Our group has used the Taqman® copy number assay (CNA) previously and here we report results of a subgroup of patients from the AGITG/MAX trial PTEN analysis exploring the concordance between pathologists assessment of PTEN IHC expression, and the relationship of Taqman CNA result and IHC.
Methods: Genomic DNA was extracted from FFPE tissue sections. The Taqman PTEN CNA was performed using 5 ng DNA in quadruplicate PCR. The results are calculated as a ratio relative to a 2-copy control using the 2-ΔΔCt method (Rotorgene software), and multiplied by 2 to give the copy number. Loss of PTEN was defined as $\leqslant$1.5 copies, no loss was >1.5 copies. IHC was performed on 60 tissue arrays and then assessed by two blinded Pathologists. Scoring was 0, 1+, 2+, 3+ for PTEN IHC expression. The two pathologists were compared directly for an IHC concordance rate, and then the scores were grouped and majority score used for IHC v Taqman CNA.
Results: Tissue arrays were analysed for PTEN staining by IHC. 95% of tissue arrays were from the primary CRC. Three were found to have no residual tissue present on the array. 18 of 57 (31.6%) had a score of zero, ie loss of PTEN expression based on IHC. Concordance of IHC PTEN loss (0 v 1+, 2+, 3+) was 63% (36/57) when comparing two pathologists. Using majority score for IHC from the two pathologists, for the 18 specimens with IHC loss of expression, 12 (66.7%) had copy number loss by Taqman. Furthermore the rate of IHC PTEN loss was 44% of those with copy number loss (12/27). There was no evidence that PTEN loss in this small subset measured by low copy number or IHC negativity was prognostic for RR, PFS or OS.
Conclusion: PTEN loss based on IHC is not directly associated with PTEN copy number loss, suggesting that there are other mechanisms such as mutation or promoter methylation, leading to loss of IHC expression. Conversely loss of just 1 PTEN allele may still allow compensatory up-regulation of PTEN to normal levels. This supports the concept that various mechanisms may lead ultimately to loss of PTEN expression. PTEN assessment by IHC alone however remains unreliable based on the lack of concordance between pathologists and robust validation will be required before routine use.
No conflict of interest.

1019 POSTER

The protective role of the vagus nerve in cancer

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Background: The evolution of tumors is multifactorial and complex. Recent research shows however, that not only do microenvironmental factors play pivotal roles in carcinogenesis, but so does the autonomic nervous system. The vagus nerve is thought to have protective roles in cancer since it reduces inflammation, oxidative stress and sympathetic activity. Vagotomized tumor-bearing animals show greater metastasis, and in contrast, activating the vagus nerve by a medication was found to reduce tumors and their metastases. In a series of retrospective studies we conducted, higher heart rate variability (HRV), a non-invasive index of vagal nerve activity, was found to predict reduced tumor burden and longer survival times, independent of important confounders including stage and treatment. Furthermore, finding modifiable prognostic factors paves the way for new treatments, which is crucial in advanced pancreatic cancer.
Methods: A 'historical prospective' study in N = 272 patients with advanced pancreatic cancer was performed. HRV was obtained retroactively from ECGs near diagnosis, and some other confounders such as age and treatments were obtained from the medical charts. Levels of C-reactive protein (CRP) were also measured as an inflammatory marker. Overall survival and survival date were obtained from medical charts and the national registry.
Results: In a Cox regression, higher initial HRV (>20 msec) significantly predicted lower risk of death, independent of confounders including age and cancer treatments. This relationship was mediated (explained) by CRP levels. Importantly, in patients who lived only one month or less from diagnosis, HRV was unrelated to CRP, while in patients surviving longer, HRV was significantly inversely related to CRP ($r = -0.20$, $p < 0.05$), suggesting that neuroimmuno-modulation let patients survive longer. Finally, chemotherapy doses increased overall survival only when vagal nerve activity was low, while patients with high vagal nerve activity survived longer, even without chemotherapy.
Conclusion: These results support vagal nerve protection in a fatal cancer, and propose that the mechanism may involve neuroimmunomodulation. Vagal nerve activity may also determine in whom certain treatments are effective and necessary.
No conflict of interest.

1020 POSTER

Prognostic value of Tubedown-100 in patients with breast cancer

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Background: Tubedown-100, also known as NAA15 and NARG1, is a large protein initially found to express at high level in testis tissues and proliferative neuronal tissues. It has been found to be expressed in certain cancer cells including thyroid cancer and lymphoma cells. Although Tubedown-100 has been indicated in the regulation of apoptosis, its biological role in cells including cancer cells remains unknown. It clinical implication in cancer is not known. In the present study, we aimed to investigate the pattern of expression of this molecule in human breast cancer and the potential prognostic value in the patients.
Materials and Methods: The levels of Tubedown-100 gene transcripts were determined using quantitative transcript analysis and PCR analysis. Human breast cancer cell lines MCF-7 and MDA MB-231 were used. Anti- Tubedown-100 transgenes were constructed and used to regulate the expression of Tubedown-100 in breast cancer cells. (cell growth and apoptosis were evaluated on the cells with differential expression of Tubedown-100) A cohort of human breast cancer tissues (n = 127) were

tested for the levels of Tubedown-100 gene transcript. Patient's clinical, pathological and outcome results were analysed against the levels of Tubedown-100.
Results: MCF-7 and MDA MB-231 cells expressed Tubedown-100 gene transcripts. Knockdown of Tubedown-100 gene in both cells resulted in cells with significantly increased growth rate and motile and adhesive to matrix (P<0.001). The Tubedown-100 knockdown cells did not have a significant difference in apoptosis compared with control cells. Levels of Tubedown-100 transcript were significantly lower in tumours from patients with a predicted poor prognosis compared with those with good prognosis (p = 0.038), although there were no significant links with tumour grade. Perhaps the most interesting observation is that levels in patients who developed distant metastasis and who died of breast cancer related complications had significantly lower levels than those who remained disease free (p = 0.016 and p = 0.022, respectively). This is reflected a significantly shorter overall survival time in patients with low levels of Tubedown-100 than those with high level.
Conclusions: It is concluded that Tubedown-100 is aberrantly expressed in patients with breast cancer and the levels are linked with the prognosis of the patients. Tubedown-100 thus is a potential prognostic indicator in patients with breast cancer.
No conflict of interest.

1021 POSTER

Expression of aquaporin 5 and its polymorphism predicts survival in patients with early breast cancer

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Background: Our previous study showed the association of aquaporin 5 (AQP5) up-regulation with cancer proliferation and migration in hormone-responsive breast cancer cell lines and with unfavorable prognosis in patients with breast cancer. Accordingly, we analyzed the prognostic impact of AQP5 expression and polymorphisms in a large number of patients with early breast cancer (EBC).
Materials and Methods: AQP5 expression was investigated based on the immunohistochemistry of tissue microarray specimens from 609 EBC patients who underwent surgery between 2003 and 2008. We scored the staining intensity (IS) and percentage of positive tumor cells (PC). The genomic DNA was extracted from paraffin-embedded tumor-free tissue and then genotyped for 3 polymorphisms (rs3736309, rs1964676, and rs74091167) using the Sequenom Mass array system.
Results: Among the 3 polymorphisms, AQP5 overexpression (IS + PC $\geqslant$6) was correlated with AQP5 rs74091167 GG genotype. AQP5 overexpression and AQP5 rs74091167 was significantly associated with disease-free survival (DFS; P <0.001 and P = 0.021, respectively). Moreover, a multivariate survival analysis revealed that AQP5 overexpression and the GG genotype of AQP5 rs74091167 were significantly associated with DFS (HR = 2.026, 95% CI 1.058–3.881, P = 0.030; HR = 0.377, 95% CI 0.179–0.793, P = 0.010, respectively), which was prominent in patients with an ER/PgR-positive tumor.
Conclusions: Consistent with our previous study of breast cancer cell lines, AQP5 expression and AQP5 rs74091167 variant can be considered as a prognostic marker in patients with EBC after curative surgery. In the future, functional relevance of this variant needs to be clarified.
No conflict of interest.

1022 POSTER

Significance of S100A7 and S100A4 expression as a prognostic biomarker for oral squamous cell carcinoma

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Oral cancer is a leading cause of cancer death worldwide. The goal of cancer-screening program is to detect tumors at early stage, enough that treatment is likely to be successful. Moreover, the screening tool must be sufficiently noninvasive and inexpensive to allow widespread applicability. A substance secreted by tumor tissue, not secreted by non-tumor tissue, and easily and cheaply detectable in saliva, serum or urine is, therefore, an ideal biomarker because the cancer is detected specifically and non-invasively. The aim of the study was to investigate the expression pattern of calcium- binding proteins S100A7 and S100A4 for patients with oral squamous cell carcinoma. The S100 Calcium binding protein family consists of at least 25 different types of low molecular weight proteins (9−13 kDa), which are characterized by two calcium-binding sites of the EF-hand type conformation and located on a cluster on human chromosome 1q21. Psoriasin (S100A7) is a member of the S100 gene family in the early stages of tumor genesis S100A7 is highly expressed in ductal carcinoma *in-situ*, but S100A7 is often up regulated in adjacent invasive carcinoma.S100A7 is thus also associated with altered and abnormal pathways of epithelial cell differentiation. The prognostic significance of S100A4 as potential biomarker for oral Cancer. The nuclear S100A4 in TNM (Tumor Node Metastasis) stage indicates the proper early diagnosis in the OSCC.S100A4 promotes metastasis and is involved in several steps of the metastatic cascade, including cell motility, invasion and angiogenesis. Disruption of calcium signaling pathways has been implicated as a central mechanism in tumor genesis and specifically in the process of invasion and metastasis The protein biomarker expression in the OSCC human tissue specimens and matching normal oral tissues from 20 patients were examined. Expression of the protein and mRNA were analyzed by Western blot, RT PCR and IFC/IHC in the human oral cancer tissue comparison with adjacent normal tissue Increased expression at mRNA level was observed in OSCC samples by quantitative real-time RT-PCR.Moreover, significantly increased mRNA ratios between malignant and normal samples were observed. From this we can predict that over expression of S100A7 and S100A4 that epithelial cells undergoing abnormal differentiation and tumor genesis, this could be the potential early detection bio-marker to prevent cancer progression and will show new direction in early stage cancer treatment.
No conflict of interest.

1023 POSTER

CRNDE (a novel marker of poor prognosis in patients with cancer) encodes the CRNDEP protein, a component of stress granules

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Background: The *CRNDE* gene, currently known as non-protein coding, has been found to negatively affect prognosis in patients with colorectal and ovarian carcinomas when overexpressed in cancer at mRNA level. Identification of a protein product encoded by this gene may be important due to its potential role in cancer prognosis and prospective usage in ovarian cancer screening.
Material and Methods: The study was performed on RNA samples isolated from normal endometrium, ovarian cancer and HeLa cells. 5' RACE/3' RACE techniques and reverse transcription PCR followed by nested PCR were employed to identify complete splice variants of the *CRNDE* gene. Next, based on bioinformatics analyses, the most promising open reading frame (ORF) was chosen and cloned into five expression vectors. These vectors were used to elicit overexpression of the hypothetical peptide in bacteria (pQE30, pET201) and HeLa cells (pCDNA3.1(+)). They were also utilised in cellular localisation studies under a fluorescence microscope (pEGFP-N1, pDsRed Monomer-C1). In addition, a polyclonal antibody against the peptide was developed in rabbits. It was used in western blot hybridisation and immunohistochemical experiments.
Results: The 5' RACE and 3' RACE experiments revealed two different splice variants of *CRNDE*. Additional PCR experiments, inspired by the results of other research teams, proved the existence of several other splice variants of *CRNDE*. Some of them seemed to be tissue-specific, but the variant recognised here as protein-coding was ubiquitous. This shortened variant encodes the CRNDEP peptide, consisting of 84 amino acids. This peptide localises to stress granules in HeLa cells, and its upregulation stimulates the formation of these granules. Given these results and the outcome of bioinformatics analyses, CRNDEP seems to exhibit oxidase activity, thus enhancing risk of oxidative stress when overexpressed. The presence of CRNDEP in the variety of human tissues was confirmed by our team with the use of immunohistochemical methods. The existence of a protein product encoded by the *CRNDE* gene has never been reported before in the literature.
Conclusions: *CRNDE* emerges as a protein-coding gene. The product of this gene, the CRNDEP peptide, was identified herein as a component of stress granules, and its overexpression seems to stimulate the formation of these granules.
No conflict of interest.

1024 POSTER

The CRNDE, VAV2 and CEBPA genes as new negative prognostic factors in ovarian cancer patients

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Background: Ovarian cancer is the leading cause of death from gynaecological malignancies. Mortality in this disease is exceptionally high due to the absence of specific symptoms at early phases and the lack of good screening methods. The majority of patients are diagnosed at late stages, characterised by poor prognosis. Identification of new molecular prognostic markers, potential targets of molecular therapy, may facilitate the fight against this neoplasm.
Material and Methods: The genes examined in this study were selected based on the results of preceding gene expression microarrays. The prognostic value of their expression at mRNA level was evaluated in ovarian cancer patients treated with either cisplatin and cyclophosphamide (the PC regimen, N = 32) or taxanes and cisplatin (the TP regimen, N = 74). *HGPRT* was chosen experimentally as a reference gene. In qPCR reactions, inventoried TaqMan assays (Life Technologies) were used, except for the *CRNDE* gene, expression of which was evaluated with two personally designed TaqMan assays, specific to two different splice variants. The amount of genomic DNA contamination was assessed and taken into account if necessary (i.e., for *CEBPA*, an intronless gene). The results were analysed statistically using univariate and multivariate Cox proportional hazards models.
Results: Elevated mRNA expression of *CRNDE* (two different splice variants), *VAV2* and *CEBPA* genes negatively influenced prognosis by significantly increasing risk of death and/or recurrence. For *CEBPA*, this association was mainly observed in a group of patients treated with PC. The clinical significance of *VAV2* overexpression seemed to be related to the TP treatment, though a negative impact of upregulation was also visible in the group of all patients analysed. Overexpression of *CRNDE* negatively affected prognosis without clear discrimination between the chemotherapies administered. In addition, some clinical associations of *CRNDE* seemed to depend on TP53 accumulation status.
Conclusions: Considering the Real-Time qPCR results, the *CRNDE*, *VAV2* and *CEBPA* genes emerged as novel cancer-promoting factors and potential molecular markers in ovarian cancer patients. The clinical meaning of their protein products should be further evaluated in a bigger, well characterised group of tumours through immunohistochemical staining.
No conflict of interest.

1025 POSTER

Characteristics of the relation between epithelial–mesenchymal transition and proliferative activity in breast carcinomas

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Background: Metastases in distant sites are the leading cause of death from breast cancer. One of the most important features of this process is epithelial–mesenchymal transition (EMT), the key marker of which is vimentin − a protein of intermediate filaments of cytoskeleton. EMT is a final stage of tumour dedifferentiation and is started by a row of signalling mechanisms responsible for adhesion and proliferation of tumour cells. The aim of the research was to study interplay between the level of proliferation of breast carcinoma tissue, detected by a proliferative index Ki-67, and realisation of EMT.
Material and Methods: 129 cases of breast carcinoma were studied. Vimentin in the tumour cells was detected by monoclonal antibodies Monoclonal Mouse Anti-Swine Vimentin (DAKO, Denmark). For detection of the cell proliferation index antibodies Mouse Anti-Human KI-67 Antigen (DAKO, Denmark) were used. Immunohistochemical tests were made in autostainer 'DAKO' (Denmark) with the use of Dako Wash Buffer, visualisation system Dako EnVision+Dual Link System-HRP, chromogen Dako Liquid DAB+ Substrate Chromogen System (DAKO, Denmark). Test evaluation was made with the microscope 'Zeiss Ymager M' (Germany). A criterion of positive expression of vimentin in the tumour cells was presence of strong cytoplasmic staining. Proliferative activity of the investigated tumour was evaluated by the percentage ratio between the stained nuclei of breast carcinoma cells and the unstained ones.
Results: A weak positive correlation between vimentin expression and Ki-67 level was found ($r = 0.39$; $p < 0.05$). Due to heterogeneity of the group of analysed carcinomas all the cases were divided into immunohistochemical subtypes: luminal A subtype − 39 cases (30.2%), luminal B (Her2-) subtype − 36 cases (27.9%), luminal B (Her2+) subtype − 15 cases (11.6%), Her-2 subtype − 15 cases (11.6%), basal-like subtype − 24 cases (18.6%). We found a strong positive correlation between proliferative activity of a tumour and vimentin expression in Her-2 positive and basal-like subtypes ($r = 0.85$; $p < 0.05$; $r = 0.87$; $p < 0.05$ respectively). A weak negative correlation was found for cases from luminal A subtype ($r = -0.38$; $p < 0.05$). For other subtypes a fairly significant correlation between studied parameters was not found.
Conclusions: EMT mainly occurs in breast carcinomas with a high level of proliferation that reflects on the correlation between the presence of the vimentin expression and a high level of Ki-67, distinctive for basal-like and Her-2+ subtypes, associated with poor prognosis and a high risk of distant metastases development.
No conflict of interest.

1026 POSTER

Prognostic significance of heme oxygenase-1, S100A4 and syndecan-1 expression in primary non-muscle invasive bladder cancers

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Background: The prognosis of non-muscle invasive bladder cancer (NMIBC) is variable and significant proportions of patients undergo tumor recurrence and progression despite clinically complete transurethral resection. We have examined whether altered protein expression for three potential biomarkers, which are associated with different BC carcinogenesis pathway and have been studied by limited number of studies, could predict tumor recurrence and progression in patients with primary NMIBC.
Materials and Methods: The study included 109 patients who were diagnosed with primary NMIBC after clinically complete transurethral resection between 2000 and 2008 at our institute. After extensive and critical literature review regarding prognostic molecular markers based on immunohistochemistry and cDNA microarray in NMIBC, three molecular markers including heme oxygenase (HO-1), S100A4 and syndecan-1 (SYND1) were selected. Protein expression was analyzed by immunohistochemistry, and the immunoreactivity for each biomarker was evaluated using a semi-quantitative scoring system for both the intensity of the stain and the percentage of positive neoplastic cells. Survival analysis was performed using Kaplan–Meier curves and Cox regression to determine the effect of each marker on recurrence-free survival (RFS) and progression-free survival (PFS).
Results: The altered expressions for each marker were noted in 36 patients (33.0%) for HO-1, 40 (36.7%) for S100A4 and 69 (63.3%) for SYND1, respectively. Abnormal expressions of HO-1 and S100A4 were significantly correlated with higher T stage and grade, whereas SYND1 alteration was significantly with lower T stage and grade (all $p < 0.001$). On multivariate analysis including clinicopathological parameters and biomarkers, the three biomarkers were significant predictors for RFS while HO-1 and S100A4 were significant predictors for PFS. A combination analysis of three markers showed that patients with multiple ($\geqslant 2$) marker alteration was associated with significantly lower 5-year RFS (43.0%) and PFS (64.0%) rates than patients with none or single marker alteration (RFS: 78.1%, $p = 0.003$; PFS: 97.0%, $p < 0.001$). In protein-protein interaction analysis using BisoGenet, there were large protein network among the three markers.
Conclusions: Our findings indicate that immunohistochemical analysis for HO-1, S100A4 and SYND1 may be useful in predicting tumor recurrence and progression, thus planning treatment strategies in NMIBC.
No conflict of interest.

1027 POSTER

Role of C-reactive protein in advanced cancer prognostication

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Background: CRP, a non-specific marker of inflammation may help cancer prognostication. CRP is secreted by liver due to interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF). It has been linked to shorter survival in some cancers. We examined associations between CRP levels and prognosis in solid tumors.
Material and Methods: Retrospective study of electronic medical records (EMR). Multiple CRP levels at a tertiary cancer center reviewed (2006–2011). Hematological cancer diagnoses excluded. Survival defined from the date with highest CRP to date of death. CRP reported as median (25^{th}, 75^{th} percentile). CRP reference range 0−10 mg/L.
Results: N = 6809 with solid tumors identified. 56% males. 83% Caucasian, 15% African American. Common cancers − genitourinary (GU) 29%, breast

14%, gastrointestinal (GI) 14%, lung 7%. Highest CRP for GI, GU, lung, breast = 8 (2, 15); 6 (2, 15); 3 (1, 8); 2 (1, 5) respectively. Median survival (months) = 13 (8, 30); 18 (11, 33); 16 (8, 27) and 25 (15, 41) respectively.
Conclusions: 1. Higher median CRP in GI, GU, lung and breast cancers.
2. Higher CRP associated with shorter prognosis across primary sites (even within reference range).
3. Inverse relationship between absolute CRP values and survival.
4. High CRP, an adverse prognostic indicator in most solid tumors.
No conflict of interest.

1028 POSTER

Atypical spitzoid melanocytic tumors versus spitzoid melanoma: Diagnostic and prognostic assessment by FISH analysis

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Background: The aim of our study was to evaluate whether molecular alterations detected by Fluorescence In Situ Hybridization (FISH) could be helpful in differentiating Atypical Melanocytic Spitzoid Tumors (AST) from Spitzoid Melanoma (SM) and performing risk assessment particularly for AST in order to a correct management of the patients.
Material and Methods: The study included 61 AST and 32 SM retrived from the files of the Department of Pathology-Istituto Nazionale Tumori-Milan from January 2010 up to March 2013. All cases were reviewed by two expert pathologists and analyzed by FISH technique using the 4-probe multicolour FISH DNA kit (Visys/Abbott Molecular®) targeting RREB1 (6p25), MYB (6q23), CCND1 (11q13) and CEP6. Sentinel lymph node biopsy (SLNB) was performed in 8 AST and 24 SM.
Results: Positive FISH result was found in 27 of 61 AST (44.6%) and 20 of 32 SM (62.5%). Gain of both CCND1 and RREB1 was the most common molecular alteration in AST (23%) and SM (75%). In AST series 4 (50%) out of 8 patients had metastatic involvement of sentinel node, 3 of them with positive FISH result. In SM series 7 (29%) out of 24 patients had metastatic involvement of sentinel node, 4 of them with positive FISH result. Furthermore molecular analysis of nonmetastasizing cases show that 1 of 4 AST (25%) and 10 of 17 SM (58.8%) had also positive FISH result. All patients affected by AST or SM with positive SLNB were alive with no evidence of disease at last follow-up.
Conclusions: Our study shows that FISH analysis is not really helpful in differential diagnosis of AST versus SM because we found positive FISH result in both lesions (AST 44.6% versus SM 62.5%) with the same molecular alteration (CCND1 and RREB1) as the most common. Furthermore we cannot use it as prognostic tool because of positive FISH result in both metastasizing and not metastasizing AST and SM. To validate the utility of FISH analysis for the differential diagnosis and risk assessment of AST versus SM a larger sample of cases of both series with prolonged follow-up need to be evaluated and tested by a broader spectrum of FISH probes.
No conflict of interest.

1029 POSTER

Evidence of cathepsin K (CTSK) in the aggressive development and invasion of pituitary adenomas

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Background: CTSK, cathepsin K, is a member of cysteine proteinases and has been found to be highly expressed in muscle and skeletal tissues. It has been indicated in the degradation of extracellular matrix and in particular protein component of bone matrix and that CTSK knockout mice has abnormalities in bone development and functions and resulted in increased fragility of bones. Pituitary adenomas are mostly benign with or without active endocrine functions. Located in the fossa of sphenoid bone, sella turcica, one of the key features of the pituitary adenomas is the invasion and destruction of the surrounding bones in the sella fossa. However, biological and molecular events involved in the destruction of sella fossa remains unclear. In the present study, we investigated expression of CTSK in pituitary adenomas with particular reference to invasion and destruction of sella fossa.
Materials and Methods: Pituitary adenomas were freshly collected immediately after surgery. Histological, radiological and biochemical analyses were carried out to provide clinical classification (Knosp's method) and to establish the endocrine status of the tumours. Expression of CTSK in pituitary tumours was determined using quantitative gene transcript analysis and analysed against tumour grade, invasion, Knosp grade, endocrine nature. Statistical analyses were conducted using Mann-Whitney U test and Kruskall-Walis method.
Results: Pituitary tumours which had invaded sphenoid sinus had a higher level of CTSK compare with those without invasion (median 14.8 vs 25.7). Likewise, using the Knosp classification, Stage 3 and 4 tumours which had signs of invasion showed high levels of CTSK than Knosp 0, 1 and 2 tumours (medians 25.7 vs 14.8). These links were independent of the size of tumours. It was interesting to observe that TSH-, gonadotrophin- and ACTH-secreting tumours had high levels of CTSK than endocrine-inactive tumours (70.1, 53.9 and 46.2 vs 20.9 respectively) and tumours secreting PRL and GH had lower levels (3.02 and 4.0 respectively). Finally, it was noteworthy that pituitary tumours with internal haemorrhage had lower levels of CTSK than those without haemorrhage (25.70 vs 4.0, $p < 0.05$), suggesting that CTSK is an unlikely candidate for this clinical condition.
Conclusions: It is concluded that Cathepsin K, an proteinase commonly involved in the regulation of bone absorption and bone pathology is over-expressed in pituitary adenomas which have aggressive pattern toward its surrounding bone tissues. This provides new direction in the understanding of the biology of pituitary tumours and their progression.
No conflict of interest.

Proffered Papers Session (Sat, 28 Sep)
Radiobiology/Radiation/PhysicsRadiotherapy Techniques

1050 ORAL

Targeting hypoxia to enhance the anti-tumour effect of a stereotactic radiation schedule

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Background: Hypoxia in tumours is well known to reduce the efficacy of radiation therapy. Recent studies suggest that such hypoxia may play a more significant role when radiation is given as large doses/fraction. The aim of this study was to use clinically relevant approaches for eliminating hypoxia to see if they could improve tumour response to a stereotactic radiation schedule.
Material and Methods: A C3H mammary carcinoma grown in the right rear foot of female CDF1 mice was used in all experiments. Treatments were performed in restrained non-anaesthetised animals when tumours had reached 200 mm^3 in size. Tumours were locally irradiated (230 kV x-rays) with 3 x 15 Gy (each fraction given with an interval of 2−3 days over a one week period). Hypoxic modification was achieved by intraperitoneally (i.p.) injecting the radiation sensitizer nimorazole (200 mg/kg), combining the oxygen modifiers nicotinamide (120 mg/kg; i.p.) and carbogen (95% O_2 + 5% CO_2) breathing, using the hypoxic cytotoxin hyperthermia (41.5^{O}C; 1-hour), or injecting the vascular disrupting agent OXi4503 (10 mg/kg; i.p.). Three days after the final irradiation the tumours were subjected to a clamped top-up dose which involved giving graded radiation doses with the tumour bearing leg clamped for 5 minutes before and during irradiation. The percentage of mice in each treatment group showing local tumour control 90 days after irradiating was recorded and the TCD50 values (radiation dose to control 50% of tumours) estimated from the clamped top-up radiation dose response curves. A Chi-squared test ($p < 0.05$) was used to determine significant differences between the TCD50 values.
Results: The clamped top-up TCD50 value (with 95% confidence intervals) following 3 x 15 Gy was 30 Gy (23−38). This was significantly reduced to 6 Gy (3−10) when nicotinamide (injected 20 minutes prior to irradiation) and carbogen breathing (starting 5 minutes before radiation and continued during each radiation period) were combined; to 9 Gy (5−17) by heating tumours 4-hours after each radiation treatment; and 12 Gy (8−19) if OXi4503 was injected 1 hour after each irradiation. The value obtained when nimorazole was injected 30 minutes prior to irradiation is currently under analysis.
Conclusions: Targeting hypoxia is a very effective method for improving the efficacy of radiation given in a stereotactic schedule. This enhancement also seemed to be relatively independent of the hypoxic modifier used.
Supported by grants from the Danish Cancer Society and the Danish Council for Independent Research: Medical Sciences.
No conflict of interest.

1051 ORAL

EGFR-amplification correlates with response to combined treatment of fractionated irradiation and EGFR-inhibition in HNSCC tumour xenografts

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Background: With the aim to identify potential biomarkers for the improvement of local tumour control by simultaneous EGFR inhibition during fractionated irradiation, 10 different HNSCC tumour xenografts are evaluated for local tumour control after irradiation with or without EGFR inhibition, while simultaneously investigating factors that might influence the response. Results of the first 5 tumour models are already published (Gurtner et al., Radiother Oncol 2011 (99):323–330) and revealed a correlation between EGFR-amplification (EGFR-CEP-7 ratio) and response to combined treatment of irradiation and EGFR-Inhibition with cetuximab. Here we present an update of the data on a total of 8 tumour models and a comparison of EGFR gene amplified versus non-amplified tumours.
Material and Methods: For evaluation of local tumour control dose 50% (TCD_{50}) 120 days after treatment tumours were treated with fractionated irradiation (RT) (30f/6 weeks) alone or combined with application of the monoclonal EGFR-antibody cetuximab (1 mg, weekly, i.p.). For tumour growth delay and relative tumor volume cetuximab was applied alone (once d0 or 4x d0, d2, d5, d7). Results were compared with molecular data on fluorescense-in situ-hybridisation (FISH) and immunohistochemistry.
Results: A significant improvement of local tumour control could be observed for the combined treatment of RT and cetuximab compared to RT alone in all 3 tumours harbouring an EGFR gene amplification. The enhancement ratios for the TCD_{50} after irradiation alone versus irradiation plus cetuximab were 2.0 (CAL-33), >40 (UT-SCC-14) and >44 (UT-SCC-8). In only 2 of the 5 tumour models without EGFR gene amplification local tumour control was improved by simultaneous Cetuximab application.
Conclusion: EGFR gene amplification appears as a promising biomarker for prediction of the improvement of local tumour control by combined fractionated irradiation and Cetuximab treatment. However, using this biomarker alone would result in some false-negative predictions, as also the group of non-amplified tumours contains some responder models. Thus, further biomarker evaluation is warranted to improve the validity especially in the latter subgroup of tumours.
Supported by Deutsche Forschungsgemeinschaft (DFG-PAK190).
No conflict of interest.

1052 ORAL

Myeloid cells as a novel determinant of hypoxic radioresponse in colorectal cancer cells through L-arginine turnover

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Introduction: Tumor-associated myeloid cells may undergo antitumor M1 or protumor M2 polarization linked to the alternative L-arginine turnover through inducible nitric oxide synthase (iNOS) and arginase-1 (ARG) respectively. The former pathway is known to result in production of nitric oxide (NO), a potent inhibitor of mitochondrial respiration. This study explored the ability of M1 versus M2 macrophages to affect radioresponse of colorectal cancer (CRC) cells in a model of metabolic hypoxia, and screened the iNOS/ARG signature of mouse and human tumor-associated myeloid cells.
Material and Methods: Mouse peritoneal macrophages were polarized to M1 and M2 types by exposure to IFN-γ/LPS or IL-4 respectively, and profiled by RT-PCR. CRC cells and macrophages were placed in a tissue-mimetic co-culture system (TMCS), wherein metabolic hypoxia was induced under limited oxygen diffusion. Oxygen depletion was assessed by fluorescence, and hypoxic radioresponse to 0−12 Gy by colony formation assay. ARG+ myeloid cells were phenotyped by FACS in colon CT26 mouse tumors and in 10 rectal cancer patients.
Results: M1 stimuli increased in macrophages the mRNA levels of iNOS, IL-6, IL-12α and IL-12β by 49700, 220, 260 and 740-fold respectively, and NO/nitrite output above 40 μM. M2 macrophages showed the up-regulation of ARG, Ym1, Fizz1 and CCL17 by 20, 499, 118 and 72-fold respectively, and no iNOS activation. In the TMCS, M2 macrophages significantly contributed to oxygen consumption and hypoxic radioprotection in CRC cells up to 2.5-fold, as compared with aerobic cells. Contrasting, M1 macrophages were able to uniformly restore impaired radioresponse of mouse CT26 and human DLD-1, HT29, HCT116, and SW480 CRC cells through NO-induced arrest of oxygen consumption resulting in oxygen sparing. The radiosensitizing effect was entirely attributed to iNOS+ macrophages since all CRC cell lines failed to activate the iNOS/NO pathway in the presence of M1 stimuli. In CT26 tumor-bearing mice, the myeloid $CD11b^+Gr\text{-}1^+$ subset underwent M2-type polarization marked by ARG activation. In cancer patients, whole blood CD15+ granulocytes displayed ARG overexpression and accelerated L-arginine turnover (up to 3-times), as compared with healthy donors.
Conclusions: CRC is associated with the predominant expansion of ARG+ myeloid cells that feature radioprotective properties and compete for L-arginine, thereby compromising the radiosensitizing potential of iNOS+ myeloid cells.
No conflict of interest.

1053 ORAL

A validated score predicting short survival (death within 30 days) after palliative radiotherapy: Better health economics and less overtreatment

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Background: Radiotherapy (RT) might palliate symptoms from incurable cancer. Its use should be tailored to prognosis and avoided in the terminal phase of disease. Survival prediction is challenging and recent reports have disclosed overutilization of RT during the final 30 days of life. Decision aids predicting short survival might help to avoid overtreatment.
Material and Methods: Uni- and multivariate analyses of factors predicting use of RT during the final 30 days of life for all palliative RT courses (metastatic or non-metastatic) administered at Nordland Hospital between 20.06.2007 (opening of radiation oncology unit) and 31.12.2009. Development of a predictive model by recursive partitioning analysis (RPA) and independent validation of its performance in patients treated during 2010 and 2011.
Results: We analysed 579 palliative RT courses given in the time period 2007–2009. Of these, 25 (4%) remained incomplete (typically because of clinical deterioration). Median survival was 6.3 months. In 53 cases (9%) RT was administered in the final 30 days of life. In univariate analysis, 19 factors were significantly associated with this endpoint. Multivariate analysis confirmed performance status (PS), primary tumour type, liver metastases, known disease progression outside the actual RT volume(s), steroid use, opioid analgesic use, serum haemoglobin, c-reactive protein and albumin levels as independent predictors for use of RT in the final 30 days of life. RPA resulted in a model consisting of 6 parameters (low haemoglobin, opioid analgesic use, ECOG PS 3−4, known progressive disease, steroid use, lung (small or non-small cell) or bladder cancer), which correctly identified 75% of RT courses administered during the final 30 days of life. Maximum survival of patients fulfilling all criteria was 69 days. Death within 40 days occurred in 83%. In the independent validation data set (2010–2011) these figures were 74% (30 days), 84% (40 days) and 100% (92 days).
Conclusions: Assigning the right patient to the right palliative approach is challenging. Based on our results, we suggest that patients with lung or bladder cancer with ECOG PS 3−4, low haemoglobin, progressive disease outside the actual RT volume, and on steroids and opioids are at high risk of dying shortly after initiation of RT. Our model facilitates decision making (best supportive care versus RT) and is the first decision aid specifically addressing RT in the final 30 days of life.
No conflict of interest.

1054 ORAL

Reduction of heart dose during left breast cancer radiotherapy: comparison between respiratory gating RT and IMRT

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Background: Breast conserving surgery and post-operative whole breast radiotherapy (RT) have proved to reduce local recurrence and improve survival of early stage breast cancer patients. It has also been demonstrated that long-term survivors have a significantly higher risk of cardiac death as a consequence of heart irradiation during left breast tangential RT. Novel technologies, such as prospective respiratory gating RT (PGRT) and intensity modulated radiotherapy (IMRT), provide a chance to reduce heart doses. This study compares the cardiac dose delivered by a standard 3D conformal tangential radiotherapy (CRT) to that delivered by PGRT or a 5 fields-IMRT.

Material and Methods: Patients with early left breast cancer, referred for adjuvant radiotherapy to our Institution, were enrolled in this study. For each patient, two simulation CT-scans were acquired: the first during free breathing and the second on prospective gating during deep inspiration breath-hold. The scans were monitored by the Varian RPM^{TM} respiratory gating system. For each patient, three treatment plans were performed: a 3D-CRT plan and an IMRT plan, based each on the free-breathing scan, and a PGRT plan based on the deep inspiration breath-hold scan. Mean heart dose (MHD), heart V_{25} and mean dose to the controlateral breast were evaluated. Dose-volume histograms were compared by the Friedman test.
Results: 44 patients were enrolled. Median age was 52 years (range 34−76), the mean breathing period was 4.3s (range 2–12.9), and the mean 4DCT scanning time was 12.5 s (range 10–16.5). Overall patients' compliance to respiratory gating technique was good. The median MHD was 3 Gy (range 1.22–7.38) in the CRT plans, 1.9 Gy (range 0.50–3.60) in the PGRT plans and 4.13 Gy (1.12−8.5) in IMRT plans ($p < 0.001$). The mean heart V_{25} was 1.06% (range 0−9.7), 0% (range 0−2.7) and 0.1% (range 0–5.82) for CRT, PGRT and IMRT plans, respectively ($p < 0.001$). Mean and maximum doses to the controlateral breast was 0.79 Gy (range 0.02−2) and 6.97 Gy (range 0.25–13.8) for the PGRT plans, and 1.98 Gy (0.05–5.38) and 9.39 Gy (range 3.73–20.57) for the IMRT plans ($p < 0.001$).
Conclusions: In this study the prospective gating tangential RT to left breast proved to offer the better dose sparing of heart, with also a lower irradiation of the controlateral breast.
No conflict of interest.

1055 ORAL

Present and future radiation therapy infrastructure in Europe: a wake up call to cut the mustard?

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Background: Radiation therapy (RT) is required in 50% of newly diagnosed cancer patients and 25% of those previously irradiated. The present status of teletherapy (TRT) units in 33 countries of Europe was recently published. To further look into a complete RT scenario with TRT and brachytherapy (BRT) units and manpower − radiation oncologist (RO), medical physicist (MP) and radiotherapy technologist (RTT) as exists today and projected needs till 2020, a comprehensive analysis for European countries was carried out.
Material and Methods: Data for 40 European countries, whose individual cancer incidences, equipments and manpower were available in the databases of GLOBOCAN and DIRAC were analysed. Norms recommended by IAEA and ESTRO-QUARTS were used to ascertain the adequacy of RT facility for each country.
Results: The combined outcome of these countries is summarized in the table.

Parameter	Total	Range (min to max)
Cancer incidence[2]	3,377,743	1,378 to 493,853
RT needed	2,111,089	861 to 308,658
TRT listed[3]	3,638	2, 529
TRT required	4,691	2, 686
Deficit in TRT	1,053	−283 to 59
BRT listed[3]	700	0 to 94
BRT required	1,055	1 to 154
Deficit in BRT	355	−95 to 7
RO listed[3]	7,399	2 to 1,218
RO required	7,037	3 to 1,029
Deficit in RO	Nil	−501 to 488
MP listed[3]	3,823	2 to 744
MP required	5,278	2 to 722
Deficit in MP	1455	−504 to 46
RTT listed[3]	13,213	4 to 4,241
RTT required	14,074	6 to 2,058
Deficit in RTT	860	−1,728 to 2,809

[2] http://globocan.iarc.fr

[3] http://www-naweb.iaea.org/nahu/dirac/default.asp

Additional number of TRT units, BRT units, RO, MP and RTT required by 2020 are 1661, 492, 550, 2138, and 2685 respectively.
Conclusions: None of the 40 countries have presently an all inclusive recommended number of RT units and manpower. While in some, the deficiencies are marginal, in others, a gross lack or imbalance of either manpower or treatment units or both were detected. This could lead to long RT waiting list, inadequate implementation of specialized RT techniques like IMRT, possibility of compromise on quality of RT and in some cases to even resort to 'fill in the RT waiting list' by chemotherapy. All these could seriously affect the treatment outcomes and nullify the efforts of early detection and treatment. Thus, in view of the imminent rise in cancer incidence during the next decade and the projected needs, adequate steps are highly desirable at individual country level or jointly to provide optimal RT access to all patients.
No conflict of interest.

Society Session (Sun, 29 Sep)
European Society for Therapeutic Radiology and Oncology (ESTRO)

1056 ORAL

VARIAN AWARD: Prediction of normal tissue morbidity in radiotherapy of prostate cancer using motion-inclusive dose distributions

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Background: In radiotherapy (RT) of prostate cancer the key organs at risk (ORs) − the rectum and the bladder − display considerable motion, which may influence the dose/volume parameters predicting for morbidity. In this study we compare motion-inclusive doses to planned doses for the rectum and the bladder and explore their associations with prospectively recorded morbidity.
Material and Methods: This study included 38 prostate cancer patients treated with hypo-fractionated image-guided intensity-modulated RT that had an average of nine (7−10) repeat CT scans acquired during treatment. These scans were registered to the respective treatment planning CT (pCT), based on rigid registration (translations) on intra-prostatic fiducial gold markers, followed by a new dose calculation on the repeat CT geometry. One motion-inclusive dose distribution was assessed for each patient and structure (rectum, rectum wall, bladder and bladder wall) by averaging over the, on average nine, motion-inclusive dose-volume histograms. The pCT volumes, the treatment course averaged volumes as well as the planned and motion-inclusive dose distributions were associated with acute and late morbidity (morbidity cut-off: $\geqslant$ Grade 2) using logistic regression.
Results: Acute rectal morbidity (observed in 11 (29%) patients) was significantly (p£0.05) associated with smaller treatment course averaged rectal volumes (population median: 75 vs. 94 cm^{-3}). Furthermore the motion-inclusive rectal wall volume receiving doses close to the prescription dose (2 Gy-equivalent dose of 76 Gy (a/b=3 Gy)) was also significantly associated with this morbidity end-point.
Conclusions: To the best of our knowledge, this study is the first to show that motion-inclusive DVH parameters are better predictors than DVH parameters based on the 'static' planning CT along. Both findings of the study are plausible − smaller rectal volumes are likely to be related to a more stable portion of this organ being located in the high-dose volume high and a high-dose relation with morbidity is in agreement with the QUANTEC study − increasing the credibility of the findings despite the modest patient number. This study indicates that the deviations between planned and delivered dose/volume parameters − caused by internal organ motion − should be accounted for to improve the ability to predict morbidity following RT.
No conflict of interest.

1057 ORAL

ACCURAY AWARD: Targeting radiation resistance in p53 mutant tumours

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Background: Increasing our understanding of how resistance to treatment occurs, helps us develop more efficient and personalised therapeutic regimes for cancer patients. Through molecular dissection of events that drive tumour initiation and progression, we have uncovered a functional connection between the most frequent oncogenic mutations that are likely to contribute to therapeutic resistance in 30% of all human tumours. Mutations activating the 'AKT/PI3K' signalling pathway and inactivation of

the '*TP53*' tumour suppressor gene are common mechanisms that cancer cells require to proliferate and escape pre-programmed cell death. Tumours employ many strategies to inactivate p53; however sequence mutations that result in mutant p53 protein (p53mut) are most often observed. p53mut tumours not only fail to respond to DNA damaging therapy, but are also described to promote therapeutic resistance by dominant negative suppression of p53 dependent promoter activity. We find that in combination these events lead to therapeutic resistance that is reversible by the AKT clinical candidate, MK-2206 and the PI3K inhibitor PI-103.
Methods: Using a combination of *in vivo* and *in vitro* techniques we have tracked the molecular mechanism for AKT mediated resistance to treatment in solid tumours. This has helped us to simultaneously derive potential biomarkers that could highlight where the greatest efficacy may be achieved in clinical practise.
Results: We demonstrate that AKT inhibition promotes reduced levels of p53mut in tumour cells via a novel regulation of the 'tumour suppressor' p14ARF and promotes re-engagement of cell cycle arrest, senescence and increased sensitivity to ionising radiation in both *in vivo* and *in vitro* systems. We also show that PI3K/AKT inhibitors- and as proof of concept, the clinical candidate AKT inhibitor, MK-2206, and PI3K inhibitor, PI-103, are effective in treatment of mice with therapeutically resistant tumours with elevated AKT and carrying p53mut.
Conclusions: We show that targeting the PI3K/AKT pathway sensitise xenografts carrying p53 mutations to DNA damaging therapy. We have also been identifying potential molecular markers to select the cohort of patient most likely to benefit best from this treatment.
No conflict of interest.

Poster Session (Mon, 30 Sep)
Radiobiology/Radiation/PhysicsRadiotherapy Techniques

1058 POSTER

CBCT-based internal gross tumor volume definition for radiotherapy of of non-small-cell lung cancer: Comparison with target volumes based on three-dimensional CT and four-dimensional CT

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Background: To evaluate the amount of respiration information included in CBCT and investigate the use of CBCT c-ombined with 3DCT and 4DCT in the definition of target volumes.
Materials and Methods: Thirty-first patients with NSCLC sequentially underwent 3DCT and 4DCT simulation scans of the thorax during free breathing. A 3D conformal treatment plan was created based on 3DCT. The first CBCT was performed and registered to the planning CT using the bony anatomy registration. All contours were performed by a radiation oncologist using the same contouring protocol. GTV-3D and GTV-50 were contoured based on 3DCT and end-expiration phase (50% phase) of 4DCT. Internal GTVs (IGTV-MIP and IGTV-CBCT) were contoured based on maximum intensity projection (MIP) of 4DCT and CBCT. The differences in the position, size, and degree of inclusion (DI) of different volumes were compared.
Results: The mean size ratio of GTV-3D, GTV-50, IGTV-MIP to IGTV-CBCT were 0.77±0.18, 0.84±0.2, 1.1±0.26 respectively for tumors in the upper lobe and 0.67±0.11, 0.65±0.18, 1.17±0.27 respectively for tumors in the middle and lower lobe. The motion vector showed a significant correlation to the ratio of GTV-50 to IGTV-CBCT ($r = -0.45$, $p = 0.012$) for all the patients. DIs of GTV-3D, GTV-50, IGTV-MIP in IGTV-CBCT were 0.65±0.27, 0.65±0.2 and 0.62±0.2, while DIs of IGTV-CBCT in GTV-3D, GTV-50, IGTV-MIP were 0.47±0.2, 0.49±0.2 and 0.67±0.19 respectively.
Conclusion: The tumor motion included in CBCT is significantly larger than 3DCT and end-expiration phase of 4DCT, but less than 4DCT MIP. CBCT can include the respiration motion information well compared to 4DCT MIP. The use of 3DCT registered to CBCT, or 4DCT registered to CBCT based on bony anatomy in radiotherapy may result in a severe target miss, which should be focused on when we perform adaptive radiotherapy and rectify treatment planning.
No conflict of interest.

1059 POSTER

Comparison of internal target volumes defined on three-dimensional CT, four-dimensional CT and cone-beam CT images of non-small-cell lung cancer

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Background: To compare positional and volumetric differences of internal target volumes defined on three-dimensional CT (3DCT), four-dimensional CT (4DCT) and cone-beam CT (CBCT) images of non-small-cell lung cancer.
Materials and Methods: Thirty-first patients with NSCLC sequentially underwent 3DCT and 4DCT simulation scans of the thorax during free breathing. A 3D conformal treatment plan was created based on 3DCT. The first CBCT was performed and registered to the planning CT using the bony anatomy registration. All contours were performed by a radiation oncologist using the same contouring protocol. GTVs were contoured based on 3DCT, maximum intensity projection (MIP) of 4DCT and CBCT. CTV3D, ITVMIP and ITVCBCT were defined with a 7 mm margin accounting for microscopic disease. ITV10 mm and ITV5 mm were defined based on CTV3D. ITV10 mm with a 5 mm margin in LR, AP directions and 10 mm in CC direction; ITV5 mm with an isotropic internal margin (IM) of 5 mm. The differences in the position, size, Dice's similarity coefficient (DSC) and inclusion relation of different volumes were compared.
Results: The median size ratio of ITV10 mm, ITV5 mm, ITVMIP to ITVCBCT were 2.33, 1.88, 1.03 respectively for tumors in the upper lobe and 2.13, 1.76, 1.1 respectively for tumors in the middle-lower lobe. The median DSC of ITV10 mm, ITV5 mm, ITVMIP and ITVCBCT were 0.6, 0.66 and 0.83 for all patients. The median percentage of ITVCBCT not included in ITV10 mm, ITV5 mm, ITVMIP were 0.1%, 1.63% and 15.21% respectively, while the median percentage of ITV10 mm, ITV5 mm, ITVMIP not included in ITVCBCT were 57.08%, 48.89%, 20.04%. The median percentage of ITVCBCT not included in ITV5 mm were 1.24% for tumors in the upper lobe and 5.8% for tumors in the middle-lower lobe ($p = 0.404$).
Conclusion: The individual ITV derived from 4DCT can't encompass the ITV from CBCT effectively, and using 4DCT ITV in radiotherapy may result in a target miss. The ITVs derived from 3DCT with istropic margins can encompass the CBCT ITV, but the size of 3DCT ITVs was far greater than the CBCT ITV. It is feasible to generate the 3DCT ITV with an isotropic IM of 5 mm for tumors in the middle-lower lobe.
No conflict of interest.

1060 POSTER

A correlation study on target displacement and its influencing factors of primary thoracic esophageal cancer during radiotherapy based on repeated four-dimensional CT scan

J.Z. Wang[1], J.B. Li[1], W. Wang[1], H.P. Qi[1], Z.F. Ma[1], Y.J. Zhang[1], T.Y. Fan[1], Q. Shao[1], M. Xu[1]. [1]*Shandong Cancer Hospital & Institute, Department of Radiation Oncology, Jinan, China*

Background: To investigate the correlation between the motion of gross tumor volume(GTV)and the tumor volume, length, the largest diameter of lesion in CT image with enhanced four dimensional computed tomography (4DCT) scanning during fractionated radiotherapy.
Material and Methods: Thirty-two patients with thoracic segment esophageal carcinoma were divided into upper (9 patients), middle (14 patients) and distal (9 patients) esophageal tumors, enhanced 4DCT were performed after every ten fractions. The gross tumor volume (GTV) were delineated by the same radiotherapist on each of the 10 4DCT phase, the displacements in left-right (LR), anterior-posterior (AP), senior-inferiors (SI) directions and three-dimensional vector were calculated, the tumor length and the largest diameter of lesion were also calculated. Then, investigate the correlation between the motion of GTV and the tumor volume, length, the largest diameter of lesion for every fraction.
Results: The correlation were not significantly between the motion of GTV and the tumor volume for the first positioning, the tenth fraction and the thirtieth fraction; but the correlation was significantly to the distal segment for the twentieth fraction in the LR direction ($r = 0.731$,$P = 0.040$). The correlation were not significantly between the motion of GTV and the tumor length for the first positioning, the tenth fraction and the thirtieth fraction; but the correlation was significantly to the upper and distal segment for the twentieth fraction in the SI direction($r = 0.714$,$p = 0.031$;$r = 0.646$,$p = 0.044$), the correlation was also significantly to the distal segment for the twentieth fraction in the LR direction($r = 0.765$,$p = 0.027$). The correlation were significantly between the motion of GTV and the largest diameter of lesion to all patients for the first positioning in LR, SI and three-dimensional vector ($r = 0.373$,$p = 0.036$;$r = 0.377$,$p = 0.033$;$r = 0.415$,$p = 0.018$), but the correlation were not significantly for the tenth fraction, the twentieth and the thirtieth fraction.

Conclusion:s There have an correlation between the primary tumor displacements and the tumor volume, length and the largest diameter of lesion during the radiotherapy, but the appearance of time and the degree of correlation were different.
No conflict of interest.

1061 POSTER

Research of primary thoracic esophageal tumor volume variance during radiotherapy based on reduplicated four-dimensional CT scan

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Background: To investigate the volume variation of primary thoracic esophagus carcinoma with enhanced four dimensional computed tomography (4DCT) scanning during fractionated radiotherapy.
Material and Methods: Thirty-two patients with thoracic segment esophageal carcinoma were divided into upper (9 patients), middle (14 patients) and distal (9 patients) esophageal tumors, enhanced 4DCT were performed after every ten fractions. The gross tumor volume (GTV) were delineated by the same radiotherapist on each of the 10 4DCT phase, $IGTV_{MIP}$ was the contour delineated from the maximum intensity projection (MIP), all 10 GTVs were combined to form $IGTV_{10}$, GTV_{mean} was the average of all 10 phases of each GTV.
Results: The majority of the GTV_{mean}, $IGTV_{MIP}$ and $IGTV_{10}$ were decreased with increasing fractions during radiotherapy based on repeated 4DCT scanning, but the volume change was different in different positions or different fractions. GTV_{mean} increased by 4.20–39.42% (1.31–7.44 cm^3) at the tenth fraction for 21.87% (7/32) patients, and the differences were significant ($t = -4.753$, $P = 0.003$). Except the upper esophageal tumors, statistical significance were existed in GTV_{mean}, $IGTV_{MIP}$ and $IGTV_{10}$ for all patients between the twentieth fraction and the first positioning (all $P<0.05$).
Conclusions: Repeated 4DCT scanning could not only observed the volume variance of primary tumor GTV without motion information, but also observed IGTV volume change. For primary middle and distal esophageal cancer, the best time to reset position should be selected at twentieth fraction when the primary tumor target volume changed significantly, and it was preferable to guide target correction and planning modification.
No conflict of interest.

1062 POSTER

Radionuclide therapy in cancer patients with bone metastases

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Aim: We submit our experience in the application of radionuclides for therapy of bone metastases of cancer patients with different foci of appearance of the cancer process: breast cancer, prostate cancer, lung cancer and others.
Material and Method: In 167 patients with bone metastases we used 32-P in 71 patients and 89-Sr in 96 patients. 93 are women and 74 are men; 90 women are with cancer of the breast, 52- with prostate cancer, 24 are with lung cancer and 1 with renal cell carcinoma. All patients are observed for side effects when an application of a radiopharmaceutical is done-32-P per os and 89-Sr intravenous, as well as for the changes of haemoglobin, leucocyte and thrombocyte count.
Results: One months after the radionuclide therapy we observed a significant reduction of the leucocyte and thrombocyte count in the group accepted 32-P ($t = 3.83$, $p < 0.05$; $t = 5.29$, $p < 0.001$). For the group, where 89-Sr is applied we did not calculate statistically significant decrease of the tested indicators mentioned above. At the same time the basic reason for which the radionuclides are applied- pain, decreases in intensity and patients return to their normal activity.This is more typical for patients with cancer of the breast and prostate cancer.
Discussion: The serious symptom 'pain' is influenced by the not so high applied activity of 2 mCi of 32-P speaks in favour of the use of this radiophar-maceutic, although there is a resistance among authors of its application with regard to myelosuppression. According to our observations it is light, the blood indicators restore quickly even without special therapy and patients can take the advantage of this additional method to overcome pain syndrome. The intravenous applicaton of 89-Sr in activity of 4 mCi also influences pain in a very good way, but the price per patient is high and all patients wait for an reimbursement from the health insurance. This is a significant factor for wider use. The use of 32-P is cheaper but the delivery stopped and for the moment we can use only 89-Sr.
Conclusion: The application of 32-P and 89-Sr is additional possibility for the treatment of pain due to bone metastases of cancer patients- easy and effective especially for those that are not treated effectively after local irradiation. The myelosuppressive effect is not statistically significant and both isotopes can be used successfully in the treatment of pain in cancer patients.
No conflict of interest.

1063 POSTER

Influence of radiotherapy on frequency of sister chromatid exchange, micronuclei and binuclear cells in breast cancer patients

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Purpose: Sister chromatid exchange (SCE) is a mutual segment exchange which occurs between two chromatids of sister chromosome that does not change the structure of the chromosome morphologically. Micronucleus (MN) is round objects stemming from nucleus and located in cytoplasm outside the main nucleus. Few studies have previously analyzed the effect of chemotherapy or radiotherapy on SCE or MN frequencies in breast cancer patients. The aim of this study was to evaluate any cytogenetic change in SCE, MN and binuclear cell (BNC) of peripheral blood lymphocytes in breast cancer patients treated with postoperative radiation therapy (RT).
Material and Methods: Frequency of the SCE, MN and BNC were examined in 22 breast cancer patients received RT and 10 healthy individuals. All parameters were measured before (RT-a), at the completion of (RT-b) and three months after the completion of (RT-c) RT. Median patient age was 47.5 (range: 29−58), and 13 (59%) were pre-menopausal, 9 (41%) were post-menopausal. Median RT dose was 50 Gy (range: 50−66). Eighteen (82%) patients received chemotherapy in addition to radiotherapy.
Results: A significant difference emerged in SCE ($p = 0.008$) and MN ($p = 0.004$) between RT-a and control groups. No statistical difference observed in BNC frequencies in breast cancer patients compared to control group.
Compared to the control group there was a significant increase in SCE frequencies in RT-b ($p = 0.008$) and RT-c ($p = 0.005$). There was also a significant increase in SCE frequencies in RT-b ($p = 0.001$) and RT-c ($p = 0.001$) values compared to RT-a measurements. There was not any statistically significant difference in the SCE frequencies between RT-b and RT-c measurements.
The frequencies of MN were also significantly higher in RT-b ($p = 0.005$) and RT-c ($p = 0.005$) than in control group. The MN frequencies were significantly increased in the RT-b compared to RT-a ($p = 0.001$). However, there was not any statistically significant difference in MN frequency between RT-a and RT-c measurements. MN levels decreased to pre-RT levels three months after completion of treatment.
No significant difference in BNC was observed between control group and any study group values.
MN decreased significantly at RT-c compared to RT-b ($p = 0.001$). No statistically significant difference was observed in MN frequencies between RT-a and RT-c (Table).
Conclusion: Increasing MN and SCE frequencies following radiotherapy is an expected situation. Decrease in MN frequency at 3-month after the completion of RT suggests that expected repair continues. Persistent SCE at the same period suggests that recovery in SCE has not completed yet and a longer period of time is needed.
No conflict of interest.

1064 POSTER

Unilobar transarterial radioembolization versus portal vein embolization for induction of contralateral liver hypertrophy in patients with secondary liver malignancies − a matched-pair analysis

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Background: Selected patients with liver malignancies can be cured by surgical resection. In case of a small-sized future liver remnant (FLR), portal vein embolization (PVE) of the tumour-bearing liver is used to induce hypertrophy of the contralateral lobe but leaves the tumour untreated and may even stimulate tumour growth. Unilobar transarterial radioembolization using Y90-labelled resin microspheres (RE) treats the tumour in the embolized lobe, reducing the risk of tumour progression, and has also been demonstrated to produce hypertrophy of the contralateral lobe. However, contralateral hypertrophy induction with these two modalities has never been compared directly. We performed a matched-pair analysis to compare the capacity for contralateral hypertrophy induction of PVE vs. RE.

Methods: Patients with secondary liver malignancies limited to the right lobe who were treated by right-lobar PVE (n = 141) or RE (n = 35) were matched according to the following criteria known to have an impact on liver regeneration following PVE: (i) Baseline FLR/Total liver volume ratio, (ii) prior platinum-containing chemotherapy, (iii) inclusion of liver segment 4 in the treatment, and (iv) baseline platelet count.The relative change in FLR volume from baseline to follow-up 4−6 weeks after treatment was calculated and compared between treatment groups using a one-way ANOVA.
Results: 21 fully matched pairs of patients were identified. After matching, minor differences between the PVE and RE groups were still seen in the interval between treatment and follow-up imaging, embolized liver volume, body weight, prevalence of colorectal cancer as the primary cancer site, and number of prior chemotherapy lines; however, covariate testing revealed none of these factors to have an impact on relative volume change in the contralateral liver lobe, indicating that the criteria used for matching resulted in two well comparable cohorts of PVE and RE patients. Baseline and post-treatment FLR volume as well as relative change in FLR volume before/after treatment are shown in table. The increase in FLR volume from baseline to follow-up was significant with both modalities but PVE produced significantly more FLR volume gain than RE (68.7 vs. 34.2%, p < 0.001). None of the RE patients demonstrated tumor progression in the embolized lobe at follow-up imaging.

	RE		PVE		
	Mean	SD	Mean	SD	
FLR baseline (mL)	246.1	115.6	287.1	84.3	
FLR post treatment (mL)	317.8	141.1	474.9	143.5	
Change from baseline (%)	+34.2	28.6	+68.7	39.0	<0.001
p(change from baseline within treatment)	<0.001		<0.001		

Conclusions: Our study shows for the first time that unilateral RE is inferior to PVE in terms of contralateral hypertrophy induction. However, contralateral hypertrophy induced by RE is substantial and RE minimizes the risk of tumour progression in the treated lobe, possibly making it a suitable modality for selected patients who need preoperative FLR hypertrophy induction and whose liver tumours are at risk of becoming locally unresectable in case of progression.
Conflict of interest: *Other substantive relationships: B. Garlipp has received lecture fees from SIRTEX Medical Ltd., J. Ricke has received lecture fees and research funding from SIRTEX Medical Ltd.*

1065 POSTER

Predictive nomogram for heterotopic ossification following radiation prophylaxis of traumatic acetabular fractures

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Purpose: To report clinically predictive model for heterotopic ossification (HO) in patients (pts) who have undergone surgical repair and radiation (RT) prophylaxis after traumatic acetabular fracture (TAF).
Methods: Between 1995 and 2010, 737 pts were eligible to our study. All underwent surgery followed by prophylactic post-operative RT after TAF. All pts received a single fraction of 7 Gy within 72 hours from surgery, prescribed to mid-plan without bone shielding. The data collected on each patient (24 categories in all) included: age, gender, race/ethnicity, body mass index (BMI), type of injury, time between injury and surgery, time between surgery and RT, use of indomethacin, NSAIDs, and narcotic, hip fracture, Brooker grade, etc.
Results: A logistic regression model was constructed and internally validated with bootstrapping techniques. Statistically significant predictors for HO were age, BMI, narcotic use, time interval between surgery and RT, and bilateral vs. unilateral acetabular fracture. The parameters in the final model were: age (coefficient=0.0211, standard deviation (SD)=0.0069, p = 0.0021), BMI (coefficient=0.0766, SD=0.0180, p < 0.0001), surgery to RT in days (coefficient = 0.1379, SD = 0.0968, p = 0.1546), bilateral vs. unilateral fracture (coefficient = −0.8683, SD = 0.3381, p = 0.0102), narcotic use (coefficient=0.7078, SD=0.2354, p = 0.0026). The area under the 'receiver operating characteristic' curve (AUC) for the predicted probability of HO was 0.731 (95% CI 0.70–0.76). The model calibrated well and demonstrated adequate discriminative ability.
Conclusion: This predictive nomogram provides a precise algorithm to predict the risk of HO. Prophylactic RT is an effective modality for HO prevention, however, patients' age should be wisely considered because of scattered RT dose to the reproductive organs (ovaries and testicles) and the risk of RT induced malignancies.
No conflict of interest.

1066 POSTER

Combination of topotecan and chronomodulated radiotherapy for treatment of xenografted human nasopharyngeal carcinoma

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Background: Nasopharyngeal carcinoma (NPC) is a malignant disease of the head/neck region and radiotherapy is the predominant treatment strategy. Here we reported a novel therapeutic strategy by combination of topotecan (TPT) and chronomodulated radiotherapy for NPC.
Materials and Methods: After a uniform biological rhythm was built through a light/dark cycle (LD 12:12). A xenografted NPC model was established by subcutaneous injection of poorly differentiated human NPC cells (CNE-2) to BALB/c (nu/nu) nude mice. Then the mice were separately administrated with: TPT (10 mg/kg), radiotherapy (RT), and TPT+RT, the anti-tumor effect was evaluated by analysis of tumor re-growth delay, the expressions of pimonidazole hydrochloride (HP-1), phosphorylated H2AX (γ-H2AX), DNA-topoisomerase I (Top I), cell cycle and apoptotic at 3, 9, 15, 21HALO (HALO means hours after light onset). The tumor-loaded mice without any treatment were used as the control.
Results: All therapeutic protocols showed the obvious tumor growth delay compared to the control at the same time point. In which, TPT+RT had the best tumor inhibitory effect and existed a remarkable increase in γ-H2AX expression and decrease in HP-1. For four time points, TPT+RT group at 15HALO showed the best inhibitory effect on tumor re-growth. The results revealed tumor hypoxia and DNA damage response varied in a time-dependent manner. The expression of Top I also showed obvious circadian rhythms with higher level at 15HALO and lower at 3HALO. Flow cytometry also appeared same trend of an increased apoptosis index and decreased proportion of S-phase cells (P<0.05).
Conclusions: Our study confirmed that combination of TPT and chronomodulated radiotherapy could enhance the radiosensitivity of xenografted NPC. TPT+RT group at 15HALO had the best therapeutic effect. The chronomodulated radiosensitization mechanisms of TPT might be related to many factors: tumor hypoxic state, cell cycle and apoptosis, DNA damage and the circadian rhythm of Top I.
No conflict of interest.

1067 POSTER

Treatment of glomus tumours with fractionated stereotactic radiation therapy and tomotherapy

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Background: Glomus are uncommon benign tumours. Treatment options are resection and radiotherapy. These tumours usually were treated using conventional RT 3D with 10−15 mm margins. Technical innovation allows to cover target volume with narrow margins. Since 2002 in our institution we use fractionated stereotactic radiation therapy (FSRT) in these tumours.
We analyze local control, clinical response and toxicity in patients treated with new techniques.
Methods and Materials: Between March 2002 and January 2013, 16 glomus were treated. 13 patients with FSRT and 3 with tomotherapy. Median follow-up was 31 months (range: 6−66.5).
Median age was 60 years (range: 46−81). 12 women and 4 men. Most common symptoms were tinnitus (13 patients) and injury lower cranial nerves (9 patients). Location: 87.5% jugular and tympanic. 93.8% unilateral. 43.8 % had surgical resection previously. Median glomus size was 30 mm (range: 10−70) and median target volume was 21.3 cc (range 6.37–464.2).
Median margins added to the gross tumour volume (GTV) to generate planning target volume (PTV) was 3 mm (range 2−10). The median total dose was 50.4 Gy (1.8 Gy per fraction) at 95% PTV coverage.
Median conformity index in FSRT was 1.2 (range: 1.18–1.47).
Results: No patients developed exacerbated symptoms or new neurological complications.
50% improved tinnitus and hearing.
No cases of clinical or radiological progression were identified.
63.7% of tumours were stable and 37.3% showed size reduction.
Conclusion: FSRT and tomotherapy are effective and safe therapies for patients with glomus. High local control rates associated with very low incidence of side effects were achieved. This treatment may be used in many patients as an alternative to surgical resection.
No conflict of interest.

1068 POSTER

Hypersensitivity of cancer cells derived from human thyroid, cervical and breast carcinomas to triptolide and its combination with radiation

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Background: Many human carcinomas are highly resistant to chemotherapy and radiotherapy; therefore, development of approaches to better therapeutic targeting such tumors is of paramount importance. In the present work, we examined the effects of triptolide (a diterpenoid triepoxide from the Chinese herb *Tripterygium wilfordii* exhibiting anticancer activity), as a mono-agent or in combination with gamma-radiation, on human carcinoma cells of different origin.
Materials and Methods: Three tumor cell lines (1) FRO (derived from human anaplastic thyroid carcinoma), (2) HeLa (derived from human cervical carcinoma) and (3) MCF-7 (derived from human breast carcinoma) were here studied. In the comparative experiments, human normal (non-tumor) epithelium cell lines 293 and HBL-100 were used as well. The cell death/survival was assessed in MTT-test, annexin-V staining and clonogenic assays. The expression of certain cell survival- and apoptosis-related proteins was explored by serial immunoblotting.
Results: We have established that the above three carcinoma cell lines are extremely sensitive to very low (0.5−5 nM) concentrations of triptolide and its combinations with low doses (2−5 Gy) of irradiation. This hypersensitivity was usually manifested in the suppressed cell proliferation, massive apoptotic cell death and sharply (100–10000-fold) impaired clonogenicity following the (co)treatment. As for the molecular basis of such enhanced cytotoxicity, the simultaneous inhibition of NF-kappaB- and Akt-mediated cytoprotective pathways in the cancer cells treated with triptolide seems to promote apoptosis that may be aggravated by such an apoptotic stimulus as radiation exposure. Besides, the known ability of triptolide to inhibit the HSF1-dependent expression of cytoprotective heat shock proteins (e.g. Hsp70 and Hsp27) may also help to kill the cancer cells treated with the drug. Importantly, when the above normal (non-tumor) cells were (co)treated by the same way, they exhibited the significantly less sensitivity as compared with the carcinoma cells.
Conclusions: The present findings allow us to hope that clinically achievable and tolerable concentrations of triptolide in the organism of patients will exert therapeutic effects upon carcinoma tumors and/or improve the outcome of anticancer radiotherapy.
No conflict of interest.

1069 POSTER

Quantitative evaluation of online correction shifts between bone and soft tissue matching for gated and non-gated stereotactic body radiation therapy (SBRT) treatments

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Purpose: To compare the use of setup marks on skin versus setup marks on immobilization device for patient positioning for SBRT. To determine the magnitude and frequency of online correction shifts between bone and soft tissue.
Methods: A retrospective study of two hundred and seven fractions of lung SBRT treatments was performed, of which one hundred and three were non-gated (group I) and one hundred and four were gated treatments (group II).
The first part of the study compared cone beam computed tomography (CBCT) localization using bone landmarks with reference to setup marks either on the patient or the immobilization device (BodyFix) in the anterior-posterior (AP), left-right (LR) and superior-inferior (SI) directions. The second part of the study evaluated if fluoroscopic localisation for moving tumours was influenced by change in tumour motion, baseline tumour position or if the internal target volume (ITV) created from treatment planning 4DCT was appropriate. Analysis of total tumour excursion was performed using CBCT for each fraction.
Results: The first part of the study demonstrated that the setup marks on the localization device yielded lower magnitude shifts in the AP and SI directions when using CBCT guided bone matching. Initial results show that 12% of gated treatments and 24% of non-gated treatments required soft tissue matching. Analysis of Group I and II showed that the ITV created from the initial 4DCT motion was adequate. The majority of soft-tissue moves were due to a change in baseline position of the tumour. An analysis of individual patients whose tumour motion was outside the norm (3 mm) will be presented. Data table 1 shows the number of total fractions and number of soft tissue shifts for gated and non gated treatments.

Table 1. The number of fractions of gated and non gated treatments where soft tissue shifts were performed. The number of shifts outside 0.3 cm is also presented.

	# Fractions	Ant	post	Sup	inf	Right	left
Gated	104	2	3	3	4	1	6
Soft tissue >0.3 cm		0	3	0	1	0	4
Non Gated	103	10	6	10	5	9	3
Soft tissue >0.3 cm		6	0	2	3	0	1

Conclusion: Based on this analysis, setup marks are placed on the immobilization (BodyFix) device for all SBRT treatments in our department. Our current motion analysis method has proven to be sufficient for creating ITVs. From our experience we have found that in most cases there may be no difference between online CBCT bone matching and soft tissue. However, the use of soft tissue verification and tumour motion using fluoroscopy is recommended on a daily basis.
No conflict of interest.

1070 POSTER

Comparison of target definition by 4DCT and 3DCT, the addition of asymmetric margins, or the addition of traditional margins for esophageal cancer

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Background: To investigate the 4DCT based planning target volume (PTV) definition compared to conventional PTV definition and PTV definition using asymmetrical margins for thoracic primary esophageal cancer.
Materials and Methods: Forty-three patients with esophageal cancer underwent 3DCT and 4DCT simulation scans during free breathing. The motion of primary tumors located in the proximal (group A), mid- (group B), and distal (group C) thoracic esophagus were obtained from the 4DCT scans. PTV3D was defined on 3DCT using the tumor motion measured based on 4DCT; PTVconv was defined on 3DCT using a 1.0 cm margin to CTV; PTV4D was defined as the union of the target volume contoured on the 10 phases of 4DCT images. PTV centroid position, volumetric differences and dice similarity coefficient (DSC) were evaluated.
Results: The median centroid shifts between PTV3D and PTV4D, PTVconv and PTV4D in the three dimensional directions were all less than 0.3 cm for the three groups. The median size ratio of PTV4D to PTV3D was 0.80, 0.88, 0.71 for group A, B and C, and for PTV4D to PTVconv was 0.67, 0.73, 0.76 respectively ($\chi 2 = -3.18, -2.98, -3.06$; $P = 0.001, 0.003, 0.002$). The DSC were 0.87, 0.90, 0.81 between PTV4D and PTV3D, with 0.80, 0.84, 0.83 between the PTV4D and PTVconv ($\chi 2 = -3.18, -2.98, -3.06$; $P = 0.001, 0.003, 0.002$). The difference between degree of inclusion of PTV4D in PTV3D and PTV4D in PTVconv was all less than 2%. Compared with PTVconv, PTV3D decreased 11.81% and 11.86% of irradiated normal tissue in group A and B respectively, but increased 2.93% for group C.
Conclusions: For proximal and mid- esophageal cancer, 3DCT-based PTV using asymmetrical margins provides a good coverage of PTV4D, meanwhile for distal esophageal cancer, 3DCT-based PTV using conventional margins provides an ideal conformity with PTV4D.
No conflict of interest.

1071 POSTER

Diffusion-weighted magnetic resonance imaging in radiotherapy: Do not forget about geometric distortion

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Background: Due to its high soft tissue contrast, MRI is commonly used in radiotherapy. Higher field strengths give higher signal-to-noise ratios and thus improved tissue contrast. Diffusion-weighted (DW) MRI enables measurement of the apparent diffusion coefficient (ADC) of the water in the field of view (FOV). Low ADCs are associated with higher cell densities and malignancy so DW MRI could improve tumour delineation. The most frequently used DW MRI technique comprises a single-shot, spin-echo

echo-planar imaging sequence. However, echo-planar images suffer from geometrical image distortions due to the relatively long gradient echo train. In theory, the incidence of these distortions increases as the field strength rises.
Material and Methods: We investigated two 3-T systems (a GE Discovery MR 750® and a Philips Achieva®) and two 1.5-T MRI systems (a Philips Intera® and a GE Signa HD®) with phased array coils. Distortion was assessed for a morphologic turbo spin-echo (TSE) T2-weighted sequence (contiguous axial slice thickness: 5 mm; matrix: 512x512; FOV: 30 cm) and DW sequences using different diffusion weightings (B values of 600, 1000 and 2000 sec/mm^2; matrix: 256x256; FOV: 30 cm). Acquisition characteristics with the phased array coil were the same for all MRI systems and distortion was measured on each ADC map. The geometric distortion was measured using a cylindrical MRI 3D Geometry Phantom® filled with an aqueous solution (dimensions: 21 x 22 x 23 cm; hole spacing: 1.5 cm; hole diameter: 0.3 cm; 196, 196 and 140 holes in the x, y and z plates, respectively). The deviation between the markers' theoretical and measured positions yielded the distortion as a function of distance to the center of the FOV. The results were compared in the Student's t-test and the threshold for statistical significance was set to $p < 0.05$. The statistical analysis was performed with SPSS software.
Results: For the two 1.5 T MRI scanners, the median ($\pm$ standard deviation (SD)) distortions on TSE T2 images were 1.2$\pm$0.3 mm, 2.4$\pm$5.2 mm and 3.2$\pm$5.4 mm at 35, 70 and 100 mm from the center of the FOV, respectively. These values were respectively 1.8$\pm$1.1 mm, 2.9$\pm$5.1 mm and 6.1$\pm$5.5 mm in ADC B600 images and 1.6$\pm$0.4 mm, 4.3$\pm$5.3 mm and 8.4$\pm$5.4 mm in ADC B1000 images.
For the two 3 T MRI scanners, the median$\pm$SD distortions on TSE T2 images were 0.2$\pm$0.1 mm, 0.7$\pm$6.7 mm and 0.9$\pm$7.2 mm at 35, 70 and 100 mm from the center of the FOV, respectively. These values were respectively 4.2$\pm$3.7 mm, 20.1$\pm$7.6 mm and 31.2$\pm$5 mm in ADC B600 images and 1.9$\pm$2.8 mm, 18.1$\pm$4.5 mm and 25$\pm$4.3 mm in ADC B1000 images. Distortion was significantly greater in 3 T scanners than in 1.5 T MRI scanners ($p < 0.01$) for DW images but not for T2 sequences. The B2000, B1000 and B600 distortions were all significantly different ($p < 0.01$).
Conclusion: Distortion is much greater in DW MRI than in morphological MRI and must be carefully assessed and corrected before it can be integrated into radiotherapy planning. The development of higher gradients and/or readout-segmented echo-planar imaging is probably a promising approach.
No conflict of interest.

1072 POSTER

A preliminary study of bone marrow metabolic response after pelvic radiation on 18F-FDG PET-CT: Correlation with hematologic toxicity

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Background: The relationship between metabolic decrease and radiation dose in irradiation field has not been revealed. The aim of this study is to evaluate the impact of pelvic radiation on bone marrow (BM) metabolic response using 18F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) and hematologic toxicity according to metabolic change.
Material and Methods: We analyzed 19 gynecologic patients treated radiotherapy with or without chemotherapy. The patients underwent baseline 18F-FDG-PET before treatment, immediate and delayed 18F-FDG-PET after treatment. Pelvic bone marrow (BM) was defined as the region extending from the iliac crests to the ischial tuberosity. On the 18F-FDG-PET, the 0.8 cm $\times$ 0.8 cm sized round region of interest (ROI) was drawn in each 10, 20, 30, and $\geqslant$40 Gy (V10, V20, V30, and V40, respectively) irradiated volume, and in the control (the first lumbar spine (L1)). Each ROI was divided by that of the liver for metabolic correction. The correlation between BM metabolic change of each dose regions, BM dose-volume metrics and hematologic nadirs (hemoglobin (Hb), white blood cell (WBC), platelet (PLT), and absolute neutrophil count (ANC)) were evaluated.
Results: The median age was 66 years old. All the patients were uterine cervical cancer except two (endometrial cancer, uterine leiomyosarcoma). The median radiation dose to pelvis was 45 Gy (range, 19.8 Gy-60.4 Gy). In comparison with pre-treatment 18F-FDG-PET, the metabolic change in the immediate 18F-FDG-PET was 29.54%, 33.43%, 36.60%, 37.41% and in the delayed 18F-FDG-PET was 31.04%, 41.73%, 44.38%, 27.71% (V10, V20, V30, V40, respectively). The metabolism of the L1 was not affected according to the hematologic change. The relationship between interval metabolic changes of V10−40 on the immediate, delayed 18F-FDG-PET and radiation dose were significant according to the linear regression model (immediate t=4.992, p<0.005, delayed t=2.167, p = 0.034). Percent hematologic changes were 15.49%, 50.96%, 39.77%, 45.21% (Hb, WBC, PLT, and ANC, respectively). Hematologic changes were not correlated with immediate and delayed metabolic response significantly.
Conclusions: The metabolic change of pelvic BM after radiotherapy was linearly correlated with irradiated dose, however, was not connected with hematologic alteration.
No conflict of interest.

1073 POSTER

Assessment of esophageal tumor motion and impact uncertainties using four-dimensional computed tomography

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Background: To access the three-dimensional motion caused by respiration and its influencing factors for radiotherapy of the thoracic primary esophageal cancer.
Methods: Sixty-five patients with esophageal cancer underwent 3DCT and 4DCT simulation scans during free breathing. The amplitude was calculated as the maximum difference between any of the 10 phases. The distance between the GTV upper/lower edges and the interesting target (carina, lower edge of aortic, the apes of the doms of the diaphragms) were measured. The motion of different thoracic groups was measured.
Results: The centroid motion of GTV were (0.15$\pm$0.10) cm, (0.12$\pm$0.15) cm, (0.34$\pm$0.15)cm in lateral(LR), anteroposterior (AP) and superioinferior (SI) directions, respectively. There were no relationship between GTV motion and patient gender, age and body mass index(BMI)($p > 0.05$). Tumor in the lower thorax had a lager displacement in LR and AP directions than tumor in the upper and mid- thorax($p = 0.036$, 0.014). Squamous carcinoma exhibited smaller motion than adenocarcinoma in all the three dimensional directions. A significant difference between the motion of the tumors with different length, and no significant difference was found in the motion of these locations in the absence or presence of the enlarged nodes. There is a negative correlation between the GTV movement and the distance between the GTV upper/lower edges and carina.
Conclusions: The greatest motion was seen in the SI direction for the thoracic esophageal cancer during free breathing. Appropriate site-specific internal target volume expansion should be consulted the tumor location and histologic type, and correlated with the distance between the GTV upper/lower edges and carina. The recommend margins might be applicable to patients with or without involved nodes.
No conflict of interest.

1074 POSTER

Comparison of the thoracic esophagus position and volume between quiet end-inspiration and end-expiration three dimensional CT assisted with active breathing control and corresponding phases in four dimensional CT

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Background: To compare the position, volume and matching index (MI) of the thoracic esophagus between quiet end-inspiration and end-expiration three dimensional CT (3DCT) assisted with active breathing control (ABC) and the corresponding phases in four dimensional CT (4DCT).
Material and Methods: Eleven patients with peripheral lung cancer underwent 4DCT simulation scan and 3DCT simulation scans in end-inspiratory hold (CT_{EIH}) and end-expiratory hold (CT_{EEH}) in succession. The 4DCT images from each respiratory cycle were sorted into 10 phases: the 0% phase was defined as end-inspiratory phase (CT_0), while the 50% phase was defined as end-expiratory phase (CT_{50}). The proximal, mid- and distal thoracic esophaguses were delineated separately on CT_0, CT_{50}, CT_{EIH} and CT_{EEH} images.
Results: In the X direction, the displacement differences of the proximal, mid- and distal thoracic esophaguses between CT_0 and CT_{EIH} were (−0.02$\pm$0.16)cm, (0.06$\pm$0.26)cm and (0.10$\pm$0.33)cm respectively, and in the Y direction, that were (0.04$\pm$0.24)cm, (0.04$\pm$0.12)cm and (0.08$\pm$0.15)cm respectively, and the displacement differences of the same direction were not statistically significant (all $P > 0.05$). In the X direction, the displacement differences of the proximal, mid- and distal thoracic esophaguses between CT_{50} and CT_{EEH} were (−0.02$\pm$0.24)cm, (0.12$\pm$0.37)cm and (0.26$\pm$0.33)cm respectively, and in the Y direction, that were (0.03$\pm$0.21)cm, (0.04$\pm$0.17)cm and (0.14$\pm$0.18)cm respectively, and the displacement differences of X and Y directions of proximal and mid-thoracic esophaguses between CT_{50} and CT_{EEH} were not statistically significant (all $P > 0.05$), while that of distal thoracic esophaguses between CT_{50} and CT_{EEH} were both statistically significant (both $P<0.05$). The volumes of the proximal, mid- and distal thoracic esophaguses were all

larger in CT_0 and CT_{50} than in CT_{EIH} and CT_{EEH}, but the differences between them were found both not statistically significant ($P > 0.05$). The MIs of the volumes of the proximal, mid- and distal thoracic esophaguses between CT_0 and CT_{EIH} were (0.50 ± 0.17), (0.50 ± 0.19) and (0.56 ± 0.08), and that between CT_{50} and CT_{EEH} were (0.50 ± 0.16), (0.47 ± 0.14) and (0.51 ± 0.15). The MI of each segment esophagus between CT_0 and CT_{EIH} was larger than that between CT_{50} and CT_{EEH} ($P > 0.05$).
Conclusions: The influence of breathing modes to the centroid positions of the proximal, mid-thoracic normal esophaguses were not significant. But there were spatial mismatches for any segment esophagus between two breathing modes.
No conflict of interest.

1075 POSTER

Low dose pre-irradiation and radio-adaptive response detected by MTT in HT29 and MRC5 cell lines

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Background: The effects of radio-adaptive response is the main interest of many studies. Adaptive response can lead to hypersensitivity or radioresistance. Both phenomena play important role in radiotherapy. The aim of this research was to examine the effects of low-dose pre-irradiation followed by two different irradiation regimes on metabolic activity in two cell lines: HT29 human colorectal adenocarcinoma and fetal fibroblasts MRC5 cell lines.
Material and Methods: The cell lines were pre-irradiated with 0.03 Gy, 0.05 Gy and 0.07 Gy and the control cell lines were not pre-irradiated. Both, control and pre-irradiated cells were irradiated two hours after priming dose and specially designed hyper- and hypofractionation regimes were applied. For the hyperfractionation the calculated doses were 1.3 Gy twice per day, with four hours period between daily fractions during four consecutive days. For the hypofractionation the calculated doses were 4.6 Gy on the first and fourth day, once per day, in order to obtain same overall treatment time as in hyperfractionation regime. The determined irradiation doses were estimated as biological equivalent (BED) to four-day treatment with 2 Gy fraction. Cell survival was tested by MTT. STATISTICA 10.0 software was applied in data processing.
Results: In hyperfractionation regime the low-dose of 0.05 Gy led to a significantly induced radioresistance in MRC5 cells compared with non pre-irradiated control. Low doses of 0.03 Gy and 0.07 Gy significantly increased radioresistance in HT29 cell line compared with non pre-irradiated control and pre-irradiation dose of 0.05 Gy. Statistically significant decrease of metabolic activity in HT29, after 0.05 Gy priming dose was observed, compared with 0.03 Gy and 0.07 Gy priming doses. In hypofractionation regime after priming dose of 0.05 Gy, as in hyperfractionation regime, statistically significant increase of cell metabolic activity in MRC5 cells was observed. In HT29 cells 0.05 Gy and 0.07 Gy priming doses led to a significance decrease of metabolic activity compred with irradiated control.
Conclusions: In both regimes, the priming dose of 0.05 Gy followed by challenging dose after two hours, led to decrease of metabolic activity in human colorectal cancer cells, and at the same time had significant radioresistance effect on the metabolic activity of human fetal lung fibroblasts. These results represent promising effect of applied low-dose pre-irradiation followed by different irradiation regimes afterwards.
No conflict of interest.

1076 POSTER

Emodin enhances growth suppression of human hepatoma cells by irradiation via enhancement of apoptosis and inhibition of cyclin D1

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Background: The application of radiotherapy of hepatocellular carcinoma (HCC) is limited due to radioresistance in tumor and radiotoxicity in nontumorous liver. Therefore, study for radioresistance mechanism and improvement of killing effect of irradiation by therapeutic insult such as radiosensitizer etc. Emodin (1,3,8-trihydroxy-6-methylanthraquinone), a family of plant derived polyphenol has been proven to have anticancer properities. There is limited data about role of emodin as radiosensitizer in human hepatoma cell line. In this study, we examined the followings: (i) whether emodin attenuated radioresitance of hepatoma cell line, (ii) what was the mechanism of radiosensitization.
Material and Methods: Methods: Two human HCC cell lines were used in this study: HepG2, and Hep3B. They were exposed to four different manners; none (control), irradiation (10 Gy, one fraction), emodin (10 μM), and irradiation combined with emodin. Cells were then subjected to MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) and immunoblotting in 24 hours and 72 hours after exposure.
Results: The growth suppression of two cell lines was significantly more enhanced compared to control group in the same order as followings; combination group, emodin, and irradiation. Treatment with irradiation combined with emodin resulted in maximal upregulation of apoptotic signaling such as poly (ADP-ribose) polymerase (PARP) and caspase-9 and downregulation of proliferation signaling such as cyclin D1.
Conclusions: Emodin enhances the activity of irradiation in tumor growth suppression of human hepatoma cells via enhancement of PARP, caspase-9 and inhibition of cyclin D1. Therefore, our findings may provide new insights into understanding the pharamacological mechanism of emodin as radiosensitizer in HCC and may aid in the design of new therapeutic strategies for the radioresistant HCC.
No conflict of interest.

1077 POSTER

Endocrinological late effects after radiotherapy

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Background: Treatment for cancer might lead to hormonal dysfunctions. Aims of this cross-sectional study were to assess the prevalence of disturbances in four hormonal axes: Hypothalamic-pituitary (HP) − thyroid axis, HP-adrenal axis, HP-gonadal axis and Growth hormone (GH), and to analyze associations between disturbances and patient- and treatment-related variables. Here we report frequency of abnormal values for all axes and analyze variables related to the thyroid- and GH axes.
Material and Methods: Eligible patients aged $\geqslant$15 years at diagnosis were treated for lymphoma, plasmacytoma, multiple myeloma or carcinoma of the epipharynx region by radiotherapy to the head and neck region or total body irradiation, with or without chemotherapy, from 1980–2006. For each axis the hormonal function was dichotomized as normal or impaired according to reference values at the laboratory. Treatment data was obtained from the patient records, and the radiation dose to the pituitary and thyroid gland was estimated. Comparisons were performed by chi-square and t-tests with $p < 0.05$ considered statistically significant (two-sided).
Results: A total of 84 males and 57 females treated for lymphoma (HL=20, NHL=103), multiple myeloma (n = 5), plasmacytoma (n = 3) and carcinoma (n = 10) were included. Observation time was 15.8 (6.4) years, age at diagnosis was 43.4 (15.1) years and at age survey 59.2 (12.9) years (mean, SD). Forty-nine (37%) survivors had biochemical hypothyroidism, 35 (25%) had abnormal GH- and/or IGF-1 values and 2 had abnormal values in the adrenal axis. Forty (48%) men had impaired gonadal function and 10 (18%) women had premature menopause (<42 years).
Survivors with hypothyroidism had received a significantly higher dose to the thyroid gland and had significantly longer observation time than those with normal thyroid function [mean 29.8 Gy (SD 16.5) vs 12.9 Gy (SD 15.8) $p < 0.001$, mean 18.3 years (SD 6.2) vs 14.1 years (SD 6.2), $p < 0.001$]. Survivors with abnormal levels in the GH-axis had received a non-significant higher radiation dose to the pituitary gland [mean 16.9 Gy (SD 18.3) vs mean 12.4 Gy (SD 11.9) ($p = 0.19$)]. There were no significant associations between gender and age at survey and abnormal levels in the GH axis and/or the thyroid axis.
Conclusions: Abnormal hormone values are frequent after radiotherapy to the head and neck region and should be measured in follow up. The clinical impact of failure in the GH axis needs further examinations.
No conflict of interest.

1078 POSTER

Do we need adaptive radiotherapy in head and neck cancer to decrease xerostomia?

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Background: Significant morphologic variations can be observed during the course of locally advanced head and neck cancer (HNC). The purposes of this study were:
– to estimate the difference between the planned dose and the actual delivered cumulative dose in the parotids (PG),
– to assess the benefit of a weekly replanning to spare the PGs.
Material and Methods: 11 patients (pts) with locally advanced HNC received IMRT to a total dose of 70 Gy. Each pt had one initial planning CT (CT0) and a weekly CT (wCT) during the treatment. The anatomical structures were manually segmented on each CT. The dose distribution corresponding to the pretreatment planning was calculated on each wCT. A new IMRT planning was also generated on each wCT, in order to spare the PGs at least as they were spared at the pretreatment planning. Each weekly dose distribution was reported on the CT0 by elastic registration. Two kinds of cumulative dose were therefore calculated on the CT0: one corresponding to the actual delivered dose and the other one to the weekly re-planned dose. The cumulative doses (with or without replanning) were compared with the planned dose for the PGs. The risk of xerostomia was estimated by the NTCP model (n = 1, m=0.4, TD50=39.9).
Results: The volume of the PGs decreased of an average of 33% [10−69 %] during the treatment. The average Dice score for the parotid registration was 0.93 (0.88–0.95). The dose in the PGs was increased in 62.5% of the PGs, by comparing the planned dose with the actual delivered dose. This increase was observed in 50% of the homo-lateral PG and 75% of the contra-lateral PGs. An increase of more than 3 Gy of the mean PG dose was observed in 31 % of PGs (up to 10 Gy for 1 PG),observed in 50% of pts and corresponding to an absolute average increase risk of xerostomia of 13% [6−24%]. A weekly replanning allows reducing the mean PGs dose at least at the same dose than at the pretreatment plan for more than 80% of the pts ($D_{mean\ Replan}$ = 31.3 Gy, $D_{mean\ initial}$ = 32.5 Gy, p = 0.013). Moreover, the doses in the CTV and PTV were not statistically different between the pre-treatment planning and the weekly re-planning.
Conclusion: A weekly re-planning can benefit significantly to one third of the PGs (and 50% of the pts), leading to decrease the absolute risk of xerostomia of at least 13%. An ongoing randomized IMRT trial comparing one planning to a weekly planning aims demonstrating the benefit on this adaptive radiotherapy strategy.
No conflict of interest.

1079 POSTER

Nodal PTV margin validation for head-and-neck cancer patients (HNC) treated with image-guided tomotherapy: Need of adaptive re planning?

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Background: Changes in dimension, shape and position of positive lymphnodes (PLs) in patients treated with image-guided RT for HNC may lead to dangerous geographical misses, especially in the case of concomitant boosting of PET PLs. Aim of this study was to assess the entity of the problem and to check if the margin (5 mm) used in our simultaneous-integrated-boost (SIB) approach with Helical Tomotherapy (HT) is appropriate.
Material and Methods: Thirty-seven HNC N2/N3 patients (pts) treated with SIB HT (delivering 54, 66 and 69 Gy in 30 fr on PTV(N), PTV($T+N^+$) and PTV of the PET-positive $T+N^+$) were considered. Regarding PLs position: 33, 7 and 2 PLs were respectively in levels II, III and V. For each patient, MVCTs taken at fr 1, 5, 10, 15, 20, 25 and 30 were matched with the planning kVCT (pl_kVCT) on bone anatomy (averaged on the treated volume). Three experts contoured 42 PLs of 30 pts on the pl_kVCT and all MVCTs (7 patients were excluded because PLs were not visible on MVCT). Intra-observer variability was assessed by blind re-delineation of 16 PLs. Volumes were normalized to fraction 1 MVCT and time-trend in volume change was assessed by Spearman's test. PLs displacement was assessed by the center of mass (CM) shift with respect to the 1st MVCT. For each PL, the % fraction of the union (UN) of all PL positions over the whole treatment that was missed by the clinical PTV (pl_kVCT PL contour+5 mm) was assessed. For pts with some missing, larger margins were tested to find UN coverage >99%.
Results: PLs were sufficiently well visible on MVCT: an acceptable intra-observer variability confirmed this impression (median DICE: 0.805±0.134). 27/42 PLs showed an average volume reduction of 70% (range: 27−94%), with significant time trend (median Spearman ρ = −0.93; range −0.78÷-1.00; p < 0.05).
Larger 3D average CM shifts on the whole population were observed in the 2nd part of the treatment toward the midline (medial shift in the 1st and 2nd part: 0.1 vs 1.6 mm, Wilcoxon test p < 0.001). Only 7/42 PLs of 7/30 pts needed margins >5 mm: respectively 6 and 7 mm for n = 2; 8, 9 and 12 mm for n = 1.
Conclusions: Results show a significant, although small, residual error in the localization of PLs during IGRT for HNC. 64% PLs show a significant shrinkage. A margin of 5 mm covers all possible positions of PLs in about 80% of pts, extending to 93−97% with a margin of 8−9 mm. Interestingly, the shrinkage seems to counterbalance the shift of PLs due to deformations, more pronounced in the last fractions. Seeing these results, adaptive re-planning aiming to avoid to miss PLs should not be recommended (excepting very selected pts) also in the case of PET PLs boost.
No conflict of interest.

1080 POSTER

Evaluation of EPID based transit dosimetry on the implementation of In-vivo dosimetry and a step forward for dose guided adaptive radiation therapy

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Background: The common practice of In-vivo dosimetry using point detectors were very successful with conformal treatment techniques but could not be extended its usage effectively for modulated treatment techniques. To address the need for an efficient in-vivo dosimetry system for the modulated treatments, Edinburgh Cancer Centre collaborated with Math Resolutions, U.S.A, to develop a 'transit dosimetry' solution using Electronic portal imaging device [EPID] based data acquisition. The algorithm used in 'Dosimetry Check' software was extended to support transit dosimetry and was extensively tested. Further studies were performed to integrate cone beam CT based dose calculations using transit dosimetry.
Material and Methods: 6MV and 10MV clinical photon beams delivered from Varian Accelerators (Clinac 21EX, Novalis − Tx and Silhouette) and amorphous silicon based EPID [aSi 1000] systems mounted on these accelerators were used in this study. For conformal and fixed gantry dmlc based modulated techniques, transit images were acquired in 'integrated' acquisition mode and for volumetric modulated [RapidArc] deliveries, 'continuous' acquisition mode is used. All the patient treatments were planned using Eclipse [Varian, U.S.A] treatment planning system. Cone beam images were taken only for RapidArc treatment deliveries using Varian On-board Imager system [Ver.15].
The transit dose calculations were initially tested using beam deliveries on water equivalent slabs and compared with 0.6cc ion chamber based measurements. Reproducibility and sensitivity of the transit dosimetry process was tested using fixed gantry dynamic MLC based modulated deliveries on an anthropomorphic thoracic phantom.
Results: The water equivalent phantom based verifications agreed within +/− 3% with the treatment planning system and the ion chamber measured values. The reproducibility study using the anthropomorphic phantom resulted within the standard deviation of +/− 0.005%. The sensitivity study with a known manual shift of the phantom by 5.0 cm shift resulted 7% dose deviation.
The agreement of transit dosimetry based dose estimations at the isocentre for 325 clinical patients so far collected from different clinical sites treated with conformal techniques agreed with the treatment planning results within a mean value of +/− 4.0%. Larger deviations of the order of +/− 10% were observed from Ca. Breast treatments especially if the mono-isocentric technique is used. For H&N and Prostate RapidArc deliveries, the results were of similar magnitude with the independent verifications using ArcCheck diode array system [Sun Nuclear, U.S.A].
Conclusions: EPID based transit dosimetry using 'Dosimetry Check' software can be reliably used for in-vivo dosimetry purposes for all co-planar treatment techniques. The reliability of the cone beam based calculation highly depends on the reproducibility of the Hounsfield units and the registration process.
No conflict of interest.

1081 POSTER

Long-term risk of ischemic heart disease following Hodgkin lymphoma treatment

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Background: Hodgkin lymphoma (HL) is the prototype of a curable malignancy. However, treatment causes excess cardiovascular morbidity and mortality in long-term survivors. The objective of this study is to identify risk factors for ischemic heart disease (IHD), defined as myocardial infarction (MI) and angina pectoris (AP) (⩾grade 2 CTCAE4.0). We quantified separate and joint effects of radiation dose to the heart, anthracycline dose, other chemotherapeutic agents, lifestyle factors and established cardiovascular risk factors.
Methods: A nested case-control study was conducted in a cohort of 2201 5-year HL survivors who were treated in the Netherlands between 1965 and 1995. Cases with IHD were matched to controls with HL who did not develop IHD (ratio 1:2 at least) on sex, age, date of HL diagnosis and duration of follow-up. Detailed treatment information was collected from medical records. Radiation dose to the heart was based on the prescribed mediastinal dose reported in the radiotherapy charts. Conditional logistic regression was used for analyses.
Results: 180 cases with IHD were identified from the cohort and matched with 499 controls. Mediastinal radiotherapy (usually performed using parallel opposed fields) was associated with an increased risk of IHD (OR: 3.0, 95% CI: 1.7−5.4). A dose-response relationship was identified (OR per 10 Gy: 1.2, $p = 0.004$). As compared to patients who did not receive mediastinal irradiation, we observed increased risks of IHD for patients who received 20−34 Gy (OR: 2.1, 95% CI: 0.97–4.63), 35−39 Gy (OR: 2.0, 95% CI: 1.10–3.51) or ⩾40 Gy on the mediastinum (OR: 3.6, 95% CI: 1.94–6.77) ($p < 0.001$), after adjusting for smoking at HL diagnosis and the presence of cardiovascular risk factors at cut-off (Diabetes Mellitus type II, hypertension, hypercholesterolemia) or obesity at diagnosis or at cut-off. No associations or interactions were found with (anthracycline-containing) chemotherapy.
Conclusions: Mediastinal irradiation is associated with a dose-dependent increased risk of IHD in patients treated for Hodgkin lymphoma.
No conflict of interest.

1082 POSTER

Multidisciplinary management of small cell cancer of non-pulmonary origin

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Background: While small cell lung cancer is managed by chemotherapy generally followed by consolidative radiation therapy, management of a non-pulmonary primary is less clear. In contrast to pulmonary small cell, aggressive surgery for these primaries is often employed. We analysed treatment technique and outcome in a large cohort of these patients to better define the role of surgery, radiation and chemotherapy.
Materials and Methods: An IRB approved chart review of all primary small cell cancers of non-pulmonary origin returned 60 (65% male, mean age 67 years) histologically confirmed cases. A total of 52 were pure small cell and the remainder were mixed with adenocarcinoma (n = 3), transitional cell carcinoma (n = 2), sarcomatoid (n = 2) and squamous cell (n = 1). Primary tumour location was head and neck (n = 20), bladder (n = 8), rectum (n = 7), prostate (n = 6), cervix (n = 5), vagina (n = 4), oesophagus (n = 4) and 1 case each from: anus, axilla, breast, ovary, trachea and inguinal. Treatment varied by anatomic site, with 31 (52%) patients undergoing definitive surgery, 51 (85%) undergoing chemotherapy, and 100% undergoing radiation therapy to the primary and regional nodes. External beam radiation therapy (XRT) was intensity modulated or 3D in almost all cases with 20 (33%) also undergoing image guided radiation therapy. The mean XRT dose to the primary was 52.4 Gray.
Results: Treatment was well tolerated. Late morbidity (grade 1/2) included 3 cases of xerostomia and 1 case each of stricture, cystitis, urinary frequency and anal incontinence. Local control was maintained in the majority of patients. Failure was most commonly distant (bones, liver, brain) with only one patient developing lung metastasis. Only 30% of patients are alive at last follow-up. Univariate analysis of local control and survival revealed that chemotherapy ($p = 0.01$) and male gender ($p = 0.007$) were statistically significant variables. Type of surgery and extent of surgery (definitive versus biopsy) did not impact outcome.
Conclusions: Small cell cancer of non-pulmonary origin is aggressive with high rates of systemic spread and poor 5 year outcome. The major treatment factor impacting survival remains chemotherapy. The addition of radiation to chemotherapy allows for control of the primary tumour. The role of surgery is limited as no benefit to survival or local control was seen. Responders may undergo consolidative XRT to the primary site. Consideration for central nervous system prophylaxis by radiation is also suggested by these data, as this is a common site of failure.
No conflict of interest.

1083 POSTER

Patterns of tumor response after stereotactic radiosurgery for vestibular schwannoma

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Background: Stereotactic radiosurgery (SRS) is commonly used for the treatment of vestibular schwannoma. In this study, we evaluate the control rates and MRI changes after treatment with Linac based SRS.
Material and Methods: 27 consecutive patients treated with 12 to 14 Gy using LINAC based SRS at a single institution from 2007–2013 were analyzed. Baseline and post-treatment tumor volumes were assessed on T1 weighted contrast enhanced magnetic resonance imaging. Local control and changes in tumor volume were calculated.
Results: Median follow up time was 35.6 months (range 1.5–61.9) and median treatment tumor volume was 1.1 cc (range: 0.1−8.7 cc). Sixty three, 33, and 4% of patients had reduction, stable, and progression in the tumor volume at their last post treatment MRI, respectively. Median SRS treatment dose was 12 Gy (range: 8−14) treated to 100% of the PTV. Median Dmin was 99.6% (range: 60.9–100), and median Dmax was 124.2% (range: 108.7–148.5%). Median number of beams was 12 (range: 8−15). 42% developed an early transient increase in their post treatment tumor volume occurring 3 to 7 months after SRS. Additionally, 19% had late transient increase in their post treatment volumes occurring 26−37 months post SRS. 7% had both an early and late transient increase in their tumor volumes. Tumor volume, treatment dose, and Dmax did not correlate with MRI changes.
Conclusions: Early and late transient increases in tumor volume occur in a significant number of patients with vestibular schwannoma treated with SRS. It is important to continue to monitor these patients with follow up imaging to avoid mistaking them for treatment failures.
No conflict of interest.

1084 POSTER

Plasmon-induced photothermal effects of gold nanoparticles on human permanent cell line T-24

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Background: Gold nanoparticles (GNPs) have been extensively used in various biomedical applications due to their advantageous biocompatibility, excellent optical scattering and photothermal properties, high chemical, photo- and thermal stability in comparison with most of molecular absorbers. The plasmon resonance for gold nanoparticles (GNPs) is at ~520 nm. The aim of the present investigation was to elucidate the effect of spherical GNPs with diameter 40nm combined with laser irradiation on permanent human tumor cell line T-24 (transitional cell bladder carcinoma).
Materials and Methods: The permanent tumor cell line T-24 was cultured in DMEM, supplemented with 10% FCS, at standard conditions. The samples were irradiated with Nd-YAG laser system, at λ=532 nm, pulse duration τ p= 15 ns and repetition rate 1 Hz. Laser pulses with energies of 20.5 J, 21.5 J, 22.5 J and 23.5 J for 5 s and 10 s were used. The potential anti-tumor effect in vitro of GNPs and laser treatment on T-24 cells were studied by MTT assay. Apoptotic changes in cell morphology were investigated with Annexin V/Propidium iodide (PI) and DAPI staining.
Results: Cells treated with GNPs or laser irradiation alone showed no significant difference from the control. Significant inhibition of tumor cell growth was detected in samples, pre-treated with GNPs and laser

beam with energy 20.5 J for 5 and 10 s (76.19±5.81 and 71.29±7.01 respectively). Similar results were obtained after irradiation with energy 21.5 J for 5 s (71.07±7.06).
Induction of apoptosis in treated with GNPs and irradiated cells was studied using Annexin V-FITC/PI and DAPI staining. Well defined morphological features of apoptosis were observed in order to interpret the results from MTT cytotoxicity assay. The results were statistically significant.
Conclusions: Based on the results obtained the combination of GNPs 40 nm and laser treatment with different characteristics of the laser beam and time of influence resulted in localized heating and causing irreversible thermal cellular destruction. This approach could have potential application in clinical practice and in experimental models in vivo for local treatment of tumors.
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No conflict of interest.

1085 POSTER

When pain restricts symptomatic radiotherapy procedure

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Background: Irradiation treatment of various cancer processes in order to achieve symptomatic relief in the shortest time and with the least toxicity to maintain or increase the quality of life of patients. The ionizing radiation is a proven therapy for the treatment of pain due to bone metastases. The pain relief is achieved in 70−80% of patients using different fractionation schemes and total dose, occurs in 2−3 days, up to 2−4 weeks. However, what do we do when the pain suffered by these patients limits the tolerance requirements for the preparation and administration of radiotherapy? The aim of this study was to evaluate the efficiency of oral transmucosal fentanyl citrate (OTFC) as an analgesic for a highly specific subgroup of cancer patients.
Material and Methods: We analyzed the influence of predictable procedural breakthrough pain (set up, mobilization, treatment administration) in candidates for symptomatic radiotherapy and control pain with the use of oral transmucosal fentanyl citrate. From January 2007 to March 2013 we retrospectively reviewed 1995 patients indicating analgesic purposes radiotherapy, patients who didn't tolerate set up requirements and treatment time for breakthrough pain, and changes in use of OTFC.
Results: Between 2007–2009 the 11.63% (111) of patients requiring radiotherapy for analgesia (954), received OTFC before receiving radiotherapy, a 27.02% (30) who indicated OTFC not tolerated not set up. Between 2010 to March 2013, after evaluation the reasons for not proceeding tolerance of palliative radiotherapy, 24.68% (257) of these patients received OTFC (out of 1041 cases), all patients tolerated the procedure set up radiotherapy. The average score premedication with OTFC with procedural breakthrough pain according to VAS was 7.5 (range 5−10). The average score after use of OTFC was 1.8 (range 1−3). The 47% required a dose of 200 mg, 23% 400 μg, 600 μg 22%, and 8% 800 μg. No patients colds undesirable effects related to the use of OTFC.
Conclusions: We can recommend the ideal analgesic OTFC as its speed and safety for the treatment of breakthrough pain in Radiation Oncology procedural. It is easy and convenient administration of short duration, allowing tolerance and administration of radiation therapy. It is important consider predictable pain control but limiting. Give the patient independence, which they can take, even if you are away from home.
No conflict of interest.

1086 POSTER

Adjuvant hypofractionated radiation therapy for breast cancer: Long term results in a cohort of 80 Cases treated at the Department of Radiation Oncology of CHUOran

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Background: To evaluate the incidence of local recurrence (LR) and locoregional recurrence (LRR) and the toxicity results (acute and late) in a group of patients with breast cancer treated with a hypofractionated schedule of adjuvant radiotherapy after surgery.
Patients and Methods: From January to December 1998, 80 patients underwent radiotherapy treatment (telecobaltotherapy) after conservative (6%) and radical (94%) surgery at our department. The dose delivered was 36 Gy (3 Gy daily fraction). The boost dose was 15 Gy (3 Gy daily fraction) in conservative surgery. To score toxicity, we used the RTOG criteria (acute) and SOMA-LENT scale (late).
Results: With a median follow-up of 77 months (9 to 113 months), 7 patients had locoregional recurrences. The 8-year actuarial rates for local recurrence (LR) and LRR were 5.4% (±2.6%) and 9.5% (±3.4%), respectively. The 8-year overall survival rate was 74.3% (±8.1%). 72 patients (90%) were tolerated this hypofractionated schedule (grade 0). We found that 14 patients had grade ⩾ II late toxicity (17.5%). No patients developed grade IV late toxicity. On univariate analysis, the grade of SBR was influenced the LR and LRR ($p = 0.023$). The tumor size ($p = 0.038$), lymph node status ($p = 0.039$) and hormone receptor status ($p = 0.0001$) were influenced the OS. On multivariate analysis, we found that tumors classified T3−4 ($p = 0.028$; HR 3.999) and hormone receptor status ($p = 0.001$; HR 14.16) were independent factors on the OS.
Conclusion: This hypfractionated RT scheme is perfectly realizable (acceptable results in terms of local control and toxicity). It permitted a short course of treatment (<3 weeks), allowing a larger number of patients to be treated per year, with a reduction in cost to the health system. However, an important number of patients and a longer follow-up are necessary to better appreciate the efficacy and the cosmetic outcome of this scheme.
No conflict of interest.

1087 POSTER

Radiotherapy and prolongation of overall treatment time: a single center case report

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Background: The prescribed total radiation dose should be administered within a specific time; however, in clinical practice, unplanned treatment interruptions are inevitable for many reasons. This fact can have a detrimental effect on the tumour local control and patient's outcome. In this report we evaluate, in a single institution, the difference between the planned and the registered overall treatment time (OTT) of a different tumour sites, due to unplanned interruptions for various reasons.
Material and Methods: A total of 3936 patients (pts) were treated between 1990 and 2010 for breast, prostate, lung, head and neck (H&N) and pelvic malignancies with radical or adjuvant intent. Palliative or symptomatic treatments were excluded. Overall there were 1032 males and 2904 females with a mean age of 66 years. For all patients [treated with single fraction (F) of 1.8 to 2 Gy administered five times per week] we registered the total radiation dose, the number of fractions and the treatment's duration (in days); than for each group we evaluated the difference between the mean planned overall treatment time (number of fractions/5 multiplied for seven days of a week) and the mean duration of the treatment.
Results: The table shows the differences (in days) between mean planned and registered overall treatment time stratified by different tumour sites; the gap represents about 10% of planned OTT for all of the groups. Causes of prolongation of OTT could be various (holydays, machine breakdowns, toxicity and others), but we can't identify them in our computerized record. Moreover this case report don't correlate the prolongation of OTT with the therapeutic outcome.

Site	Pts	Gender (M/F)	Total dose	Dose/F (Gy)	Number of Fs	OTT (days) planned	OTT(days) observed	Difference (days)
Breast								
no boost	902	0/902	50 Gy	2	25	35	39	**4**
+ boost	1597	0/1597	60 Gy	2	30	42	47	**5**
Pelvis	219	67/152	45 Gy	1.8	25	35	38	**3**
Prostate	184	184/0	76 Gy	2	38	53	58	**5**
Lung	76	66/10	61.2 Gy	1.8	34	48	51	**3**
H&N								
no CT	741	494/247	58.7 Gy (mean)	1.8−2	31	43	46.5	**3.5**
+ CT	217	145/72	63.7 Gy (mean)	1.8−2	33.5	47	50	**3**

Conclusions: This is a simple picture of an unselected case report of patients treated at a single institution. Our results shows a moderate gap between planned and observed OTT. Efforts should be done to minimize the unforeseen prolongation of the overall treatment time through different methods for compensation (weekend treatments, increase number of daily fractions, increased dose per fraction, delivering extra fractions). In our centre, according with a codified internal protocol, we add a fraction on Saturday if unplanned break caused by machine breakdowns exceeds two consecutive days; we generally don't operate a compensation if the prolongation of the treatment is due to other causes (e.g. holydays, toxicity). If the break has occurred late in the schedule, we sometimes deliver one or more extra fractions.
No conflict of interest.

1088 POSTER

The impact of intensity modulated radiotherapy on skin dose for deep sited tumours

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Abstract: Background: The purpose of this study was to investigate the impact of IMRT on surface doses for brain, abdomen and pelvis deep located tumours treated with 6 MV photon and to evaluate the skin dose calculation accuracy of the XIO, 4.04 treatment planning system.
Materials: More investigations for the influences of IMRT on skin doses will increase its applications for many treatment sites. Measuring skin doses in real treatment situations will reduce the uncertainty of skin dose prediction. In this work a paediatric human phantom was covered by a layer of 1 mm bolus at three treatment sites and thermolumencent dosimeter TLD chips were inserted into the bolus at each treatment site before CT scan. Two different treatment plans (3-DCRT and IMRT) for each treatment sites were performed on XIO, 4.04 treatment planning system using superposition algorism.
Results: The results showed that the surface doses for 3DCRT were higher than the surface doses in IMRT by 1.6%, 2.5% and 3.2% for brain, abdomen and pelvis sites respectively. The results showed that there were good agreement between measured and calculated surface doses, where the calculated surface dose were 15.5% for brain tumour calculated with 3DCRT whereas the measured surface dose were 12.1%. For abdomen site the calculated surface dose for IMRT treatment plan was 16.5% whereas the measured surface dose was 12.6%.
Conclusions: The skin dose in IMRT for deep sited tumours is lower than in 3DCRT which is another advantage for the IMRT. The TLD readings showed that the difference between the calculated and measured point dose is negligible. The Superposition calculation algorism of the XIO, 4.04 treatment planning system modelled the superficial dose well.
No conflict of interest.

1089 POSTER

Development of pretreatment 'truly' patient-specific quality assurance procedure for stereotactic body radiotherapy using volumetric modulated arc radiotherapy

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Background: In stereotactic body radiotherapy (SBRT) using volumetric modulated arc therapy (VMAT), pretreatment patient-specific quality assurance (QA) is essential. But radiochromic films suitable for larger doses (10−25 Gy) than conventional fraction dose (~2 Gy) are hardly available now. In some institutions, VMAT-SBRT verification with radiochromic film have been performed by rescaling dose prescribed to be inside the optimal dose range from the calibration curve (c.g. ~2 Gy). This is not patient-specific QA. We have developed a new patient-specific QA procedure for VMAT-SBRT patient pretreatment verification.
Materials: Co-planar VMAT plans (one arc VMAT plans, the total dose 55 Gy in five fractions) were created in five patients, who have been already treated using breath-holding conventional seven non-coplanar static SBRT (55 Gy in five fractions). We constructed a script to export VMAT plan from Pinnacle^3 (Philips Medical Systems, Best, The Netherlands) and to divide it into three partial arcs VMAT plans and then to import the divided three arcs plan into Pinnacle^3. We compared digitally reconstructed radiographs (DRRs) of all control points, 2D/3D dose distribution and DVH parameters of targets and organs at risk (OARs) of original plans and divided plans. And then we performed gamma analysis on both plan with the DD-System (R-TEC. Inc., Tokyo, Japan) using 1 mm/1% with a dose threshold of 5%. We moreover divided the divided plans into three plans with one partial arc VMAT in Pinnacle^3 and performed patient-specific QA measurements for each partial arc plans as routinely performed in conventional fraction dose (e.g. 2 Gy).
Results: All original plans and divided plans are completely coincided on DRRs of all control points, 2D/3D dose distribution and DVH parameters of targets and OARs. Gamma analysis showed original plans and revised plans are perfectly matched. In all five patients, gamma evaluation of three plans with one partial arc VMAT plans showed more than 95 % of passing rate using 3 %/3 mm criteria.
Conclusions: New pretreatment 'truly' patient-specific QA procedure was developed using a script in Pinnacle^3. New procedure enables us true QA for VMAT-SBRT patient plans using the same procedures as ones in VMAT plan using conventional fraction doses.
No conflict of interest.

1090 POSTER

Quality improvement project streamlines workflow performance and results in improved access to cancer care

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Objectives: Our Team performed a work-flow performance quality improvement project in our Radiosurgery Center. Our primary endpoint was to decrease the mean time between initial consult and first radiosurgery treatment. Our goal was to determine our current performance level and then implement a systematic performance quality improvement process to streamline workflow through the center, which in turn would result in improved access to care, patient satisfaction, and clinical outcomes without compromising patient safety.
Methods: We retrospectively reviewed our workflow performance for 248 patients treated in 2010 using 15 metrics to evaluate delays in the workflow and identify variables we could modify to improve performance. We assigned times to each metric as a benchmark. Time limits were assigned to each step in the process. Our evaluation resulted in a goal of 23 days between consult and treatment. In 2011−12, we prospectively evaluated 539 patients using the above metrics to evaluate the effect of our interventions.
Results: In 2010, the Radiosurgery Center treated 248 patients; mean time between consultation and treatment was 36 days. Access to dedicated CT Simulation time was a cause for delay and for 2011 we increased dedicated slot times which reduced our mean time between consultation and start by 4.52 days to 31.48 days with 245 patients treated. We determined the largest barrier to care was insurance carrier approval. To improve the insurance approval process, our Radiation Oncologists developed site specific templates which provided specific carrier approval requirements depending on the type of radiosurgical intervention. Contouring of target volumes and treatment planning times were rigorously monitored and timelines were enforced for each step to prevent treatment planning delays. In 2012 we treated 294 patients and our mean time between consult and initial treatment dropped to 22.92 days, this was an 8.56 day improvement over 2011, and a 13 day improvement over 2010, while patient volume in the center increased by over 17%, between 2011 and 2012. Patient safety was monitored throughout the process to ensure there was no compromise in patient treatment from changes in the process.
Conclusion: This research demonstrates that proactive interventions can result in a significant reduction in time between consult and initial treatment. Improved access provides more agility within our center while allowing for scheduling flexibility to triage more acute lesions with expedited start times. Protected treatment planning time has been demonstrated to decrease the potential for errors in treatment plans while ensuring adequate quality assurance and plan review prior to treatment. This model is a low-cost high-yield example of the quality improvement process which could easily be adapted to fit many practice models and yield significant improvement in the delivery and access of safe patient care.
No conflict of interest.

1091 POSTER

Deliberated errors caused by shifts in an ionization chamber bidimensional array. How far can we get without realizing?

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Background: One of the issues we have to take into account about bidimensional ionization chamber arrays for IMRT pre-verification is the low spatial resolution and sample frequency. In this study we analyze PTW 2DARRAYSeven29® (2DARRAY) which consists of 729 ionization chambers with a center to center distance of 1 cm and a volume of 0.125 cm^3.
Our goal in this study is to find a pattern between systematic misalignments and the values of the gamma index criteria for IMRT verification. We also hope to determine if a spatial resolution of 1 cm is small enough to detect misalignments less or equal *to* 5 mm.
Materials and Methods: We carried out several deliberated shifts (±1 mm, ±3 mm and ±5 mm) in three orthogonal directions (head-feet, left-right and

up-down) on 2DARRAY placed inside PTW Octavius® Phantom (Octavius). Both of them were irradiated in a Siemens Oncor Impression® 6MV Linear Accelerator equipped with a 160 multileaf collimator. Previously, a real prostate plan was transferred on to 2DARRAY and Octavius CT and it was computed by Pinnacle® v9.2 Treatment Planning System without any shift, providing us with a reference dose map and a reference central chamber dose. We also measured the dose at the isocenter for 5x5, 10x10 and 20x20 cm^2 fields to check the constancy of the dose along the whole study. Finally, we compared the doses at the 2DARRAY central chamber and reference map doses computed by TPS to those measured in each shift using the 2DARRAY. The comparison between the reference dose map computed by TPS and 2DARRAY measured planar doses was performed by PTW Verisoft® Software drawing on a gamma index (3 mm, 3%, local dose).
Results: There is an agreement between the TPS reference dose in the central chamber and those obtained by 2DARRAY whatever the shift performed. In all cases the differences were less than 2.5%. However, we obtained unacceptable values for gamma index when shifts were greater than 1 mm. For 1 mm shifts, the drop in the gamma index was from 100% to 98.8%, the mean gamma index for 3 mm shifts was 81.1% and for 5 mm it was 70.4%.
Conclusions: Misalignments greater than 1 mm can be detected by map dose measured by 2DARRAY when using a gamma index criteria (3%, 3 mm, local dose) but no useful information is provided by dose at the 2DARRAY central chamber as we have a tolerance level of 3%. On the other hand, these results could be achieved once we had planned and verified about 30 prostate cases and the values of the gamma index were always approximate 100%.
No conflict of interest.

1092 POSTER

An approach to individualized tolerance level of patient-specific QA depending on treatment planning using dosimetric metrics

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Background: The aim of this study is to compare and evaluate the individualized tolerance levels of patient-specific DQA depending on the delivery techniques (IMRT vs VMAT) and treatment sites using a variety of dosimetric tools.
Material and Methods: Dosimetric QA (DQA) measurements were performed with (1) a point dose by an ion chamber, (2) 2D dose distribution by radiochromic film (EBT2) for an axial plane and an ion chamber array (Matrixx®) for a coronal plane, and (3) 3D dose distribution by a head-mounted ionchamber array (COMPASS®). The two criteria (2 mm/2% and 3 mm/3%) were used to analyze the gamma passing rate of 2D and 3D dose distributions. Several statistics were applied to analysis the normality of each group (IMRT, VMAT, Prostate, and H&N), significance between the comparisons (IMRT vs VMAT and Prostate vs H&N), and correlation between dosimetric tools. The concept of confidence limit was applied to approach to the tolerance levels.

Measurement		2D (Matrixx)	2D (Film)	3D w/Body	3D w/PTV
Point dose	*r*-value	−0.33	0.17	0.35	−0.32
	p-value	<0.05	0.29	<0.05	<0.05
	N	40	40	40	40
2D (Matrixx)	*r*-value		−0.11	−0.02	0.42
	p-value		0.49	0.89	<0.05
	N		40	40	40
2D (Film)	*r*-value			0.17	0.31
	p-value			0.30	0.05
	N			40	40
3D w/ Body	*r*-value				0.02
	p-value				0.90
	N				40

Results: The difference between IMRT and VMAT showed in the point dose, axial plane dose, coronal plane dose and 3D volume dose measurements. The difference between prostate and H&N showed in the axial plane dose, coronal plane dose, and 3D volume dose measurements. There was no the correlation between the dosimetric tools as shown in table.
Conclusions: 3D volumetric verification is required to assess the dose discrepancy in VMAT and the multi-institutional study is required to have confidence in the implementation of individualized tolerance level based on the approach and results in this study.
No conflict of interest.

1093 POSTER

Quality assurance in IMRT: A comparison between two-dimensional ionization chamber array and film dosimetry

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Background: Two dimensional ionization chamber arrays are an easy-handling and fast real-time method for pre-treatment verification in IMRT. However, spatial resolution can be an issue because the sample frequency in the Fourier domain must be at least 0.2 mm^{-1} (5 mm in coordinate space) when using 1 cm^2 beamlets. Commercial devices provide smaller resolutions PTW 2DARRAY Seven29® (2DARRAY): 0.1 mm^{-1}, Scanditronix Wellhofer IMRT Matrixx®: 0.13 mm^{-1} and Sun Nuclear Mapcheck2®: 0.14 mm^{-1}). Together with Merge 27® software, a 5 mm shift for 2DARRAY in orthogonal directions can provide a 5 mm spatial resolution map dose. Although this is not a good settlement as it triples the time for verification. The aim of this study is to check if 2DARRAY has a good gamma index agreement to Film dosimetry using Gafchromic® EBT2 when IMRT Step and shoot beamlets have a minimum area of 4 cm^2.
Materials and Methods: All the measurements were performed in a Siemens Oncor Impression® 6 MV Linear Accelerator that was equipped with a Multileaf Collimator of 160 leaves whose width was 5 mm at the isocenter. Planar doses were computed by Pinnacle v9.2 Treatment Planning System (TPS) and measured planar doses were carried out in Octavius® Phantom both for films and 2DARRAY. Also, computed doses at isocenter from the TPS were compared to measured doses using a PTW 31003 Semiflex® Chamber for film verification and the central ionization chamber from 2DARRAY.
The comparison between planar doses was made using gamma index (3 mm, 3%, local dose) for Head and Neck and Prostate cancer cases. We used FILM QA PRO® and PTW Verisoft® software for these purposes.
Results: In all prostate cases (N = 32) we obtained a gamma index higher than 95% for 2DARRAY or film verifications when comparing to the TPS planar doses. For head and neck cases (N = 13) we achieved a gamma index higher than 91% for all cases for film dosimetry and even better results for 2DARRAY verification as the results were never less than 95%. For dose at the isocenter the difference between TPS and semiflex® chamber or central chamber from 2DARRAY was never higher than 3% for both sets of cases.
Conclusions: PTW 2DARRAYSeven29® has proved to be a reliable and accurate method for IMRT pre-treatment verifications compared to film dosimetry and has the great advantage of real-time measurements for quality assurance purposes.
No conflict of interest.

1094 POSTER

Impact of inhomogeneous tissue on IMRT QA for MatriXX

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Purpose: By applying self-made inhomogeneous phantom to MatriXX, a group of IMRT plans were delivered, to observe the impact on test results on different measuring condition.
Method: Two foam planks were made to simulate the low density tissue, e.g lung tissue, in the size of (40×40×3)cm, identical to virtual water. Three measurement condition using MatriXX were arranged: A. virtual water only (8 cm top and 5 cm bottom); B. one foam plank was used plus 5 cm virtual water on its top, and 5 cm virtual water on MatriXX bottom; C. two planks were used on the top and at the bottom of MatriXX, with 5 cm and 2 cm virtual water planks wrapped. The sizes in B and C condition were same to A. CT scanning images for three conditions were obtained and transmitted to TPS. QA plans were generated to the scanning images respectively. The original plans were 10 esophageal cancer IMRT plans. The QA plans were calculated in CMS xio-release 4.62, using superposition algorithm, and then delivered to Elekta Synergy. Each plan was measured in different condition, and the gamma passing rate was obtained.
Results: There was no significant difference (P<0.05) among the three measuring condition. The results, i.e gamma passing rate, in B (98.78±1.34)% and C (98.71±1.16)% were as good as in A (98.49±1.10)%.

Conclusion: The results showed that there is no difference in MatriXX measurement, no matter what phantom is used. However, more measurements should be needed to make it confirmed.
No conflict of interest.

1095 POSTER

Multi-dimensional patient-specific plan QA on IMRT and RapidArc techniques

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Background: The aim was to distinguish the differences between IMRT and RapidArc delivery, through comparison of plan QA results in three modalities.
Materials: 20 cases with NPC were enrolled. Based on the simulation CT images and targets, IMRT and RapidArc plans were both generated for every case in Eclipse v8.6. The plans, after approved, were converted into QA plans, using virtual water as phantom. 1. A cylindric ion chamber (PTW 0.125cc) was applied to point dose QA. The selected point was set to iso-center. 2. MatriXX (IBA v1.76) was applied to plane QA. The plane through iso-center was the measurement plane. 3. COMPASS system (IBA v2.0) was applied to volumetric QA. Each QA plan was delivered to Varian Trilogy to be measured. For point measurement, the QA result was the ratio of measurement to planning. For plane and volumetric measurement, the QA results were gamma passing rate with the criteria of 3%&3 mm. The paired *t*-test was used as statistical analysis.
Results: Point QA: the average result was (96.83±0.84)% for IMRT and (97.03±0.46)% for RapidArc, $P = 0.275$.
Plane QA: the gamma passing rate on average was (97.59±0.61) for IMRT and (97.98±0.36)% for RapidArc, $P = 0.055$.
Volumetric QA: the gamma passing rate on average was (96.70±0.81) for IMRT and (97.03±0.83)% for RapidArc, $P = 0.068$.
Conclusion: Although the differences among three QA modalities were not significant, it's found that almost RapidArc plan measurement results were better than IMRT's. The study indicated that RapidArc technique could be as good and safe as IMRT in plan delivery.
No conflict of interest.

1096 POSTER

Dosimetric shield evaluation with tungsten sheet in 4, 6, and 9 MeV electron beams

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Purpose: In electron radiotherapy, shielding material is required for attenuate beam and scatter. A newly introduced shielding material, *Tungsten functional paper* (TFP), has been expected as much useful device which is lead-free, light, flexible and easily processed containing very tiny tungsten powders as much as 80 percent by weight. The purpose of this study is to investigate the dosimetric changes due to TFP shielding for electron beams.
Method and Materials: TFP (thickness 0−15 mm) was placed on water or water equivalent phantom. Percentage depth ionization and transmission were measured by 4, 6, and 9 MeV electron beams with 10×10 and 20×20 cm^2 field size. Off-center ratio was also measured using the film at depth of dmax under the similar condition. Then, beam profiles, transmission with two shielding materials, TFP and Lead, were evaluated.

Table 1. TFP thickness required for 95% and 98% reduction.

Depth	Attenuation	TFP thickness required (mm)					
Energy (MeV)		4		6		9	
Field size (cm^2)		10×10	20×20	10×10	20×20	10×10	20×20
0.0 cm	95%	5.56	5.63	10.57	10.74	15.61*	15.65*
	98%	5.87	6.09	11.86	11.93	16.20*	16.18*
0.5 cm	95%	4.31	4.30	8.88	8.94	15.06*	15.20*
	98%	5.11	5.08	11.14	11.34	15.91*	16.02*
1.5 cm	95%	0.98	0.98	5.67	5.70	11.49	11.99
	98%	1.33	1.34	7.63	7.91	15.12*	15.55*
3.0 cm	95%	–	–	–	–	4.99	5.49
	98%	–	–	0.66	0.77	8.63	11.47

*Estimation value by linear interpolation with transmission curve.

Results: TFP thickness required for 95 % and 98 % reduction is shown Table 1. The reduction of 95% by use of TFP at 0.5 cm depth were 4, 9, and 15 mm with 4, 6, and 9 MeV electron beams, respectively. Beam profile of TFP intended to increase dose at the field edge that might be influenced by thickness. The energy of 4, 6 MeV were not problem in the clinical situation since the transmission under shielding were less than 2%.
Conclusions: This study shows the characteristic of new radiation-shield-material (TFP) was evaluated by using the electron beams. It has several unique features and is highly expected to be useful for radiation protection for the electron beam.
Conflict of interest: *Other substantive relationships: Device (Tungsten functional paper) used in this study was provided by TOPPAN PRINTING CO., LTD, Tokyo, Japan.*

1097 POSTER

Dosimetric comparison of postoperative whole pelvic lymph node intensity-modulated radiotherapy for cervical cancer patients with multiple pelvic lymph node metastases

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Background: To study the dosimetric difference among RapidArc (RA) and intensity-modulated radiotherapy (IMRT) plans applying different energy x-ray for whole pelvic lymph node irradiation.
Material and Methods: Ten cases of cervical cancer who underwent radical surgery and demonstrated multiple pelvic lymph node metastases were treated with radiotherapy. Three plans were generated for each case: 7-field IMRT, one-arc RapidArc (RA1 = 358º)and two-arc RapidArc (RA2 = 716º). For each plan, 6 MV and 15 MV X-ray were applied respectively. The dosimetric differences were compared among different plans.
Results: All plans could meet the clinical requirement. The CI, HI and EVI of IMRT and RA2 were better than RA1 with significantly difference ($p < 0.05$), while the differences between IMRT and RA2 were not significant. There were no significant differences were found in irradiation dose of organs at risk except for small bowel V40 (IMRT < RA2).
Conclusions: Compared to IMRT, there were no significant dosimetric benefits were found except treatment time and monitor unit in radiotherapy for whole pelvic lymph node applying RaidArc. Additionally, the 6MV photons may be the prudent choice.
No conflict of interest.

1098 POSTER

Dosimetric test of collapsed cone convolution superposition algorithm in a heterogeneous media

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Background: The Collapsed Cone Convolution Superposition (CCCS) algorithm is used for dose calculation and intrinsically considers the effects of patient heterogeneities. The purpose of this study is to evaluate the accuracy of Prowess 5.0 (Prowess Inc., Concord, Ca) TPS dose calculations using CCCS algorithm in a heterogeneous phantom.
Material and Methods: Dose calculations of Prowess 5.0 TPS using the CCCS algorithm were compared against measured data. Measurements were performed with ionization chamber (0.057 cm^3) and EBT2 radiochromic films. The films were calibrated in 10 points between 0 and 320 cGy and scanned in Epson Expression 1000 XL scanner. Two different phantoms were used in the measurements and both were imaged using CT scanner for TPS dose calculation at corresponding detector locations. Both phantoms are composed of an 8.6 cm depth cork slab between two 7 cm depth solid water slabs. Each phantom has a rounded shape volume (≈95.5 cm^3) made of wax inside the cork. One wax volume is split at central plane for film placement and the other is made with the ionization chamber build up cap at volume's center. The irradiations were carried out with 6 MV Primus Siemens linear accelerator (Siemens Medical Solutions, Concord, Ca) photon beam with 20x20 cm^2 field size, 90 cm SSD and 200 MU. Three field profiles were acquired with film dosimetry in the crossplane direction: 1) at 7 cm depth (in solid water/cork transition); 2) at the central plane of wax volume; and 3) at 15.6 cm depth (in cork/solid water transition). Further, absolute dosimetry with ionization chamber was performed at two positions: 1) inside the wax volume with the build up cap; and 2) at 16.6 cm depth, inside a solid water ion chamber slot (1 cm deeper than cork slab).
Results: The field profiles did not present dose differences higher than 2% or 2 mm DTA. Inside the wax volume, the dose calculated was 2.6% higher than the measured dose, and in deeper position was 4.5% higher.
Conclusions: The field profiles relative dosimetry presented acceptable agreement with TPS calculation. The absolute measured doses showed

that collapsed convolution superposition algorithm can overestimate dose (up to 4.5%) in a heterogeneous media. Further study is necessary for CCCS algorithm clinical implementation.
No conflict of interest.

1099 POSTER

MicroRNA-regulated pathways of radiotherapy sensitivity and resistance in breast cancer

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Background: Radiotherapy is an important component of primary, adjuvant, and palliative treatment for many types of solid tumour. Because of this, innate or acquired resistance is a major clinical problem. These issues are compounded by the fact that effective biomarkers that can identify this subset of resistant tumours or can otherwise accurately target radiotherapy to those patients who would most benefit from it have yet to be discovered. We previously identified 3 microRNAs (miRs) able to regulate genes associated with key mechanisms of the cellular response to radiotherapy, and which could trigger resistance or sensitivity to clinically-relevant doses of radiation in breast cancer cells *in vitro*. We aimed in the current study to characterise these miRs.
Materials and Methods: Bioinformatics analyses were used to identify putative gene targets of each miR of interest. Target binding was confirmed by luciferase assay, QPCR analyses, and Western blotting. Knockdown of several targets themselves also triggered significant sensitivity or resistance to radiation in breast cancer cells *in vitro*. Four key targets, NEK1, PLK2, SKP2, and RAG1 were further tested as biomarkers predictive of relapse following radiotherapy in two independent early-stage breast cancer patient cohorts.
Results: Microarray gene expression profiling of miR mimic-transfected MCF7 cells together with miR binding sequence prediction algorithms were used to identify putative miR targets. Mimics were confirmed to bind to specific 3′ UTR sequences of several key genes, including those associated with DNA repair, cell cycle control, and reactive oxygen species defence, resulting in their downregulation at both the mRNA and protein level. For radiotherapy-sensitizing mimics this also had functional significance, with impaired DNA repair and increased sensitivity to both radiation and DNA damaging chemotherapy agents. Several miR targets were also effective biomarkers − the expression of miR targets NEK1, PLK2, SKP2, and RAG1 were significantly correlated to relapse-free survival in 435 early-stage breast cancer patients.
Conclusion: The expression of several genes important to the cellular response to radiation are miRNA-regulated, which suggests that the dysregulation of these miRs could lead to resistance to radiotherapy in patients. Furthermore, several of these genes correlate to survival in radiotherapy-treated patients, and could be used to guide treatment in the clinic.
No conflict of interest.

1100 POSTER

Identification of compounds modifying radiation-therapy using a 3D-microtissue technology

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Background: The two key issues in chemo- and radiation therapy is the development of tumor resistance as well as toxic effects on normal tissue. In this sense new strategies are required to increase efficacy of radiation to improve the therapeutic impact and reduce toxicological side effects**.** Material and Methods: The performance of 3D cell culture systems over classical 2D culture systems has been shown to provide a closer representation of tissue-level biology. This has led to the rapid adoption of 3D systems for both drug discovery and toxicology. InSphero has developed a highly reproducible hanging drop technology able to generate monotypic cell spheroids called microtissues in a 96-well format.
Results: The innovative 3D-microtissue plate technology has been adapted for analysis of the cellular response of radioresistant T47D breast cancer and FaDu head and neck cancer cells to combined radio-chemotherapy (RCTx). We have validated the model by comparing the treatment of microtissues with 10 different chemotherapeutic compounds, each tested alone and in combination with an acute 2 Gy radiation exposure. The cancer cells were stably transduced with GFP-lentiviral vector enabling faster high throughput quantification of 3D microtissue growth assessment using an Operetta working Software and detection system 'Harmony 3.0' (PerkinElmer, USA). We studied the ability of RCTx to modify 3D-microtissue growth 3, 6 and 10 days after treatment. Results for five compounds (Actinomycin D, Staurosporine, Docetaxel, Doxorubicin and Vinblastine) showed that the IC50 values were improved by the addition of the single 2 Gy radiation dose, indicating that they are capable of inducing a radiosensitation effect on radioresistant breast cancer cells. Panels of commercial secondary functional assays were adapted to the 3D-microtissue high throughput assay. Cellular viability and cytotoxicity were measured directly in microtissues using the CellTiter-Glo Reagent (Promega, USA). Apoptosis was measured using an ELISA based M30-Apoptosense assay (TECOmedical AG, Switzerland).
Conclusions: These results confirm that the assay operated with the 3D-microtissue model system is able to detect compounds that modulate tumor cell survival after irradiation.
No conflict of interest.

1101 POSTER

Identification of the mechanisms of radiosensitization by human papillomavirus (HPV) in head and neck cancer cell lines

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Background: Several clinical studies have shown that HPV+ head and neck squamous cell carcinomas (HNSCC) present a more favorable outcome and greater response to radiotherapy. Although there are some data supporting the hypothesis that HPV-related tumors have a better survival due to a higher sensitivity to radiation therapy, it is difficult to conclude that the improved clinical outcome of HPV-related HNSCC is only attributable to intrinsic radiosensitivity of the HPV+ cells. As an hypothesis, it is postulated that a complex interaction concurs between intrinsic mechanisms of radio-response and the tumor micro-environment including cells of the immune system stimulated by the presence of HPV. The objectives are to identify the mechanisms of radiosensitization by HPV and to investigate whether irradiated HPV+ cells exhibit an increased immunogenic cell death signal.
Material and Methods: We determined radiosensitivity by clonogenic survival of two HPV+ HNSCC cell lines (UPCI-SCC-154 & UPCI-SCC90) compared to two HPV− (SCC61 & SQD9). Cell cycle distribution and G2/M checkpoint were assessed by flow cytometry. DNA damage repair was evaluated by gamma-H2Ax assay. In addition, apoptosis was investigated in the four cell lines together with immunogenic cell death signal, through membrane exposure of calreticulin.
Results: The surviving fraction at 2 Gy (SF2) for the two HPV+ cell lines was 0.13 and 0.15 for UPCI-SCC-90 and UPCI-SCC-154, respectively while SF2 for SQD9 was 0.49 and 0.16 for SCC-61 a head and neck squamous cell line known to be radiosensitive. Cell cycle distribution indicated a block in G2/M 24 h after irradiation for the HPV+ cell lines not observed in the HPV− cells. In HPV+ cells, gH2Ax kinetics after a single dose of 5 Gy correlated the SF2 data with a slower clearance of gH2Ax for HPV+ cells, compared to HPV− cells. At 24 h after irradiation, less than 5% of cells in all groups were apoptotic, but further time points still need to be analysed. Finally, calreticulin membrane translocation as a measure of immunogenic death signal after irradiation was detected in HPV+ but not in HPV− cell lines, before and 24 h after irradiation.
Conclusions: These results confirm the increased radiosensitivity of HPV+ cells although at this point further investigation is needed to elucidate what are the mechanisms implicated. However, detection of calreticuline translocation as a marker of immunogenic death signal in HPV+ cells opens an interesting hypothesis.
No conflict of interest.

1102 POSTER

Antitumor effect induced by combination of radiation with a novel HSP70 inhibitor pifithrin-mu on human prostate cancer

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Background: Heat shock proteins (HSPs) are constitutively expressed in many cancers, and various types of stress increase their expression. HSPs are thought to make cancer cells resistant to anti-cancer therapy, with HSP70 playing a central role in the resistance. Recently, pifithrin-μ (PFT-μ) has been revealed as a new HSP70 inhibitor. In this study, we investigated the antitumor effects of the combination therapy of radiation with PFT-μ on

human prostate cancer cell lines and attempted to elucidate the underlying mechanisms.
Material and Methods: Three human prostate cancer cell lines (LNCaP, PC-3, and DU145) and a human normal prostate epithelial cell line (PrEC) were used. HSP70 expression in the cancer cells was examined by immunoblot. HSP70 was knocked down with siRNA. Cell viability was evaluated using a WST-8 assay. Clonogenic capacity of cancer cells was determined using a colony formation assay. Cell proliferation was examined by flow cytometry after labeling with carboxyfluorescein succinimidyl ester (CFSE).
Results: Knockdown of HSP70 significantly decreased the viability and the colony-forming capacity of all three prostate cell lines, suggesting a crucial role of HSP70 in the survival of cancer cells. The combination of low-dose radiation (2 Gy) and low-dose PFT-μ (3 μM) significantly decreased the viability and the colony-forming capacity of three prostate cancer cell lines compared with either treatment alone. Importantly, this combination effect was not observed on PrEC cells. In addition, CFSE assay revealed that the combined therapy decreased proliferation of cancer cells, suggesting the induction of growth arrest.
Conclusions: These data indicate that the combination therapy with radiation and PFT-μ can decrease the growth of human prostate cancer cells, and that PFT-μ is a promising reagent to enhance the antitumor effect of radiation.
No conflict of interest.

1103 POSTER

In vitro prediction of radiation sensitivity in patients treated with external beam radiation therapy

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Background: Understanding the basis of clinical radiosensitivity (RS) is considered by many to be a key goal of radiation research. Numerous cellular and molecular assays have been applied to cells from RS cancer patients in an attempt to predict which patients may suffer excessive RS reactions. In this study, a correlation between transformed lymphoblasts and clinical response was investigated using the limiting dilution assay (LDA) in cell lines from clinically RS individuals with seven tumour varieties, compared with controls.
Material and Methods: Lymphoblasts from 29 cancer patients (19 RS patients, 10 controls) who had or had not experienced severe normal tissue reactions, and one ataxia telangiectasia positive control cell line, were exposed to graded doses of gamma-radiation *in vitro* and cell survival assessed via LDA. RS cell lines were created from patients who had RTOG Grade 2 (n = 1), Grade 3 (n = 12) or Grade 4 (n = 3) skin reactions. Cell survival was expressed as the surviving fraction at 2 Gy (SF_2), and data fitted with non-linear regression analysis.
Results: SF_2 ranged from 0.0245–0.3894, with variability in cell survival evident both among and within individuals, with a coefficient of variation of 31% overall. A non-significant trend was seen between acute or consequential late reaction cell lines and LCL radiosensitivity ($p = 0.10$ and $p = 0.08$ respectively). Two RS outliers were detected in the assay corresponding clinically to Grade 3 late breast fibrosis, and Grade 4 osteoradionecrosis of the jaw and odynophagia in a patient treated for head and neck cancer.
Conclusions: The presence of cell survival heterogeneity and a number of patients demonstrating *in vitro* sensitivity but normal clinical response, among other reasons, may limit the usefulness of this assay for predictive purposes. However, two radiosensitive outliers were observed in the present study which may yield insight into the genetic basis of RS, for example by utilizing deep DNA sequencing.
No conflict of interest.

1104 POSTER

Ionizing radiation of esophageal carcinomas leads to alterations of the hyaluronan matrix and stroma mediated cell death of tumor cells in vitro

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Background: Radiotherapy is commonly used in the management of esophageal carcinomas (OSC). Hyaluronan (HA), a major carbohydrate constituent of the extracellular matrix, forms a complex, biologically active matrix with its binding partners (e.g. versican). This network plays an important role in tumor–stroma interactions. However, little is known about the response of the HA-matrix to radiation.
Material and Methods: To investigate changes of the HA-matrix induced by ionizing radiation, mono-cultures of OSC and fibroblasts were irradiated with 10 Gy. The HA matrix was analysed by qRT-PCR, ELSA and immunocytochemistry. Cell cycle was investigated by FACS. Cells were observed by time lapse microscopy. Cell death was measured by visual analysis. The results were confirmed by PARP and Annexin-V analysis. The role of ROS in matrix changes was assessed by the ROS inhibitor N-acetylcysteine (NAC).
Results: Mono-cultures of OSCs and fibroblasts show an altered expression of HA-matrix related genes. The main HA-synthase (HAS)-isoform of OSCs, HAS3, was up-regulated (2.02 ± 0.25 fold of control), whereas the main isoform in fibroblasts, HAS2, was reduced (0.21 ± 0.02 fold of control). Versican mRNA is induced in fibroblasts (1.64 ± 0.08 fold of control), however not changed in OSCs. HA quantification by ELSA showed no differences in irradiated cells, although structural changes evident by the formation of HA-cables were observed in irradiated OSCs. Immunocytochemistry confirmed the increase of versican in irradiated fibroblast. To evaluate functional consequences of the altered HA-matrix in the tumor–stroma interaction, fibroblasts were seeded on OSCs 24 h after irradiation. A significantly increased amount of dead OSCs was observed in co-culture with irradiated fibroblasts compared co-culture with mock-irradiated fibroblasts (36.72 ± 9.68 fold of control). This effect could be abolished by pre-incubation of fibroblasts with NAC. PARP western blots and flow cytometry confirmed the microscopic results.
Conclusions: In conclusion, ionizing radiation leads to stromal cell mediated, ROS dependent, cell death of tumor cells. These effects might be associated with changes of the HA-matrix. In particular, versican has been shown to exhibit pleiotropic effects concerning cell death. Therefore, it might protect stromal cells while driving tumor cells into apoptosis. This effect will be subject to further investigations, thereby possibly leading to new innovative strategies in tumor chemoradiotherapy.
No conflict of interest.

1105 POSTER

Relationship between the quantity of serum reactive oxygen metabolites and skin toxicity grade in the irradiated rat model

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Background: Adverse effects of radiation are one of the major problems for the patients undergoing radiotherapy. It is known that radiation causes damage by producing free radicals or oxidative stress. The aim of this study was to analyze relationship between oxidative stress and reactions to radiation in vivo using rats.
Materials and Methods: 4 MeV Electron beams were irradiated to the skin of the right leg of 6-week-old female Wistar rats. Serum ROMs were quantified with d-ROMs test using blood collecting from caudal vein.
Serum ROMs (reactive oxygen metabolites) were quantified with d-ROMs test instead of directly measuring free radicals, which have extremely short life-time.

1. Rats were divided into 3 groups according to irradiated dose (0 Gy [control], 2 Gy/fraction, 30 Gy/fr.), and then time course of serum ROMs of each group was quantified using blood sample at certain intervals.
2. In another experiment, rats were divided into 4 groups according to irradiated dose (0 Gy [control], 30 Gy/fr., 50 Gy/fr., 70 Gy/fr.). Serum ROMs and skin reactions were examined at certain intervals. Skin reactions to radiation (inflammation, erosion, ulcer, necrosis, etc.) were graded according to the grading system.

Results:

1. In the analysis of time-course of measured serum ROMs, there was a significant difference among the 3 groups (0 Gy, 2 Gy, 30 Gy), and measured serum ROMs were higher in the 2 Gy group than other groups ($p = 0.018$, repeated-measure ANOVA). At 3 days after irradiation, the serum ROMs of 2 Gy-group were significantly higher than those of the other groups (0 Gy vs. 2 Gy: $p = 0.034$, 2 Gy vs. 30 Gy: $p = 0.028$).
2. There were significant differences among 4 groups in the time-course of skin grade in each group ($p < 0.001$), the skin grades were high in the order of 70 Gy-group, 50 Gy-group, 30 Gy-group (and control-group). In the analysis of time-course of measured serum ROMs, there were significant differences among the 4 groups. Measured serum ROMs of 70 Gy-group and 50 Gy-group were significantly higher than that of 30 Gy-group and control-group. The peak of serum ROMs and appearance of skin toxicity were emerged around the same time.

Conclusion: Increasing the amount of serum ROMs by irradiation has been shown. Moreover, it has been shown that an increase in serum ROMs and occurrence time of the skin reactions to radiation were almost concurrent. Measuring serum ROMs may be useful for predicting radiation damage.
No conflict of interest.

1106 POSTER

Inhibition of tumor growth and metastasis in mice by pulsed low-dose (<0.5 Gy) X-ray irradiation

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Background: Main radiotherapy problem of cancer treatment is side-effect as a result of high dose of radiation. All of the modern apparatus for cancer therapy are not enough efficient when low-doses applying. It related with the biological sensitivity and reaction of tumor cells on radiation. Biological effects could be increased by using pulse-modulated radiation. It could allowed to significantly decrease radiation dose with saving antitumor efficacy. The source of low-dose repetitively pulsed X-ray radiation was first developed and created at the Institute of high-current electronics (Russia).
Material and Methods: 'Sinus-150' as a generator of pulse periodic X-ray was applied. A high-voltage pulse had a half-height duration of 4 ns and amplitude of 260 kV. The calculated photon energy spectrum had a maximum at 90 keV, and most of the quantum flux was the 60–200 keV range. Dose per pulse was 0.3 mR, absorbed dose were 0.12; 0.2 and 0.5 Gy for 2-time irradiation (day 6 and 9). Solid-type of Lewis lung carcinoma was prepared by intramuscularly transplantation of 3×10^6 cells into the hind limb of C57BL/6 female mice. Tumor volumes were measured with calipers and a volume calculated (L+W+W/2). The metastases of the lung were counted using a stereoscopic microscope.
Results: Low-dose pulsed X-ray inhibits growth of Lewis lung carcinoma cells at all experimental groups. Irradiation with absorbed dose 0.12 Gy affects 69% of tumor inhibition, 0.2 Gy – 56% and 0.5 Gy up to 46% compare to control group. Inhibition of metastasis growth (by square of colonies) was highest in group 0.5 Gy (72%) and lowest at 0.2 Gy absorbed dose (58%). Applying 0.12 Gy produced 68% decreasing of metastatic colonies square. Same time, index inhibition of metastasis (by number of colonies) was highest both in groups irradiated with 0.12 and 0.5 Gy (84−85%) and only 67% observed in group with absorbed dose 0.2 Gy.
Conclusions: Pulse regime increase antitumor efficacy of low dose X-ray up to 50−70% and antimetastatic action up to 60−80%. Similar effects of non-pulsed X-ray achieved when the absorbed dose exceed 10−20 Gy.
No conflict of interest.

1107 POSTER

X radiation effects on small cell lung cancer and non-small lung cancer − an in vitro study

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Background: Lung Cancer (LC) is one of the most diagnosed cancers, with higher mortality in men and among women is the fourth most commonly diagnosed and the second with highest mortality rate. This cancer is associated with smoking, which is the cause of about 80% in men and 50% in women worldwide. Radiation therapy may be used in all stages of LC, enabling better the disease site control, as well as the reduction of metastatization, however, tumor radioresistance is often a barrier to therapeutic effectiveness. The aim of this study was to assess the effects of ionizing radiation (X-rays) in three LC cell lines, two of non-small cell lung cancer (NSCLC) (H1299 and A549 cells) and one of small cell lung cancer (SCLC) (H69 cells), namely in cell proliferation and viability.
Materials and Methods: To attain the purposed objectives we submitted the 3 LC cells to X irradiation (0.5, 15 and 30 Gy**)** and used spectrophotometry, clonogenic assays and flow cytometry after 48 hours, to analyze the effect on cell proliferation, viability and death, and also to evaluated the effect on cell cycle.
Results: X-rays induces a decrease in cell proliferation and viability in a dose, time and cell line dependent manner, inducing cell death preferentially by apoptosis. These anti-proliferative and cytotoxic effects are in agreement with the observed cell cycle arrest. However, our results show that A549 and H69 cells are more sensitive to cell death induced by radiation, being the H1299 cells more resistant. These results may be related with differences in the p53 expression or stress oxidative response and could contribute to radiotherapy failure in this type of cancer.
Conclusion: Our preliminary results suggest that X-rays leads to a decrease in LC cells viability inducing cell death mainly by initial apoptosis. However, the sensibility and/or resistance to radiation may be dependent on molecular LC characteristics which could influence the response to radiotherapy and consequently treatment success.
No conflict of interest.

1108 POSTER

Prognostic value of the nodal ratio and Ki-67 expression in breast cancer patients treated with postmastectomy radiotherapy

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Background: This study was performed to evaluate the prognostic or predictive factors in breast cancer patients having postmastectomy radiotherapy (PMRT).
Material and Methods: A total of 113 patients received PMRT between 2003 and 2009: 61 patients underwent preoperative systemic therapy, and 52 patients received postoperative systemic therapy. Baseline molecular parameters were evaluated by immunohistochemical analysis, using biopsy specimens (preoperative systemic therapy group) or surgical specimens (postoperative systemic therapy group).
Results: The median follow up time was 72.3 (34.0–109.4) months for surviving patients. The median number of excised lymph nodes was 22 (1−55), and the median nodal ratio (NR) was 0.19 (range, 0−1). Higher NR had association with worse disease-free survival (DFS; relative risk (RR), 3.589; $p = 0.003$), and overall survival (OS; RR, 3.444; $p = 0.019$). Higher baseline Ki-67 was associated with poor locoregional progression-free survival (LRPFS; RR, 2.944; $p = 0.041$), DFS (RR, 3.274; $p = 0.002$), and OS (RR, 3.133; $p = 0.015$). Patients were classified into three subgroups: low risk (NR $\leqslant$0.2 and baseline Ki-67 $\leqslant$20%; n = 34), intermediate risk (NR >0.2 or baseline Ki-67 >20%; n = 63), and high risk (NR >0.2 and baseline Ki-67 >20%; n = 16). At the time of analysis, all the low risk group patients survived. Comparing the high and the low risk group patients, there was a significant difference in LRPFS ($p = 0.040$) and DFS ($p < 0.001$). Between the intermediate and the low risk group, significant difference was observed in DFS ($p = 0.022$), while not in LRPFS ($p = 0.204$).
Conclusions: In breast cancer patients having PMRT, higher NR and baseline Ki-67 were associated with worse outcomes. A prognostic model using these two factors can discern the patients with poor prognosis regardless the setting of systemic therapy, preoperative or postoperative.
No conflict of interest.

1109 POSTER

Spinal cord tolerance dose in fractionated stereotactic body radiation therapy for spinal metastases − can biologic equivalent dose according to linear quadratic model be applied?

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Background: Stereotactic body radiation therapy (SBRT) has been increasingly adopted to treat spinal metastases with its merit of rapid dose fall off near spinal cord. The authors hypothesized that irradiated dose to spinal cord is low (<6 Gy) enough to adopt the linear-quadratic (LQ) model in biological effective dose (BED) calculation with modern SBRT technique. This study is to verify this hypothesis, and report clinical outcome in patients with spinal metastases treated by fractionated SBRT.
Material and Methods: Between January 2010 and March 2012, 44 cases of spinal metastases in 33 patients were treated with Novalis Tx® at Gachon University Gil Medical Center. Spinal cord was contoured starting from 6 mm above the superior of the planning target volume(PTV) to 6 mm below the inferior of the PTV. Spinal cord itself was always excluded from PTV. Prescription doses to PTV ranged from 26−44 Gy in 4−6 fractions. The most common prescription dose was 40 Gy in 5 fractions.
Results: The rapid dose fall off from PTV resulted in much decreased dose to spinal cord. The maximum irradiated dose to spinal cord per fraction varied from 2.8 to 5.5 Gy (average, 4.3 Gy) depending on each PTV shape and fraction size. The maximum dose to spinal cord were 12.1–33.2 Gy (20.5 Gy) which were equivalent to 15.0–62.4 Gy2/2 (2 Gy per fraction with α/β ratio of 2, EQD2/2) (32.9 Gy2/2) according to LQ model. The average maximum dose to spinal cord was 54.8% of prescription dose to PTV. The average dose to the 10% spinal cord volume was 14.3 Gy (18.5 Gy2/2). The mean follow-up period was 9.8 months (0.3–37.4 months). No patient developed radiation-induced myelopathy or other neurologic deficit. During follow-up period, radiological local control rate and pain control rate were 80.1% and 90.9%, respectively. There were 3 compression fractures at 2, 3 and 4 months after treatment, respectively.

Conclusions: The dose to spinal cord was low enough to apply LQ model when spinal cord dose was constrained to 55% of prescription. The clinical outcome until now supports its validity without any radiation-induced myelopathy.
No conflict of interest.

1110 POSTER

Stereotactic body radiotherapy for spinal metastases using CyberKnife

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Background: To evaluate local control and pain and quality of life improvement for spinal metastases, with and without prior irradiation, treated with Stereotactic Body Radiotherapy (SBRT) using CyberKnife.
Material and Methods: Between August 2008 and December 2011, 47 lesions in 30 patients were treated with SBRT for spinal metastases. Twelve lesions (25.5%) were given re-irradiation for recurrence after prior radiotherapy. Patients were treated with a median dose of 27 Gy (range, 18−33 Gy) in a median of three fractions (range, 1−5) by CyberKnife Xsight™ Spine tracking system. The median target volume of 47 spinal metastatic lesions was 28.28 cm^3 (range, 0.9–301.45 cm^3). Radiation was prescribed to the 73−83% isodose line that encompassed at least 90 % of the tumor volume except one re-cyberknife case. SBRT dose was calculated with linear-quadratic model and normalized to a 2-Gy equivalent dose (nBED, α/β=2 Gy for spinal cord, α/β=10 Gy for tumor). Doses to a point within the spinal cord that received the maximum dose (Pmax) were checked. Local failure was defined as progression by imaging and/or clinically or the case that other therapy was given such as surgery or re-irradiation. Pain relief was assessed by Revised Oswestry Disability Index (ODI) Questionnaire before and 3 months after completion of SBRT. The median follow-up period was 6.3 months (range, 1.2–26.4 months).
Results: The local progression-free survival (LPFS) rate at 12 months was 84.7%, and 4 out of 47 (8.5%) tumors have progressed radiologically. Two of 4 treatment failure occurred in re-irradiated tumors, which received 21 Gy in 3 fractions, and their time to local progression was short (3.2 and 3.8 months). The median spinal cord Pmax nBED was 51.1 Gy2/2 (range, 3.4–75.1) in retreatment and 62.6 Gy2/2 (range, 3.2–84.9) in initial treatment. No neuropathy or myelopathy was observed during follow-up periods. No one suffered from spinal compression fracture. All patients experienced pain relief with SBRT. The ODI score dropped significantly from 51.5 to 18.4 on an average.
Conclusions: SBRT using CyberKnife is a safe and effective modality to achieve local control. SBRT offers significant pain relief in spinal metastases and maintenance of quality of life even after previous radiotherapy.
No conflict of interest.

1111 POSTER

Partial breast irradiation margins with two deep-inspiratory breath-hold techniques

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Background: Partial external beam breast irradiation (PBI) is being investigated in a number of ongoing phase III trials (IMPORT LOW, NSABP B-39, RAPID) and it is hoped that this technique will, in selected patients, reduce normal tissue doses whilst maintaining local control rates. Deep-inspiratory breath-hold with the active breathing coordinator™ (ABC_DIBH) significantly reduces the volume of heart irradiated, and voluntary deep-inspiratory breath-hold (v_DIBH) significantly reduces median heart and LAD volumes receiving >50% of the prescription dose. These dosimetric savings are projected to equate to a 10-fold reduction in cardiac deaths. Combining PBI with DIBH would be expected to reduce normal tissue doses yet further, however, data is lacking on suitable PBI margins to account for setup error and organ motion with DIBH. This study aimed to estimate appropriate CTV-PTV margins for using DIBH in combination with PBI.
Material and Methods: The UK HeartSpare Study (Stage IA) compared v_DIBH with ABC_DIBH in terms of positional reproducibility and normal tissue sparing. Patients were randomised to receive one technique for fractions 1−7 and the second technique for fractions 8−15 (40 Gy/15 fractions total). Cone-beam CT (CBCT) images were acquired for 6/15 fractions and matched to planning-CT data. Using clip-based matches, population systematic (Σ) and random errors (σ) were estimated. By applying the margin recipe proposed by van Herk (2.5 Σ + 0.7 σ), appropriate CTV-PTV margins were estimated for both DIBH techniques.
Results: Twenty-three patients were recruited between February and August 2012. Twenty-two patients underwent CBCTs and clip-based matches were possible in 18 (4 patients underwent mastectomy). In all, 126 CBCTs were analysed and uncorrected data was used. Σ for v_DIBH were 2.4 mm (right-left (R-L)), 3.6 (superior-inferior (S-I)), 3.0 mm (anterior-posterior (A-P)) and σ were 2.3 mm (R-L), 2.7 mm (S-I) and 2.7 mm (A-P). Σ for ABC_DIBH were 3.2 mm (R-L), 2.9 (S-I), 2.7 mm (A-P) and σ were 2.3 mm (R-L), 3.4 mm (S-I) and 3.5 mm (A-P). Estimated CTV-PTV margins for v_DIBH were 8 mm (R-L), 11 mm (S-I) and 9 mm (A-P) and for ABC_DIBH were 10 mm (R-L), 10 mm (S-I) and 9 mm (A-P).
Conclusions: Using either DIBH technique, a minimum uniform CTV-PTV margin of 10 mm is suggested for PBI.
No conflict of interest.

1112 POSTER

Accelerated partial breast irradiation (APBI) with tomotherapy HI-ART on 85 patients treated at San Giovanni-Addolorata Hospital Rome: Preliminary report

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Background: Partial Breast Irradiation (PBI) after conservative breast surgery is a novel radiation technique for the tumor bed area where the most frequent local recurrences occur. The smaller irradiated volume allows a higher dose fractionation with shorter overall treatment time (APBI) and reduced total dose to the breast as well as nearby structures. It was recently reported that exposure of the heart to ionizing radiation during whole breast irradiation (WBRT) increases the subsequent rate of ischemic heart disease and the increase is proportional to the mean dose to the heart.
Material and Methods: At the Breast Unit of our Hospital, since April 2011 we have enrolled 85 women in a phase II study on APBI, using a highly conformal external beam approach with Tomotherapy HI ART linac. Inclusion criteria were: age $\geqslant$50 years, unicentric and unifocal tumors smaller than 3 cm diameter, negative surgical margins without an extensive intraductal component (EIC) and lympho-vascular invasion (LVI). Patients received a total dose 38.5 Gy in 3.85 Gy fractions daily per 2 weeks. The clinical target volume (CTV) was the tumor bed and the close area defined by surgical markers. The primary end points of the study are local control and acute and late toxicity. Secondary end points are survival, cosmetic outcome, QoL and patient compliance.
Results: We present a preliminary report in terms of local control, toxicities, cosmetic outcome, QoL and patient compliance. In all cases the treatment was well tolerated and no acute or subacute side effects (according RTOG scale no toxicity > grade 1). Physical examination at 3−6−12 months and bilateral mammogram, US, MRI at 1 year, were performed on all women after radiotherapy. With a median follow-up of 10.5 months (range 3−22), there were no ipsilateral breast tumors and no loco-regional recurrences. The overall average of the mean doses to the whole heart was 0.9 Gy: much lower than that reported in cases of standard WBRT (>4.9 Gy).
Conclusions: The growing evidence obtained from phase I−II studies supports the use of APBI for selected early stage breast cancer. In our experience patients with tumor-related cautionary features will benefit from careful selection and a highly conformal external beam approach with Tomotherapy HI ART linac can significantly decrease the mean dose to the whole heart by at least 1/5 with respect to WBRT. These preliminary results need to be confirmed by longer-term follow-up data.
No conflict of interest.

1113 POSTER

Application of IMRT technique in treatment of malignant gliomas: Assessment of treatment tolerance

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Background: Assessment of tolerance of combined modality therapy of patients with malignant gliomas irradiated using IMRT technique. We compared dose distribution in IMRT and conformal 3D treatment plans.
Materials and Methods: Between 2009 and 2012 in the Oncology Center in Krakow 50 patients with malignant gliomas received combined modality treatment. Mean age was 53 years (range 24−70 years). All patients were in good performance status (WHO 0−1). There were 38 patients with glioblastoma multiforme and 12 with anaplastic astrycytoma.

38 patients underwent complete resection and 12 partial resection. Patient were irradiated using IMRT technique with a total dose of 60 Gy in 30 fractions. All patients concurrently received temozolamide in the dose of 75 mg/m^2. In all patients we performed additional plans using 3D conformal radiotherapy (3D-CRT) techniques and compared with IMRT plans. The 3D-CRT plans were prepared using 3−4 fields and IMRT plans consisted of 7−8 fields. The primary objective was to treat the planning target volume and to minimize the dose to organs at risk (OAR). Volumetric analysis, target coverage and conformity of prescribed doses were used in plan comparison.
Results: Treatment tolerance was vary good in all patients. Only 12 patients needed steroids during treatment. Adjustment of the dose distribution to the target volume was improved and the critical structures were better spared in the IMRT plans than in 3D-CRT plans. For all patients the mean dose and the maximum dose to OAR were significantly reduced in IMRT plans. With respect to target volume, IMRT technique reduced the maximum dose while increasing the minimum dose, resulting in improved conformity. In same patients with tumors located very close to OAR it was impossible to give 60 Gy for target volume with 3D-CRT technique because of not acceptable doses in OAR.
Conclusions: The IMRT technique combined with concurrent temozolamide is well tolerated and offers significant advantages comparing to 3D-CRT. Application of IMRT allows dose reduction at OAR without compromising target coverage.
No conflict of interest.

1114 POSTER

Feasibility of simultaneous integrated boost by helical tomotherapy in whole-pelvis radiation for prostate cancer: From a standpoint of acute toxicity

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Background: The validity of simultaneous integrated boost (SIB) by helical tomotherapy (TOMO) was assessed in terms of acute intestinal and urinary toxicity by comparing with 3-dimensional conformal radiotherapy (3DCRT) in the cases of whole-pelvis radiotherapy (WPRT) for prostate cancer.
Material and Methods: Thirty-eight consecutive patients who underwent curative WPRT were retrospectively reviewed. The pathologic diagnoses for all patients were adenocarcinoma, and median age was 68 years (range, 50−79 years). Twelve patients (31.6%) were treated by TOMO SIB method. A local boost for the prostate circumferential area was added to WPRT sequentially for 3DCRT and concomitantly for TOMO SIB. The median prostate dose was 64.8 Gy (range, 59.4 to 75.6 Gy) for 3DCRT and 66.6 Gy (range, 66.0 to 75.0 Gy) for TOMO SIB. The WPRT dose was 45.0 Gy (range, 41.4 to 45.0) for 3DCRT and 51.0 Gy (range, 50.4 to 54.0 Gy) for TOMO SIB. Acute toxicities were assessed according to RTOG criteria.
Results: The ratio of Grade 2 or higher acute intestinal toxicity was lower in the TOMO SIB group ($p = 0.008$). When intestinal toxicity was analyzed separately for rectum and bowel except rectum (BXR), TOMO SIB showed no significant difference in rectal toxicity ($p = 0.191$) with borderline superiority only in BXR toxicity ($p = 0.047$). The proportion of acute urinary toxicity of Grade 2 or higher was 55.3% (21 patients) with no significant difference in the proportion between the two groups ($p = 0.796$). On dosimetric analysis for the rectum, the mean dose ($p < 0.001$), dose delivered to 80% of the rectum (D80) ($p < 0.001$), V15 Gy ($p = 0.001$), V25 Gy ($p < 0.001$), V40 Gy ($p < 0.001$), and V45 Gy ($p = 0.029$) were higher in 3DCRT. For the bladder, dose delivered to 80% of the bladder (D80) ($p < 0.001$), V25 Gy ($p < 0.001$), V40 Gy ($p < 0.001$), and V45 Gy ($p < 0.001$) were higher in 3DCRT. For BXR, overall dosimetric data did not show significant difference between TOMO SIB and 3DCRT except maximum dose ($p < 0.001$).
Conclusions: Acceptable acute intestinal toxicity results by TOMO SIB should be verified with more detailed anatomic categorization such as rectum and BXR. TOMO SIB could not reduce acute urinary toxicity because of the inevitable high prostatic urethral dose exposure. Current dosimetry system did not reflect acute toxicities directly at the time of full dose WPRT, especially in urinary toxicity assessment. Proper dosimetric guidelines need to be determined in TOMO SIB.
No conflict of interest.

1115 POSTER

Is there a role for IMRT in bilateral breast cancer? Dosimetric comparison of IMRT and standard 3D conformal radiation therapy

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Background: The incidence of bilateral breast cancer is increasing due to improved diagnosis test and methods of screening. In addition, recent trials have shown the benefit of regional nodal irradiation. The aim of our study is to demonstrate if there is dosimetric benefit in women diagnosed with bilateral breast cancer that undergoing bilateral breast and regional nodal irradiation comparing standard 3D conformal radiation therapy 3DCRT) versus IMRT technique after breast conserving surgery.
Material and Methods: Volumes were delineated on 6 patients. The 3DCRT technique involved bilateral tangential fields for breast and two oblique, one anterior and one posterior, to the supraclavicular fossa. IMRT technique involved several multifield coplanar inverse planning. The prescription dose was 50 Gy in 25 fractions. Dose- volume histograms, dose homogeneity and dose to OAR were evaluated.
Results: See Table 1.

	V95 (%)		D99-D1 (%)		Mean dose (Gy)		V20 Gy (%)		V30 Gy (%)	
	IMRT	3D	IMRT	3D	IMRT	3D	IMRT	3D	IMRT	3D
Breast	96	96.6	(92.7–107.1)	(92.5–108.2)						
Supra	96.4	97.8	(91.7–106.4)	(91.1–107.5)						
R-Lung					15.8	18.3	25.7	35.3		
L-Lung					15.7	18.3	25.3	35.8		
R+L							25.5	32.5		
Heart					12.3	9.9			2.3	11.3

IMRT was superior to 3DCRT with improvements in reducing the volume of heart and lung in the high dose region (V30 and V20 respectively) and achieving lower mean lung dose (IMRT: 15.8 Gy versus 3DCRT: 18.3 Gy), although for some patients we would find within the limits of tolerance for lungs with both techniques. This can be explained due to larger gaps between inner parts of both breasts.
However, there were no significant improvements covering the planning target volume. Both techniques are adequate with good coverage in the V95 with no differences in PTV dose homogeneity.
Conclusions: IMRT provided reduction in the high dose heart volume without differences for improvements in coverage. We recommend this technique for cases with breasts showing a big concavity which embraces the heart and there is a high probability of superimposed beams on the skin in the gap between inner parts of both breasts.
No conflict of interest.

1116 POSTER

Impact of 6DOF robotic couch in high volume radiotherapy centre, surveying geometrical, dosimetric, management parameters

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Background: To investigate the impact of Protura 6 degree of freedom (DOF) Robotic Patient Positioning System (CIVCO Medical Solution) in high volume radiotherapy centre, surveying geometrical, dosimetric, management and parameters.
Material and Methods: We enrolled patients with pelvic, brain and head and neck cancer treated by 3D-CRT, RapidArc, IMRT and SRT radiotherapy.
A daily CBCT was acquired before dose delivery. The translational and rotational displacements obtained by 3D automatch were applied on the Protura Robotic Couch to obtain a more accurate alignment. The initial treatment plan was copied to a translated CT (TPt) and a rototranslated CT (TPtr) according by MIM 5.5.2 software according to the collected displacements.
Finally the daily dose volume histogram (DVH) was calculated for both the treatment plans and the dosimetric parameters were compared.
Results: From October 2012 to April 2013, we enrolled 23 patients: 8 affected by prostate cancer treated by RapidArc, 11 by brain cancer treated by RapidArc or 3D-CRT or SRT, 3 by H&N cancer, treated with IMRT.

The mean (±SD) interfraction displacement in vertical, lateral, longitudinal direction and the mean (±SD) interfraction rotations (Pitch, Roll and Yaw) were reported in the table 1.
Regarding prostate displacement, 85% of the translational shifts were <5 mm and 6% of the rotation were >2º. H&N translational shift smaller than 5 mm were 94% while 9% of the rotational shift were bigger than 2º. For brain and SBRT brain displacement, the translational shift <5 mm were 94% and 96% respectively, the rotational displacement >2º were 9% in both the group.
No correlation was observed between the magnitude of translational and rotational shifts.
The mean time for all treatment procedures take just one minute more respect to conventional IGRT without displacement correction by 6DOF robotic couch. The applied protocol is anyway no time consuming and it does not required any extension of staff.
Preliminary linear correlations were observed in a selected subgroup of prostate patients, between shifts and dosimetric parameters differences (PTt vs PTtr): yaw rotation with the V20 femoral heads and pitch rotation with V50 rectum.
Conclusion: Our preliminary results underline the feasibility of IGRT workflow with Protura system in daily clinical practice and in standard patient care. The data show the relevance of the translational and rotational errors and their dosimetric effect analysis is ongoing.
No conflict of interest.

Table 1. mean (±SD) interfraction translational and rotational patient setup error

Location	Translational error (mm)			Rotational error (º)		
	x axis (Lat)	y axis (Lng)	z axis (Vrt)	Pitch	Roll	Yaw
Prostate (n = 223)	0.5±3.8	−1.8±4.4	−1.8±4.1	−0.4±1.1	−0.2±1.1	−0.1±0.7
Brain (n = 164)	1.2±2.6	0.5±3.4	−1.2±1.8	−0.2±1.2	0.7±1.2	0.9±1.0
H&N (n = 24)	0.8±2.8	0.5±3.1	0.7±2.8	−0.3±0.7	0.95±1.2	0.1±0.5
SBRT brain (n = 17)	0.7±3.7	−1.2±3.4	−1.2±2.3	−0.5±1.0	−0.1±1.2	0.9±1.3

1117 POSTER

The role of stereotactic body radiation therapy for hepatocellular carcinoma refractory or unsuitable for other therapeutic modalities

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Background: The aim of this study was to evaluate safety and efficacy of stereotactic body radiotherapy (SBRT) for the naive or salvage treatment of inoperable hepatocellular carcinoma (HCC) were unsuitable for other therapies.
Methods: The authors reviewed the medical records of 30 patients that were treated by SBRT in our institution when they had HCC without another standard treatment option or complete response of loco-regional therapy between August 2010 and November 2013. All patients SBRT dosages (24−60 Gy from two to five fractions) were administered according to tumor volume. Survival, response, and toxicities were evaluated. Response evaluation was performed according to modified Response Evaluation Criteria for Solid Tumors.
Results: Twenty-five patients had Child-Pugh class A disease, 5 patients had class B disease. Eleven patients had macrovascular invasion (7 portal vein thrombosis, 2 hepatic vein thrombosis, 2 both venous thrombosis), 2 patients had bile duct invasion. The median greatest tumor dimenstion was 32.5 mm (range, 10–170 mm). The median survival was 9.5 months (range 4−28 months) and the median progression-free survival was 6 months (range 1−19 months). Twenty-five patients (83.3%) achieved complete response within 6 months after complete SBRT, 2 patients (6.7%) had a partial response, 3 patients had stable disease, and 1 patients had progression disease. Infield local recurrence was observed in 3 patients, and outfield failure was 13 patients. Three patients (10%) experienced grade 3 gastrointestinal toxicity, 1 patients (3.3%) experienced grade 4 gastric ulcer perforation, and 4 patients (13.3%) experienced pneumonitis.
Conclusions: This study suggests that SBRT can be effective and safe modality that achieves promising rates of local control in inoperable HCC, even with vascular invasion. A further well controlled, large scaled study to reduce gastrointestinal and pulmonary toxicity is recommended.
No conflict of interest.

1118 POSTER

A comparison study utilizing portable bladder scanner versus cone beam computed tomography (CBCT) to measure bladder volumes in post-prostatectomy patients having radiotherapy

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Background: In post-prostatectomy radiotherapy to the prostatic bed, consistent bladder volume is essential to minimize the amount of small bowel within the radiotherapy fields and to maintain the position of the treatment target volume. We assessed the differences between bladder volume readings from a portable bladder scanner (BS-V) and those obtained from planning CT (CT-V) or cone-beam CTs (CBCT-V). Inter-fraction bladder volume variation was also determined.
Material and Methods: BS-Vs were recorded in the treatment position pre- and post-planning CT or CBCT. The percentage difference between the readings using the two imaging modalities, standard deviations, and 95% confidence intervals were determined. Data was analyzed for the whole patient cohort and separately for the older BladderScan™ BVI3000 and newer BVI9400 model. Inter-fraction bladder volume variation was evaluated by determining the percentage differences between the CT-V with CBCT-V. Treatment duration incorporating the BS and CBCTs were determined.
Results: Fourteen patients were enrolled into this study, producing 133 datasets for analysis. BS-Vs were taken using the BVI9400 in four patients (43 datasets). The mean BS-V and CT-V or CBCT-V was 253.2 mls and 199 cm^3, respectively. The mean percentage difference between the two modalities was 19.7% (SD 42.2; 95% CI 12.4 to 26.9). BVI9400 model (n = 43) produced more consistent readings with a mean percentage difference of −6.2% (SD 27.8; 95% CI −14.7 to −2.4%). The mean percentage difference between CT-V and CBCT-V was 31.3% (range −48%- 199.4%). Treatment duration from time of first BS reading to CBCT was on average 12 minutes (range 6−27).
Conclusions: The BS produces bladder volume readings of an average of 19.7% difference from those CT-V or CBCT-V and can potentially be used to screen for large inter-fraction bladder volume variations in radiotherapy to prostatic bed. The observed inter-fraction bladder volume variation suggests the need to improve bladder consistency. Incorporating the BS into practice is feasible.
No conflict of interest.

1119 POSTER

Verification of mechanical accuracy of new irradiation technique with simultaneous gantry and ring rotation

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Background: Vero4DRT (MHI-TM2000) is a unique image-guided radiotherapy system consisting of an O-ring gantry that is designed to rotate ±185º around the patient (gantry rotation) and ±60º around its vertical axis (ring rotation). This system can realize a new irradiation technique called Dynamic WaveArc (DWA) which employs continuous and simultaneous gantry and ring motion during dose delivery. The purpose of this study is to verify the mechanical accuracy of DWA irradiation.
Material and Methods: (1) Verification of irradiation accuracy during ring rotation. Beam outputs during ring rotation (rotation range were −30º − +10º and −10º − +30º for clockwise (CW) and counter clockwise (CCW) directions) were measured using a farmer type ionization chamber and cylindrical phantom, and compared to the output at static irradiation. At the same time, the mechanical accuracy was assessed by analyzing linac log files. The ring position error (E_R) and accumulated MU error (E_{MU}) at each time were evaluated as the differences between planned and actual values recorded in the log files.
(2) Mechanical verification of DWA irradiation by log file analysis. Some test pattern irradiations with different ring rotational range (±5º, 10º, 15º, 30º), direction (CW, CCW), and speed (1.0, 2.0, 3.0 deg/s) were examined. In all test plan, gantry were rotated from 270º to 90º (CW direction). The E_R, E_{MU}, and gantry position error (E_G) at each time were evaluated by the log files.
Results: (1) Beam output variation with ring rotation was less than 0.2% on the basis of the output value with static irradiation. E_R and E_{MU} were less than 0.03º, 1.1 MU, respectively.
(2) E_R, E_{MU}, and E_G were less than 0.11º,3.4 MU, and 0.13º, respectively. Although each mechanical motion were stopped momentarily (for about 0.5

sec) in the turning point of gantry and ring speed, the beam irradiation was continued with low dose rate (nearly 100 MU/min) in the actual treatment machine. Therefore, maximum E_{MU} were occurred in the suspended time. These differences were compensated in about 2 seconds after that time. Except these time, E_{MU} were less than 1.5 MU.
Conclusions: As an initial experiment, we have demonstrated that Vero4DRT has sufficient mechanical accuracy and beam output constancy during gantry and ring rotation. A more quantitative evaluation of irradiation accuracy, treatment planning and dose distribution are needed to apply this new irradiation technique in clinical settings.
Conflict of interest: *Advisory board: T. Mizowaki and M. Hiraoka have consultancy agreement with Mitsubishi Heavy Industries Ltd., Japan. Other substantive relationships: M. Yamada and S. Kaneko have substantive relationship with Mitsubishi Heavy Industries Ltd., Japan.*

1120 POSTER

Development and evaluation of a system based on 3D printer to create IMRT compensator blocks

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Background: In this study a system based on 3D printer was developed and evaluated to manufacture IMRT compensator blocks from a fluency map. This alternative method doesn't use milling machines.
Material and Methods: A fluency map of a prostate case planned with Jaws-Only IMRT (Prowess Panther®, v.5.01) was selected from our clinical database. An algorithm was developed to convert an image file into a STL file (used by 3D printers). The gray levels of the intensity map were converted into a STL file representing a mold thicknesses. The mold was printed by a 3D printer (Z Corp®, model 310). It was made with a thermal resistant powder. Then it was filled with *cerrobend* alloy. After solidification time, the mold was removed and the final IMRT block was achieved. The algorithm considered previously data acquired about linear attenuation coefficient and beam hardening factor of the *cerrobend* alloy. In order to evaluate the block, dosimetric tests were performed in 3 planes of a phantom (depths: 2.5, 6.5 and 15.5 cm) using a matrix with 729 ionization chambers (PTW®, 2D Array). Phantom/detector CTs were used to generate reference dose distributions of the selected beam (intensity map) predicted by a Jaws-Only IMRT algorithm. The gamma-index function (3%, 3 mm) was used for dose distribution evaluation. The Monitor Units (MU) were defined carrying out measurements of absolute dose using a thimble ionization chamber in a homogeneous dose region. It was used the MU that provided a dose closer than that predicted by TPS. Further, absolute doses were measured in 7 depths for 2 regions of the IMRT block. The block thicknesses of these regions were 0 and 1.5 cm.
Results: The mold printing and the manufacturing of the IMRT block took 4 h. The MU used was 10% less than that reported by the same Jaws-Only IMRT plan. The MU used was 60 (dose deviation of 0.66%). The measurements using a matrix of detectors provided 81, 99 and 91% of the assessed points approved for the depths of 2.5, 6.5 and 15.5 cm respectively. The absolute doses measurements presented dose deviations up to 1.8% for all depths and both regions.
Conclusions: The 3D printers can be effectively used to manufacture IMRT compensator blocks. The advantages to this approach are: it can be fully conducted inside a radiotherapy facility; 3D printers are easier to operate. The results suggest lower cost and production time. Further investigations are in progress to permit the clinical use.
No conflict of interest.

1121 POSTER

Dosimetric relevance in prostate cancer of 6 degree of freedom patient correction with Protura Robotic Couch System

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Background: To investigate the magnitude and the dosimetric relevance of translational and rotational errors on IGRT prostate RapidArc using Protura 6 degree of freedom (DOF) Robotic Patient Positioning System (CIVCO Medical Solution).
Material and Methods: We enrolled patients with cT3aN0M0 low risk for nodal involvement prostate cancer, treated with RapidArc simultaneous integrated boost. PTV2 was obtained adding margins of 0.7 cm to seminal vesicle base (CTV2), while PTV1 adding to prostate (CTV1) a margin of 0.7 cm in all directions and 1.2 cm as lower margin. A daily CBCT was acquired before dose delivery. The translational and rotational displacements obtained by 3D automatch were applied on the Protura Robotic Couch to obtain a more accurate alignment. The initial treatment plan was copied to a translated CT (TPt) and a rototranslated CT (TPtr) according by MIM 5.5.2 software according to the collected displacements. Finally the daily dose volume histogram (DVH) was calculated for both the treatment plans and the dosimetric parameters were compared.
Results: From October 2012 to April 2013, we enrolled 8 patients with a median age of 76 yrs (range 72−77). We performed 223 CBCT studies, 223 TPt and 223 TPtr. The mean (±SD) interfraction displacement in vertical, lateral and longitudinal direction was-1.8±4.1 mm, 0.5±3.8 mm and −1.8±4.4 mm respectively, with 85% of the shifts <5 mm. The mean (±SD) interfraction rotations were: Pitch= −0.4±1.1º, Roll = −0.2±1.1º and Yaw = −0.1±0.7º, with 6% of the rotations >2º. No correlation was observed between the magnitude of translational and rotational shifts. Dosimetric evaluation is ongoing and nowadays it was carried out on 4/8 patients We observed two linear correlations between yaw rotation and the V20 femoral heads and pitch rotation with V50 rectum. Regarding prostate target coverage, V95% and V105%, no significant difference between the TPt and TPtr was observed.
Conclusion: Our preliminary data show the relevance of rotational shift in prostate patients. The preliminary dosimetric data underlines that the used PTV margins are such as to compensate all translational and rotational shifts detected before treatment. Dosimetric effects evaluation of PTV reduction margins, useful to promote dose escalation studies, is ongoing.
No conflict of interest.

1122 POSTER

Stereotactic Body Radiotherapy (SBRT): Are we improving? Trends from a single Brazilian institution

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Background: The possibility of delivering radiation in a single high dose or with short radiation courses of high doses/fraction is very attractive. Though, the use of SBRT for curative or palliative purposes is becoming very popular besides the fact that the technology needed for such treatment is not yet worldwide available. The objective of this study was to make a profile of the indications of SBRT in the institution.
Materials and Methods: Data of patients' registers from May/2007 (first treated patient) to February/2013 were retrospectively collected. We extracted data on patients' characteristics (demographics), treatment sites, data processing, fractionation, dose and technique. Two independent researchers performed data collection.
Results: 74 patients, 47 (63.5%) men, with histologically proven primary or metastatic tumors (clinical/radiographic or by biopsy) were treated. The median age was 73 years (31−96 years). Ten (13.5%) patients were treated from 2007 to 2009 (period 1), and 64 (86.5%) from 2010 to 2013 (period 2). The most usual treated site was lung, comprising 51/74 (70%) lesions (39 primary and 12 metastatic tumors). In period 1, all 10 treated patients had primary lung tumors. In period 2, there was still a predominance of lung tumors 41/64 (64.1%). In addition, 17 (26.5%) bone tumors (4 primary and 13 metastatic), 5 (7.9%) liver metastases and 1 (1.5%) primary prostate cancer was also treated. One to five fractions from 7 Gy to 24 Gy with total doses ranging from 12 to 60 Gy were delivered. Single doses were mostly used for bone tumors, and lung and hepatic lesions were treated with 3 or 5 fractions. Prescription ranged from 78 to 85% isodose. The equivalent dose to 2 Gy fractions (EQD2) was calculated for evaluation of the different fractionation schemes. Three-dimensional (3D) conformal technique was the most widely used (81%), followed by volumetric arc therapy (VMAT) (15%) and intensity modulated radiotherapy (IMRT) (4%). In period 1, only 3D techniques was used, and in period 2, 78.2% of patients were treated with 3D, 15.6% with DA, and 6.2% with IMRT. In all cases, prescription and plan analysis was based on already published protocols (RTOG and others) on the respective disease site, and 74% of the plans respected absolutely all the required dosimetric criteria of the protocols; the others were approved with minor deviations.
Conclusion: In this private institution, there has been an increase in the use of SBRT along the years. A progressive indication for different tumor sites, as well as an evolution of the treatment technique was observed. All treatments were based in already published and well-established protocols.
No conflict of interest.

1123 POSTER

Is what you move what you get? A phantom study of surrounding tissue importance in delineation of moving targets

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Purpose: To evaluate the relevance of surrounding tissues for contouring of moving targets in tissue specific phantoms.
Material and Methods: The xy-table (SunNuclear®) was combined with a self-developed equipment. This equipment enabled programable motion in 3 dimensions of a 3.4l PMMA box. A water filled table tennis ball simulated the tumor. The ball was placed centrally in PMMA box, surrounded by three different materials: corkboards to simulate lung tissue (lung phantom − LunPh), animal fat to simulate fatty soft tissue (fatty tissue phantom − FatPh) and water with contrast medium with hounsfield units (HU) adapted to simulate liver tissue (liver phantom − LivPh). Slow planning 3D-CT (Somatom Emotion, Siemens Medical Solutions®) were aquired with and without phantom movements (period 3s). Linear motions in y direction (cranio caudal) with amplitudes of 5 and 10 mm, respectively and complex motions with simultaneous amplitudes in x, y and z directions (2.5, 10, 2.5 mm, period 3 s) were performed. Additionally, patients' tumour trajectory were reconstructed using the center of mass motion of GTVs delineated in the 10 phases of 4D-CT scans. These movements were simulated with each phantom. The ball volume was contoured using the Eclipse 10 planning system (Varian Medical systems®) in optimal window settings The contoured ball volumes were compared with the mathematically calculated ball volumes.
Results: The relative differences between contoured and calculated volumes are presented in the table. The volume of the ball without movement was overestimated in LunPh and FatPh and underestimated in the LivPh. As compared to the real volume, the volume of the moving target was underestimated in all phantoms. Further, significant differences were found between the three phantoms.

Table: Relative difference between contoured and calculated ball volume

Motion amplitude	Relative difference [%]		
	LunPh	FatPh	LivPh
0	111.9	112.4	96.4
y 5 mm	94.4	97.4	69.6
y 10 mm	91.2	90.8	70.3
x 2.5 mm, y 10 mm, z 2.5 mm	89.7	91.5	82.7
Patients (average ± standard deviation)	93.7±7.0	91.9±2.8	71.5±5.4

Conclusion: Our results showed relevant differences between real target volumes and contoured target volumes if the target is surrounded by different tissues. This could have an impact on the gross tumor volume and internal target volume delineation, and needs to be critically considered when delineating target volumes at different anatomical sites.
No conflict of interest.

1124 POSTER

Quantitative study of 18F-fluorodeoxyglucose and 18F-fluorothymidine PET characteristics in esophageal squamous cell carcinoma staging

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Objective: PET has become the key investigative tool with its expanding role in esophageal carcinoma diagnosis, staging and assessing or predicting response to therapy. Until recently, early detection and surgery are the hope of cure for patient with esophageal carcinoma. In addition, precise pretherapeutic staging is crucial in choosing best available therapy for esophageal carcinoma patient. The aim of this study was to quantitatively evaluate the value of diagnostic information provided by both ^{18}F-FDG and ^{18}F-FLT PET and quantitatively investigated whether ^{18}F-FLT PET had a better performance compared with ^{18}F-FDG PET in esophageal squamous cell carcinoma (ESCC) staging and delineation.
Materials and Methods: 26 patients with newly diagnosed ESCC and underwent pretreatment ^{18}F-FDG and ^{18}F-FLT PET were included in this study. The indices such as the standardized uptake value (SUV), gross tumor length and extracted texture parameters between ^{18}F-FDG and ^{18}F-FLT PET were compared, respectively. Moreover, the indices' relationship between ^{18}F-FDG and ^{18}F-FLT PET mentioned above, were analyzed using Spearman's correlation coefficient and Paired T-test. Subsequently all patients received esophagectomy and the extracted PET indices' capability in ESCC pathological staging were assessed by Kruskal-Wallis test and Mann-Whitney test. In addition, tumor delineation length on ^{18}F-FDG (SUV threshold 2.5) and ^{18}F-FLT (SUV threshold 1.4) PET were validated by pathologic gross tumor length.
Results: ^{18}F-FDG highly correlated with ^{18}F-FLT possessing a high correlation coefficient value r approximate 0.8 and $p < 0.001$ in SUVmax or SUVmean. ^{18}F-FDG uptake was significantly higher than ^{18}F-FLT with respect to average SUVmax(^{18}F-FDG: 11.48, ^{18}F-FLT: 6.07) or average SUVmean (^{18}F-FDG: 6.09, ^{18}F-FLT: 3.80), with Paired T-test result $p < 0.001$. In terms of texture parameters' relationship Entropy and Correlation (two derived texture parameters) showed statistically significant difference. Both of ^{18}F-FDG and ^{18}F-FLT PET SUV, some of texture parameters,gross tumor length and shape feature showed statistically significant difference with respect to their feasibility in ESCC staging. The mean±standard deviation pathologic longitudinal tumor length was 5.52±2.56 cm and delineation length for ^{18}F-FDG and ^{18}F-FLT were 5.60±2.32 cm and 5.49±2.43 cm, respectively.
Conclusion: The ^{18}F-FDG and ^{18}F-FLT PET scans have their own advantages in ESCC staging and tumors were well identified as the nonphysiologic distribution of radiotracers intensity typically higher than normal tissues on either PET scans. Delineation on the two types of PET with proper threshold can both provide accuracy estimation of pathologic tumor length. Those different indices extracted from PET scans can be potentially employed to differentiate AJCC and TNM in ESCC stage.
No conflict of interest.

1125 POSTER

Comparative study of the position and/or volume of diaphragm dome, lung and heart between quiet end-inspiration and end-expiration three dimensional CT assisted with active breathing control and corresponding phases in four dimensional CT

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Background: To compare the position and/or volume of diaphragm dome, lung and heart between quiet end-inspiration and end-expiration three dimensional CT (3DCT) assisted with active breathing control (ABC) and the corresponding phases in four dimensional CT (4DCT).
Material and Methods: Eighteen patients with peripheral lung cancer underwent 4DCT simulation scan during free breathing and 3D-CT simulation scans in end-inspiratory hold (CT_{EIH}) and end-expiratory hold (CT_{EEH}) in succession. The 4DCT images from each respiratory cycle were sorted into 10 phases: the 0% phase was defined as end-inspiratory phase (CT_0), while the 50% phase was defined as end-expiratory phase (CT_{50}). The left and right lungs, heart and both diaphragm domes were delineated separately on CT_0, CT_{50}, CT_{EIH} and CT_{EEH} images.
Results: In the cranio-caudal direction, between CT_{EIH} and CT_{EEH}, CT_0 and CT_{50}, the mean displacement differences of both diaphragm domes were not larger than 1.5 mm and were not statistically significant (P = 0.228, 0.106). Between CT_{EIH} and CT_0, CT_{EEH} and CT_{50}, the centroid position differences of two lungs and heart were found all statistically significant (P = 0.001–0.047) in the cranio-caudal direction, and not statistically significant (P = 0.128–0.798) in the radial directions. The volumes of two lungs were both larger in CT_{EIH} and CT_{EEH} than in CT_0 and CT_{50}, and the differences between them were found both statistically significant (P = 0.000–0.041); while the volume of heart was larger in CT_0 and CT_{50} than in CT_{EIH} and CT_{EEH}, but the differences between them were found both not statistically significant (P = 0.054, 0.085).
Conclusions: Compared with quiet end-inspiration and end-expiration 3DCT assisted with ABC, in the corresponding limited phases of 4DCT, the centroid positions of lungs and heart had obvious hysteresis in the cranio-caudal direction, and the volumes of lungs were obviously larger in the former than in the latter, while the volumes of heart were smaller in the former than in the latter. From the point of view to protect lung and heart, when other conditions were same, gated radiotherapy in quiet end inspiration was better basing on ABC than basing on 4DCT.
No conflict of interest.

1126 POSTER

Impact of anatomic changes and treatment parameters on simulated dose to lung tumors in a patient cohort with sequential 4D-CTs

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Background: The physical and biological properties of scanned carbon ion beam therapy potentially permit more conformal irradiation than photons. Range sensitivity and interplay renders treatment of moving tumors complex. Purpose of this study is to investigate adaptive treatment of lung cancer in a sequential 4DCT planning study.
Materials and Methods: For 7 NSCLC lung tumor patients from MDACC (The University of Texas MD Anderson Cancer Center), a total of 55 weekly 4DCT datasets were available. Reference phases of each subsequent CT were registered rigidly (based on bony anatomy or the complete CT) to mimic patient setup. Motion phases of each 4DCT were registered non-rigidly. Gating plans were simulated using the GSI treatment planning system TRiP4D, including 4D-dose reconstructions. For each calculation, the target point was set as the center of the CTV in reference phase. The impact on dose coverage (V95) of variations in focus size and length of the gating window was analyzed. Three beam foci (6, 10 and 15 mm) and three gating windows (11.9%, 30% and 50% of the amplitude) were investigated. To assess the need of margins, these initial simulations were performed without a CTV-PTV extension.
Results: The largest effects on dose coverage were caused by anatomic variations such as tumor shrinkage but also different patient positioning with deformed soft tissue. For three patients, such variations reduced V95 below 75% for all studied parameter combinations.
A larger beam focus and a shorter gating window increased the homogeneity of the dose, but not sufficiently to compensate the anatomic changes. The worst configuration of longest gating window and smaller focus yielded a mean V95 value of 79.7% (from 51.3% to 96.3%) while the best configuration of shortest gating window and largest focus yielded V95 = 91.2% (71.0% to 99.7%).
Conclusions: Dose coverage deteriorated due to anatomic changes as well as patient setup. Gating window and beam focus size could partially recover 4D-dose coverage, but adaptive treatment schemes appear necessary. CTV-PTV margins will most likely cover patient setup uncertainties and will be included as a next step.
No conflict of interest.

1127 POSTER

External beam radiotherapy target volumes for cervical cancer: A multi-institutional study assessing contouring variability on magnetic resonance imaging (MRI) and computer tomography (CT)

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Background: Accurate delineation of target volumes for external beam radiotherapy is a crucial step in radiation treatment, but can be associated with considerable uncertainty and variability. The aim of this study was to compare target volume delineation on MRI vs CT and to compare Simultaneous Truth and Performance Level Estimation (STAPLE) generated contours with expert clinician contours.
Material and Methods: Nine expert clinicians from five tertiary centres across Australia participated. Each clinician independently contoured target volumes on a CT and a T2 weighted 3T MRI dataset for a cervical cancer patient. Gross tumour volume (GTV), uterus, cervix, vagina and parametrium were each delineated using RTOG consensus guidelines and the individual volumes were combined into a clinical target volume 'CTV'. To evaluate inter-observer agreement the kappa co-efficient was calculated for each structure, and the dice similarity co-efficient (DSC) was used to compare individual clinician contours for each structure to both a clinician determined consensus contour and the STAPLE contour.
Results: The GTV_{staple} volume for MRI was 2.5 times smaller than the CT volume (17.1 cm^3 vs. 43.1 cm^3) and there was less inter-observer variation when contouring the GTV on MRI compared to CT, with a kappa coefficient (corrected for chance) of 0.70 for MRI and 0.52 for CT. The MRI contours for the uterus and CTV demonstrated substantial agreement between clinicians with kappa coefficients 0.64 and 0.68 respectively. The remaining contours completed on MRI showed moderate agreement with kappa coefficient of 0.41 for cervix, 0.43 for vagina and 0.56 for parametrium. The cervix contours on CT only showed slight agreement with a kappa coefficient of 0.31. The remaining structures on CT (uterus, vagina, parametrium and GTV) demonstrated moderate or substantial agreement. The MRI GTV_{staple} contour demonstrated a high concordance with the clinician determined consensus contour with a DSC of 0.77. The MRI $uterus_{staple}$, $parametrium_{staple}$ and CTV_{staple} contours all exhibited a good concordance with the clinician determined consensus contour with DSCs of 0.74, 0.69 and 0.80 respectively.
Conclusions: GTV delineation on MRI resulted in a smaller GTV and reduced inter-observer variability compared to CT. STAPLE contours on MRI demonstrated a high level of agreement with expert clinician consensus contours for GTV, uterus, parametrium and CTV.
No conflict of interest.

1128 POSTER

Dosimetric comparison of four different irradiation techniques to treat mediastinum in Hodgkin Disease (HD) and lung cancer (LC) patients (pt): Three dimensional conformal radiation therapy (3D-CRT), intensity modulated radiation therapy (IMRT), volumetric modulated arc therapy (VMAT) and helical tomotherapy (HT)

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Aims: To compare PTV coverage and OAR dose distribution in the treatment of mediastinum in HD and LC pt, treated with 30 Gy and 50 Gy using 3DCRT, IMRT, VMAT, HT.
Methods and Materials: 10 HD and 9 LC pt were treated with 3D-CRT. 2 experimental plans were calculated for each technique and patient: 1 optimized for PTV coverage, 1 for lung dose reduction. IMRT static fields(f) plans were evaluated with a 5 and 8f combination. In LC pt, for HT plans optimized for the reduction of lung doses, 2 plans were calculated (collimation width 1 cm /2.5 cm); 9 and 10 plans were calculated for each pt. Optimal PTV coverage required D95 and D98 >95% of prescribed dose; lungs constraints were V20 <20% and V5<50%. PTV and OAR doses were directly compared. The best experimental plan was compared with that used to treat the pt. Differences were considered significant if $p < 0.01$ (T-student).
Results: Results are presented in the tables. LC: a statistical direct correlation was shown between 'PTV length/lung length' ratio (PLLLR) and lungs V5 for 3D-CRT, IMRT with 8f and VMAT plans and an inverse correlation between PLLLR and PTV D95 for HT plans.
Conclusion: For HD the dose distributions obtained with HT seem to be better than with the other techniques, with a high target coverage and a reduction of high doses to lungs and spinal cord. For LC IMRT with 8f is the best technique to obtain the goals of the optimization. VMAT can be used when the PLLLR is low. HT doesn't allow to maintain an acceptable PTV coverage if the ratio is high. Clinical correlates needed to evaluate impact on practice of the findings.
No conflict of interest.

Table 1 (abstract 1128). Dose optimization in HD

		5f vs 8f IMRT		8f IMRT vs VMAT		VMAT vs TOMO		3D-RT vs TOMO	
		target	lung	target	lung	target	lung	target	lung
PTV	D(95%), Gy	ns	ns	$p < 0.005$	ns	ns	ns	$p < 0.005$	$p < 0.01$
	D(98%), Gy	$p < 0.001$	ns	$p < 0.01$	ns	ns	ns	$p < 0.005$	ns
Lung	V(5 Gy), %	$p < 0.01$	ns	ns	ns	ns	ns	$p < 0.005$	$p < 0.01$
	V(20 Gy), %	ns	ns	ns	ns	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$
	D_{avg}, Gy	$p < 0.005$	$p < 0.005$	ns	ns	$p < 0.005$	$p < 0.005$	ns	ns
CI		ns	ns	ns	ns	ns	ns	$p < 0.005$	$p < 0.005$

Table 2 (abstract 1128). Lung reduction dose optimization in LC

		5f vs 8f IMRT			8f IMRT vs VMAT			8f IMRT vs HT			3D-CRT vs HT		
		5IMRT	8IMRT	p	8IMRT	VMAT	p	8 IMRT	HT	p	3D-CRT	HT	p
PTV	D(95%)	47.2 Gy	48.1 Gy	ns	48.1 Gy	46.8 Gy	0.005	48.1 Gy	45.7 Gy	ns	46.5 Gy	45.7 Gy	ns
	D(98%)	45.4 Gy	46.7 Gy	ns	46.7 Gy	44.9 Gy	0.005	46.7 Gy	43.5 Gy	0.005	45 Gy	43.5 Gy	ns
Lungs	V(5 Gy)	48.5%	46.3%	ns	46.3%	44.2%	ns	46.3%	50%	0.01	53.3%	50%	ns
	V(20 Gy)	18.3%	17.9%	ns	17.9%	14.9%	0.005	17.9%	16.2%	0.01	17%	16.2%	ns
	D_{avg}	10.5 Gy	10.5 Gy	ns	10.5 Gy	9.1 Gy	0.005	10.5 Gy	9.6 Gy	0.005	10.7 Gy	9.6 Gy	ns
CI		1.7	1.9	ns	1.9	1.6	0.005	1.9	1.3	0.005	1.8	1.3	0.005

1129 POSTER

Study of usefulness of megavoltage computed tomography for electron beam treatment planning for conjunctival MALT − lymphoma

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Background: The Kilo-voltage Computed Tomography (KVCT) has been used for electron beam treatment planning for conjunctival MALT − lymphoma; however, its lens shielding generates metal artifact, leading to inaccurate treatment planning. This study assessed the usefulness of treatment planning using the Mega-voltage Computed Tomography (MVCT) images without metal artifact.
Material and Methods: The Image Value-to-Density Table (IVDT) of KVCT and the IVDT of MVCT was calculated by using the Cheese Phantom® and then the density values calculated from the above process was input in the Pinnacle®. As establishing the treatment planning and measuring the projection result with the EBT3 Film® using the Elekta Infinity™, the $10\times10\,cm^2$ electron cone with 6-MeV and 9-MeV electron beam was used at 300 MU and the Source-Skin Distance (SSD) was set to 100 cm.
First, the treatment planning of KVCT was matched to that of MVCT using the IVDT to measure the consistency.
Second, the error of treatment planning of KVCT caused by metal artifact (generated by lens shielding) was compared with that of MVCT caused under the same conditions.
Third, the treatment planning with the above settings was executed for the MVCT image (with lens shielding) matched using the IVDT and the electron beam was projected to the EBT3 Film® (with lens shielding) with the same conditions. The acquired MVCT result and EBT3 Film® result were analyzed using Axial PDD, D_{max} beam profile, and R_{50} beam profile.
Results: First, for the γ − index between the KVCT and the MVCT matched using IVDT, the γ value has not exceeded 1 at 3%/3 mm and 2%/2 mm.
Second, the radiation difference between the KVCT and the MVCT with the lens shielding was calculated as about 74%.
And third, after analyzing the level of radiation projected to the MVCT image for treatment planning and to the EBT3 film, the γ value has not exceeded 1 at 3%/3 mm for 6-MeV and 9-MeV. However, for 6-MeV at 2%/2 mm, 6.34% of axial PDD and beam profiles of D_{max} and R_{50} were 3.48% and 4.21%, respectively, and for 9-MeV at 2%/2 mm, 10.68% of axial PDD and beam profiles of D_{max} and R_{50} were 3.85% and 6.23%, respectively.
Conclusion: This study assessed the error rate of treatment planning, caused by metal artifact, between the KVCT and the MVCT.
As shown in the study, metal artifact was generated at the MVCT, too. However, as comparing to the result of EBT3 Film®, the error rate was lower than 3%, within the clinically-allowable range.
The result showed that the MVCT could get images which allowed establishment of an accurate treatment planning without being affected by metal artifact. It will be a new solution to improve the weak point of KVCT, inaccurate electron beam treatment planning for conjunctival MALT − lymphoma.
No conflict of interest.

1130 POSTER

Evaluation of dose distribution using positioning CT in IMRT for prostate cancer

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Background: In room cone beam CT (CBCT) is a popular modality for reducing setup errors nowadays, however, CT numbers of CBCT is not reliable enough for the dose calculation by treatment planning system (TPS). We analyzed dose distribution using conventional diagnostic CT image that was obtained for checking the patient position just before the treatment.
Material and Methods: The dose distributions for 8 patients with prostate cancer were evaluated. IMRT was done by step and shoot technique with 7 fields. The CTV included prostate and proximal seminal vesicle, and the PTV margin was 5 to 7 mm in the posterior direction, and 10 mm in other directions. The prescribed dose was 72 Gy at D50 of the PTV. We daily used Linac graphy to check setup errors and also used CT image that was acquired in the separate CT room weekly. Patients were transported to the treatment room in the fixation device after CT scanning and underwent radiotherapy. After the CT image dataset for verifying the isocenter was transferred to TPS (Xio), the target volumes and organs at risk (OARs) were contoured by the same physician in the same manner as done on planning CT image. The IMRT plan was imported as a QA plan, and dose calculation and evaluation of dose for contours were performed.
Results: The dose evaluation could be done with total of 41 CT datasets, 1 to 8 (median of 6) times for each patient. For the CTV, the mean EUD was 2.00 Gy, ranged from 1.97 to 2.03 Gy and D98 was more than 1.92 Gy except for one CT. For the PTV, the mean EUD was 1.93 Gy (range, 1.31–2.01 Gy) and D98 was more than 1.9 Gy only in two CT, which implied the presence of low dose area in the PTV. Regarding OARs, the dose constraint was not satisfied only in one CT for the rectum because of large rectal volume, and in four for the bladder by reason of small bladder volume.
Conclusions: It was verified that the dose for the CTV was sufficient mostly without exceeding dose constraint for the OARs and the PTV margin used in this study was considered to be appropriate.
No conflict of interest.

1131 POSTER

First experience with rotational IMRT with a Siemens accelerator ARTISTE®

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Introduction: In our departement, since February 2012 a Siemens accelerator ARTISTE ® is in clinical use. The dose rate with flattened beam is 300MU/min for 6MV photons respectively 500MU/min for 15MV photons. Simultaneaously with the start of patient treatment at this accelerator also IMRT with the step&shoot method was established in our department. Although Siemens quit the business in radiation therapy, we got the opportunity to test the already developed method of rotational IMRT (mARC®) at our accelerator. In this investigation, both methods of IMRT will be compared according treatment time, monitor units and plan quality.
Material and Method: In this new variation of rotational IMRT, the dose is delivered during a continuous rotation of the gantry in bursts over short gantry angels with static leaf positions. Between these dose bursts, the MLC leaves are moving rapidly in order to form the next field segment.
For IMRT treatment planning a direct aperture optimization algorithm DAO (Panther® DAO, Prowess Inc.) is used. The planning of the rotational IMRT is done with the proarc option of the Panther treatment planning system.
In a retrospective study, this new technique will be compared with the step&shoot IMRT used for treatment. For both methods the same dose prescription to the target volumes and the same dose limits for organs at risk is used. The IMRT techniques are compared regarding the nessescary monitor units (MU), the treatment time, target coverage (TC), conformity index (CI) and conformity number (CN).

	MU/Gy	Treatment time [s]	TC	CI	CN
Prostate					
Step&Shoot	212.6±10.3	283.0±22.3	0.91±0.03	0.84±0.07	0.88±0.33
mARC	222.0±20.9	217.3±18.4	0.94±0.03	0.92±0.70	0.99±0.34
difference [%]	+ 4.4	−23.0%	+ 3.6	+ 9.6	+ 12.7
p	0.22 (n.s.)	0.0000013	0.11 (n.s.)	0.09 (n.s.)	0.58 (n.s.)
Head & Neck					
Step&Shoot	185.5±15.5	386.8±33.8	0.921± 0.03	0.884±0.02	0.814±0.03
mARC	169.6±10.6	306.4±24.5	0.957±0.11	0.887± 0.02	0.849±0.02
difference [%]	−8.5	−20.8	+3.8	+0.3	+4.2
p	0.103 (n.s.)	0.0032	0.036	0.85 (n.s.)	0.061 (n.s.)

Results: The first results for patientes treated for prostate cancer indicate a slight increase (+4.4%) in monitor units (212.6±10 MU/Gy for Step&Shoot

IMRT compared to 222±21 MU for the mARC-technique) while the treatment time is reduced significantly by 23% (283±22sec (Step&Shoot) vs 217±18sec (mARC)).
For head&neck cases, with the mARC-technique a reduction of monitor units and treatment time could be observed. The monitor units could be reduced by 8.5% from 185.5±16 MU/Gy (Step&Shoot) to 169.6±11 MU/Gy (mARC). The mean treatment time of 306±25sec with the mARC method has been reduced by 20% compared to a mean treatment time if 387±34sec in case of step&shoot IMRT.
In all cases, the plan quality according to TC, CI and CN could be improved slightly with the mARC techniqe.
Conclusion: In comparison to Step&Shoot IMRT, the mARC technique shows comparable or improved dose distributions with a significant reduction in treatment time even for an accelerator with flattening filter. A further reduction of treatment time might be expected in case of flattening filter free beams with a higher dose rate.
No conflict of interest.

Poster Session (Sun, 29 Sep)
Imaging

1150 POSTER

Monitoring of pulmonary tumors in computed tomography: Thresholds for volume-based response assessments and target lesions selection

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Background: The change in lung tumor volume is an emerging imaging biomarker that is expected to be more sensitive to disease evolution than Longest Axial Diameter (LAD). Several aspects of volume-based response assessment are still under investigation. One aspect to be clarified is the magnitude of significant volume changes that allows classifying response as Progressive Disease (PD), Partial Response (PR) and Stable Disease (SD). A second aspect is the selection of target lesions usually restricted to 'Measurable' lesions (ML). So far, no precise definition of volume measurability is available despite its probable impact on the response performance. This study proposes a solution for volume-based response assessments that provides, thresholds for volume-based response of ML and a method for automatic identification of ML.
Material and Method: Our study relies on data published by the Quantitative Imaging Biomarker Alliance (QIBA) which reports an inter reader (IR) Limits of Agreement (LoA) of repeated ML assessments of +/−30%. Considering a standard deviation of measurement as 15%, we used Geary Hinkley (GH) transformation to model response thresholds with a 5% Type I error. We used Training (Tr) and Testing (Te) datasets of respectively 99 and 100 pulmonary lesions. Tr and Te data were segmented twice by two imaging scientists (IS) and two expert radiologists (ER). 79 image-based lesion features, such as statistics of intensities and morphology, were computed from segmentations. We labelled all data as 'Non Measurable Lesions' (NML) when repeated measurements exceeded the LoA. We used labels and computed features to train a Support Vector Machine (SVM) classification system. Sensibility (Se) and Specificity (Sp) at detecting ML have been computed.
Results: With 95% confidence, our model gives response thresholds for volume as: 35% reduction reports for PR, 55% increase for PD and SD otherwise. Our Tr and Te datasets disclose 27.3% and 27% of NML respectively. Performance in classifying ML was Se=91.3%; Sp=48.2%.
Conclusions: We provide response thresholds and validate an approach to detect ML. In documenting these two aspects we make the volume a usable biomarker applicable to lung tumors in CT imaging. However, these aspects of the biomarker cover only metrological considerations, and should be validated by clinical investigations.
No conflict of interest.

1151 POSTER

Characteristics of lung cancer diagnosed with low dose chest CT

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Background: Role of chest CT in early diagnosis of lung cancer is controversial between benefits revealed by recent NLST study showing mortality reduction in high risk gruop and harms of overdiagnosis and radiation hazards. The clinical characteristics of lung cancer detected on CT screening is not well known in general population. This study aimed to find clinical characteristics of lung cancers with screening chest.
Material and Methods: This study included 15,615 Korean adults who received low dose chest CT(LDCT) screening in voluntary health checkup program in Healthcare system gangnam center from October 2003 to June 2010. Patients of lung cancer diagnosed on CT screening were reviewed retrospectively about clinical parameters such as sex, smoking et al and final pathology and stage of lung cancers.
Results: 35 lung cancer patients occured. Lung cancer detection rates were 0.22%(crude annual incidence rate 61.0/100,000/year). Male and female ratio was 6:4. 18 of 35 lung cancers occurred in high-risk groups(>20 pack-year smoking) and 17 in low risk groups(ex-smokers and non-smokers). 76% of lung cancers showed less than 3 cm tumor size(<1 cm − 30%, <2 cm −22%, <3 cm − 24%).
The comparison of clinical characteristics with Korean national cancer registry showed that features of lung cancers detected in CT screening showed less advanced stage(stage I 60.6% vs 17.5%), more frequent histology of adenocarcinoma(68.6% vs 36.1%) and relatively higher rates in non-smokers and females than general population.
Of 35 patients with lung cancer, VATS segmentectomy or VATS lobectomy was performed in 10 patients and lobectomy in 16. 4 patients underwent chemotherapy and 5 patients was managed with supportive therapy because of advanced stage or old age. 2 patients died despite of treatment and other patients survived during followup. Adenocarcinoma including BAC histology was more prevalent and most of patients could be treated in early stage.
Conclusion: Lung cancers detected in CT screening had somewhat different clinical characteristics such as less advanced stage and higher frequency of adenocarcinoma from general population. LDCT screening may be useful in diagnosis of lung cancer in early, operable stage.
No conflict of interest.

1152 POSTER

Comparison of moving target delineation using slow 3D-CT, CBCT and MVCT: A phantom study

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Background: To assess whether different motion patterns have an impact on delineation of a moving target in a lung phantom using planning CTs (slow 3D-CT) and IGRT-CTs (Conebeam-CT [CBCT], Megavoltage-CT [MVCT]).
Material and Methods: A commercial xy-table (SunNuclear®) was combined with a self-made installation wich enables programable motion of a box in 3 dimensions. The box was filled with corkboards and a water-filled table tennis ball to simulate a tumour inside the lung. Using this phantom, slow 3D-CT (Somatom Emotion, Siemens Medical Solutions®), CBCT (Clinac DHX, Varian Medical Systems®) and MVCT image series (Tomotherapy, Accuray Inc.®) were acquired during phantom movement. Different motion patterns were performed: linear motion in x or y direction with amplitudes of 5 and 10 mm, each with periods of 3 and 5 s. Additionally, a linear motion with amplitudes in x, y and z directions (2.5, 10, 2.5 mm, periode 5 s) and a patient tumour trajectory was applied. The latter was constructed using the center of mass motion of GTVs delineated in a 4D-CT scan.
The ball volume was contoured in all image series (Eclipse 10, Varian Medical systems®) using a lung window setting of 200 HU to −1000 HU. The contoured ball volumes were compared with the mathematically calculated ball volumes.

Table: Relative difference between contoured and calculated ball volume

Motion amplitude	slow 3D-CT		CBCT		MVCT	
	period 3 s	period 5 s	period 3 s	period 5 s	period 3 s	period 5 s
0	11.9	11.9	2.4	2.4	21.5	21.5
x 5 mm	−0.5	−8.5	−8.1	−18.0	−30.2	−14.5
x 10 mm	−6.0	−18.4	−10.9	−19.1	−63.5	−43.9
y 5 mm	−5.6	−7.4	−4.6	−8.3	−8.0	−13.4
y 10 mm	−9.2	−18.8	−7.5	−11.4	−10.9	−25.2

Results: The relative differences between contoured and calculated volumes are presented in the table. The static ball volume was overestimated in all imaging methods. Ball motion resulted in underestimation of the contoured volumes. The volume decreases as the motion amplitude increases. In the slow 3D-CT and CBCT smaller differences were determined for motion period of 3 s than for 5 s. For CBCT and MVCT

ball motion in y direction resulted in smaller deviations than motion in x direction. The largest underestimation of volumes was found for MVCT. Complex motion patterns resulted also in underestimated volumes (−4% to −29%).
Conclusion: Due to movement, contoured volumes were underestimated for all imaging methods. Depending on the applied technique, motion amplitude, direction and period have different impact on target delineation which has to be considered in clinical assessments.
No conflict of interest.

1153 POSTER

Evaluation of a cloud-based local read paradigm for imaging evaluations in oncology clinical trials

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Background: Imaging evaluation in oncology heavily depends on human readers performances. In clinical trials, regulatory authorities have been recommending an Independent Central Review (ICR) with several readers to mitigate potential biases resulting from variance between investigator sites. Based on recent publications promoting site based evaluation as imaging endpoint, they currently investigate an alternative to ICR. The goal of this study is to evaluate a cloud-based paradigm implementing software solutions and services that standardize the imaging evaluations among international investigator sites.
Material and Methods: 10 patients, who received chemotherapy for lung cancer and for which chest CT scans were available at 3 different time points, were retrospectively selected. CT scans were evaluated according to the RECIST 1.1 criteria by two oncologists (Saga University) and one radiologist (Nice University Hospital) independently, through web software solutions (MEDIAN Technologies). Such solutions were hosted by the data center (Canon IT Solutions, Japan) and used by readers and data managers (CANON and MEDIAN Technologies) for de-identification, quality control and centralization of the images and their evaluations. The study compared evaluations between readers and analyzed the reasons for discordances.
Results: Readers with different medical training and education, working at distant locations were able to reliably perform radiological evaluations from the same cloud system. The cloud quality control service detected2 non-conformances in applying RECIST 1.1 and had the readers changed their evaluations, resolving discrepancies. Between the oncologists and the radiologist, a discordance rate of 35 % (14/40 evaluations) was observed when considering RECIST overall response (CR, PR, SD, PD) at all time points.
The main reason for discordance in RECIST overall response was a difference in the selection of the target lesions (50%, 7/14). Those discordances represented 78% (7/9) in the group where target selection was different.
Conclusions: The study shows the feasibility of imaging evaluation based on cloud services for clinical studies involving multiple international sites. Centralization of data made possible the on-going monitoring of evaluations through specialized services reducing variability among sites. Analysis of discordances between readers identified areas of improvement for cloud-based services such as consensus process for target selection at baseline evaluation to reduce discrepancies.
Conflict of interest: *Corporate-sponsored research: MEDIAN Technologies, France. Canon Inc, Japan*

1154 POSTER

Hepatic blood flow using CT perfusion as a prognostic imaging biomarker for esophageal squamous cell carcinoma

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Background: According to the previous reports, preoperative evaluation of hepatic blood flow by diagnostic imaging such as ultrasound and scintigraphy could identify patients of malignancy with high risk of recurrence. CT perfusion (CTP) is a noninvasive, functional imaging which can quantify a perfusion of the target organ, by an analysis of the time-density curve of the injected contrast material. The purpose of this research is to evaluate the change of hepatic blood flow measured by preoperative CTP and identify the high-risk group of postoperative recurrence in patients with esophageal squamous cell carcinoma.
Material and Methods: Forty-five consecutive patients with esophageal squamous cell carcinoma treated with surgical resection at Chiba University Hospital from Jun 2010 to December 2012 were enrolled in this study. Radical subesophagectomy with field lymphadenectomy was performed. Prior to surgery, hepatic CTP images were obtained using a 320-row area detector CT with a 0.5-mm slice thickness. Data was analyzed by perfusion software (Body Perfusion; Toshiba, Tokyo, Japan), based on the dual input maximum slope method. Perfusion parameters, arterial blood flow (AF: ml/min/100g tissue), portal blood flow (PF: ml/min/100g tissue) and %AF (AF/AF+PF x100), were measured by placing region of interest in the abdominal aorta, in the portal vein, in the right hepatic lobe and spleen excluding vessels. We compared the perfusion parameters with pathological Stage (TNM classification of UICC) and postoperative course.
Results: The following factors, which might have influence to a hemodynamics, had no significant correlation with the hepatic perfusion parameters; age, sex, systolic blood pressure, body surface area, hematocrit, serum asparate aminotransferase (AST) and estimated glomerular filtration rate (eGFR). In the comparison of the mean values of the parameters for each stage, there was no significant difference. The postoperative recurrence has observed in 8. The types of recurrence were as follows: hematogenous metastases 9, lymph node 6 and peritoneum 2 (duplication was included). %AF of recurrent cases was significantly higher than that of without recurrence (24.8 vs. 15.9, $P = 0.0027$). The predictive value of recurrence have a sensitivity of 87.5% and a specificity of 88.9%, by setting a cut-off %AF value (%AF=20) from Receiver Operating Characteristic analysis. If divided into two groups, high %AF group ($\geqslant$20) and low %AF (<20), the relapse free survival rate of low %AF group was significantly better than that of high group ($P<0.0001$). Multivariate analysis using the Cox proportional hazards model showed that preoperative high %AF was an independent risk factor of recurrence (Hazard ratio: 26.1, P value: 0.0029).
Conclusions: Hepatic CTP is a non-invasive modality and a valid functioning tool to predict postoperative recurrence of esophageal squamous cell carcinoma, furthermore its potentiality, as an imaging biomarker, has been suggested.
No conflict of interest.

1155 POSTER

A novel approach for cancer risk reduction associated serial CT scanning in pediatric hydrocephalus

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Background: Pediatric patients with acute hydrocephalus are frequently subjected to serial CT studies to confirm ventricular shunt patency. It is not uncommon for patients to receive up to 12 CT scans/year resulting in substantially increased lifetime cancer risk. This feasibility study investigates the ability to accurately measure ventricle size as CT dose is reduced far below the child-size CT protocols routinely employed in pediatric imaging. By narrowing the diagnostic goal to a determination of ventricle size, rather than exclusion of all possible pathology within the scan region, the radiation dose can be greatly reduced.
Materials and Methods: Initial studies were performed using phantom materials with CT Hounsfield units matched for brain and CSF. These images were acquired at various kVp, mAs, slice thickness using axial and helical acquisitions. The data was processed using a variety of reconstruction kernels and several post-processing and volume rendering algorithms to modulate image smoothing.
In addition, three fresh cadaver heads were imaged using a wide range of CT protocol parameters, varying kVp, mAs, axial versus helical, and reconstruction kernels to maximize the dataset for analysis. Raw and reconstructed data was saved for post-processing using 3D workstations as well as image processing toolkits provided in commercial image analysis software.
Results: Ventricular dimensions were estimated using both 3D volume rendered and 2D image analysis algorithms. Concordance of measurements between the maximum dose and reduced dose datasets was analysed and confirmed as was the lack of significant interobserver variability between three experienced neuroradiologists.
The range of CTDI from the maximum dose protocol to the minimum dose protocol was greater than two orders of magnitude. In spite of this extreme dose reduction, ventricular dimensions could still be accurately measured despite a 100X dose reduction.
Conclusions: Radiation exposure from serial CT scans results in an increased risk of malignancy later in life and is particularly significant in young patients. CT scanning required to exclude acute obstructive hydrocephalus related to shunt malfunction may result in radiation exposure levels known to significantly increase cancer risk in pediatric patients. A method to reduce dose by more than 2 orders of magnitude associated with

serial CT scanning in suspected shunt malfunction has been developed that can reliably exclude acute hydrocephalus in a vulnerable pediatric population. This strategy to maximally reduce dose while maintaining accurate identification of key image parameters to reliably resolve a specific clinical question can reduce cancer risk while ensuring appropriate patient management.
No conflict of interest.

1156 POSTER

Predictive value of optimal morphologic response to first-line chemotherapy in patients with colorectal liver metastases

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Background: It was reported that morphologic response to preoperative chemotherapy was an independent prognostic factor in patients who underwent hepatic resection of colorectal liver metastases (CLM). The aim of this study was to evaluate the predictive value of morphologic response to first-line chemotherapy in patients with CLM.
Methods: We assessed 41 patients with CLM who received fluorouracil-based chemotherapy with or without bevacizumab as first-line chemotherapy at two institutions between April 2006 and June 2012. All patients underwent enhanced computed tomography (CT) at the start of chemotherapy and then every 2−3 months. Three blinded radiologists evaluated CT images and classified as optimal, incomplete or none response according to the morphologic criteria (Chun YS, et al. JAMA 302: 2338−44, 2009). Response to systemic chemotherapy was also evaluated according to RECIST. Progression-free survival (PFS) was calculated with the Kaplan–Meier method and statistical differences with survival curves were determined by the log-rank test. Predictive factors associated with PFS were identified in multivariate analysis.
Results: Patients characteristics were as follows: median age=67 years (range 52−80); Male: female=29:12; PS 0:1:2:3=24:13:3:1. Thirty two patients had synchronous liver metastases and 9 had metachronous liver metastases. Five patients had solitary liver lesions and 36 had multiple liver lesions. Twenty three patients (54%) received chemotherapy with bevacizumab, while 18 patients (46%) received chemotherapy without bevacizumab. Optimal morphologic response was observed in 11 patients (48%) treated with bevacizumab and, in 5 patients (28%) treated without bevacizumab. Eight patients (20%) underwent hepatic resection after chemotherapy. The median follow-up period was 31.3 months. The median PFS was 12.7 months for patients with optical morphologic response and 8.1 months in those with incomplete/none morphologic response ($p = 0.0026$). On multivariate analysis, PS and morphologic response were significant independent predictors of PFS. Morphologic response was superior to RECIST for prediction of PFS.
Conclusions: Optimal morphologic response was significantly associated with PFS in patients with CLM who were treated with fluorouracil-based chemotherapy as first-line chemotherapy. Chemotherapy with bevacizumab tends to have a higher optimal morphologic response than chemotherapy without bevacizumab.
No conflict of interest.

1157 POSTER

The value of chest CT for prediction of breast tumor size: Comparison with pathology measurement

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Background: To date, no data are available on the use of chest CT for prediction of breast tumor size in patients with breast cancer, although the CT examinations are increasing in practice. The purpose of this study is to evaluate the value of chest CT for prediction of breast tumor size using the pathology measurement as the reference standard.
Materials and Methods: We retrospectively analyzed the tumor size on the preoperative chest CT for 295 patients with surgically proven unifocal, invasive carcinoma. The maximal diameter of tumor on chest CT and pathologic result were compared by linear regression and Spearman's rho correlation coefficient. Concordance between CT and pathology was defined as a difference $\leqslant 0.5$ mm. Sub-groups analysis was also performed by tumor size (<2 cm and $\geqslant 2$ cm), and histologic grades.
Results: CT and pathology tumor size showed positive correlation (Pathology tumor size = 1.092 × CT tumor size − 1.013, Spearman's rho correlation coefficient = 0.84, P <0.001). Most of the tumors (n = 232, 78.6%) showed concordance in the size on chest CT and pathology, and 42 of tumors (14.2%) showed underestimation (average underestimation of CT = 12 mm) and 21 of tumors (7.1%) showed overestimation on CT (average overestimation of CT = 10 mm) compared with that of pathology. No significant difference was found in the sub-group analysis by tumor size and the histological subtypes.
Conclusion: CT tumor size positively correlates with pathology size in the breast cancer patients. Most of the tumors show difference less than 5 mm in the size on chest CT and pathology. The tumor size and histological subtype is no significant indicator for prediction of tumor size on CT.
No conflict of interest.

1158 POSTER

Geographical analysis of hypoxic subvolumes in locally advanced head and neck tumours during primary chemoradiotherapy in serial 18-F-MISO-PET imaging

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Background: Hypoxic subvolumes (HV) in head and neck tumours are associated with worse prognosis and outcome, attributed to increased chemo- and radioresistance. Tumour hypoxia can be visualized using PET imaging. 18-Fluoro-Misonidazole (F-MISO) was the first tracer clinically validated and established. The incorporation of biological information into radiotherapy planning can personalize radiotherapy (e.g. by dose painting).
Material and Methods: In a pilot project, 16 patients with locally advanced head and neck tumours underwent 3 consecutive F-MISO-PET scans (weeks 0, 2, 5) before and during primary chemoradiotherapy (70 Gy, Cisplatin) in addition to FDG-PET, CT and MRI (week 0). Tumour localisations included oral cavity (n = 1), oropharnynx (n = 7), hypopharynx (n = 5) and larynx (n = 3). Normalised standardized-uptake-values (SUV) were generated for all patients (index SUVmax Tumour/SUVmax Muscle) on the F-MISO-PET scans. The size, localisation and overlap of the HV between the F-MISO-PET scans were analysed. Volume delineation was done using a semi-automatic algorithm: A sphere containing normal tissue contralateral to the tumour site was defined for all patients and scans. A 1.5-fold increase over the spheres' mean SUV yielded the threshold for the HV for each scan and patient. Analyses were carried out with BrainLAB iplan.
Results: For an index of 1.5, quantitative evaluation showed tumour hypoxia in week 0 in 16/16, in week 2 in 5/14 and in week 5 in 0/11 patients. The mean tumour volume − as defined on FDG-PET and validated with CT and MRI − was 28 cm^3, the mean HV initially 28 % with a decrease to 15 % after 2 weeks. HV completely resolved in the majority of patients. Mean HV overlap between the first and second scans was 55 % of the HV on the first scan, indicating a relatively high proportion of initial hypoxia persisting at the same localisation in later scans. Similarly, the majority of the later HV have already been hypoxic before (mean overlap 72 % of the HV on the second scan). Descriptive analyses showed both stationary and dynamic components in those patients with persisting hypoxia (decreased or increased).
Conclusions: Tumour hypoxia decreased or regressed in a majority of patients; however, HV showed a geographically relatively stable conformation in those patients with persistent hypoxia. This might make an inclusion into radiation treatment planning possible.
No conflict of interest.

1159 POSTER

Visualization of solid tumors and metastasis using an uPAR specific NIR fluorescent-labeled antibody

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Real-time near-infrared (NIR) fluorescent imaging is a novel technique used to intraoperatively visualize tumor cells. For this purpose NIR light-emitting fluorophores are conjugated to tumor protein-specific tracers. One potential target for tracing tumors is the urokinase plasminogen activator receptor (uPAR). uPAR plays an important role in the development of cancer, tumor invasion, angiogenesis and metastasis. Over-expression of uPAR is found on the majority of human carcinomas, in cancer as well as stromal cells.

This study investigates the potential of an uPAR-specific NIR fluorescent antibody for the *in vivo* identification of tumor cells.
The NIR fluorophore MSAP-ZW800−1 was conjugated to a humanized monoclonal uPAR specific antibody and an isotype IgG control. The conjugation and binding capacity of both compounds were validated *in vitro* using photospectrometry and plate-assay analyses on tumor cells. Athymic mice were subcutaneously injected with human colon adenocarcinoma HT-29 cells or orthotopicly in the tongue with OSC-19 cells, a metastasizing human squamous cell carcinoma. After establishment of the tumors, 1 nmol of either uPAR-specific or control IgG were injected intravenously (IV). At sequential time points up-to 120 h after injection images were obtained with the PEARL Impulse small animal imager and the intraoperative FLARE™ imaging system. A dose-range study was performed with doses of 150 μg (1 nmol), 100 μg, or 50 μg per mouse. *Ex vivo* fluorescence imaging and histology was performed to demonstrate distribution of the compounds and tumor specificity.
In vivo, the tumors were clearly fluorescently delineated, with the highest tumor-to-background ratios (TBR) at 72 hours after injection of 3.6±0.4 in the HT-29 model and 2.3±0.1 in the OSC-19 model respectively (n = 3). The control compound showed a mean TBR of 1.8±0.2 in the HT-29 model and 1.1±0.2 in the OSC-19 model, whereas injection of the fluorophore alone showed a mean TBR of 0.8 ±0.1 in both animal models. Unexpected fluorescent spots were found in the cervical region of the OSC-19 tumor-specific compound group, which histologically turned out to be cervical lymph node metastases. Two-way repeated measurements ANOVA analysis showed significant differences between the tumor-specific compound and control groups in the HT-29 model at all time points later than 24 hours ($p < 0.01$) and for the OSC-19 model at every time point from the start ($p < 0.01$). *Ex vivo* evaluation showed a tumor-specific signal in both the OSC-19 primary tumors and lymph node metastases. No significant differences were found among the dose groups, indicating the potential of this anti-uPAR compound to be used in the lower micro-dose range.
In conclusion, this study describes a new tumor-specific fluorescent probe, targeting uPAR, which provides visualization of solid tumors including their metastases in real time using a NIR fluorescence imaging system.
Conflict of interest: *Ownership: Kuppen stockholder of Antibodies for Research Applications BV; Mazar stockholder and consultant of Tactic Pharma.*

1160 POSTER

Non-invasive monitoring of pharmacodynamics and -kinetics of a death receptor 5 antibody: Induction of apoptosis depends on treatment schedule

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Background: Using fluorescence- and bioluminescence-imaging, the pharmacodynamics and tumor saturation kinetics of a Death Receptor 5 antibody (anti-DR5) was examined to optimize treatment schedules of a combination therapy with Doxorubicin (DOX).
Material and Methods: Split-luciferase based bioluminescence imaging allowed us to monitoring apoptosis non-invasively in living mice. A human glioblastoma cell line stably transfected with the split-luciferase apoptosis reporter was applied to screen various chemotherapeutics and anti-DR5 on their ability to induce apoptosis in cells *in vitro* as well as *in vivo*. Fluorescence labeled anti-DR5 was injected i.v. and tumor saturation kinetics was monitored.
Results: We found that DOX treatment *in vitro* led to significant apoptosis induction within 48 hours and to a 2.3-fold increased anti-DR5 binding to the cell surface in contrast to Cisplatin and 5-FU treatment. Induction of apoptosis by treatment with anti-DR5 was dose- and time-dependent (both *in vitro* and *in vivo*). Simultaneous visualization of fluorescence labeled anti-DR5 in tumor tissue and apoptosis revealed maximal apoptosis induction immediately after the compound had reached tumor site. Regarding combination therapy of anti-DR5 and DOX, we found that the sequential application of DOX before anti-DR5 resulted in synergistically enhanced apoptosis reporter activity. In striking contrast, anti-DR5 given before DOX did not lead to increased apoptosis induction.
Conclusions: We suggest that DOX-induced recruitment of DR5 to the cell surface impacts the enhanced apoptotic effect which can be longitudinally monitored by apoptosis imaging. This study demonstrates that the combination of apoptosis and antibody accumulation imaging is an excellent method for optimizing dosing and treatment schedules in preclinical cancer models.
Conflict of interest: *Ownership: Roche Diagnostics GmbH*

1161 POSTER

The hybrid tracer ICG-99mTc-nanocolloid aids surgical visualization of the sentinel node in different basins

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Background: Conventionally a combination of radiocolloid and blue dye is used for sentinel node (SN) biopsy. The radiocolloid allows preoperative lymphoscintigraphy and SPECT/CT for SN mapping, and intraoperatively its gamma rays can be traced to guide the surgeon to the SN. An intraoperative injection with blue dye prior to the start of the procedure is then used to enable optical lymphatic ducts and SN identification. Recently we introduced the hybrid, radioactive and fluorescent, tracer indocyanine green (ICG)-99mTc-nanocolloid. With this tracer, one single injection enables preoperative SN mapping and intraoperative radio- and fluorescence guidance to the SN. In this study we compared SN identification via the fluorescence component of the hybrid tracer to SN identification using blue dye in different drainage basins.
Materials and Methods: One-hundred-ninety-six patients were included in the study: 77 patients with melanoma and 119 patients with squamous cell carcinoma of the penis or vulva. The hybrid tracer was injected surrounding the lesion/scar 3−27 hours prior to surgery. The SNs were then preoperatively identified using lymphoscintigraphy and SPECT/CT imaging. Prior to the start of the operation vital blue dye was injected. The SNs were intraoperatively traced using a gamma ray detection probe and a portable gamma camera. In addition, SNs were visualized via fluorescence imaging and/or blue dye detection.
Results: Intraoperatively, a total 342 basins was explored from which a total of 609 SNs were excised. In the 43 neck-basins a total of 68 SNs were excised with 33% of these SNs being blue and 97% being fluorescent. In the 247 inguinal basins (461 SNs), 54% of the SNs was blue whilst 92% was fluorescent. Eigthy-one percent of the SNs located in the 44 axillary basins was blue while 92% was fluorescent. Three SNs were located in other basins namely the elbow (n = 1) and supraclavicular (n = 2); here 20% of the SNs was stained blue vs. 100% being fluorescent. Aberrant drainage to the periscapular region was found in 4 patients, to 4 basins with a total of 4 SNs. Drainage to one prepubic SN was found in a patient with penile carcinoma. Of these abberant draining 5 SNs only 1 SN was blue whereas they were all fluorescent.
Discussion: For all nodal basins studied significantly ($p < 0.001$) more fluorescent SNs (average 93%) than blue stained SNs (average 54%) were visualized and removed. This was especially true for the neck. The fluorescence feature of the hybrid tracer might, therefore, provide a valuable alternative for optical SN identification in the operating room.
No conflict of interest.

1162 POSTER

89Zr-cetuximab imaging in advanced colorectal cancer patients: A feasibility study

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Systemic treatment for advanced, wild type K-RAS colorectal cancer (wtK-RAS CRC) includes Epidermal Growth Factor Receptor (EGFR)-inhibition with anti-EGFR antibodies like cetuximab. Only 40% of patients with wtK-RAS CRC do benefit from this treatment, while the others are intrinsically resistant. Potential causes of resistance include aberrations in the EGFR-signaling cascade and/or differential pharmacokinetics of anti-EGFR-antibodies in patients. We hypothesize that high EGFR-expression in normal tissues, such as in normal liver, may lead to insufficient availability of anti-EGFR-antibodies to effectively target tumor lesions and thereby may be responsible for resistance.
To study our hypothesis, we first investigated whether accumulation of cetuximab can be detected in tumor lesions of patients with wtK-RAS CRC by PET-imaging with radiolabeled cetuximab (^{89}Zr-cetuximab).
Six patients with histopathologically confirmed advanced wtK-RAS CRC who were candidates for monotherapy with cetuximab, were included in this feasibility study upon their consent. Based on a 40% clinical benefit

rate of cetuximab we hypothesized that 40% of patients would show ^{89}Zr-cetuximab tumor uptake. This hypothesis would be correctly accepted if uptake would be present in $\geqslant$1 and $\leqslant$7 of 10 patients (power >90%, type I error <5%). ^{89}Zr-cetuximab PET-imaging was performed at the start of treatment with cetuximab. ^{89}Zr-labelled cetuximab (10 mg, 37MBq) was injected within 2 hours after the first dose of cold cetuximab (500 mg/m^2) on day 1. PET scans were performed on days 1, 2, 3, 4 and 7. While high liver uptake of ^{89}Zr-cetuximab was found in all patients, specific uptake of ^{89}Zr-cetuximab was detected in non-hepatic metastases of 3 patients during subsequent scans (standard uptake values between 3 and 8.5 on day 7). In the other 3 patients, no specific ^{89}Zr-cetuximab uptake was detected in non-hepatic metastases. Detailed imaging results will be shown at the meeting. The patients did not experience any toxicity related to ^{89}Zr-cetuximab; only known adverse events to cetuximab were observed, none exceeding grade 2. We conclude that PET imaging with ^{89}Zr-cetuximab for determination of tumor uptake in non-hepatic lesions is feasible. Based on these results, we initiated a clinical trial in which we will investigate the correlation between tumor uptake and tumor response by ^{89}Zr-cetuximab PET-imaging of non-hepatic lesions. Our goal is to guide treatment decisions for the individual patient with wtK-RAS CRC using a non-invasive, patient-friendly imaging strategy.

No conflict of interest.

1163 POSTER

Segmentation of subsequent FMISO-PET before and during radiochemotherapy: Experts versus swarm intelligence

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Background: Researchers in the field of imaging diagnostics for oncology increasingly incorporate new PET tracers such as [18F]fluoromisonidazole (FMISO) for hypoxia localisation in clinical trials to determine its prognostic potential. However, reproducibility of FMISO-PET measurements is part of controversy. To determine the stability of contours derived from subsequent FMISO PET imaging, we compared manually-outlined target object definitions and automatically-created contours.
Material and Methods: As part of a prognostic trial on head and neck cancer, 35 patients were imaged using FMISO-PET before and after the first week of combined radiochemotherapy. Three experts outlined FMISO-positive objects related to the primary tumor in the PET data sets. The analogously applied automatic routine is based on swarm intelligence. The algorithm utilizes virtual ants that move in the PET image stack and accumulate inside potential target objects.
Comparison of manually- and automatically-created contours was performed using the Jaccard-Index for determination of the degree of contour overlap. The comparison included expert-versus-expert analysis on both single FMISO-PET scans to estimate inter-observer-similarity, inter-scan-comparison to determine intra-observer- and intra-algorithm-similarity and algorithm-versus-expert comparison to estimate the reliability of automatic contours.
Results: In 25 of the 35 data sets, all experts consistently outlined objects which were further analysed. The Jaccard-Index was JI_{EvE}=45% and JI_{iE}=36% for inter- and intra-observer-similarity, respectively. Automatically-generated contours matched to the manually-created contours with JI_{AvE}=45%, which was a similar measurement as inter-observer-similarity. However, automatic contours matched by JI_{iA}=53% between both scans.
Conclusions: Automatically delineated contours matched to manually-created contours to a degree as the latter matched to each other. However, the similarity of contours between both scans was significantly increased using automatic delineation. The fact that intra-observer-similarity between the scans was decreased may partly be explained by changes of the tumour microenvironment during the first week of therapy between both PET scans.

No conflict of interest.

1164 POSTER

Diffusion-weighted imaging in the follow-up of patients after primary surgical and non-surgical treatment for rectal cancer

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Background: Accurate detection of recurrent disease after primary rectal cancer treatment is crucial to allow for curative salvage surgery. Standard imaging is known to experience difficulties in differentiating between post-treatment effects (inflammation and fibrotic scar tissue) and recurrent disease. Diffusion-weighted MRI (DWI) is a technique that analyses differences in tissue cellular density to differentiate between hypercellular (tumour) and low or normocellular tissues (fibrosis and inflammation). Aim of this study was to evaluate the value of DWI in the follow-up of patients after primary surgical or non-surgical treatment for rectal cancer.
Material and Methods: The study group (n = 117) consisted of 36 patients who had previously undergone rectal cancer treatment, consisting of either standard surgical resection with or without neoadjuvant (chemo-)radiotherapy (n = 36), a local transanal excision (n = 40, of which 15 after chemoradiotherapy) or a non-operative 'wait-and-see'-policy (n = 41). During clinical follow-up (FU) patients underwent on or more FU-MRIs (1.5T) including DWI (highest b-value b1000), as part of routine FU or because of a suspected local recurrence after surgery. Two readers in consensus evaluated each MRI and scored the b1000 DWI-images as 'no high signal', 'high signal suspected of recurrence' or 'not adequately assessable due to artefacts'.
Results: Patients underwent a mean number of 3 FU-scans (range 1−11) with a mean FU-time of 44 months (4–144). 27/117 patients developed a local recurrence, of which 23 (85%) were accurately detected on DWI. The other 90 patients (without recurrence) together underwent a total of 261 FU scans, of which 194 (74%) remained consistently true negative (no high signal) on DWI. 57 DWI-scans (19%) could not adequately be assessed due to artefacts. 14 DWI-scans were false positive (mainly at the first FU-scan after surgery/local excision), of which 50% again normalised during further FU.
Conclusion: DWI is a useful tool in the follow-up of patients after primary rectal cancer treatment. False positives may occur immediately after surgery, but the DWI signal normalises again during follow-up.

No conflict of interest.

1165 POSTER

Superparamagnetic iron oxide nanoparticles (SPIONs) modified with epidermal growth factor (EGF) or heat shock protein Hsp70 for targeting the brain tumor

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Background: Superparamagnetic iron oxide nanoparticles (SPIONs) due to their high magnetic moment have the ability to function as theranostic agents. They could be used both as contrast agents for MRI, and as delivery vehicles for anti-cancer agents and thermotherapy. In series of *in vitro* and *in vivo* experiments we analyzed brain tumor targeting with magnetic nanoparticles conjugated with epidermal growth factor (EGF) or heat shock protein Hsp70.
Material and Methods: Human recombinant Hsp70, EGF were produced by genetic engineering. SPIONs modified by EGF, Hsp70 were characterized by spectrophotometry, ELISA assays, atomic-force microscopy and NMR. Proton magnetic relaxation times T_2 were measured with the help of the NMR-spectrometer (CXP-300, Bruker) in magnetic field of 7.1 T. The *in vitro* binding and uptake of SPIONs, Hsp70-SPIONs and EGF-SPIONs conjugates were assessed on the C6 glioma cells culture by confocal and electron microscopy. The *in vivo* traffic was analyzed in the model of intracranial C6 glioma. MR images (gradient echo (FLASH), T_1- and T_2-weighted, multi-sc and multi-echo (MSME T_2-map) of rat glioma were obtained by Bruker Avance II NMR spectrometer 11 T.
Results: SPIONs measured relaxivity corresponded to properties of negative contrast agents with a hypointensive change of resonance signal

in MR imaging. According to *in vitro* studies SPIONs were incorporated into C6 cells mostly by endocytosis pathway. Intriguingly, the conjugation of protein Hsp70 or EGF to the SPIONs increased the internalization of nanoparticles as was demonstrated by confocal microscopy (by the receptor-mediated endocytosis: EGFR for EGF-SPIONs, CD40 for Hsp70-SPIONs). *In vivo* studies confirmed the tumor targeting ability of modified SPIONs. I.v. injected nanoparticles accumulated inside C6 glioma tumor with a significant decrease of signal on T2-weighted images. Confocal microscopy images confirmed the Hsp70-SPIONs or EGF-SPIONs accumulation within the tumor cells cytoplasm.
Conclusions: Modified SPIONs application represents a promising approach for the targeted therapy and imaging of malignant tumors. SPIONs conjugated with heat shock protein Hsp70 or EGF increased the uptake of nanoparticles by glioma cells and, what is more important, − provided the selectivity of tumor targeting in *in vivo* conditions. The proposed technology based on magnetic nanoparticles exhibits the high diagnostic potential and could be further transferred to the thermotherapy of brain tumors.
No conflict of interest.

1166 POSTER

Diffusion-weighted magnetic resonance imaging at 3.0-T versus fluorine 18 fluorodeoxyglucose positron emission tomography/computed tomography for detection of pulmonary malignant tumors

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Background: Emerging evidences suggest that diffusion-weighted magnetic resonance imaging (DW MRI) at 1.5-T could be useful for tumor detection, together with N and M staging in patients with lung cancer, especially non-small cell lung cancer (NSCLC), in place of fluorine 18 fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) most recently. This investigation prospectively examined whether DW MRI at 3.0-T might be as useful as FDG PET/CT for detection of pulmonary malignant tumors.
Material and Methods: This study was approved by the institutional review board, and written informed consent was obtained from all patients. DW MRI and FDG PET/CT were performed before therapy in 113 patients with pulmonary nodules, including lung cancer, lung metastases, and benign lesions, diagnosed by pathological examination. Mean apparent diffusion coefficient (ADC), maximal standardized uptake value (SUV_{max}), and five-point visual scoring were assessed. Immunohistochemical staining for Ki-67 was performed in 36 patients with lung cancer, and Ki-67 score was evaluated. Receiver operating characteristic (ROC) curve analysis was used to determine feasible threshold values. Diagnostic capabilities for detection of pulmonary malignant tumors were compared with the McNemar test on a per-patient basis, and correlation between malignant degree of lung cancer and ADC or SUV_{max} was analyzed by Spearman rank test.
Results: As for diagnostic capability, area under ROC curve (A_z) for ADC (0.91) were significantly higher than that for SUV_{max} (0.78, P <0.05), and A_z value for DW MRI (0.94) were not significantly different from that for FDG PET/CT (0.92, P >0.05). For quantitative assessment, specificity and accuracy of ADC (91.7%, 92.9%) proved to be significantly higher than those of SUV_{max} (66.7%, 77.9%, P <0.05), although sensitivity of ADC (93.5%) was not significantly different from that of SUV_{max} (83.1%, P >0.05). When feasible threshold values were used to assess qualitatively, sensitivity, specificity, and accuracy of DW MRI (96.1%, 83.3%, 92.0%) were also not significantly different from that of FDG PET/CT (88.3%, 83.3%, 86.7%, P >0.05). Significant correlation was found between Ki-67 score and ADC (Spearman coefficient r = −0.66, P <0.05), as well as ADC and SUV_{max} (r = −0.37, P <0.05). On the contrary, Spearman coefficient was −0.11 between Ki-67 score and SUV_{max} (P >0.05).
Conclusions: Quantitative and qualitative assessments for detection of pulmonary malignant tumors obtained with DW MRI at 3.0-T are as useful as, even superior to, those obtained with FDG PET/CT. Furthermore, another significant outcome of this study was that ADC in DW MRI at 3.0-T can also play a role in prediction for malignant degree of lung cancer in particular, but SUV_{max} did not in FDG PET/CT.
No conflict of interest.

1167 POSTER

Associations between BRCA mutation status, pathologic findings, and MR imaging features in patients with breast cancer who have high risk factors for mutation

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Background: We investigated the relationship between BRCA mutation, pathologic findings, and magnetic resonance imaging (MRI) findings in patients with breast cancer who had risk factors for the mutation.
Materials and Methods: Genetic testing for BRCA mutation was performed in 275 breast cancer patients with at least one risk factor for the mutation. Using the Breast Imaging Reporting and Data System MR lexicon, morphologic and kinetic features on MRI were reviewed for 230 tumors in 209 of these patients. The relationship between BRCA mutation, pathologic findings, and MRI findings was examined by chi-square or Fisher's exact tests. Disease recurrence was also estimated.
Results: BRCA mutation was detected in 48 (23.0%) patients; BRCA1, 21 (10.0%); BRCA2, 25 (12.0%). Two (1.0%) patients had mutations in both genes. Tumors in patients with BRCA1 mutations more frequently showed a high nuclear grade (p = 0.0041) and triple-negative (TN) phenotype (p <0.0001). On MRI, the tumors were seen as mass types in 182 (79.1%) of 230 lesions and non-mass types in 48 (20.9%) cases. Among the MRI features, rim enhancement was significantly associated with molecular subtypes based on immunohistochemistry (p <0.0001) and nuclear grade (p = 0.0387) in multiple logistic regression. Rim enhancement on MRI, along with advanced pathologic T stage, was associated with increased disease recurrence (p = 0.0023) in multivariate analysis. However, the proportion of mass types and non-mass types and the distribution of morphologic shape, margin, internal enhancement, or kinetic features on MRI were not different according to BRCA mutation status.
Conclusion: In patients with high-risk breast cancer, BRCA1 mutation was associated with aggressive pathologic characteristics and TN phenotype. However, direct association between BRCA mutations and MRI features was not observed. Rim enhancement was frequently seen on MRI in high grade tumors and in TN phenotype, and a significant predictor of increased early recurrence of disease.
No conflict of interest.

1168 POSTER

Preoperative assessment of gastrointestinal stromal tumors using diffusion-weighted magnetic resonance imaging

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Background: In Gastrointestinalstromal tumors (GIST), surgical indication is an important problem, and the malignant evaluation is indispensable. In recently modified Flecher risk classification and UICC-TNM are made, and it was shown for the risk evaluation that mitosis count and the tumor size are index. The establishment of clinical diagnostics count is a problem.
Method and Materials: Fifty-three patients who underwent surgical resection were enrolled (esophagus 1, stomach 39, small bowel 7, Cecum 1 and rectum 5; mean size 4.3 cm, range 1.4−21 cm). Prior to surgery, Diffusion-weighted Magnetic Resonance imaging was performed by SENSE-STIR-EPI TR/TE 10000/75 ms, 4 mm, b=0, 1000 sec/mm2. Apparent diffusion coefficient Minimum value (ADCmin) of the tumor was measured Histological diagnosis was made by immunohistological staining of c-kit and CD34. Risk groups were estimated by assessing tumor size and mitosis per 50 HPFs based on modified Flecher risk classification and UICC-TNM. We evaluated the relationship between ADCmin and risk groups, Stage.
Results: There was a correlation of ADCmin between tumor size (R = −0.43, P = 0.001). There was a correlation of ADCmin between mitosis (R = −0.41, P = 0.003). ADCmin of very low-risk group was 1.39 +/− 0.26, low-risk 1.23 +/− 0.29, intermediate-risk 1.29 +/− 0.18 and high-risk 0.80 +/− 0.23. ADCmin of high-risk group was higher than that of other groups with a statistical difference (P = 0.001). The cut-off ADCmin level of 0.8 revealed 76.9% sensitivity and 97.5% specificity and 90.9% positive predictive value and 92.9% negative predictive value and 92.5% accuracy in predicting high-risk group. ADCmin of Stage I group was 1.28 +/− 0.29, Stage II 1.20 +/− 0.18 and Stage III/IV 0.75 +/− 0.23. ADCmin of Stage

III/IV group was higher than that of other groups with a statistical difference (P = 0.001).
Conclusion: Diffusion-weighted MRI may enable to discriminate high-risk group from other groups with high accuracy and to discriminate high-risk group from others with high specificity. It may become one of the valid clinical modalities for evaluating risk group of GIST.
No conflict of interest.

1169 POSTER

Diagnostic performance of the combination of multi detector-row computed tomography and fluorodeoxyglucose-positron emission tomography and diffusion-weighted magnetic resonance imaging for preoperative esophageal squamous cancer lymph node

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Background: Lymph node diagnosis is important in determining the course of treatment of esophageal cancer. Lymph node metastasis of esophageal cancer currently is done by multi detector-row computed tomography (MDCT) mainly, it is made in the size and morphological evaluation to identify the lymph nodes. MDCT is superior in resolution, but is poor in qualitative diagnosis. fluorodeoxyglucose-positron emission tomography (FDG-PET) and diffusion-weighted magnetic resonance imaging (DWI) is superior to qualitative diagnosis, utility as a diagnostic aid in the lymph nodes of esophageal cancer is not being considered.
We use MDCT and DWI and FDG-PET to the lymph nodes preoperative diagnosis. We examined the usefulness of DWI and FDG-PET in the diagnosis.
Material and Methods: 47 cases of esophageal squamous cell carcinoma resection with no preoperative treatment. 2813 lymph nodes were dissected, it was identified in the CT 252(9.0%). The meta diagnosed with a case in which diameter more than 10 mm and ratio of the major axis and single size 0.7 or more are met. Further, diagnosis of metastasis even if it meets rim enchancement. Line of SUV value was set to 3.0 and Line of ADC value was set to 1.5 than the data in the normal tissue and abnormal tissue of esophageal cancer. Line of ADC was possible to distinguish 100% non-metastatic lymph nodes allow the diffusion suppression and metastatic lymph nodes that allow the diffusion suppression in DWI.
Result: Sensitivity 19.1% positive predictive value 69.2% correct diagnosis rate 83.3% in the CT diagnosis. Sensitivity decreases to 12.8% by the addition of PET to CT diagnosis, PPV was increased to 100%, overall accuracy rate was increased to 83.7%. By adding a DWI in CT diagnosis sensitivity is next to 48.9%, PPV is next 100%, overall accuracy rate became 90.2%. DWI was increased the sensitivity significantly.
Conclusions: Combination of the PET is not lead to increase overall accuracy rate, but it is suitable to maintain a high PPV.
Combination of DWI increased sensitivity and overall accuracy, and improve the diagnostic performance of the lymph nodes.
No conflict of interest.

1170 POSTER

Diffusion weighted magnetic resonance imaging for assessing the early response to chemoradiotherapy for advanced esophageal squamous cell carcinoma

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Background: Predicting the outcome at an early stage of chemoradiotherapy (CRT) for advanced esophageal squamous cell carcinoma (eSCC) can prevent the exposure of the patient to ineffective and unnecessary toxicity. Identifying the patients of which treatment is effective could increase the probability of surgical resection and finally improve the prognosis of locally advanced eSCC. The aim of this study was to investigate the utility of the apparent diffusion coefficient (ADC) value in Diffusion Weighted Magnetic Resonance Imaging (DWMRI) for prediction and early detection of treatment response in advanced eSCC.
Materials and Methods: Twenty-seven consecutive patients with primary cT4 eSCC who underwent CRT were retrospectively evaluated. Patients received accelerated radiotherapy. Chemotherapy was performed with 5FU and CDDP. CRT response was assessed at the end of a 40 Gy the clinical response of the primary tumor was discriminated as follows: CR; disappearance of tumor, PR; at least a 30% decrease, PD; at least a 20% increase, SD; not CR, PR nor PD. CR and PR were estimated as responders. Responder patients underwent operation. Meanwhile, non-responder patients underwent definitive CRT until the 60 Gy. DWMRI was performed before CRT, after 20 Gy and after 40 Gy. We measured tumor ADCs (x 10^{-3} mm^2/s) and compared with the therapeutic effect between responders and non-responders.
Results: The ADC value of 20 Gy was significantly higher in responders in comparison to non-responders (1.13 vs. 0.93; P = 0.005). Cut-off ADC value was set at 1.00 by Receiver Operating Characteristic analysis. The ADC predicted the responders with a sensitivity, positive predictive value(PPV), and accuracy of 79%, 73%, and 74%, respectively. The increase rate of the ADC at the period of 20 Gy (ADC20) was also significantly higher in the responders in comparison to the non-responders (35.4% vs. 1.5% P = 0.0007). An ADC cut-off value for ADC20 of 15% predicted the responders with a sensitivity, PPV, and accuracy of 71%, 100%, and 85%, respectively. At the time of 20 Gy, eleven patients were less than cut-off level both of ADC and ADC (Group A). Ten patients of Group A were non-responders. Sixteen patients were more than cut-off level either ADC or ADC (Group B). Thirteen patients of Group B were responders.
Conclusion: DWMRI is a non-invasive functional modality for eSCC. It may predict early response after CRT and also available to estimate outcome after the treatment.
No conflict of interest.

1171 POSTER

Detection of liver tumors by indocyanine green fluorescence: Results and perspectives

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Introduction: The gold standard to treat liver metastases from colorectal cancer is a parenchymal resection that achieves a R0 result at final pathology. This is a major determinant for a prolonged disease free survival. However the current imaging techniques can face a limit of resolution. Possibly one could find an intraoperative staging worse that expected from the preoperative workup. Recently Indocyanine green fluorescence has been proposed to improve the intraoperative staging of liver tumors in synergy with IOUS.
Material and Methods: 30 patients with colorectal metastases were enrolled. Each had a i.v. bolus of ICG (0.5 mg pro kg of body weight) 24 hrs before surgery. ICG fluorescence, which accumulates around neoplastic lesions as a result of defected biliary clearance, was detected intraoperatively with a specifically near-infrared camera system (PDE-PhotoDynamicEye). We recorded the total number of lesions detected by PDE-ICG, intraoperative ultrasound (IOUS) and preoperative computed tomography (CT).
Results: The combined use of PDE-ICG and IOUS revealed 30% more nodules than IOUS alone and 40% more than preoperative CT. While the detection was quite similar for nodules >3 mm, PDE-IOUS was significantly superior for nodules <3 mm.
Conclusions: The chase for radical liver surgery requires imaging techniques that effectively detect all the tumor deposits. PDE-IOUS detection could foster our ability to find and remove undiscovered liver nodules. Probably we should recognize that current imaging still has limits. New technologies will probably modify our approach to liver resections.
No conflict of interest.

1172 POSTER

Evaluation of malignancy for esophageal cancer by dynamic FDG-PET

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Background: One of the basic characteristics of cancer includes heterogeneity. The heterogeneity of the tumor tissue is thought to have influence to the sensitivity such as an anticancer agent or the radiation and is thought one of the treatment-resistant causes. In late years a cancer stem cell hypothesis is advocated, and the biological malignancy evaluation of cancer by the molecular biologic technique attracts attention. It is the present conditions that there are few reports about the heterogeneity of the tumor tissue. We analyze heterogeneity in the glucose metabolism of the esophageal cancer by applying a fractal dimensional analysis using Dynamic FDG-PET (dPET). The fractal dimension is a parameter of heterogeneity and it is used for brain blood flow evaluations by the SPECT, for example.
Methods and Materials: Our evaluation study included 30 patients of esophageal cancer. dPET studies were performed after intravenous injection of 370 MBq ^{18}F-FDGfor 60 min. All patients were examined with

a 23-frame protocol (10 frames of 1 min, 5 frames of 2 min, and 8 frames of 5 min). The evaluation of the dynamic PET was performed using the software package PMod (PMod Ltd.,Zurich, Switzerland). Fractal dimension (FD) was calculated for the time-activity-data in each individual voxel of a VOI (volume of interest, consists of several regions of interest (ROI) over the target area). We compared SUVmax with FD and examined a change of FD in before and after preoperative adjuvant therapy, and an association of clinical progress.
Results: FD has a stronger correlation in a clinical progression than SUVmax; by the comparison between a clinical depth of tumor invasion (cT) and FD, $r^2 = 0.603$ whereas $r^2 = 0.5733$ SUVmax, and by the comparison between a clinical stage of tumor (cStage) and FD, $r^2 = 0.5929$ whereas $r^2 = 0.5694$ SUVmax. When we compared a rate of decline of FD before and after treatment in preoperation, in the case with the rate of decline of FD more than 10 the effect was grade 2 or 3 in postoperative pathology, in the rate of decline of FD is less than 10 the grade was 1. It was confirmed pathologically that the rate of decline of FD reflected an effect of the treatment more exactly than that of SUV in preoperation.
Conclusions: FD is a useful parameter for indicating heterogeneity of tumor, and clinically it is thought that FD is feasible for a preoperative curative effect judgment. It becomes the new parameter for the SUV.
No conflict of interest.

1173 POSTER

Optical coherence tomography (OCT) in pigmented lesions

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Background: Cutaneous malignant melanomas are diagnosed worldwide in about 200,000 patients per year. To reduce mortality, early diagnosis is important. Optical coherence tomography (OCT) measures backscattered light like ultrasound measures backscattered sound waves. The backscattered light versus the depth is described by the attenuation coefficient (μ_{oct}). We hypothesize that OCT images of benign nevi will differ qualitatively and quantitatively from malignant melanomas, enabling the dermatologist real time, non-invasive measurement of suspicious lesions.
Material and Methods: Forty lesions from thirty-three consecutive patients were imaged with OCT. Directly after data acquisition, excision was performed. Images were studied with attention to morphological details. Epidermal layer thickness was measured and values of μ_{oct} were extracted from 200 OCT images of pigmented lesions.
Result: Morphologically, absence of the lower border of the lesion was characteristic for melanoma ($p = 0.02$). Also, the attenuation coefficient (μ_{oct}) was different between benign and malignant lesions ($p = 0.02$). There were no differences in epidermal layer thickness of benign lesions and malignant melanoma.
Conclusion: This study shows that quantitative analysis of OCT images in pigmented skin lesions gives valuable additional information about lesions characteristics. When using the attenuation coefficient, it becomes possible to distinguish between benign lesions and malignant melanoma.
No conflict of interest.

1174 POSTER

Optical coherence tomography (OCT) in neoplasia of the penis

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Introduction: Optical coherence tomography (OCT) measures backscattered light like ultrasound measures backscattered sound waves. With OCT, non-invasive micro-scale resolution volumetric *in vivo* image datasets of (pre)malignant lesions of the penis are obtained, closely resembling histopathology. Moreover, the OCT-signal can be quantified, using the attenuation coefficient (μ_{oct}). This quantitative parameter, μ_{oct}, may vary along different histological types of tissue. We hypothesize that qualitative and quantitative measurements of penile skin with OCT can differentiate between benign and (pre)malignant tissue.
Material and Methods: OCT-imaging was performed at the outpatient clinic of the NKI-AvL. All OCT- images were conducted in vivo and were analyzed afterwards. Directly after imaging, a punch biopsy was performed. One investigator, blinded for pathology, performed the qualitative analysis as well as the quantitative analysis. Qualitative analysis consisted of epidermal layer thickness measurements and determination of visible lower border of the lesions, quantitative analysis comprised of determination of the μ_{oct} of the suspicious lesions. All results were grouped according to histopathology reports.
Results: OCT images of 18 penile lesions of 18 patients were analyzed. Qualitative analysis showed a statistically significant difference ($p = 0.047$) between benign and (pre)malignant lesions with regard to the visibility of the lower border of the lesions. Also, epidermal layer thickness was significantly different between benign and (pre)malignant tissue ($p = 0.001$). Quantitative analysis showed an attenuation coefficient (μ_{oct}) of benign and (pre)malignant lesions of 2.47 mm^{-1} (SE 0.52 mm^{-1}) and 5.23 mm^{-1} (SE 0.32 mm^{-1}) respectively ($p < 0.001$).
Conclusion: OCT imaging and quantitative analysis of suspicious penile lesions has the ability to differentiate benign penile lesions from (pre)malignant penile lesions.
No conflict of interest.

1175 POSTER

Optical coherence tomography in vulvar squamous cell carcinoma

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Objective: Vulvar squamous cell carcinoma (VSCC) is the fourth most common gynaecological type of cancer with an incidence of approximately 2−3 per 100,000 women. VSCC arises from, and occasionally next to, premalignant lesions called vulvar intraepithelial neoplasia (VIN). VIN and VSCC are diagnosed through painful and invasive punch biopsy.
Optical coherence tomography (OCT) is a non invasive optical imaging technique that measures backscattered light like ultrasound measures backscattered sound waves. OCT has the potential to reduce the number of biopsies by performing an optical tissue diagnosis. We hypothesize that: a) specific qualitative features in the OCT images will differ between normal vulvar skin, VIN and VSCC, b) thickness of the epidermal layer of the skin, measured in the OCT-images, is different for normal, VIN and VSCC tissue, c) quantitative measurements of the OCT image, such as the attenuation coefficient (μ_{oct}), can differentiate between VIN, VSCC and normal vulvar tissue.
Material and Methods: Eighteen consecutive patients diagnosed with VSCC were included. Tumour and surrounding tissues were imaged with OCT. Directly after data acquisition, biopsies for histopathological correlation were taken. Thereafter, wide local excision or (partial) vulvectomy was performed. In the OCT-images specific features were sought, that could differentiate between the different tissue types In addition, epidermal layer thickness was measured and values of μ_{oct} were extracted from the OCT data. For both methods, statistical analysis was performed using mixed effects regression analysis.
Results: Qualitative analysis of OCT images showed an evident epidermal layer and a clear basement membrane layer in normal vulvar skin, while these layers could not be observed in VIN and VSCC ($p < 0.0001$).
OCT images showed a significant difference in epidermal layer thickness between normal vulvar tissue and VIN or VSCC ($p < 0.0001$) where no difference was found between VIN and VSCC. When looking at μ_{OCT}, a statistically significant difference between normal skin and VIN ($p = 0.001$) was found as well as between normal vulvar skin and VSCC ($p < 0.0001$). There was no difference between the μ_{OCT} of VIN and VSCC ($p = 0.68$).
Conclusion: This study demonstrates that OCT may be a helpful technique for non invasive tissue diagnosis of vulver malignancies.
No conflict of interest.

1176 POSTER

Pitfalls with the independent audit method of PFS endpoint trials with central imaging

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ODAC (Oncologic Drug Advisory Committee) discussed the pros and cons of implementing subpopulation audit methods of sites' assessments in oncology trials with PFS endpoints. Instead of a complete Blinded Independent Central Review (BICR) of the entire study population only a few selected cases should undergo BICR.
The discussion is about two potential methods introduced by Dodd et al., 2008, 2011 and Amit et al., 2011. In these papers the authors attempt to reduce the risk of informative censoring, optimize trial conduct and reduce associated cost.
Both authors describe novel and interesting methods to be used. Based on logistical and even more so statistical complexities inherent in both these methods in their current form neither will achieve any of the proposed goals of reducing cost, complexity, time and data censoring challenges.
This paper challenges the proposed processes and provides detailed explanations to introduced obstacles logistically, timewise, statistically, operationally. Either method increases the complexity of conducting Oncology trials. The study duration can not be shorter but rather longer in most study designs. The statistical impact of any such discrepancy between Local Evaluations (LE) and BICR are not foreseeable and not at all defined. Operationally, any sponsor and imaging CRO together need to invent new and abandon well established and industry-accepted processes. This will increase the risk, complexity and as such any chance for errors.
Based on experience and daily practice this paper gives examples why the proposed audit methods of PFS endpoints with central imaging are not feasible for Oncology trials and rather put patients, sponsors and study data at risk.
No conflict of interest.

1177 POSTER

3D SPECT/CT-based navigation to the sentinel node in the groin

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Background: To further improve the accuracy with which a sentinel node can be detected, the Declipse® SPECT-system (SurgicEye, Munich, Germany) was introduced. This navigation system can incorporate preoperatively acquired SPECT/CT information. By projecting this dataset onto the patient, it enables intraoperative mixed-reality-based navigation to the area of interest (e.g. the sentinel node). This study evaluated the accuracy of the navigation during open- and laparoscopic sentinel node biopsy in urological malignancies.
Materials and Methods: Following tracer injection in penile carcinoma (n = 10) patients, who were scheduled for sentinel node biopsy, preoperative SPECT/CT was performed with a reference target fixed on the patient. For the penile carcinoma patients the reference target was placed on the pubic bone. The location of the reference target was then marked with indelible ink. Prior ot the start of the operation a sterile ReT was repositioned on the patient. A second reference target was positioned on the gamma probe as such allowing the surgeon to navigate the tip of the gamma probe to the sentinel node. Navigation was provided in mixed-reality, in 3D, based on the preoperatively acquired SPECT/CT imaging. The accuracy of the navigation approach was determined in relation to the location pointed out by the conventional gamma probe (coronal plane). The depth measured on the axial CT slices (distance skin surface − sentinel node; sagittal plane) was compared to the depth measured with the navigation system.
Results: In the 10 penile cancer patients, compared to the results obtained with the conventional approach, the average error of navigation was 5.0 mm and 5.3 mm in the coronal and sagital plane, respectively. During exploration, the inaccuracy of navigation could be overcome by both gamma tracing and fluorescence imaging of the sentinel node.
Conclusion: 3D SPECT/CT mixed-reality-based navigation allowed the identification of the sentinel node and has the potential to more accurately guide the surgeon to the area of interest.
No conflict of interest.

1178 POSTER

Quantification of baseline [18]F-FDG PET images predicts treatment response and progression free survival in Hodgkin's lymphoma

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Background: Efforts to tailor treatments to individual prognosis are important in Hodgkin's Lymphoma (HL) which has a relatively good prognosis but a high chance of developing late treatment effects. Interim PET has shown prognostic value in HL but baseline PET has not yet been extensively studied. In solid tumours the heterogeneity of ^{18}F-FDG uptake as measured by image heterogeneity analysis has been associated with treatment response and survival.
We investigated whether similar predictive and prognostic value exists in the baseline pre-treatment ^{18}F-FDG images.
Material and Methods: 55 consecutive patients with stage II−IV disease were retrospectively analysed (median FU 34 months), defining the nodal mass with the highest SUV on the baseline scan. Interval and end-of-chemotherapy responses were classified according the Deauville Score (Responders = Deauville 1, 2 & 3; Non-responders = Deauville 4 & 5); Overall treatment response was assessed using the Deauville Score combined with clinical evaluation. In-house software was used to measure Standard Uptake Value (SUV) parameters alongside 1st and 2nd order heterogeneity features.
Results: SUVmean, max and peak from the baseline ^{18}F-FDG PET image predicted the end of chemotherapy and overall treatment response ($p \leqslant 0.01$), as well as progression free survival (SUVmax $p = 0.016$, SUVpeak $p = 0.028$). 1st order parameters from region of interest (ROI) analysis similarly predicted end of chemotherapy Deauville response and overall treatment response ($p \leqslant 0.05$).
The 2nd order heterogeneity parameters measured on the baseline PET image using grey-level co-occurrence matrices (GLCM) and grey-level run length (GLRL) analyses predicted the Deauville response of the early interval PET ($p = 0.01$–0.05).
Conclusions: Baseline ^{18}F-FDG PET SUV and other 1st order parameters predict end of chemotherapy Deauville response, overall treatment response and PFS. GLCM and GLRL 2nd order heterogeneity measures of the baseline PET image predicts early response to chemotherapy.
No conflict of interest.

1179 POSTER

Preoperative assessment of gastrointestinal stromal tumors using positron emission tomography

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Background: In Gastrointestinalstromal tumors (GIST), surgical indication is an important problem, and the malignant evaluation is indispensable. In recently modified Flecher risk classification and UICC-TNM are made, and it was shown for the risk evaluation that mitosis count and the tumor size are index. The establishment of clinical diagnostics count is a problem.
Method and Materials: Eighty-seven patients who underwent surgical resection were enrolled (esophagus 5, stomach 66, small bowel 8, Cecum 2 and rectum 6; mean size 5.0 cm, range 2.0−30 cm). Prior to surgery, PET imaging was performed after injection of 370MBq of F18-DG tracer. Standardized uptake value (SUV) of the tumor was measured for evaluation. Histological diagnosis was made by immunohistological staining of c-kit and CD34. Risk groups were estimated by assessing tumor size and mitosis per 50 HPFs based on modified Flecher risk classification and UICC-TNM. We evaluated the relationship between SUV and risk groups, Stage.
Results: There was a correlation of SUV between tumor size (R=0.41, P = 0.0007). There was a correlation of SUV between mitosis (R=0.52, P = 0.0000002). SUV of very low-risk group was 4.0 +/− 2.1, low-risk 3.6 +/− 1.6, intermediate-risk 3.5 +/− 2.0 and high-risk 8.9 +/− 5.4. SUV level of high-risk group was higher than that of other groups with a statistical difference (P = 0.000001). The cut-off SUV level of 5.5 revealed 76.0% sensitivity and 87.5% specificity and 73.1% positive predictive value and 90.2% negative predictive value and 85.1% accuracy in predicting high-risk group. SUV of Stage I group was 3.5 +/− 1.6, Stage II 4.3 +/− 2.3 and

Stage III/IV 10.1 +/− 5.0. SUV level of Stage III/IV group was higher than that of other groups with a statistical difference (P = 0.000001).
Conclusion: FDG PET may enable to discriminate high-risk group from other groups with high accuracy and to discriminate high-risk group from others with high specificity. It may become one of the valid clinical modalities for evaluating risk group of GIST.
No conflict of interest.

1180 POSTER

Utility of PET scan for early diagnosis of bleomycin induced pneumonitis in Hodgkin's Lymphoma

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Background: Bleomycin induced pneumonitis (BIP) is an inflammatory process occurring with use of Bleomycin. This is an idiosyncratic response which is debilitating, potentially irreversible and at times ending in fatality (up to 27%). Currently, signs & symptoms, pulmonary function tests (PFT), carbon monoxide diffusion capacity (DLCO) and high resolution computed tomography (HRCT) are used to diagnose BIP. Unfortunately these reveal only manifest BIP. Positron emission tomography (PET), has shown promise to detect early inflammatory changes, a key feature of early BIP. We prospectively explored the abilty of PET scan to detect early BIP and compared it with standard existing modalities such as DLCO and HRCT in our patients with Hodgkin's Lymphoma (HL).
Methods: This was a prospective observational single centre study wherein, newly diagnosed HL treated with the ABVD regimen from November 2011 to July 2012 was included. After baseline staging evaluation, ABVD was instituted. All were monitored every two cycles for any form of BIP, assessed clinically, by PFT, DLCO and PET scan with HRCT chest. If none occurred they were followed up until completion of treatment. Data was analyzed using SPSS v 16.0.
Results: 75 patients were enrolled in the study and 59 were evaluable for final analysis. 49% had advanced stage, 12% were smokers and 8% had history of tuberculosis. 25 (40%) had features suggestive of BIP based on any one or combination of tests. 11/25 showed PET positivity but 14/25 were negative on PET while showing clinical or PFT findings indicative of BIP. 7/25 and 9/25 were positive only on PET and DLCO respectively but were asymptomatic for BIP. 5/25 were clinically symptomatic for BIP and correlated with DLCO but were negative on PET. Only 2/25 was both positive by PET and PFT combined but remained asymptomatic. 2/25 patients had all three parameters indicative of BIP. 34 patients remained negative.
Conclusions: Our study showed PET scan to be sensitive in detecting early BIP but did not directly correlate with standard testing and clinical suspicion. It however provides a platform for exploring its utility for detecting early BIP.
No conflict of interest.

Table 1. Bleomycin induced pneumonitis in 25 patients

	Number	Percentage
CLINICAL +PFT	5	8.5
PFT	9	15.3
PET	7	11.9
PET+PFT	2	3.4
CLINICAL+PFT+PET	2	3.4

1181 POSTER

Role of the maximal standardized uptake value on fluorine 18 fluorodeoxyglucose positron emission tomography /computed tomography for predicting malignancy grade of operable breast cancer

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Background: [18F]-fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT) is potentially useful not only in examining and assessing metastasis but also in predicting prognosis of recurrent breast cancer and measuring treatment effects.
Material and Methods: Stage I−III breast cancer patients who underwent preoperative FDG-PET/CT and who were able to undergo radical surgery at the Hiroshima University Department of Breast Surgery and the Shikoku Cancer Center between January 2006 and December 2011, were included in this study (median follow up period:26.5 months). The maximal standardized uptake value (SUVmax) were assessed for predicting disease free survival (DFS). For the evaluation of relationship between SUVmax values and prognostic factors such as hormone receptors, human epidermal growth factor receptor 2 (HER2), nuclear grade, lymph node metastasis and tumor size, statistical analyses were performed using Student t test and log-rank test, and p values of less than 0.05 were considered to indicate statistically significant differences.
Results: Clinical Stage included were I (n = 194, 56.4%), II (n = 134, 39.0%) and III (n = 16, 4.7%). Tumors with estrogen receptor (ER) positive were 292 (84.9%) and negative were 52 (15.1%). All patients were divided into two groups according to cut-off SUVmax established on the basis of receiver operating characteristic (ROC) analysis ($\leqslant$3.0 vs >3.0, AUC=0.713). There was a significant difference in DFS between two groups (p = 0.001) and, hormone receptor, HER2, nuclear grade, lymph node metastasis were found strong relation to SUVmax values. SUVmax and ER status were predictive factors with multivariable analysis using cox proportional hazard regression model (p = 0.033 and p = 0.004, respectively). Furthermore patients were categorized in 3 subtypes including luminal (ER+, HER2−, n = 260), HER2 (ER− or ER+, HER2+, n = 47), and triple-negative type (ER−, HER2−, n = 37), and average SUVmax in each subtype were 3.24$\pm$2.64 in luminal, 5.01$\pm$3.60 in HER2, 4.99$\pm$4.57 in triple-negative type.
Conclusions: Our results suggest that SUVmax on preoperative FDG PET/CT have a predictive value for high-grade malignancy and prognosis in clinical Stage I−III breast cancer and it might be necessary to determine the cut-off points for each subtype for use in determining the course of treatment.
No conflict of interest.

Subtype	n (total 344)	SUVmax (average$\pm$SD)	Recurrence (total 17)
Luminal	260	3.24$\pm$2.64	6
HER2	47	5.01$\pm$3.60	5
Triple-negative	37	4.99$\pm$4.57	6

1182 POSTER

Nuclear medicine in the study of multidrug resistance in hepatocellular carcinoma: Studies with 18F-FDG and 99mTC-MIBI

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Background: Hepatocellular carcinoma (HCC) is known to be resistant to chemotherapy, which is due in part to overexpression of multidrug resistance proteins (MDR). A method to evaluate the function of these proteins involves the measure of radiolabeled substrate ^{99m}Tc-MIBI uptake. Studies have demonstrated that ^{18}F-FDG uptake is associated with MDR proteins expression in HCC. Other studies have demonstrated that ^{18}F-FDG uptake by HCC is associated with p53 expression since tumors with lower expression or mutated expression of this protein has a higher uptake of this tracer. This study aims evaluate the uptake and retention of ^{18}F-FDG and ^{99m}Tc-MIBI in three human HCC cell lines and to correlate them with the expression of three MDR proteins and with p53 expression.
Methods: Cell lines used were HepG2 (wp53), HuH7 (mp53) and Hep3B2.1−7 (p53null). Uptake and retention studies with ^{18}F-FDG or ^{99m}Tc-MIBI were performed. Cells grown were evaluated in low glucose medium (5mM) and in high glucose medium (25mM) in order to verify the influence of the glucose on ^{18}F-FDG and ^{99m}Tc-MIBI uptake and retention. Pgp, MRP1 and LRP proteins were determined by flow cytometry. To evaluate MDR modulation, retention studies were performed in the presence of verapamil (Pgp inhibitor) prior to incubation with ^{18}F-FDG or ^{99m}Tc-MIBI.

Results: For all cell lines used, ^{18}F-FDG and ^{99m}Tc-MIBI uptake and retention were higher when cells grown on low glucose medium. For both media formulations Hep3B2.1−7 cell line has higher uptake and retention of ^{18}F-FDG and ^{99m}Tc-MIBI. HepG2 cell line has a lower uptake and retention and a higher expression of MRP1. Through modulation studies with cells incubation with verapamil, a considerable increase of ^{18}F-FDG and ^{99m}Tc-MIBI retention in all cell lines were obtained.
Conclusions: It is concluded that medium glucose concentration influences the uptake and retention of both radiopharmaceuticals. There is an inverse relationship between MRP1 expressions and uptake and retention of ^{99m}Tc-MIBI and ^{18}F-FDG. Through modulation studies it was found that Pgp has an active role on MDR in HCC. Uptake and retention profiles for the two radiopharmaceuticals are similar, showing that the ^{18}F-FDG can be used to study the MDR proteins function in HCC cells, being an alternative to ^{99m}Tc-MIBI. We also conclude that p53 expression influences ^{18}F-FDG uptake once Hep3B2.1−7 and HuH7 cell lines have higher uptake than HepG2.
No conflict of interest.

1183 POSTER

The effect of respiratory motion of diaphragm on positron emission tomography/computed tomography for patients with clinical stage IA lung adenocarcinoma

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Objectives: F-18-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) is reported to be a surrogate marker of tumor malignancy grade in lung adenocarcinoma. However, respiratory motion of diaphragm resulted in reducing the measured maximal standardized uptake value (max SUV) on PET/CT of tumors, especially localized in the lower zone of lung. The purpose of this study is to compare the diagnostic significance of max SUV to predict tumor malignancy and prognosis between upper zone (UZ, containing segment 1, 2, 3 and 6) and lower zone (LZ, containing segment 4, 5, 7, 8, 9 and 10) in patients with early stage lung adenocarcinoma.
Methods: 608 consecutive patients with clinical stage IA lung adenocarcinoma who had undergone preoperative PET/CT were enrolled in this study. Tumor location (UZ, n = 383; LZ, n = 225) and surgical results were retrospectively analyzed for all patients.
Results: Although there were no significant differences between UZ and LZ patients in terms of sex, age, whole tumor size, solid component size, lymphatic invasion (ly), vascular invasion (v), pleural invasion (pl) and lymph nodes metastasis (n), max SUV in UZ is significantly higher than that in LZ (2.4±2.4 VS 2.0±1.8, respectively, p = 0.013). All receiver operating characteristics area under the curve of max SUV for predicting ly, v, pl, n, and high-grade malignancy (ly, v, pl or n) were larger for UZ tumor than those for LZ tumor. Moreover cut-off values of maxSUV for predicting pathological malignancy (ly, v, pl, and n) in UZ and LZ were 2.4 vs 1.9, 2.5 vs 1.9, 2.3 vs 1.9, and 2.8 vs 1.6, respectively. The predictability of all outcomes on the basis of maxSUV in UZ seemed to be better than that in LZ and cut-off values in UZ tended to be higher than that in LZ.
Conclusion: MaxSUV of tumors in LZ may be affected by the respiratory motion of diaphragm and apparently become lower than those which reflects a real malignant potential of tumors. Deep-inspiration breath-hold PET/CT could resolve this problem in order to precisely evaluate the malignant grade of tumors and to select appropriate surgical procedures.
No conflict of interest.

1184 POSTER

Characterization and analysis of tumorous 18F FDG-avidity in deaths due to differentiated thyroid cancer

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One of the major roles of ^{18}F Fluorodeoxyglucose (FDG) PET in managing differentiated thyroid cancer (DTC) is to detect the non-iodine avid or dedifferentiated tumors and therefore, the FDG avidity is regarded as a poor prognostic indicator. In this study we attempt to characterize and analyze the FDG PET findings in patients dying due to advanced DTC.
Material and Methods: Our institutional cancer registry has maintained a record of all patients with malignancies seen at our hospital since 1983. The registry was searched for cases with diagnosis of thyroid cancers and also to determine if the death cause is thyroid cancer-related. A total of 290 such patients were known to be expired during the follow-up and 67.9% (197/290) were ascribed to die from thyroid cancer after exclusion of non-cancer related deaths (N = 60) or dying due to other known (N = 21) or unknown (N = 12) malignancies. We further review the cases ever assessed by FDG PET within 2 years before their death and categorized the patterns and FDG avidity of tumors on PET. The lesional FDG avidity of each case was measured from the most dominant ones and presented as maximal standardized uptake value (SUVmax).
Result: Totally 38 cases of FDG PET were analyzed and 26 of them showed extensive FDG-avid metastatic lesions including: lung (N = 17), bone (N = 9), liver (N = 3), brains (N = 2), neck and mediastinal lymph nodes (N = 11) and other soft tissues (N = 6). There were 8 cases with only loco-regional tumor with FDG uptake at neck region. The SUVmax of bony lesions were significantly higher than the others whereas the pulmonary and cervical lesional FDG-avidity could vary greatly, 1.1 to 8.4 (lung), 1.3 to 7.2. However, there were four cases showing none of obvious FDG-avid lesion demonstrated on the PET.
Conclusion: As FDG PET begins to gain its role in assessing advanced DTC and our observation of FDG-avid metastasis pattern as well as tumor FDG uptake of different tumor sites in these DTC patients with imminent death might raise more clinical interests in related investigation.
No conflict of interest.

1185 POSTER

Predictive outcome assessment of extravasation injuries by use of indocyanine green video angiography

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Background: Extravasation of cytotoxic drugs constitutes a serious complication of cancer treatment. So far, a reliable method for early and predictive assessment of tissue damage is still missing. In this study, we evaluated superficial blood flow as assessed by indocyanine green (ICG) angiography in the affected extravasation area as a possible prognostic parameter. Aim of the study was to discriminate between sufficiency of conservative treatment versus the need for later surgical intervention.
Material and Methods: 29 patients were evaluated by ICG angiography after extravasation of vesicant or highly irritant cytotoxic drugs administered by peripheral i.v. infusion. To this aim, 0.2 mg/kg ICG (Pulsion Medical Systems, Germany), tricarbocyanine dye that binds quantitatively to plasma proteins, were injected i.v. to the contralateral extremity. Perfusion index and maximum pixel intensity of the defined regions of interest were individually calculated after recording by dynamic laser-fluorescence-videography (IC-VIEW®).
Results: The perfusion index at the site of extravasation differed significantly between patients (n = 22) with reversible tissue damage and thus healing under conservative management versus those (n = 7) who needed surgical intervention due to the development of necrosis (p = 0.0001). In patients benefiting from conservative management, the perfusion index was significantly higher in the central extravasation area denoting hyperaemia, when compared with the peripheral area (p = 0.0001).
Conclusions: In this cohort of 29 patients, ICG angiography as indicator of superficial local perfusion within the extravasation area of i.v. cytotoxic drugs was of prognostic value for tissue damage. ICG angiography could thus be used for the early identification of patients at risk discriminating between the appropriateness of conservative or surgical measures in the management of extravasation of cytotoxic drugs.
No conflict of interest.

1186 POSTER

Early esophageal squamous cell cancer by high-barium esophagography using flat panel X-ray detector in comparison with histological findings

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Background: In Japan Esophageal squamous cell cancer (eSCC) has been increasing and 5-year survival rate has prolonged in recent years due to standard operation, subtotal esophagectomy with thoraco-abdominal radical lymphadenectomy. Also endoscopic submucosal dissection (ESD) for superficial cancer has become not rare treatment. Because curative operation for eSCC is one of the high invasive surgical treatments of gastro-intestinal diseases, the therapeutic indications for surgery or ESD should be assessed precisely. The aim of this study is to evaluate the depth of tumor

invasion of superficial esophageal cancer by high-barium esophagography (HBE) using flat panel X-ray detector(FPD) and to compare X-ray findings with histological results.
Methods and Materials: 146 lesions of 132 consecutive patients of superficial eSCC in 2007–2012 who underwent esophagectomy or ESD were included. Histological tumor depth was classified into three groups; A (pT1a-EP/pT1a-LPM): carcinoma in situ/invades lamina propria, B (pT1a-MM/pSM1): invades muscularis mucosae/upper 1/3 in submucosa, C (pSM2/pSM3): invades middle 1/3 submucosa or extended to under 1/3. Group A: N = 68, Group B: N = 42, Group C: N = 36. HBE was performed with double-contrast method with 200–230w/v% of bariumsulfate using FPD by three experienced gastroenterologists. X-ray findings were evaluated by morphological findings as follows: longitudinal fold, aggregated nodularity, degree of the depression, roughness, double line in the lateral image, deformation and compliance of wall.
In early period (2007–2010, N = 101 lesions) we compared these X-ray findings with histological results postoperatively, and established new X-ray criteria according to these findings. The accuracy rate was re-evaluated according to the new criteria. In latter period (2011–2012, N = 48 lesions) we examined the criteria prospectively.
Results: The detectability of superficial cancer was 97.0%. **In early period** we scored on each X-ray findings from 0 to 6 point, and by the total number of points, we divided to three clinical groups; 0−5 points was group 'a' (cT1a-EP/cT1a-LPM), 6−12 points was 'b' (cT1a-MM/cSM1), and 13−21 was 'c' (cSM2/cSM3). We establishednew criteria retrospectively, and its accuracy rate was 81.2%. Then in latter period, we tried the new criteria prospectively, the accuracy rate was 79.2%.
Conclusions: HBE with FPD may enable to assess the depth of superficial eSCC in detail. These morphological findings are valid features to discriminate between T1a and T1b of superficial eSCC lesions. This study is clinically available for appropriate selection of the treatment, surgery for T1a cancer or endoscopic resection for T1b cancer.
No conflict of interest.

1187 POSTER

Prognostic factors of ductal carcinoma in situ in association with mammographic characteristics

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Background: The aim of this study is to examine the correlation between mammographic characteristics of Ductal Carcinoma In Situ (DCIS) and tumor Grade, Estrogen(ER)/Progesterone Receptors(PR) expression and amplification of the oncogene HER2/neu respectively.
Material and Methods: During 2012, 145 women with microcalcifications in their mammogram, classified as BIRADS$\geqslant$4, underwent Vacuum Assisted Breast Biopsy(VABB) with the use of radiofrequency device(Breast Lesion Excision System).
Histology identified 92 Ductal Carcinomas In Situ, which were classified as high, intermediate or low nuclear grade, as positive(+) or negative(−) for immunohistochemical (IHC) expression of ER and PR receptors and as low(1+), intermediate(2+) or high(3+) HER2/neu protein expression, respectively.
We studied the biopsy results and we evaluated the former prognostic histological parameters in association with the mammogrpahic pattern of microcalcifications.
Results: From 92 DCIS, 35 were evaluated as high, 31 as intermediate and 26 as low nuclear grade respectively. The expression of ER and PR was positive in 77% of DCIS, and HER2 overexpression was observed in 72% of the tissue samples. Casting/linear calcifications were present in 54% of high grade DCIS, in 25% of intermediate grade and in 21% of low grade respectively. Casting/linear and granular/irregular calcifications were also present in 92% of DCIS with intermediate and high HER2 expression. No correlation was established between morphologic characteristics of calcifications and ER/PR receptors status.
Conclusions: Mammographic manifestation of casting/linear and/or granular/irregular calcifications in a biopsy confirmed DCIS, might be associated with poor prognosis, since it seems to correlate with high tumor Grade and intermediate and high HER2 expression.
No conflict of interest.

1188 POSTER

Microinvasive ductal carcinoma in situ: mammographic features and pathologic findings

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Background: The aim of our study was to evaluate the mammographic and pathologic findings of microinvasive Ductal Carcinoma In Situ.
Material and Methods: Our study sample consists of 15 patients who underwent Vacuum Assisted Breast Biopsy(VABB) with the use of radiofrequency device(Breast Lesion Excision System) for excision of suspicious non palpable mammographic lesions, presenting as microcalcifications. These 15 patients were diagnosed with microinvasive Ductal Carcinoma In Situ. We retrospectively evaluated the BIRADS classification, the tumor size and nuclear grade as well as the biological pattern of the tumors.
Results: The mammographic lesions, as interpreted in our Breast Unit, by two separate radiologists, were classified as BIRADS 4b in 8 cases and BIRADS 4c in 7. The mean tumor size was 7.95 mm(range 4−15 mm). Based on the pathologic report, 4 carcinomas were non-high grade while 11 were high grade. Furthermore, 10 out of 15 tumors were ER(+), 9 out of 15 were PR(+) and 10 were Her2(+).
Conclusions: The presence of suspicious for malignancy microcalcifications (classified as BIRADS 4b and 4c) might be indicative of microinvasive Ductal Carcinoma In Situ. Such tumors often present with an aggressive phenotype, as indicated from their high nuclear grade and the increased rate of Her2 positiveness.
No conflict of interest.

1189 POSTER

About techniques to choose the right anti-cancer agents and determine the proper dosage

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Background: Various anti-cancer drugs have been developed. Suitable agent and dose for each patient should differ. I announce that we have developed a way to achieve that goal.
Material and Methods: *New-Matsumoto method*:
1. Using the MH method, living blood cells are taken from a patient, and divided into two layers, upper and lower (i.e., ULRBC(U) and LLRBC(L): hereinafter referred to as 'U' and 'L'). Then, each layer of blood cells is put into 3 ml of RPMI-1640 solution and cultured at 37degrees in a 5%-CO_2 incubator.
2. Assuming that the entire amount of daily dosage of drug administered to the patient (α) is absorbed in 5L of blood, the absorbed amount of drug in 3 ml of blood (X) is calculated as follows: X:3=α:5000, i.e. X=3$\times\alpha$/5000.
3. The calculated amount of drug (X) is added to 3 ml of saline, solution and fully mingled together.
4. The solution, which the drug is dissolved, is sterilized by filtering twice in a clean bench.
5. The sterilized solution is put into U and L, and cultured at 37degrees in a 5%-CO_2 incubator.
6. They are monitored with an inverted scanning microscope, and recorded on photos and VTRs.
7. U and L which the drug-dissolved solution is not added to are used as the control groups.

Judgment method: The most appropriate medicine is that alteration and deformation of blood cells is less, also life span of the cell is increased, compared with the control without the addition of drug.
Results:
1. The conventional dose is overdose in most cases. 1/3 is a proper equivalent in many medications.
2. Inappropriate drug will be easily clear.

Conclusions: This method is very beneficial to the patient and should be used widely from now on.
No conflict of interest.

1190 POSTER

Implementation of hybrid PET/MRI for target volume delineation in stereotactic body radiotherapy of liver tumors

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Background: Stereotactic treatment of liver metastases is a treatment option for patients not eligible for surgical resection. PET/MRI could be a helpful tool to improve target volume delineation. The aim of this report is to assess whether hybrid (^{18}F)FDG PET/MRI is feasible for stereotactic treatment planning and whether the implementation of this new imaging modality into the daily routine wokflow is effective.
Material and Methods: Patients diagnosed with liver metastases or recurrent primary tumor of the liver, who were not eligible for surgical resection, were referred to stereotactic body radiation by a multidisciplinary tumor board. Prior to the start of stereotactic treatment all patients were imaged in the same session on a hybrid 3-Tesla MRI with an integrated PET scanner as well as a on a conventional PET/CT-scanner. For stereotactic treatment planning, patients were positioned using a vacuum couch and scanned with a conventional slow CT as well as a respiratory gated 4-D CT. Images were then transferred to a commercial treatment planning system and a rigid co-registration was performed. Gross target volume (GTV) was delineated in PET-CT(GTV_{PETCT}), slow planning CT (GTV_{CT}), SUV corrected PET(GTV_{PET}) and T2-weighted MRT (GTV_{MRT}). Liver Volume was delineated in PET-CT($Liver_{PETCT}$), slow planning CT ($Liver_{CT}$), respiratory gated CT at maximum expiration(GTV_{4-D}) and MRI (GTV_{MRI}).
Results: There was a good reproducibility concerning liver volume delineated on PET/CT compared to PET/MRI, with the volume delineated on MRT being slightly smaller ($Liver_{MRT}$/$LiverPET_{CT}$ = 93.0% $\pm$ 5.5%). The best correlation was seen between liver volume delineated on PET/CT and respiratory gated 4-D CT ($Liver_{4-D}$/$Liver_{PETCT}$ = 97.8% $\pm$ 3.6%). The average GTV_{CT} was 84.2 ml, GTV_{PETCT} 82.6 ml, GTV_{PET} 108.8 ml and GTV_{MRT}.75.8 ml. Volumes delineated in PET were in average 32.8% larger than volumes delineated on contrast enhanced CT.
Conclusions: Combining MRI and FDG-PET imaging using a hybrid PET/MRI scanner lead to increased patient comfort by acquiring both modalities at a single appointment. First experiences demonstrated a good reproducibility of target volume delineation. Further studies will be performed to test whether the precision of target volume delineation might be increased by using PET/MRI images.
No conflict of interest.

1191 POSTER

Simultaneous cone beam CT scans captured during prostate radiotherapy; image quality and the effect of arc delivery time

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Background: Cone beam CT (CBCT) images can be acquired during delivery of VMAT rotational radiotherapy. We assessed the quality of simultaneous CBCTs captured during prostate radiotherapy and studied the effects of arc delivery time on image quality.
Methods: Fifty patients with localised prostate cancer were treated with radical VMAT radiotherapy. Treatment was delivered using a single 8 or 10 MV arc on an Elekta linear accelerator fitted with a Synergy CBCT system. Standard and simultaneous CBCT's were captured on fractions 1, 6, 11 and 16. The simultaneous CBCT data was reconstructed and image quality was improved using in-house software. The quality of the CBCT images was assessed using a previously validated scoring tool. The scoring clinician was blinded to whether the scan was a standard or simultaneous CBCT.
Results: 392 CBCT scans were performed on 49 patients who completed radical radiotherapy. 74 simultaneous CBCT scans were performed with a mean arc delivery time of 120 seconds during which 688 CBCT frames were acquired (slow simultaneous CBCT). Following a software upgrade the subsequent 122 simultaneous CBCTs were obtained with a mean arc delivery time of 83 seconds during which 502 CBCT frames were acquired (fast simultaneous CBCT).
All standard CBCTs and 101 (52%) simultaneous CBCTs were deemed to be clinically useful. Further results are given in table 1.
The image quality from fast simultaneous CBCT was significantly worse than image quality from slow simultaneous CBCT (chi squared test, $p < 0.001$).
Conclusion: Simultaneous CBCT scanning can produce images which are clinically useful. Prostate CBCTs are affected by bowel gas and patient habitus. Simultaneous CBCTs are also affected by scatter from the MV beam. Reducing the arc delivery time and the number of CBCT frames acquired by approximately one-third reduces the quality of simultaneous CBCTs.
Further development and refinement of simultaneous CBCT scanning is warranted. Future reductions in arc delivery times may have to be limited if the use of simultaneous CBCT scanning becomes standard practice.
No conflict of interest.

Table 1. The quality of cone beam CT scans (CBCTs) according to method of acquisition and arc delivery time.

	Standard CBCT	Slow simultaneous CBCT	Fast simultaneous CBCT
Low quality CBCT (Not clinically useful)	0	12 (16%)	83 (58%)
Intermediate quality CBCT (Clinically useful)	86 (44%)	62 (84%)	39 (32%)
Good quality CBCT (Clinically useful)	110 (56%)	0	0

1192 POSTER

Visualization of the left anterior descending coronary artery on computed tomographic images used for breast radiotherapy planning

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Background: The purpose of this study was to assess the visualization of the left anterior descending coronary artery (LAD) on CT images that were used for breast radiation treatment planning.
Material and Methods: Twenty-five breast cancer patients (including 11 left breast tumors) had radiation treatment. The delineation of the LAD artery was achieved by one radiologist and one radiation oncologist independently on 2 sets of images for each patient: one set was a pre-operative CT scan using intravenous contrast media (IV) to determine the primary gross tumor volume (GTV) and the second set was a post-operative CT scan used for treatment planning. A Student's paired t-test was used to compare the number of CT slices in which the LAD was visible for each patient in the 2 series by each observer. Interpolations and extrapolations of the LAD volume were performed for the left-sided cases using a published heart atlas. Doses to the interpolated LAD structure and the heart were reported for the group of left-sided cancer patients.
Results: There was a non-significant difference between the results with and without IV ($p = 0.34$ for the radiologist; $p = 0.90$ for the radiation oncologist). The visible LAD corresponded to a 30% portion (range 12−47%) of the interpolated structure. The maximum dose to the left artery as measured by D2% varied widely, from 2.7 Gy to 41.7 Gy, in the group of patients with left breast tumors. The largest values (>25 Gy) corresponded to those patients in whom the LAD distal extremity lay inside the breast fields.
Conclusions: With the current planning CT protocol, only one-third of the LAD could objectively be visualized. Contrast-enhanced imaging used for GTV delineation before the breast surgery did not improve the visualization of the artery. The large range of doses to the LAD artery reported in the literature may be partially due to imaging artifacts and consequently to variations in the delineation. This study has revealed the lack of consistency that may be encountered when contouring heart vessels, thereby questioning the reliability of dose reporting.
No conflict of interest.

1193 POSTER

Validation of biomarkers to predict PFS with DCE-US in 539 patients treated with different anti-angiogenic drugs: analysis of sub-groups

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Background: The prospective multicentre French National Program for the Evaluation of DCE-US has studied the technique in different tumor types and anti-angiogenic treatments.

The aim was identify perfusion parameters to predict tumor response to different anti-angiogenic treatments.
Methods: DCE-US was performed at baseline and at 4 time-points (Day 7, 15, 30, 60). At each examination, we quantified 7 DCE-US parameters. We also estimated the variation between baseline and each post-baseline time-point. The main endpoint was freedom from progression assessed according to RECIST. We first selected the best parameters: for each parameter and each time point, we studied the trend between the parameter value and freedom from progression. After, the best cut-points were searched through a grid search. The best single cut-point was that with the lowest P-value for progression free survival. We performed analyses according to the treatment and type of tumor, looking for the groups of patients that contribute the most to the heterogeneity.
Results: A total of 1968 DCE-US were performed in 539 patients. The median follow-up was 1.65 year. The mean transit time (MTT) was the only significant parameter at day 7 (P = 0.002). The best cut-point to predict tumor progression was 12 seconds (P = 0.02), a MTT >12s being of good prognosis. Variations from baseline were significant at day 30 for several parameters. The area under the curve (AUC) was the parameter with the lowest P-value (P = 0.00004); Patient with a decrease of more than 40 % had a better prognosis. The groups defined accordingly were different for both FFP (P = 0.009) and OS (0.03).
The analyses according to treatment suggested heterogeneity. We performed a separate analysis for RCC treated with Sunitinib: the best cutoff for AUC at 30 days was 0.1, corresponding to a decrease of 90%. A total of 122 patients treated with Bevacizumab were analysed: 61 Metastatic colon cancer and 61 breast cancer. The median follow-up was 1.65 year. The MTT was the only significant parameter at day 7 (P = 0.002). PFS was significantly different according to MTT < or >12S for 122 patients (<0.05), breast cancers (P<0.05) and Colon cancers (P<0.001).
Conclusion: DCE-US is the first functional imaging technique that validated predictors of tumor progression in a large multicentric cohort. This study confirms the potential of DCE-US as imaging biomarker to monitor different anti-angiogenic treatments in different type of tumors.
No conflict of interest.

Poster Session (Sun, 29 Sep)
Oncotechnology

1200 POSTER

Isolation of stem cells and production of extracellular matrix powder from fat tissue for tissue engineering: in vivo and in vitro tests

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Objectives: Due to easy accessibility and large amounts, adipose tissue could provide ideal source of stem cells. The aim of this study is to isolate stem cells and produce natural matrix from human fat tissue and then examine the growth potential of stem cells on that natural scaffold with in vivo and in vitro tests.
Material and Methods: Adipose tissue is obtained on sterile conditions from waste materials of liposuction in plastic surgery clinic of Tehran. Stem cells are isolated, examined by flow-cytometry, cultured on DMEM plate with or without acellular matrix powder (natural scaffold). In order to provide appropriate scaffold, we use physical, chemical and enzymatic digestion of abdominopelvic fat. To proof decellurisation and structure of the scaffold, hematoxylin and eosin and immunochemistry staining is used. We compare the proliferation, adhesion and differentiation potential of cultured stem cells as well as transplantation of that as graft on the subcutaneous tissue on the back of immunosupressed rats.
Results: The mesenchymal nature of the stem cells was confirmed by expression of CD90, CD105, CD166, and lack of expression of haematopoietic markers of CD34, CD31, and CD45. IHC markers showed heavy staining for collagen IV and no staining for vimentin, laminin, or S100. Assessment of stained stem cells by electron-microscopy and live cell counting examination showed increased proliferation and adhesion of adipose stem cells after adding scaffold to medium for one week (in vitro test). After six weeks of animal phase of study, increased graft size, surface vascularisation, more differentiation of stem cells, neovascularisation, and penetration of host cells to the graft and less necrosis or fibrosis were seen with microscopic examination of stained scaffold contained grafts in comparison to stem cells alone (in vitro test).
Conclusion: Our study shows that our produced human decelluralised fat scaffold provided a suitable environment to increase proliferation, differentiation, adhesion and migration of stem cells. This product should be checked in clinical setting for tissue repair.
No conflict of interest.

1201 POSTER

KRAS, BRAF and TP53 deep-sequencing for colorectal carcinoma patient diagnostics

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Background: In colorectal carcinoma, *KRAS* and *BRAF* mutations have emerged as predictors of resistance to anti-EGFR antibody treatment and worse patient outcome, respectively. In this study, we aimed to establish a high-throughput deep-sequencing workflow based on 454 pyrosequencing technology to cope with the increasing demand for sequence information at medical institutions.
Material and Methods: A cohort of 81 patients with known *KRAS* mutation status detected by Sanger sequencing was chosen for deep-sequencing. The workflow allowed us to analyze seven amplicons (1 *BRAF*, 2 *KRAS*, 4 *TP53* exons) of nine patients in parallel in one deep-sequencing run.
Results: Target amplification and variant calling demonstrated reproducible results with input DNA derived from FFPE tissue ranging from 0.4 to 50 ng using different targets and multiplex identifiers (MIDs). Equimolar pooling of each amplicon in a deep-sequencing run was necessary to counterbalance differences in patient's tissue quality. Five *BRAF* and 49 *TP53* mutations with functional consequences were detected. The lowest mutation frequency detected in a patients tumor population was 5% in *TP53* exon 5. This low frequency mutation was successfully verified in a 2nd PCR and deep-sequencing run.
Conclusion: Our workflow allows to process 315 targets a week and provides the quality, flexibility, and speed needed to be integrated as standard procedure for mutational analysis in diagnostics.
No conflict of interest.

1202 POSTER

An interactive web portal for patient empowerment in cancer survivorship

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Background: Interactive web portals are an effective tool to enhance patient empowerment, but they are still uncommon in oncology. In the Netherlands Cancer Institute we are developing such a portal for breast and lung cancer survivors. The aim of this abstract is to report on the developmental phase of this project.
Material and Methods: We determined the portal requirements based on a literature review and on focus group discussions. We used 3 electronic databases to identify relevant literature on interactive, web-based interventions for various chronic diseases, and used available guidelines for cancer survivorship care to evaluate the relevance of these interventions for the oncology setting. We held focus groups with cancer survivors and health care professionals to better identify the desired features of a web portal from the perspective of these stakeholders.
Results: The literature review indicated that although the content, duration and frequency of interventions varied considerably, features commonly used included education, self-monitoring, feedback, self-management training, individualized exercise, and communication with either health care providers or patients. These elements were evaluated as appropriate to fulfil recommendations for survivorship care. The analysis of the focus group transcripts indicated that breast and lung cancer survivors were primarily interested in those features of a web-base portal that could fulfil their information needs: a survivorship care plan, access to their medical record, and an overview of their medical appointments. Health care professionals considered telemonitoring, patient-reported outcomes plus related feedback and a rehabilitation program as the most useful elements of such a portal. Cancer survivors and health care providers agreed on the potential value of a survivorship care plan and on the possible risks of a patient forum, but were less in agreement with regard to access to medical records and e-consultation (survivors were positive).
Conclusions: Based on our literature study and focus group discussions we were able to formulate requirements for an interactive web portal to empower breast and lung cancer survivors: information provision by means of a survivorship care plan and interactivity by providing feedback on patient- reported outcomes (on, for example, quality of life and physical

activity behaviour). These features will be transformed into prototypes of the web-based portal and will be evaluated. We will present screenshots, algorithms for automated feedback, and additional patient and professional views on late draft versions of the portal. The final version of the web portal will be implemented and evaluated.
No conflict of interest.

1203 POSTER

ARRACT − a real-time randomisation application for clinical trials

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Introduction: In investigator-initiated trials there is a need for affordable and adequate tools to randomise patients.
Relational database technology and internet-based connectivity offer resources to facilitate development of efficient randomisation procedures.
At Department of Oncology, Herlev University Hospital, we have developed a web application – ARRACT. The system covers the following aspect of the randomisation process:
- **Various Algorithms:** Range based n-factors stratified balanced randomisation and permuted block randomisation.
- **Patient Randomisation:** Enables randomisation of patients.
- **Monitoring and Management of data:** Enables on-going data management and monitoring in step by step visual logging.
- **Administration:** Allows overall system oversight, configuration, and reporting by administrators.
- **Test Environment:** Allows simulations on existing treatments with fixed factors and levels and performs generic multiple n-trials simulations.
- **Integration:** Use of web services to integrate with clinical remote data entry system (OpenClinica) − Under development.

Materials and Methods: Commercial and Open Source randomisation systems were investigated as well as relevant literature to establish the minimum requirements in respect of the systems functionality and security.
A data model for the system was designed and a database was created in Microsoft SQL server 2005. The web application was programed in Visual Studio 2010.
As part of the implementation plan we tested:
- IQ – Installation Qualification
- OQ – Operational Qualification
- PQ – Performance Qualification
- Site Standard Operational Procedure (SSOP)

Results: We have conducted several simulation with the system especially the Stratified Balanced Allocation Method: A test study was setup and simulated 1000 times with satisfactory results in respect of imbalance.
At least two clinical trials are awaiting the final implementation.
Discussion: Many investigators are still forced to use inadequate methods such as 'envelope method' for randomisation in their trials.
An option might be to turn to the Open Source programs available. Currently, however, we have not found any Open Source programs suitable for our needs.
To succeed in developing and implementing systems one needs: adequate resources, funding, an implementation plan and a skilled staff.
The final aim is to use ARRACT for all investigator-initiated randomised studies.
No conflict of interest.

1204 POSTER

National online portal for registration of chemotherapy side effects

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Background: The department of oncology at Roskilde Hospital has a large outpatient ward for patients treated for CRC, Breast cancer, lung cancer and ovarian cancer. At the clinical research unit we wanted to capture data regarding the side effects of chemotherapy. Currently we register the side effects of chemotherapy by contacting the patients and register data in a pre-printed paper template. This can be biased as no current instructions currently are available on how to interview the patient.
The best sources of information about how patients experience their side effects are the patients themselves, so it seemed as an obvious choice to get patients to register themselves It has been shown that easy access to relevant information regarding disease improves patient empowerment. Studies also indicate that the better the patients handle the adverse reactions to chemotherapy, the more they are able to complete a course of treatment as planned.
Aim: The first aim was to create an online portal that could provide patients with better access to relevant guidance and accurate information about side effects in real time. By providing the patients with relevant information in due time they will increase their empowerment to act and comply with the treatment.
The second aim was the development of a database based on all the registrations done by the patients for further research and developmental projects.
Methods: We created an online portal for patients to record their side effects. This portal is part of a national project for patient based registration of data and observations linked to their chronic diseases. Roskilde is pilot site for cancer treatments.
Each side effect reported is graded according to CTCAE standards, and users get practical advice on how to act precisely to what they are experiencing. In addition, patients will be able to get a visual overview of the development of their own side effects, and compare with other patients receiving similar treatments.
Result: Patients have increased their abilities to manage their cancer treatment and compliance and are more likely to respond to the side effects accordingly and therefore more likely to complete their treatment as planned.
Conclusion: The portal and the database enable us to capture side effects for all treatments currently given to patients at our department, potentially the whole country. As this is the first time an attempt to capture this much data is made in Denmark, it will hopefully facilitate research within oncology.
No conflict of interest.

1205 POSTER

Evaluation of the metastatic potential of human tumor cells by means of 3D culture on silicon microstructures

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Background: − The ability of cancer cells to spread and give rise to a secondary tumor is dependent of their physical interactions with microenvironment. Tumor cells must undergo elastic deformations and aberrant activation of the epithelial–mesenchymal transition (EMT). A better understanding of the role of cell mechanical properties will provide new insights in cancer progression and identification of new prognostic biomarkers and therapeutic targets. We investigate cell plasticity as an indicator of the tumor cell behaviour, strongly related to the metastatic potential.
Methods: − Silicon micromachined structures (SmS) were exploited as 3D microincubators (Carpignano F, PLOS ONE, 2012) able to host tumor cells. The 3D microstructure consists in periodic arrays of silicon walls separated by empty gaps, fabricated by electrochemical micromachining of a small silicon dice. After incubation, samples were washed, fixed overnight in 10% formalin and stained for fluorescence microscopy analysis. Experiments were performed in triplicate with human cell lines, with high (A375, MDA-MB-231, RPMI-7951) or low (MCF-7, CAPAN-1) metastatic potential. The K562 leukaemia cells were utilized as control.
Results: − Fluorescence microscopy analysis of the SmS populated by cells evidenced: a) cells with low metastatic potential are largely unable to grow on the SmS surface; only few of them can survive on top of the walls. Their nuclei showed a round shape typical of cell growing on flat surfaces; b) cells with high metastatic potential can grow on the SmS thanks to their ability to colonize the deep, narrow gaps between the silicon walls. These cells showed stretched nuclei aligned along the wall direction, proving that are deep inside the extremely limited empty spaces of the SmS.
Conclusions: − Data indicate that mechanical stiffness grades the metastatic potential (Swaminatan V, Cancer Res, 2011); on these basis, the less stiffed cytoskeleton/membrane of the cells grown inside the SmS can be related to their bio-mechanical ability to undergo dynamic changes in EMT. Applied to the study of circulating tumor cells (CTCs), the SmS could provide rapid and significant insights into functional readouts of EMT-oriented CTC subpopulations characterized by different levels of aggressiveness. Thus, SmS could be implemented in a lab-on-microchip clinically applicable to predict the metastatic potential. Work was partially supp. by Fondazione Cariplo, grant nº 2011–0308.
No conflict of interest.

1206 POSTER

Preclinical antitumor activity of a nanoparticulate SN38-polymeric micelle formulation in mouse xenograft models

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Background: Irinotecan (CPT-11) functions as a prodrug which is converted into SN38 (active metabolite of CPT-11) in liver or tumor tissues through carboxylesterases. In general, only 2−8% of CPT-11 can be converted into SN38 and to considerable patient-to-patient variability. Although SN38 is very effective against various human cancer cells, its extreme hydrophobicity also prevents the clinical use. In the current study, we demonstrated that SN38 was able to be efficiently incorporated into polymeric micelles (PM) fabricated using the synthesized block copolymers.
Material and Methods: Fifteen human cancer cell lines were testing and treated with CPT11, SN38, and SN38-PM respectively, and the cell viability was determined by MTT assay. The in vivo pharmacodynamics efficacy was performed in BALB/c nude mice. NCI-H460 (human large cell lung cancer) and RPMI-2650 (human nasal septum squamous cell carcinoma) were subcutaneously (sc.) implanted into mice and to develop xenografts tumor models.
Results: Compared with the IC_{50} of cytotoxicity concentration, SN38 and SN38-PM were 217-fold activity than CPT-11 in NCI-H460 cell and 1200-fold more potent than CPT-11 in RPMI-2650 cell. In NCI-H460 xenograft model, SN38-PM was administered to mice at doses of 20 mg/kg (iv., twice week) for three weeks, the tumor inhibition rate (TIR) was 87% at day 37. In contrast, for mice given CPT-11 at the dose of 50 mg/kg, 80% of TIR was achieved at the same time. In tumor growth delay (TGD), the mean tumor volume reaching to 1000 mm^3 was as compared with control group. The TGD of SN38-PM (iv., 20 mg/kg, twice week) were 28 days. In sc. xenograft of RPMI2650 tumor models, SN38-PM at doses of 20 mg/kg and 30 mg/kg (iv., twice week) for five doses, the TIR were 95% and 99% at day 40. On the other hand, treatment with CPT-11 at the dose of 20 mg/kg and 50 mg/kg, the TIR of RPMI2650 tumor were 54% and 80% respectively.
Conclusions: We have successfully developed a PM formulations that can efficiently incorporate with SN38 and markedly increase the solubility. Furthermore, SN38-PM exhibited significantly pronounced anti-tumor activities against both NCI-H460 and RPMI-2650 xenografts. These results suggest that SN38-PM has the potential to become a modality of anticancer treatments.
No conflict of interest.

1207 POSTER

Diffuse reflectance spectroscopy (DRS) for identification of breast cancer in lumpectomy specimen

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Background: Breast-conserving surgery (BCS) is an effective treatment provided adequate surgical margins can be obtained. Irradicality is a risk factor for an adverse clinical outcome. Even in highly specialized cancer centers often over 10% of BCS patients are left with a positive resection margin after initial surgery. Here we investigate whether DRS based on altered spectroscopic properties in malignant tissue can provide guidance in differentiating benign from malignant tissue.
Materials and Methods: The lamellae of 15 lumpectomy specimens were measured at the pathology department. Using an optical probe, spectra between 400 and 1600nm of macroscopically malignant, benign and borderline tissue were obtained. At each measurement location three optical spectra were acquired as well as histology. By fitting the spectra with a mathematical model fat and water content was registered.
Results: On the fifteen ex-vivo lumpectomy specimen a total of 95 locations were measured. Of these locations 56 were benign and 39 were malignant tissue. Comparing the shape of the tumor spectra with the benign spectra clear differences were seen, especially in the 1000–1200nm wavelength region where fat and water are the dominant absorbers. By calculating fat and water ratios for all 95 locations (Fat/Water >1 for benign and Fat/Water <1 for malignant tissue) malignant tissue can be distinguished from benign tissue with a sensitivity of 97% and a specificity of 92%. Based on this ratio not all of the locations were classified correctly in three patients. When these specimens were evaluated individually, in two of the three specimens all measurement locations were classified correctly when the cut-off of the ratio was shifted to a lower value. In a clinical setting where each patient is their own control and DRS spectra of suspicious tissue is compared to benign spectra of the individual patient even better results are to be expected.
Conclusions: DRS was able to distinguish benign tissue from malignant tissue based on fat and water content. This makes it a promising tool for the future.
Conflict of interest: *Corporate-sponsored research: Philips Research*

1208 POSTER

Transcatheter oily chemoembolisation (TOCE) in the treatment of patients with locally advanced adenocarcinoma of the stomach: First experience

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Purpose: To study the first results of new treatment for local advanced unresectable gastric cancer.
Materials and Methods: We performed 26 TOCEs in 18 pts with histologically proven adenocarcinoma of the stomach (T_{3-4}, N_{1-2}, M_{0-X}). TOCE of branches of the left gastric (LGA, n = 17), gastroduodenal (GDA, n = 5) and right gastric (n = 4) supplying the tumour was made using bolus injection of 5−10 mg mitomycin C (n = 14) or 40–100 mg irinotecan (n = 12) emulsified in 3−10 ml lipidiol. In 5 cases, when selective catheterisation of feeding arteries was impossible, we used redistribution embolisation of the right gastroepiploic and right gastric arteries with steel coils. Every TOCE was added with celiac arterial infusion of oxaliplatin, docetaxel, 5FU, gemcitabin in different combinations. The follow-up included clinical exemenation, repeat biopsy, CT and PET. In 8 pts, TOCEs was repeated with 1- mo interval.
Results: There were no treatment-related complications. The post-embolisation syndrome included mild upper abdominal pain and nausea; these symptoms disappeared within 1 day of symptomatic therapy.
After first TOCE partial tumour response was seen in 9 (50 %), stabilisation in 6 (33%), and progression in 3 (17%) pts. All 9 responders became resectable and were operated (R0 resection). Of them, 7 are alive 2−40 (mean 17.4) mo, 2 pts died in 7 and 26 mo from disseminated tumour progression. The 1-, 2- and 3 yr survival was 66%, 33 % and 33 % respectively.
At present three non-operated pts are alive during 2, 5 and 21 mo. Mean survival of 6 pts who died was 9.9 ± 2.3 mo.
Conclusion: TOCE of gastric arteries is a well-tolerated procedure that causes partial tumour response and promising down-staging effect in 50% patients with advanced unrespectable adenocarcinoma of the stomach.
No conflict of interest.

1209 POSTER

Spanish radiotherapy department experience with volumetric modulated arc therapy (VMAT)

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Introduction: Technology in Radiation Oncology is rapidly expanding. The Volumetric Modulated Arc Therapy (VMAT) designed by Elekta® is a more efficient way to administer Intensity Modulated Radiation Therapy (IMRT). This study reports the experience of a Spanish Radiotherapy Department by using this technique in several pathologies.
Patients and Methods: Between January 2010 and March 2013, we have analysed the experience in 171 consecutive radical radiotherapy treatments in 142 patients with 114 VMAT (66.67%) and57 IMRT step-and-shoot (33.33%).
All cases were planned with VMAT and Step-and-Shoot IMRT and dosimetric distributions have been compared and selected individually.
The proportion of cases that had been prescribed with each technique (VMAT/IMRT) regarding tumour localization was analysed by the application of a c2 independence test (1) = 19.00; $p < 0.001$.
To find difference between the techniques in terms of time, and monitor units (MU) per dose units we applied a multivariate analysis of covariance (MANCOVA).
Results: The most frequently treated pathologies were:
Prostate 49: 38 VMAT and 11 IMRT c2 (1) = 14.88; $p < 0.001$
Lung 20: 13 VMAT and 7 IMRT

Brain metastases 24: 15 VMAT y 9 IMRT
Head and Neck 22: 8 VMAT y 14 IMRT
Abdominal tumours 18: 15 VMAT y 3 IMRT c2 (1) = 8; $p < 0.01$.
Bone 7: 3 VMAT y 4 IMRT
Mean time (minutes per Gy) of treatment with VMAT = 1.92 (0.83) vs. IMRT Step and Shoot = 2.85 (1.17)
Mean Monitor Units per Gy (MU/Gy) with VMAT = 18.16 (10.61) vs. IMRT Step and Shoot = 25.34 (32.55)
Significant differences in time/Gy between the techniques were found F(1, 93 = 10.44; $p = 0.02$; h2 $p = 0.02$). Nevertheless, there were not significant differences in MU/Gy.
Conclusions: In all cases the dosimetric quality of the VMAT was similar to the IMRT Step-and-Shoot but the treatment time was shortened considerably.
In our series, VMAT is a more efficient therapeutic option in prostate and abdominal tumours, specifically in treatment time.
MU is not statistically different between both techniques in our series.
No conflict of interest.

1210 POSTER

Frameless stereotactic radiosurgery for brain metastases using image guided radiotherapy (IGRT)

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Introduction: Stereotactic radiosurgery (SRS) has become increasingly used for treatment of brain metastases. A non-invasive mask system plus Image Guided Radiotherapy (IGRT) is a very attractive and comfortable alternative for patients (Elekta® system).
Objective: To assess the clinical outcomes of frameless radiosurgery combined with IGRT for brain metastases.
Patients and Methods: In ONCOSUR and CROASA between January 2010 and December 2012 we have treated 16 patients (50% female) with 45 brain metastases and mean age of 53.63 years (33−68). A total of 21 treatments have been performed. Our GTV margin was of 2−3 mm.
We have evaluated clinical, therapeutic data and acute toxicity.
Results: Primary tumours were 7 breasts, 3 melanomas, 5 lungs, and 1 esophagus.
Only 6 patients were also treated with whole brain radiotherapy (WBRT).
The radiotherapy techniques used were: 15 Volume Modulated Arc Therapy (VMAT); 1 Intense Modulated Therapy Step and Shoot (IMRT S-S); 1 Dynamic Arc Therapy (DART); and 4 3D Conformal Radiotherapy (3DCRT).
The hypofractionated schemes that we used more often were: 6 Gy x 6 fractions (fx) (4 cases) and 10 Gy x 3 fx (6 cases). All patients received 2 fx per week.
A variable positioning accuracy of 1−4 mm has been reported for frameless stereotactic systems. In our series, IGRT repositioning mean accuracy was: X = 0.18 mm (0.01–0.44); Y = 0.23 mm (0.06–0.66); and Z = 0.20 mm (0.01–0.40).
Acute side effects were not detected.
With a mean follow-up of 10.35 months (2−30), 6 patients are alive, and 10 are dead. The causes of death were progression in: brain: 2 patients (no WBRT); lung: 3 patients; liver: 1 patient; unknown: 3 patients and general deterioration: 1 patient.
Conclusions: Frameless SRS is an effective and comfortable treatment in the management of brain metastases.
Non-invasive mask fixation system plus IGRT is associated with a high repositioning accuracy with no errors up to 3 mm.
No conflict of interest.

1211 POSTER

Establishing a clinical trial support system using open source software

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Background: To conduct an effective clinical study, reliable screening of the study candidates and information sharing of the study is important. IT technologies resolve these problems effectively. Specific software purchase or a service provider contract is needed but it is sometime difficult for academic institute due to the maintenance load and cost issues. As such, we have established a clinical study support system using an open source software (OSS) to meet the objective of solving the above issues.
Material and Methods: The system is based on Netcommons, an open source content management system (CMS), adding two implementations which are the eligibility check upon registration for subjects and information offering and sharing functions. Regarding the eligibility check function, we implemented mandatory and range check functions and examined measures to avoid non-qualified subjects registrations. Also implemented the interface which transfers the user information and registration details considering data coordination with other systems.
Results: The system operation started in our hospital server since 2011 and is now used as a registration system for multi institutions' trials on the Cloud server. The system is mainly operated for registration of the subjects for Phase 1 trial and early development of devices, and has been used in 7 trials registering 90 subjects. Recently, the information sharing functions utilizing CMS is also being used. The coordination with EDC developed by our hospital has also been implemented using data transfer interface.
Conclusions: In our hospital, the effective trial operation has become possible with the advanced IT introduction in subject registrations and information sharing. The rapid implementation of necessary functions for each clinical study is the strength of CMS allowing various allocations of information transfers and file sharing functions. Also, as the software that consists the system is OSS, modifications are possible with the introduced institutions. This indicates the possibility of the user community to come up with new ideas for improved functions and its actual implementations in the future. As such, with the wide use of the system, reasonable costs for clinical studies are expected by offering efficient clinical studies using IT technology in Academia.
No conflict of interest.

1212 POSTER

Does an automated self check-in process improve the quality and patient satisfaction associated with chemotherapy administration?

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Background: The Odette Cancer Centre (OCC) is the sixth largest cancer institution in North America and currently manages over 24,000 chemotherapy patient visits per year (and over 100 chemotherapy patients per day). We initiated an automated kiosk system whereby patients can 'actively' self-identify for check-in to the chemotherapy unit.
Materials and Methods: From January 1 to May 1, 2012, consecutive patients receiving >2 cycles of chemotherapy were randomly assigned to either radio-frequency identification (RFID) or barcode technologies to facilitate self check-in and time-in-motion studies. In parallel, the former manual check-in system (guided by OCC staff) continued for all patients. The primary outcome was the proportion of patients with more 3 or more scheduled appointments who used the self-check system at lest 3 times (compliance). Patient satisfaction was attained with a baseline and post-study survey instrument.
Results: The study accrued 81 patients (43 patients using RFID and 38 patients using barcode technology). Mean age was 59 (range 20 to 81 years). Sixty-four individuals completed baseline survey instruments at the time of analysis. Mean age of patients was 56 (range 21−81). The majority of patients at baseline had regular access to a computer (87.5%) and used the internet at least >1 hour/day (50%). With implementation of the study, 24 of 81 patients (29%) have used the kiosk only once. Of individuals with multiple scheduled chemotherapy appointments (at least 3), 50% assigned to the RFID group and 52.6% assigned to the barcode group used the kiosk at least 3 times ($p = 0.827$; Fisher's exact test). Thirty-eight patients completed patient satisfaction surveys; 95% found the system easy to use. More than half (53%) indicated the self check-in process would have positive effects on their overall cancer care, although only a third (34%) indicated that the efficiency of their care improved.
Conclusions: An automated check-in process is feasible for a diverse population of patients receiving chemotherapy. Multiple uses of the kiosk technology suggest appropriate uptake and retention of the technology. Continued use of the system was not different between RFID and barcode technologies. Patient satisfaction was high and indicated a positive contribution to the delivery of cancer care.
No conflict of interest.

1213 POSTER

Oncolex.org − a web based cancer encyclopedia for health care providers

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www.Oncolex.org is a free, comprehensive online resource for cancer diagnostics, treatment and supportive care. Oncolex contains extensive material for each cancer type including explanatory articles, illustrations,

animations, photos and video footage. As a resource in continual progress, it keeps track of novel procedures and technology transforming the field of cancer diagnostics and treatment.
Background: Few hospitals and medical centers have cancer specialists on site. Oncolex acts as an accessible resource for health care providers worldwide in need of specialized information when treating and informing their cancer patients. Acclaimed medical specialists in Norway and the US would like to share their knowledge, providing up-to date and detailed information on cancer care to health care professionals worldwide.
The accessibility of Oncolex will aid in increasing the quality of patient treatment, which again will ultimately increase cancer survival rates. The content of Oncolex is constantly being revised and reviewed, and as current information becomes available, it occurs on the site.
Content: Oncolex contains theoretical and practical information on the various forms of cancer, as well as descriptions of procedures within diagnostics, prognostics, surgery, drugs, radiation and adjuvant therapy. Details such as surgical instruments used and cell level histological analyses are included. All types of cancer and stages are covered, so health care providers can consult Oncolex when treating a patient with a possible cancerous disease.
Customizable search options are available on the site, and users can filter out specific information such as surgical procedures or radiation therapy.
Surgical treatments have been filmed at the Norwegian Radium Hospital using a custom made camera crane, capturing the details of the operation without disturbing the surgical team or contaminating the operating theatre.
Conclusions: Since 2006 the Norwegian version of Oncolex has been available, and more than a 1000 Norwegian health care providers consult the site on a daily basis (Norway has a total population of 5 million). In 2012 all texts and articles were translated in to English to supply information for a larger audience. More than 100 cancer specialists from the Norwegian Radium Hospital at Oslo University Hospital and MD Anderson Cancer Center in Houston have contributed to the content of Oncolex.
Oncolex aims to become one of the worlds preferred cancer resources online.
No conflict of interest.

1214 POSTER

Advances in data capture in oncology outpatients with modern mobile technology

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Introduction: The HITECH (Health Information Technology for Economic and Clinical Health) Act, enacted as part of the American Recovery and Reinvestment Act of 2009, was implemented to improve health and management of complex conditions like cancer through appropriate technology. Benefits of electronic health records, patient portals etc has been recognized in Oncology. The ease and portability of iPads (© 2013 Apple Inc, Cupertino, California) is attractive for use in outpatients. Compliance with HIPAA privacy is necessary; REDCap (Research Electronic Data Capture; REDCap Software − Version 5.1.3 − © 2013 Vanderbilt University) allows users to build and manage secure online databases.
Objectives: To assess the feasibility and acceptability of an iPad as an electronic self-report symptom assessment instrument in Oncology outpatients.
Methods: Consent obtained from participants. Electronic symptom assessment instrument on iPad given to the participants prior to their first Oncology visit. The instrument with 34 questions (symptoms, quality of life) was adopted from European Palliative Care Cancer Symptom Study. Responses downloaded to REDCap simultaneously. Printed assessment results given to the Oncologist for effective symptom management.
Results: Sample size = 50; Participation rate = 72%; Mean age = 65 yrs; Males = 64%; College education = 53%; Completion rate = 100%; Self-completed = 65%; Mean time to complete = 10 minutes.
Conclusions:
1. Self completion = 65% in an older population group with college education of 53%
2. Secure web-based data collection ensured compliance with patient privacy regulations
3. New technologies may offer practical comprehensive symptom assessment in complex illness

No conflict of interest.

1215 POSTER

Automating clinical protocol monitoring: A model from developing countries

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Background: Clinical Research is thought to be a luxury science that needs a lot of investment in implementing policies, protocols and building teams for taking care of patients and their close follow up. With aim of building the first autonomous clinical research unit we tried to reduce the number of staff needed for conducting clinical research and ensuring delivery of patients' best care. We will discuss using the mentioned system in the management of Retinoblastoma as model for other diseases conducted at our center.
Material and Methods: We have developed a standardized treatment protocols for Retinoblastoma diagnosis and treatment based on best available evidence. Clinical research specialist (CRS) job was initiated to handle the different processes in protocol development, monitoring and patients' enrollment. Electronic clinical research system was used to capture different protocol data and registering patients on studies. CRS started to extract data from hospital electronic and paper-based medical record system. The Clinical Research System was integrated with Electronic Medical Records (EMR) to automatically import new patients to the hospital cancer registry. Electronic Hospital Cancer Registry was sorted and revised collaboratively between many departments to distinguish tumours histology and topography. Patients with potential retinoblastoma lesions were also filtered by an electronic routine process from hospital EMR and sent weekly in automatic way to the CR who verifies diagnosis and put the patient on follow-up. With every visit or surgical operation the electronic routine process sends the details to be tracked in the patient clinical research profile.
Results: Clinical protocol management office Retinoblastoma service became fail-proof without missing patients with diagnosis. Moreover, All the visits of the patients were tracked in real time. Patients with missing investigations or data for some visits could be tracked promptly for instantaneous resolution of such incidences. Only one CRS job became needed for monitoring the whole process. The net result of this experience was proper patient monitoring for ensuring that best care is delivered to the patient and maximized control of clinical research subjects enrolled on clinical trials.
Conclusions: The above mentioned integration reduced the need for huge investments in complex processes and reduced the staff needed for implementing clinical research in limited resource settings. We believe that this experience can be replicated in other centers where resources could jeopardize the quality of care.
No conflict of interest.

1216 POSTER

The effect of telephone consultation and triage for outpatients with early breast cancer during chemotherapy

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Background: In recent years, there has been an increase in the number of cancer patients who receive outpatient chemotherapy. In order to detect adverse events early and provide appropriate care to outpatients, we have offered telephone consultation service for outpatients during chemotherapy since May 2011. We assessed the effect of telephone consultation and triage on unplanned hospital visits and admissions.
Material and Methods: Retrospective date was collected for patients with operable breast cancer who received primary systemic chemotherapy in our outpatient chemotherapy clinic. The chemotherapy regimen included anthracycline-containing regimen or anthracycline followed by taxane. Among patients with HER2-positive cancer, trastuzumab was also administered.
Results: Between May 2011 and March 2012, 166 patients were included. The median age was 51 years (range, 27−78), and all of patients were female. 73 patients (44.0%) were treated with anthracycline-containing regimen only, and 93 (56.0%) with anthracycline followed by taxane regimen. 52 patients (32.0%) accessed the telephone consultation service during chemotherapy, and the median number of service usage was 1 (1−30). A total of 139 complaints, frequently reported symptoms were pain (14.4%), fever (11.5%), chemotherapy-induced nausea and vomiting (CINV) (5.8%), and confirmation of dosing instruction for supportive care drugs (6.5%). Approximately three fourth of consultation subjects were concentrated during first 4 cycles of chemotherapy. After consultation, 12 patients were advised to visit the hospital, 2 of whom ended up with

hospitalization. Patients with telephone consultation had lower rate of unplanned hospital visits compared to patients with no consultation (7.2% vs 31.3%; P = 0.06); but trend toward that of unplanned admissions (1.2% vs 7.2%; P=.151). No treatment-related mortality was observed.
Conclusions: The telephone consultation may reduce unplanned hospital visits during outpatient chemotherapy and decreases the burden on busy hospital clinics. Further evaluation to assess safety and patients' satisfaction is needed.
No conflict of interest.

Poster Session (Sat, 28 Sep)
Surgical Techniques

1250 POSTER

Near-infrared fluorescence sentinel lymph node mapping in breast cancer: A multicenter experience

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Background: The sentinel lymph node procedure plays an important role in the diagnosis and treatment of breast cancer. Currently, blue dyes or radiotracers or a combination are used as standard of care. However, these modalities have several disadvantages, including the use of ionizing radiation and tattooing of the breast up till several months after blue dye injection. Near-infrared fluorescence (NIRF) imaging has the potential to improve the sentinel lymph node (SLN) procedure by facilitating percutaneous and intraoperative identification of lymphatic channels and sentinel lymph nodes. Indocyanine green (ICG) is currently the only FDA and EMEA approved NIRF probe that can be used as a lymphatic tracer. Previous studies indicated that a dose of 500 μM ICG is optimal for SLN mapping in breast cancer. The current multicenter study validates these results in a large patient cohort.
Material and Methods: 95 breast cancer patients planned to undergo SLN procedure were included at the Dana-Farber/Harvard Cancer Center (Boston, MA, USA) and the Leiden University Medical Center (Leiden, Netherlands) between July 2010 and January 2013. Patients underwent standard of care SLN procedure with lymphatic mapping using ^{99}Technetium-colloid, and in 33 patients patent blue dye was also injected. In addition, the optimal dose of 500 μM ICG (1.6 mL) was administered directly before surgical draping. For NIRF imaging the Mini-FLARE™ camera system was used, which is capable of displaying real-time NIR signal and visible image simultaneously.
Results: SLN mapping was successful in 94 of 95 patients using NIRF imaging or a combination of both NIRF imaging and radioactive guidance. A total of 175 sentinel lymph nodes (mean: 1.9, range: 1−5) were detected: 96% hot, 98% fluorescent, and 76% blue. In one patient, the SLN was found only by fluorescence imaging. Time between skin incision and detection of SLN was 8±4 minutes and was correlated to BMI (P <0.001). In all patients that were treated with patent blue, the NIRF signal in the SLN was detected through the axillary fat considerably earlier than blue staining. The NIRF signal of the SLN was on average 11.7±6.7 times higher than the surrounding axillary fat tissue. No adverse events related to ICG injection were noted.
Conclusions: This study demonstrates the safe and feasible introduction of NIRF imaging in order to detect the SLN in breast cancer patients with high identification rate using 500 μM ICG and the Mini-FLARE™ camera system.
Conflict of interest: *Ownership: FLARE™ technology is owned by Beth Israel Deaconess Medical Center, a teaching hospital of Harvard Medical School. It has been licensed to the FLARE™ Foundation, a non-profit organization focused on promoting the dissemination of medical imaging technology for research and clinical use. Dr. Frangioni is the founder and chairman of the FLARE™ Foundation. The Beth Israel Deaconess Medical Center will receive royalties for sale of FLARE™ Technology. Dr. Frangioni has elected to surrender post-market royalties to which he would otherwise be entitled as inventor, and has elected to donate pre-market proceeds to the FLARE™ Foundation. Dr. Frangioni has started three for-profit companies, Curadel, Curadel Medical Devices, and Curadel In Vivo Diagnostics, which may someday be non-exclusive sub-licensees of FLARE™ technology.*

1251 POSTER

Intraoperative ultrasound guided lumpectomy versus mammographically needle localization for breast cancer patients after neoadjuvant treatment

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Background: The use of intraoperative ultrasound (IOUS) to localize and guide breast cancer tumors excision has advantages over other techniques. It has been reported to improve negative margins and to reduce the resection of large excision volumes of breast. However, the use of IOUS has not been explored after neoadjuvant treatments (NAC). We aimed to compare IOUS guided surgery with needle localization (NL) guided surgery in breast cancer patients after neoadjuvant chemotherapy.
Material and Methods: In this study, patients with T1−3 and N0−2 who underwent NAC and where considered for breast conservative surgery after treatment were included in the study between July 2008 and April 2011. All patients had a clip placed at the beginning of systemic treatments for tumor localization after NAC. IOUS guided surgery was used in patients with a visible clip or tumor under ultrasound. If the clip was not visible under US, a NL guided surgery was performed.
Results: A total of 84 patients were included. IOUS was performed in 37 (44%) and in 47 (56%) a NL was performed. Mean age in IOUS was 59 years (range, 33−83) and in the NL was 52 years (range, 27−88), (p = 0.8). Tumoral volume at diagnosis measured by mammogram was 31.7 cc^3 in the IOUS group and 37.1 cc^3 in the NL group (p = 0.54). There were no statistically differences in the lumpectomy volume between groups (p = 0.84). Thirty-two patients (39%) had no tumor on the lumpectomy or microscopic foci of invasive tumor after NAC (11 patients in the IOUS and 21 patients in the NL group). When considering this group with better response to NAC, lumpectomy volume was smaller in the IOUS group 24.5 [SD 14] vs 41 [20] cc^3; p = 0.02. Tumor free margins were obtained in 95% with IOUS and in 94% in the NL group (p = 0.7).
There were no differences in rates of recurrences between groups with a median follow-up of 42 months (range, 24−57).
Conclusions: Compared to NL guided surgery, IOUS surgery lowers the volume of resection in patients with complete pathologic response or minimal microscopic disease after NAC. IOUS reduces the resection of healthy tissue without compromising margins and local recurrences. Breast conservative surgery can easily be achieved with IOUS guided lumpectomy in patients with good response after NAC.
No conflict of interest.

1252 POSTER

The SentiMag Study: Sentinel node biopsy with superparamagnetic iron oxide (SPIO) vs. radioisotope

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Background: The SentiMag study compares the 'gold standard' ^{99m}Tc with a new technique, which employs superparamagnetic iron oxide particles (SPIO) for localisation of sentinel lymph nodes. Aim of this study is to investigate the potential equivalency of the SentiMag® technique in comparison to the gold standard of sentinel lymph node biopsy (SLNB).
Materials and Methods: In a prospective, multicentre and multinational 2-arm study, 150 patients with histologically verified breast carcinoma are examined. For comparison, SLNs are marked initially with radioisotope following a 1- or 2-day protocol. Additionally, SPIO (Sienna+®) is injected at least 20 minutes before SLNB into the subareolar interstitial tissue, followed by 5 minutes massage. SLN-detection is carried out using a magnetometer (SentiMag®) and a gamma probe. Preparation and excision of lymph nodes is conducted using both techniques in a parallel manner. All lymph nodes marked with either tracer are excised.
Results: Interim analysis of 96 patients resulted in a detection rate concordance per patient of 98% (94/96). An average of 1.9 (radioisotope) and 2.0 (SPIO) lymph nodes were collected per patient. Nodal detection rate was 92% (173/188) for the radioisotope vs. 99% (185/188) for the SPIO tracer with magnetometer detection. The proportion of pathologically positive lymph nodes was 21/173 (12%) vs. 22/185 (12%). All pathologically positive lymph nodes detected with the conventional technique (radioisotope) were also detected with the new technique (SentiMag®).

Conclusions: The SentiMag® provides an easy technique which can be rapidly implemented into daily routine. Due to the simple handling, preoperative efforts can be reduced to a minimum. If further and consistent results prove its efficacy, this technique may ultimately replace the standard of care.
No conflict of interest.

1253 POSTER

Laparoscopic near-infrared fluorescence imaging of hepatic uveal melanoma metastases using indocyanine green: A technical note

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Background: Uveal melanoma is the most common primary intraocular tumor in adults and up to 50% of patients will develop liver metastases. Complete surgical resection of these metastases can improve 5-year survival, but only a few patients are eligible for a radical surgical treatment. Therefore, it is of great importance to select and treat these patients carefully, to prevent unnecessary laparotomies. Near-infrared fluorescence (NIRF) imaging using indocyanine green (ICG) is a promising technique to assist in the intraoperative identification of liver metastases in real time. However, all published cases concerning intraoperative detection of liver metastases using NIRF and ICG were performed in open procedures. A laparoscopic operation is preferable for patients with liver metastases from uveal melanoma, due to the high risk of multiple small metastases. The aim of this study was to introduce a novel, high definition, NIRF laparoscope during minimal invasive surgery for intraoperative identification of uveal melanoma liver metastases and to provide guidance during resection.
Methods: Two patients previously treated for uveal melanoma, both pre-operatively diagnosed with one solitary liver metastasis are presented. Patients received 10 mg indocyanine green (ICG) intravenously 24 hours before surgery (optimal timing based on a dose-finding study performed in 16 patients with colorectal liver metastases and an open imaging system). A high definition NIR fluorescence laparoscope (Karl Storz, Germany) was used to detect malignant liver lesions. After resection, ex-vivo imaging and fluorescence microscopy was performed for histological validation.
Results: In both patients, laparoscopic NIRF imaging using ICG successfully identified uveal melanoma liver metastases. A clear fluorescent rim around the tumor was observed. In patient 1, seven additional lesions in both left and right liver lobe, not seen with computer tomography (CT), were identified by inspection and NIR fluorescence imaging. In patient 2, one additional lesion, not identified by CT, magnetic resonance imaging, laparoscopic ultrasonography and inspection, was seen with NIR fluorescence imaging. Importantly, NIR fluorescence imaging provided guidance during resection of these metastases. A clear fluorescent rim around the metastases was seen with fluorescence microscopy.
Conclusions: This study describes the successful use of laparoscopic identification and resection of uveal melanoma liver metastases using NIR fluorescence imaging and ICG. This procedure is minimal invasive, and should be used as complementary to conventional techniques for the detection and resection of liver metastases.
No conflict of interest.

1254 POSTER

Liver metastases in close contact to supra-hepatic veins ablated under vascular exclusion

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Background: Liver metastases (LM) in close contact to suprahepatic veins (SHV) is a frequent cause of unresectability. Radical approach involving hepatic vein resection have been published using demanding grafting techniques for reconstruction. Otherwise experimental data have shown high resistance of SHV to heat. Intraoperative radiofrequency ablation (IRFA) with vascular exclusion (VE) may be a useful approach.
Material and Methods: Out of 358 patients operated for LM, 22 with LM close to a SHV treated by IRFA under VE with at least one year of follow-up were included in this retrospective study. Complications and outcomes are reported.
Results: There were 9 females and 13 males with median ASA of 2 (range: 1−3). All patients received IRFA with VE, combined or not with a parenchymal resection. One patient received two IRFA with VE. One patient received a 2-stage procedure. Median age was 67.5 years [range: 38−80]. Eighteen (81.8%) patients had a primary colorectal tumour and all except four received neoadjuvant chemotherapy.
Median number of metastases was 4.5 [range: 1−12]. They were bilateral for 17 patients. Median size of ablated lesions was 2 cm [range: 1−5.5]. Seven complications occurred (4 Grade IVa), and no mortality. At 4 months, no recurrence of ablated lesions was detected. Median overall survival for colorectal patients was 40 months 95% CI [17.5–not reached]. The OS at 2 years was 72.2%, 95% CI [45.6–87.4].
Conclusions: Resection of the metastasis only leaving clear margins is not so much a technical concern, but an oncological one, representing potential under-treatment. Indeed this resection is at high risk of leaving at least microscopic tumoral residue on the vessel's wall (R1 resection) increasing the risk of recurrence. To resect the lesion plus the vessel *en-bloc* is a good oncological solution, but this presents real technical difficulties for reconstruction. Despite some publications originating from transplant liver surgeons, resection of SHV followed by different grafting reconstructions has not gained widespread acceptance and cannot be advocated as a routine procedure. IRFA plus VE for LM in close contact to a SHV is a safe and effective technique which can extend the applications of liver metastases surgery.
No conflict of interest.

1255 POSTER

Extended indications for skin sparing mastectomy and immediate breast reconstruction without compromising oncological safety

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Background: Skin sparing mastectomy (SSM) and immediate breast reconstruction (IBR) is the treatment of choice for early breast cancer patients who need or desire a mastectomy. Considering SSM and IBR in patients after neoadjuvant chemotherapy (NAC) or after breast conservative recurrences is more controversial. The aim of this study was to evaluate the oncological safety and risk of complications in this group of patients.
Material and Methods: Outcomes from a multicentric prospectively maintained database of patients undergoing SSM and IBR from 2001 to 2011 were reviewed.
Results: SSM and IBR were performed on 261 breasts in 235 patients. Eleven (4.2%) patients underwent bilateral prophylactic SSM for high risk or mutation carrier, 7 (2.7%) patients underwent contralateral elective SSM at the same time of the breast cancer mastectomy, 22 (8.4%) patients were for local recurrences after breast conservative treatment (BCT) and 221 (84.7%) patients for a diagnosis of breast cancer who need or desire a mastectomy, including 15 patients (6%) who had received NAC. Tumor characteristic included 49 breasts (20%) with ductal carcinoma in situ, 167 breast (70%) with invasive ductal carcinoma, and 25 breasts (10%) with invasive lobular carcinoma. Pathological stage was 0 in 43 (16.5%) patients, I in 77 (29.5%) patients, II in 105 (40%) patients and III in 21 (8%).
IBR were performed with autologous tissue-latissimus dorsi (LD) in 3 (1%) patients, LD + implant in 76 (29%), and implants only in 182 (70%) patients. 45 (19%) patients had post mastectomy radiotherapy (RT) (15 with LD + implant and 30 with implants).
Complications after surgery were more frequent with autologous tissue than implants (27% vs 14%) $p = 0.013$, and this was not influenced by the pathological tumor stage ($p = 0.8$), NAC ($p = 0.6$) or RT ($p = 0.7$). Complications included 13 cases (5%) with partial skin flap necrosis and 15 with hematoma (5.7%). There was no delay in starting adjuvant treatment in any group.
Locoregional recurrence rate was 5.5%, 8 patients developed recurrence in the skin, 4 patients in the locoregional lymph nodes and one in the pectoral muscle; after median follow-up of 46 months (range 5–135). The 5-year disease free survival and 5-year overall survival was 88% and 96%, respectively.
Conclusion: Based on this study, SSM with IBR is an oncologically safe treatment regardless of tumor stage or neoadjuvant chemotherapy. Local recurrences are low and similar to conventional mastectomies series.
No conflict of interest.

1256 POSTER

Modified gastroesophageal anastomosis in proximal gastrectomy

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Background: Radical proximal gastrectomy is remaining to be a widely applied operative method, especially for gastric cancer, that involves mucosa and submucosa layer of the stomach wall. Postgastrectomy syndromes after proximal gastrectomy are still more or less unavoidable.

That is why, the selecting an ideal alimentary canal reconstructive pattern to elevate the quality of life has become more critical.
Methods: Three hundred twenty-four patients with upper third gastric and gastroesophageal cancers were admitted consecutively with curative intent in a clinic of National Cancer Institute of Ukraine, between May 2007 and May 2012. All patients were randomized in three groups by type of gastroesofageal anastomosis use during proximal gastrectomy (stapler anastomosis (SA), hand-sutured anastomosis by Ivor Lewis (HSA) or modified antireflux hand-sutured anastomosis (MAHSA)).
Results: Endoscopic control at 1 year follow-up of SA group showed reflux esophagitis with the following distributions: 40.6%, 30.2% and 13.2%; the same control in HSA group show 17.3%, 13.5 % and 8.6% for grade A, B and C respectively (according to Los Angeles Classification of Esophagitis). In contrast endoscopic control of MAHSA group showed reflux disease grade A and B only in 14.1% and 1.7% respectively.
The evaluation scores measured by the EORTC QOL gastric cancer-specific questionnaire (QLQ-25) for eating solid, liquid food and enjoying of meals were better in group MAHSA than in SA group patients: 2.1±0.1; 1.3±0.1 and 1.1±0.05 vs 2.4±0.2; 1.7±0.2; 1.8±0.2 respectively. The evaluation scores for acid indigestion or heartburn and acid or bile coming into mouth in main group MAHSA were 1.2±0.08; 1.2±0.08 whereas in groups HSA and SA they were 1.8±0.1; 1.8±0.2 and 2.2±0.2; 1.8±0.1 respectively ($p < 0.05$).
Conclusions: Our data showed that the presented modified method of esophagogastric anastomosis forming is a safe, easy to implement and effective in preventing the development of reflux after PGE for cancer of the upper third of the stomach.
No conflict of interest.

1257 POSTER

Oncoplastic breast-conserving surgery using latissimus dorsi miniflap

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Background: Breast-conserving surgery plus radiotherapy is firmly established as a good and safe option for most women with early breast cancer. Cosmoses after Breast-conserving surgery depend on two main factors; the site of the lesion and the breast volume excised in relation to total breast volume. Latissimus dorsi miniflap is one of the various autologous tissue reconstructions that can replenish loss of more than 25% of breast volume. The aim of our study is to evaluate the aesthetic outcome and complications of breast reconstruction using latissimus dorsi miniflap augmentation after wide local excision of the tumor combined with axillary lymph node dissection.
Patients and Methods: From January 2008 till January 2010 twenty eight patients with breast cancer were carefully selected from out-patient clinic of surgical oncology department, South Egypt Cancer Institute and underwent conservative breast surgery in the form of wide local excision with safety margin with immediate reconstruction using latissimus dorsi miniflap either by muscle only or musculocutaneous flap. Neoadjuvant chemotherapy was given in some patients to reduce the tumor size and after surgery; all cases received eligible adjuvant therapy. The aesthetic results were assessed independently by the patients and two surgeons. The aesthetic results have been ranked into three categories by the surgeons: good, satisfactory and fair and satisfaction of patients has been classified into three levels: deeply satisfied, satisfied and poorly satisfied. Follow-up of the patients ranging from 24 to 48 months (median 28 months) was done.
Results: Most of the patients (71.4%) were having T2 tumor, while (14.3%) of the patients had T1 tumor and (14.3%) had T3 tumor. Neoadjuvant chemotherapy was given for 14 patients with overall response rate about 76.7%. Wide local excision with safety margin with immediate reconstruction using latissimus dorsi mini-flap was done. Seventeen patients had reconstruction with muscle only, while 11 patients had reconstruction by musculocutaneous flap. A deeply satisfying cosmetic result was achieved in (82.1%) and none of them subsequently required mastectomy. After median follow up of 28 month, the progression free survival was 92.9% and the over all survival was 96.4%. No local recurrence was recorded.
Conclusion: Breast augmentation with autologous tissue comes into play by reducing the resultant deformity when the breast volume excised is significant. The Latissimus dorsi miniflap is the mainstay of oncoplastic breast surgery after partial mastectomy and it has low donor site morbidity, deep patient satisfaction with low and temporary postradiation effects.
No conflict of interest.

1258 POSTER

Oncoplastic surgery as an indispensible technique in breast conserving surgery

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Background: In breast cancer surgery more and more weight is lent to the cosmetic effects of operations whose aim is to decrease the psychic trauma of female patients and minimize mutilation resulting from the surgical treatment. The recent fundamental achievements in breast cancer surgery include the increasing use of breast conserving surgery, performing of breast reconstruction operations in such cases when breast conserving surgery is impossible and resignation from the excision of all the axillary lymph nodes in favour of sentinel node biopsy. This direction of changes in the treatment methods has begun to appear in Poland as well. Oncoplastic surgery is a new element of the therapy. It is the connection of classic oncological surgery associated with tumour resection with plastic surgery procedures. The essence of oncoplastic surgery is supplementing of the defect after tumour excision with healthy surrounding tissues. It is especially important in the case of an unfavourable proportion of a tumour size to the breast size, when a considerable tissue defect causes a negative cosmetic effect.
The aim of the study was to examine whether oncoplastic surgery has significantly increased the percentage of patients undergoing breast conserving surgery.
Material and Methods: 1468 patients with breast cancer were operated in the Oncological Surgery Department in 2010–2011. There were performed 648 simple amputations with sentinel node biopsy /SNB/, or axillary lymph node dissection /ALND/. Breast conserving surgery with SNB or ALND was conducted in 820 cases, including 58 oncoplastic operations. There was performed the translocation of breast tissues to suplement the defect after tumour excision with a margin in 45 patients, and subcutaneous amputation with a simultaneous prosthetic restoration was conducted in 13 patients.
Results: After introduction of oncoplastic surgery increase in the use of BCT techniques compared to simple mastectomy reached approximately 9%.
Conclusions: Oncoplastic surgery significantly increases the possibility of performing breast conserving surgery and thus constitute an indispensible technique which is a must for oncological surgeons specializing in breast cancer treatment. The survey of female patients' satisfaction revealed a high level of satisfaction from the aesthetic effects − 87%.
No conflict of interest.

1259 POSTER

Strategy for synchronous and multiple liver metastasis

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Background: Surgical indications for resection of synchronous metastasis from colorectal cancer (CRC) and the optimal timing of hepatectomy are still controversial and widely debated.
Patients: Synchronous and multiple metastatic liver tumours were detected in 57 since May 2005. Our treatment policy has been to perform hepatectomy first, if the resection can be done with no limit on size and number of tumours. However, if curative resection is not, chemotherapy is begun first and timing for the possibility of a radical operation is planned immediately. In 37 patients whose tumours were located only in the liver, primary tumour resection was performed first in 16 patients, and after tumour-decreasing by chemotherapy, operation was performed in 7 patients. In 20 patients in whom chemotherapy was performed first, after controlling the distant metastasis, hepatectomy was performed in 3 patients, and staged hepatectomy was performed in 10 patients.
Results: 1) Recurrence was detected after hepatectomy in 75.0% of simultaneous resection cases and in 70.0% of staged cases. In the recurrence cases, early detection (within 6 months) after tumour resection occurred in 58.3% of the simultaneous and 14.2% of the staged. 2) No differences in results of pre- and postoperative liver function tests were found, and duration of hepatectomy and blood loss were also similar. 3) Median survival time (MST) and 2-year survival rate were significantly better in the hepatic resection cases than in the non-operated cases. There was no significant difference in MST or 2-year survival rate between simultaneous and staged cases. 4) In 10 staged cases, length of chemotherapy had no effect on pre- or postoperative liver function test results, and survival curves.

Conclusion: The present data show that neoadjuvant chemotherapy does not increase the risk of postoperative complications or the surgical difficulties of hepatectomy for colorectal metastases.
No conflict of interest.

1260 POSTER

Randomized control trial for non-palpable breast lesions: Comparing radio-guided occult lesion localization (ROLL) vs. wire guided lesion localization (WGLL)

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Background: Non-palpable breast lesions have been localized using a variety of methodologies. Wire guided lesion localization (WGLL) has been employed for many years with successful outcomes. Recently, with the development of radio-pharmaceutical agents, radio-guided occult lesion localization (ROLL) has been implemented in actual worldwide practice. We conducted a study intended to determine the most effective method to localize and excise these non-palpable lesions in our countries biggest neoplastic referral center.
Material and Methods: A randomized control trial was designed and conducted in the National Cancer Institute of Colombia located in Bogota, from March 2006 to June 2011. We included 129 patients of which 64 (49.6%) were allocated to the ROLL group and 65 to the WGLL (51.4%). The intention was to compare effective lesion localization in these two groups of patients with lesions at risk evaluated by mammography or ultrasonography that previously had non-diagnosis percutaneous biopsy.
Results: In our study ROLL technique demonstrated a statistically significantly better median of centricity of the lesion (ROLL= 11.7; WGL = 15.4 p = 0.038). No differences were found for other variables studied between the two groups regarding the demographical characteristics, the surgical specimen, need of widening margins, surgical complications, difficulty of procedure and patient/surgeon satisfaction.
Conclusions: Using the ROLL technique is as equally as effective as WGLL in identifying the non-palpable lesions of the breast. In our study the ROLL technique demonstrated a better centricity of the lesions leading us to conclude that this technique could be applied as routine in localizing non-palpable breast lesions in centers of expertise.
No conflict of interest.

Table 1.

	WGL	ROLL	Total
Clinical and radiological characteristics			
Number of patients	65 (51.4%)	64 (49.6%)	
Age (standard deviation)	56.9 (9.6)	57.3 (10.7)	
Asymmetry	6 (9.23%)	8 (12.50%)	
Mass	28 (43.07%)	20 (31.25%)	
Microcalcifications	29 (44.61%)	35 (54.68%)	
Asymmetry and Microcalcifications	1 (1.53%)	0 (0%)	
Mass and Microcalcifications	1 (1.53%)	1 (1.56%)	
Outcome			
Localization rate	65/65 (100%)	62/64 (96.9%)	
Centricity (Median mm)**	15.4	11.7	
Minutes until skin closure	31.9 (12.5)	33.9 (15.1)	
Volume	20.7 mL (18.0)	18.3 mL (19.4)	
Weight	10.4 g (9.1)	9.3 g (8.1)	
Pain (VAS)*	3.7 (2.1)	3.0 (2.0)	
Difficulty (Likert) *	4.5 (1.4)	4.1 (1.5)	
Compromised borders	39 (60%)	38 (59.37%)	
Malignancy	11 (16.92%)	12 (18.75%)	
Patient satisfaction	63 (96.92%)	62 (96.87%)	
Surgeon Satisfaction	63 (96.92%)	63 (98.43%)	
Procedure complications			
Infection	1 (1.56%)	3 (4.61%)	
Seroma	0 (0%)	2 (3.07%)	
None	63 (98.43%)	60 (92.30%)	
Margin widening required	16 (24.61%)	12 (18.75%)	
Pathology			
Histology			
In situ Carcinoma	9 (13.84%)	5 (7.81%)	14 (10.85%)
Invasive Carcinoma	5 (7.69%)	7 (10.93%)	12 (9.30%)
Non proliferating lesions	15 (23.07%)	27 (42.18%)	42 (32.55%)
Proliferating lesions w/o atypia	25 (38.46%)	22 (34.37%)	47 (36.43%)
Proliferating lesions w/ atypia	11 (16.92%)	3 (4.68%)	14 (10.85%)
Total	65 (100%)	64 (100%)	129 (100%)

*Reported in medians (intercuartilic range).
**Sum Rank test, Z = −2.06, p = 0.038.

1261 POSTER

Lymphatic mapping and sentinel lymph node biopsy in an in-vivo porcine model using a novel magnetic technique

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Background and Objective: A novel magnetic technique for sentinel lymph node biopsy (SLNB) was developed and is currently being evaluated in an international multicentre trial (SentiMag). In this European collaborative project, we evaluated the optimal dose of magnetic tracer required for performing sentinel lymph node biopsy (SLNB). We also quantified the iron content of nodes and correlated this with readings with handheld magnetometer readings.
Materials and Methods: Local ethics approval was obtained (IR-CAD Ethical Committee, Strasbourg, France). SLNB was performed in anaesthetized pigs (Strasbourg, France) following the subcutaneous administration (deep to the third nipple in the inguinal mammary glands) of magnetic tracer (Sienna+, Endomagnetics Ltd) using a magnetometer (SentiMag, Endomagnetics Ltd.) to localize and excise groin lymph nodes. Procedures were undertaken bilaterally and using a range of different concentrations of magnetic tracer injected (0.1−2.0 ml). First hot spot measurements were performed within minutes post injection. Second hot spot measurements and SLN dissection were performed four hours post injection. Further ex-vivo counts were obtained with the handheld magnetometer. In addition, total quantity of iron in the SLNs was estimated using quantitative magnetometry (University of Twente, the Netherlands). High field ex-vivo MRI (14.1 T) and histological examination (H&E and Perl's staining) was undertaken to demonstrate the intra-nodal presence and distribution of magnetic dye.
Results: The magnetic dye drained from injection site to SLNs in the groin area in all cases. A total of 31 SLNB procedures (16 pigs) were successfully undertaken and 76 nodes (74 sentinel; 2 non-sentinel) were retrieved. Magnetic dye was proven to be present in the SLNs when measured with quantitative magnetometry, on MRI and on histological examination.
Conclusion: SLNB with the magnetic technique is feasible after subcutaneous injection of magnetic tracer, with all doses used. This magnetic tracer is ideally suited for SLNB.
No conflict of interest.

1262 POSTER

Adding imaging to the clinical picture improves BRONJ case adjudication and staging classification: Results of the "MISSION" study

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Background: Osteonecrosis of the jaw (BRONJ) is an adverse event of bisphosphonate therapy, which can significantly affect the quality of life of patients. Osteonecrosis of the jaw vary widely among studies and remain unclear. Recent data suggest that a defective definition and staging classification, based only on evidence of necrotic jawbone exposure, purulent discharge and pain could represent the main culprit of current under-diagnosis and inconsistent results of treatment.
Material and Methods: The Multicentre study on phenotype, defInition and claSSification of osteonecrosIs of the jaws associated with bisphosphONates (MISSION) was designed to investigate a well characterised large cohort of individuals diagnosed with BRONJ so to test their clinical and radiological phenotype against staging system of the American Association of Oral and Maxillofacial Surgeons (AAOMS), which relies on clinical data alone. The extent of jawbone involvement at computed tomography (CT) was measured based on the density of bone (osteosclerosis). Two patterns were analysed: focal sclerosis against diffuse sclerosis, with the former showing exclusive involvement of the alveolar bone process.

Thirteen European centres (the Institutions here represented plus UCL Eastman Dental Institute of London, Hospital 'S. Anna' of Como and the University of Pisa) contributed to MISSION collecting detailed clinical data of 799 individuals, the largest osteonecrosis cohort ever reported.
The main outcome measure was the proportion of individuals within the cohort who escape the standard AAOMS stage classification when CT is added to describe the extent of bone disease.
The local ethical Committee of each contributing Center approved the study protocol.
Results: Testing the present cohort against AAOMS staging system led to the adjudication of a total of 192 individuals (24.0%) as having non-exposed BRONJ (stage 0), and 605 with exposed BRONJ (stage 1 =72; stage 2 = 405; Stage 3 = 130). When bone disease extent as measured on CT was tested against each AAOMS stage, 57% of Stage 0 patients had already diffuse bone disease to the jaw despite the absence of frank bone exposure. In addition, more than half of AAOMS stage 1 patients had diffuse disease at CT, while 1/3 of AAOMS Stage 2 (35.1%) patients had focal disease at CT. Instead, inclusion of CT signs of bone involvement did not change distribution of AAOMS Stage 3 patients.
Conclusions: We have shown that clinical adjudication of ONJ patients based on current AOOMS staging system would potentially translate into underdiagnosis of stage 0 patients and defective treatment allocation for the entire population. The addition of imaging to the clinical picture of ONJ may become paramount in the future to anticipate diagnosis, categorize patients more consistently and offer successful treatments.
No conflict of interest.

1263 POSTER

Evaluation of surgical outcomes in conventional endoscopic thyroidectomy compared with single incision endoscopic thyroidectomy through the axillary approach for papillary thyroid carcinoma

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Background: Minimally invasive thyroidectomy has been researched extensively, and even now, the operative technique for this procedure is being further developed through research. Endoscopic thyroidectomy is performed by many techniques, using the axillary, breast, and anterior chest approaches. We have introduced single incision endoscopic thyroidectomy (SIET) as an alternative method for conventional endoscopic thyroidectomy (CET). The purpose of this study is to compare the surgical outcomes of CET and SIET for papillary thyroid microcarcinoma.
Material and Methods: This study included 118 patients with thyroid microcarcinoma who underwent CET and SIET through the axillary approach from Oct 2002 through June 2012. The surgical outcomes were retrospectively analyzed. The assessment included the size of tumor, operation time, complications, length of hospital stay, postoperative pain, and patient satisfaction.
Results: There was no conversion to conventional open thyroidectomy. The mean age of the patients was 42.3±7.6 years for CET and 38.0±9.0 years for SIET ($p = 0.551$). The mean size of the tumor were 0.5±0.23 cm in CET and 0.56±0.297 cm in SIET ($p = 0.051$). The operation time for SIET was not greater than that for CET (138.4±36.9 min vs. 128.3±36.55 min, $p = 0.794$). Postoperative pain was scored using the Visual Analog Scale (VAS). Postoperative pain was lesser in SIET than in CET (VAS 1: 4.7±1.7 vs. 3.7±1.2, $p < 0.001$; VAS 7: 2.6±1.9 vs. 2.0±1.4, $p < 0.04$). Cosmetic satisfaction was evaluated using a numeric system that ranged from 1 (extremely satisfactory) to 4 (not satisfied at all). Postoperative cosmesis appeared to have no difference between both groups (1.43±0.55 vs. 1.3±0.49, $p = 0.058$).
Conclusions: Endoscopic thyroidectomy is safe and feasible using both CET and SIET. However, SIET results in less postoperative pain with no increase in operation time. Although SIET has not been evaluated in a prospective clinical trial or large study, our results suggest that SIET reduces the postoperative pain by reducing the invasiveness of the procedure.
No conflict of interest.

1264 POSTER

A prospective study of surgical decompression and spinal reconstruction in vertebral metastases

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Background: The present prospective study aims to investigate the role of surgical decompression and spinal reconstruction in vertebral metastases.
Material and Methods: Patients with vertebral metastases were enrolled. Surgical approaches include laminectomy or laminectomy plus corpectomy. Pedicle screws stabilization was performed in all cases. Corpectomies and stabilization were performed via a single stage posterolateral approach. Expandable titanium cages were used to reconstruct spinal column after corpectomy. All patients were followed up until death.
Results: 21 patients (11 males, 10 females) with a mean age of 58 years were enrolled. 12 patients had a history of cancer, whereas vertebral metastases were first presentation of cancer in 9 patients. There were 3 cases in cervical spine, 14 in thoracic and 4 in lumbar spine. The most common primary cancers were lung, breast, colorectal and renal. Laminectomy and stabilization were performed in 3 cases, and corpectomy and cage reconstruction were performed in 18 cases. No peri-operative death or major complication was encountered. All patients were discharged to home or rehabilitation after surgery. Visual analog scale (VAS) pain score was significantly reduced from 8.8 to 3.2. In patients who had non-surgical treatment of vertebral metastasis, VAS decreased significantly from 8.3 to 3.1. There was no neurological deterioration after surgery. In patients with pre-operative neurological deficits, most patients (10/12) improved after surgery. Cobb angles were significantly reduced from 15 to 10 degree, and vertebral height was significantly increased from 28 to 34 mm after surgery. Mean survival was 5.3 months.
Conclusions: Surgical decompression and spinal reconstruction appeared to be safe in patients with vertebral metastases. Surgery reduced pain in patients with vertebral metastases, and likely to be more effective than non-surgical treatments alone. Surgery improved neurological deficit and spinal deformity.
No conflict of interest.

1265 POSTER

Diagnostic accuracy of preoperative CT scan and 18F-FDG PET/CT in patients with peritoneal carcinomatosis undergoing hyperthermic intraperitoneal chemotherapy (HIPEC) following cytoreductive surgery

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Background: The peritoneum is a preferred site of metastasis from several primary malignancies, and peritoneal carcinomatosis (PC) is one of the most significant negative prognostic indicators in patients with metastatic disease. A survival benefit has been observed for patients with PC from colorectal, ovarian, and gastric carcinomas treated by cytoreductive surgery (CRS) followed by intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC). In patients undergoing this treatment modality, the strongest determinant of outcome is the residual tumor extension after CRS. Unfortunately, PC can be difficult to diagnose preoperatively by imaging studies. The aim of our study was to evaluate the role of 18F-2-deoxy-fluoro-D-glucose positron emission tomography/computed tomography (PET/CT) scanning in detecting the presence and the extent of PC in patients with various malignancies.
Patients and Methods: A group of 47 patients (median age 61.4±11.5 years) with advanced or recurrent cancers (colorectal=26, ovarian=21, gastric=8, pseudomyxoma peritonei=3) scheduled for CRS and HIPEC underwent preoperative CT-scan and PET/CT. The results were expressed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. The negative (NLR) and positive (PLR) likelihood ratio were also obtained. The receiver operator characteristic (ROC) curves were drawn, and areas under the curve (AUC) were measured. The 95% confidence interval (95% CI) was also calculated, when appropriate. The results of CT scan and PET/CT were compared with the final surgical findings.
Results: The sensitivity, specificity, PPV, NPV, accuracy were 91%, 33%, 93%, 29% and 69% for CT-scan, and 82%, 67%, 95%, 33% and 71% for PET/CT, respectively. NLR and PLR were 0.27 (95% CI 0.07–1.09) and 1.37 (95% CI 0.76–2.44), while the AUC was 62 and 74 for CT-scan and PET/CT, respectively (p=NS).
Conclusions: In patients with PC undergoing surgery both PET/CT and CT showed low sensitivity, and thus they seem not to be a reliable tool in view of treatment planning. Our study demonstrates that PET/CT does not add substantially to CT scanning in the detection of this disease.
No conflict of interest.

1266 POSTER

Surgical management for gallbladder cancer

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The effect of radical resection in gallbladder cancer(GC) is still area of debate. Gallbladder carcinoma is rare and associated with dismal outcomes. Radical surgery is the only curative treatment and options for adjuvant therapy remain limited. Gallbladder cancer is a highly lethal disease. It is an aggressive disease with extremely poor outcome after surgical treatment and a poor prognosis. This study aimed to determine the factors influencing outcome of treatment in patients with gallbladder carcinoma and to identify the patients who might benefit from radical surgery and adjuvant therapy.
A retrospective analysis was conducted of 57 patients with the gallbladder carcinoma and the results of surgical treatment of patients with pathologically confirmed gallbladder cancer were identified. There were 57 cases (43 females, 14 males) with a mean age of 58 (range 36−84) years, treated surgically between 1995–2012.
57 patients were assessable for this study. Simple cholecystectomy was the only procedure performed in 44 of T2 and 4 of T3 cases. Radical cholecystectomy was performed as the primary procedure for 5 of the T2 and T3 cases in each. Palliative by-pass procedure or exploration was performed in 4 patients with unresectable tumours. Adenocarcinoma was the most frequent histological type and squamous cell carcinoma in 15 percent. The three-year survival rate was 70%(40 cases) and five-year survival rate was 30%(17 cases).
Favourable survival rate can be achieved after curative resection, even for selected patients with advanced disease. Adjuvant therapy may improve the survival of patients with gallbladder carcinoma. Gallbladder cancer continues to carry a poor prognosis. Very few patients underwent aggressive surgery. En bloc resection and lymphadenectomy may have stage-specific effects on survival. The only consistent curative therapy for gallbladder cancer is surgical resection. A subset of patients with peripancreatic positive nodes or invasion of adjacent organs seems to benefit from a synchronous pancreaticoduodenectomy. Radiotherapy and chemotherapy have not been found effective as an adjuvant or palliative therapy in gallbladder cancer. Because flat infiltrating gallbladder cancer and cancer with cholecystitis and numerous stones are difficult to diagnose preoperatively, we recommend taking frozen sections from patients who are of advanced age, have a long history of stones, or have a thickened gallbladder wall.
No conflict of interest.

1267 POSTER

Ruptured hepatocellular carcinoma in cirrhotic patients: Treatment options and survival outcome

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Background: Spontaneous rupture of hepatocellular carcinoma (HCC) is reported to be 3−15% of cases, with an increased incidence in Asian countries. It is considered the third leading cause of hepatocellular carcinoma-related death, after tumour progression and liver failure.
Materials and Methods: We retrospectively analysed data of 12 patients with ruptured HCC who were surgically or conservatively treated and evaluated the treatment modalities, the complications and the survival outcome.
Results: All the patients had histologic evidence of underlying cirrhosis. The median age of the patients was 65 years. There was a male predominance. Eight of these patients were hemodynamically unstable at presentation. Five patients had multifocal disease. Transcatheter arterial chemoembolisation was performed in 5 patients and 7 patients were subjected to emergency surgery. Patients with cirrhosis who received transcatheter arterial chemoembolisation had a median survival rate of 32 days. The patients who subjected to hepatectomy had higher survival rates 28.5% at 1 year.
Conclusion: The treatment of ruptured HCC should be individualised for each patient. Even though transcatheter arterial chemoembolisation is a promising treatment option still surgery is associated with better survival.
No conflict of interest.

1268 POSTER

Laparoscopic surgery of pancreatic endocrine tumors: Is there a benefit?

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Background: Although minimally invasive surgery is widely adopted for the treatment of many surgical diseases, results of laparoscopic procedures for pancreatic endocrine tumors (PET) are published only in small series. Objective of the study was to reveal and estimate the benefits of laparoscopic resection of PET and to compare it with the open approach by reviewing the available data.
Methods: Medline search for the words laparoscopic resection and pancreatic endocrine tumors was performed. 52 relevant papers were identified and studied from 2000 till 2012.
Results: Four non-randomized studies compared laparoscopic and open approach for resection of PET comprising totally 384 patients − 81 laparoscopic and 303 open. There were no cases of postoperative mortality. Mean operative time was estimated in three studies where there has been a significant difference ($p < 0.5$) in favor of open technique (121 min. vs 92 min) in one study, in favor of laparoscopic technique in the other study (188 min. vs 305 min.) and with no difference in the third study. Mean hospital stay was estimated in four studies, where it reached a significant difference ($p < 0.05$) in one study in favor of laparoscopic group (11 days vs. 14 days). Rate of postoperative pancreatic fistula was significantly higher in open group in two studies reaching up to 100% in comparison to only 14.2% in laparoscopic groups ($p < 0.05$).
Conclusion: Laparoscopic resection of PET is at least as feasible and safe as open surgery with possible benefits in terms of operative time, length of stay and rate of pancreatic fistula.
No conflict of interest.

1269 POSTER

Experience with the implant of vascular access devices by medical oncologist in a non-surgical scenery

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Background: Totally implantable central venous catheters are widely used in the management of patients (pts) with malignant diseases in order to facilitate drug delivery for the new therapeutic protocols. These are based on continuous administration and higher doses of chemotherapeutic agents with relative phlebitis problems and supportive treatment. Staff of our department, specially trained on the routinely implant of central venous accesses were in charge of the procedure. The technique was carried out under local anaesthetic in a special location of day hospital or in hospitalization environment, under strict aseptic measures without fluoroscopic control.
Material and Methods: From Sep 94 to March 2013, 1240 devices (port-a-cath systems [PS]) were implanted in 1212 pts, with age 50.5 yr (14−81) (mean, range), and K.I. 70% (50–100), female 738/male 474. Venous access: right interior jugular 715, left subclavian 245, right subclavian 252, left interior jugular 28. A thorax X-ray was performed after each procedure and in 168 pts prophylactic antibiotics were given.
Results: The venous access remained implanted a median of 438 days (1- +2210). Complications occurred in 195 placements (16%): Infections 87 (7%); deep venous thrombosis 50 (4%) Left placements 26, right placements 24; obstruction 7 (0.6%); malpositioned 25 (2%); fractures/migration 21 (1.7%); pneumothorax 4 (0.32%); local skin necrosis 7 (0.6%). Five hundred and twenty devices were removed, 347 (28%) after completing planned therapy and 173 (14%) due to complications [Infections (92), migration (22), malposition (12), venous thrombosis (26), obstruction (11) and skin necrosis (10)].
Conclusions: The best access route in order to avoid complications seems to be the right internal jugular vein. Our results are comparable with those obtained when the venous access is placed in the operating room, under fluoroscopic control and prophylactic antibiotics are administrated. This procedure, on top of being more comfortable for the patients is more affordable for the public health system.
No conflict of interest.

1270 POSTER

Needle oophoropexy: A new simple technique for ovarian transposition prior to pelvic irradiation

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Background: Irradiation of the pelvis in the treatment of cancers will result in ovarian failure unless the ovaries are shielded adequately. To protect

the ovaries, an oophoropexy may be performed. Our aim was to evaluate the feasibility, morbidity, and efficacy of laparoscopic ovarian transposition using a simple percutaneous needle technique.
Material and Methods: Fifteen patients (ten with rectal cancer and five with Hodgkin's disease) underwent the new laparoscopic oophoropexy technique. Laparoscopic releasing of the ovary was performed by cutting the utero-ovarian ligament followed by placing the ovaries on the anterior abdominal wall. A percutaneous straight needle was introduced through a 2-mm skin incision at the site of fixation. Repositioning of the ovaries was done on an outpatient basis without the need for readmission to the operating theatre.
Results: The technique was effective, reliable, and simple with no morbidities. Repositioning was performed simply in the outpatient clinic. At follow-up, 11 patients had evidence of ovarian function.
Conclusion: Percutaneous needle transposition of the ovaries is a simple, effective, reliable, and easy-to-perform technique. It has short learning curve and can be done by less experienced laparoscopic surgeons.
No conflict of interest.

1271 POSTER

From simple to complicated: The first 400 consecutive cases of oncologic robotic surgery

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Background: During the recent years, robotic surgery has known a tremendous growth although it is still not as widespread in general surgery as it was expected. However, the robot has changed the preferred approach even in oncologic surgery, in the centers that own the device. The aim of the present study is to globally assess the use of the robot for general oncology cases and to establish major indications.
Material and Methods: The first 400 consecutive cases of oncologic robotic surgery performed in our center were analyzed. The cases span on almost five years since the 1st of January 2008 until the 3rd of December 2012. Data was prospectively assembled in a Robotic Surgery Registry. Personal, clinical and surgical variables were recorded and analyzed using parametric and non-parametric tests under SPSS 16.0 software (SPSS Inc., Chicago IL, USA) for Windows. A p-value <0.05 was considered significant.
Results: In the study group there were 164 males (41%) and 236 females (59%) with an average age of 57 years (±12.53). The operations were performed by three main surgeons in stable surgical teams, with 30% of the cases performed in 2012. Indications included primary neoplasms, relapses and metastases of all major abdominal organs and some thoracic organs (thymus and esophagus). Only 17 patients needed thoracic surgery. 243 cases (60.8%) were pelvic surgery cases (gynecological cancers, rectal cancers, pelvic invasions or recurrences). In time, surgeons showed a stronger preference for pelvic robotic surgery compared to other indications ($p = 0.007$). A surgical complexity score showed a mild increase of the procedures' complexity in time and a correlation with the operative time ($p < 0.05$). Whilst pelvectomies where approached from the first year of experience, major hepatectomies and major pancreatic surgery were only addressed after an experience of 200 cases. There were only 11 conversions (2.8%) and 104 (26%) various Clavien-grade postoperative complications. The pathologic reports were satisfactory. Survival data was not available for all patients.
Conclusions: Robotic surgery in oncologic indications is feasible and safe. Pelvic cancers and pelvic recurrences are one of the most interesting indications due to a combination of factors favoring robotics: a narrow working space, complex procedures and difficult operating positions.
No conflict of interest.

Poster Discussion Session (Mon, 30 Sep)
Symptom Science

1300 POSTER DISCUSSION

Efficacy and safety of NEPA, a fixed-dose combination of netupitant and palonosetron, in preventing chemotherapy-induced nausea and vomiting (CINV) in patients receiving moderately emetogenic chemotherapy (MEC)

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Background: Targeting multiple molecular pathways is important for maximizing control of CINV. This is supported by antiemetic guidelines which recommend agents that target different pathways involved in emesis. NEPA is a fixed-dose combination of netupitant (NETU), a highly-selective NK_1 receptor antagonist (RA) and palonosetron (PALO), a pharmacologically and clinically distinct 5-HT_3 RA, that targets dual antiemetic pathways.
Materials and Methods: This multinational, randomized, double-blind, parallel group study (NETU-08−18; NCT01339260) evaluated the efficacy and safety of a single oral dose of NEPA (NETU 300 mg + PALO 0.50 mg) versus a single oral 0.50 mg dose of PALO in chemotherapy-naive patients receiving anthracycline-based chemotherapy. All patients also received oral dexamethasone (DEX) on Day 1 (12 mg in the NEPA arm and 20 mg in the PALO arm). The primary efficacy endpoint was complete response (CR: no emesis, no rescue medication) during the delayed (25–120 h) phase. Secondary efficacy endpoints included complete protection (CR plus no significant nausea), no emesis, and no significant nausea. Safety was assessed through reporting of adverse events, ECGs and cardiac troponin levels.
Results: 1455 patients were randomized. Treatment groups had comparable baseline characteristics with the overall population being predominantly female (98%) and white (80%), with a mean age of 54 years.
NEPA was superior to PALO for the primary CR endpoint as well as secondary efficacy endpoints during the delayed and overall phases following chemotherapy.

	Overall (0–120 h) % Patients	
	NEPA (N = 724)	PALO (N = 725)
Complete response	74.3*	66.6
Complete protection	63.8*	57.9
No emesis	79.8*	72.1
No significant nausea	74.6*	69.1

*p-value <0.05.

The type, frequency, and severity of AEs were comparable between groups. Most frequently reported treatment-related adverse events (TRAEs) for NEPA included headache (3.3%) and constipation (2.1%). The majority of adverse events were mild/moderate intensity and there were very few (0.7%) severe TRAEs for NEPA-treated patients. There was no evidence of any cardiac safety concerns for either NEPA or PALO.
Conclusions: NEPA is superior to PALO in preventing CINV following MEC. As a fixed-dose antiemetic drug combination it offers guideline-based prophylaxis with a convenient, single-day dose.
This study was sponsored by Helsinn Healthcare, S.A.
Conflict of interest: *Advisory board: Grunberg: Helsinn Healthcare, Eisai, Merck, AP Pharma, Redhill Biopharma Aapro: Helsinn Healthcare. Other substantive relationships: Rossi, Rizzi and Borroni: Employed by Helsinn Healthcare*

1301 POSTER DISCUSSION

Improved control of nausea and vomiting (CINV) with a fixed-dose combination of netupitant and palonosetron (NEPA) following highly emetogenic chemotherapy (HEC): Results from a pivotal study

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Background: Further progress in preventing CINV will require the introduction of novel agents with improved efficacy for nausea as well as vomiting. NEPA is a fixed-dose combination of netupitant (NETU), a new NK_1 receptor antagonist (RA) and palonosetron (PALO), a pharmacologically and clinically distinct 5-HT_3 RA.
Materials and Methods: This randomized, double-blind, parallel group study (NETU-07−07) in 694 chemotherapy-naive patients undergoing cisplatin-based HEC compared 3 oral doses of NEPA (100 mg, 200 mg and 300 mg NETU + 0.5 mg PALO) with oral PALO 0.50 mg, all given on day 1. A standard IV ondansetron (OND) 32 mg + 3-day aprepitant (APR) regimen was also included as an exploratory arm. All patients received oral dexamethasone (DEX) days 1−4. The primary efficacy endpoint was complete response (CR: no emesis, no rescue medication) during the overall (0–120 h) phase.
Results: Treatment groups were comparable: male (57%), median age 55. Most frequent cancers were respiratory (27%) and head & neck (21%). Median cisplatin dose: 75 mg/m^2.
All NEPA doses met the primary endpoint of superior overall CR rates compared with PALO (87.4%, 87.6%, 89.6% for $NEPA_{100,}$ $NEPA_{200,}$ and $NEPA_{300}$, respectively vs 76.5% PALO) with the highest NEPA dose studied ($NEPA_{300}$) showing an incremental benefit over lower NEPA doses for all efficacy endpoints. $NEPA_{300}$ was also more effective than PALO for all secondary efficacy endpoints: no emesis, no significant nausea and complete protection (CR plus no significant nausea) rates during the acute, delayed and overall phases.

	Overall (0–120 h) % Patients		
	$NEPA_{300}$ (N = 135)	APR+OND* (N = 134)	PALO (N = 136)
No emesis	91.1	87.3	76.5
No significant nausea	89.6	85.8	79.4
Complete protection	83.0	78.4	69.9

*Exploratory arm.

The type, frequency, and severity of AEs and % of patients who developed ECG changes were comparable between groups.
Conclusions: NEPA, a convenient single-day oral combination targeting dual antiemetic pathways, is superior to PALO for preventing CINV. $NEPA_{300}$ was more effective than PALO for all efficacy endpoints including nausea control following HEC. All NEPA doses were well tolerated with a similar safety profile to PALO and APR.
This study was sponsored by Helsinn Healthcare, S.A.
Conflict of interest: *Advisory board: Gralla: Helsinn Healthcare Hesketh: Helsinn Healthcare. Other substantive relationships: Rossi, Rizzi, and Palmas: Employed by Helsinn Healthcare*

1302 POSTER DISCUSSION

Prevention of chemotherapy-induced nausea and vomiting (CINV) over repeated chemotherapy cycles: Results of a phase 3 trial of NEPA, a fixed oral dose combination of netupitant and palonosetron

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Background: Safe, effective and convenient antiemetic regimens that preserve benefit over repeated chemotherapy (CT) cycles are key for enhanced cancer treatment. This study tested a single oral dose of a fixed combination, NEPA (netupitant (NETU), a highly selective NK_1 receptor antagonist (RA) and palonosetron (PALO), a pharmacologically distinct 5-HT_3 RA) over up to 6 CT cycles. Recent trials have demonstrated the safety and efficacy of NEPA in a single cycle of highly (HEC) or moderately (MEC) emetic CT. Therefore, this study (NETU-10−29; NCT01376297) was intended to assess the safety and describe the efficacy of NEPA over multiple cycles of HEC and MEC. Major efforts were made in this study to retain patients (pts) on trial as many multi-cycle antiemetic studies are of questionable validity due to high patient drop out.
Materials and Methods: This multinational, randomized, double-blind, active-controlled, parallel group study assessed the safety and efficacy of a single oral dose of NEPA (NETU 300 mg + PALO 0.50 mg) given only on day 1 of repeated cycles of HEC or MEC with oral dexamethasone (DEX) [days 1−4 (HEC) or day 1 (MEC)]. A 3-day regimen with oral aprepitant (APR) + PALO + DEX was included as a control in an unbalanced 3:1 (NEPA:APR) randomization. Safety was assessed primarily by adverse events; efficacy by complete response (CR: no emesis, no rescue medication).
Results: 413 pts were randomized with 309 (NEPA) and 103 (APR) included in the efficacy analysis. Groups were comparable with: male (50%), mean age 57, 24% HEC, 76% MEC. Percent of all patients remaining on study (balanced among groups) and overall CR rates by cycle are in the table:

Cycle #	% Pts remaining on study	% CR	
		NEPA+DEX	APR+PALO+DEX
1	100%	81%	76%
2	92%	86%	81%
3	85%	91%	87%
4	76%	90%	88%
5	52%	92%	86%
6	41%	91%	86%

The type/frequency of AEs were comparable for both groups. The most frequent patient-reported treatment-related AEs (TRAEs) for NEPA included constipation (3.6%) and headache (1.0%); there was no indication of increasing TRAEs over multiple cycles. The majority of TRAEs were mild or moderate; few were serious (1.3% NEPA, 0% APR).
Conclusions: With 75% of patients completing 4 CT cycles, it can be concluded that NEPA, a convenient single-dose oral antiemetic targeting dual pathways, was well tolerated and highly effective over multiple cycles of HEC/MEC.
This study was sponsored by Helsinn Healthcare, S.A.
Conflict of interest: *Ownership: None. Advisory board: Gralla: Helsinn Healthcare Jordan: Helsinn Healthcare and Merck Balser: Roche Pharma. Board of directors: None. Corporate-sponsored research: None. Other substantive relationships: Rizzi, Borroni, and Rossi are employees of Helsinn Healthcare*

1303 POSTER DISCUSSION

A randomized phase III trial of palonosetron plus dexamethasone (day 1) versus palonosetron plus dexamethasone (day 1−3) in the prevention of nausea and vomiting induced by moderately emetogenic chemotherapy, not including a combination of anthracycline plus cyclophosphamide

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Background: The objective of this trial was to evaluate the efficacy of palonosetron plus dexamethasone (day 1) single administration for the prevention of chemotherapy induced nausea and vomiting (CINV) in patents receiving moderately emetogenic chemotherapy, not including a combination of anthracycline plus cyclophosphamide (non-AC MEC).
Methods: This trial was conducted as a multi-center, randomized non-inferiority phase III trial. Patients who received non-AC MEC as the first line chemotherapy were randomized by minimization for palonosetron (0.75 mg, IV) plus dexamethasone (9.9 mg, IV) administered before that

chemotherapy (PALO + DEX day1 arm) and PALO + DEX day1 plus day2−3 dexamethasone (8 mg, IV or PO) (PALO + DEX day1−3 arm).
Primary endpoint was complete response (CR; no emesis and no rescue antiemetics) during the overall 5-day study period. The difference of CR rate in overall between two arms and 95% confidence interval (95% CI) were calculated by logistic regression model which includes chemotherapy, sex and age as covariates. Non-inferiority margin was estimated as 15%, and the degree of lower limit of 95% CI in the difference of CR rate in overall was verified whether it include less than 15%.
Results: From April 2011 to March 2013, 305 patients who received non-AC MEC were randomized. Oxaliplatin-containing regimen was the most common non-AC MEC regimen (72.8%), followed by irinotecan-containing regimen (13.4%), carboplatin-containing regimen (12.1%), and other regimens (1.7%). Overall CR rate was 68.2% in PALO+DEX day1 arm (n = 151) and 64.7% in PALO+DEX day1−3 arm (n = 154). PALO+DEX day1 was non-inferior to PALO+DEX day1−3 (difference 3.6%, 95% CI, −6.6% to 13.9%; $p = 0.0002$). There were no differences between two arms on CR rate in acute and delayed phase (Table).

	CR (%)		
	Overall: 0–120 h	Acute: 0−24 h	Delayed: 24–120 h
PALO+DEX day 1	68.2	95.3	68.9
PALO+DEX day 1−3	64.7	94.7	66.0

Conclusions: This is the first report of the study that demonstrated the prevention of CINV induced by non-AC MEC. PALO+DEX day1 was non-inferior to the standard regimen: PALO+DEX day1−3 for the prevention of CINV induced by non-AC MEC. In conclusion, the administration of dexamethasone on day2 and 3 can be omitted in the prevention of CINV for patents receiving non-AC MEC.
No conflict of interest.

1304 POSTER DISCUSSION

Skeletal-related events in patients with solid tumors receiving denosumab or zoledronic acid by baseline pain status: Results from three phase 3 trials

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Background: Pain is often a consequence of bone metastases in patients with solid tumors, and may precede skeletal-related events (SREs) (Saad, et al, J Urol 2010). Previous integrated results from three phase 3 trials demonstrated superiority of denosumab compared with zoledronic acid (ZA) for preventing SREs in patients with breast, prostate, or other solid tumors. Here, we asked if it was possible to identify patients who would benefit, or not benefit, from bone-targeted treatment on the basis of clinical pain symptoms at baseline.
Materials and Methods: Patients with solid tumors and bone metastases including breast and prostate cancers and other solid tumors received either SC denosumab 120 mg and IV placebo (n = 2775) or IV ZA 4 mg (adjusted for renal function) and SC placebo Q4W (n = 2768) in the double-blinded treatment phase. Time to first on-study SRE and to first and subsequent SRE were evaluated in patients with no/mild pain or moderate/severe pain at baseline as measured by the Brief Pain Inventory − Short Form (BPI-SF).
Results: Among patients with no/mild pain at baseline, 828 (31%) patients had experienced an SRE at the time of primary analysis compared with 1000 (40%) among those with moderate/severe pain at baseline. Denosumab significantly delayed time to first SRE compared with ZA in patients with no/mild baseline pain (HR = 0.84 [95% CI: 0.73, 0.96]; p = 0.01) and in patients with moderate/severe baseline pain (HR = 0.83 [95% CI: 0.73, 0.94]; p = 0.003). The time to first and subsequent SREs was similarly significantly delayed compared with ZA in the no/mild baseline pain group (RR = 0.81 [95% CI: 0.70, 0.92]; p = 0.002) and in the moderate/severe baseline pain group (RR = 0.82 [95% CI: 0.73, 0.93]; p = 0.001).
Conclusions: In a combined analysis of three phase 3 trials of patients with solid tumors and bone metastases, the presence of pain at baseline was not a reliable predictor of response to bone-targeted therapy. In this analysis, denosumab significantly delayed time to first and multiple SREs compared with ZA. This difference in treatment effect was similar in magnitude regardless of patients' pain status at baseline.
Trials sponsored by Amgen, Inc. ClinicalTrials.gov registration numbers NCT00321464, NCT00321620, NCT00330759.
Conflict of interest: *Advisory board: RvM-Amgen, Novartis, Roche, Merck Sharp Dome (MSD), Bristol-Meyers Squibb (BMS) AS-Amgen KF-Amgen JEB-Amgen, Novartis, Bristol-Meyers Squibb SO-Janssen, Novartis, Bayer, Pfizer, Sanofi LC-Amgen, Novartis, Roche, Janssen. Corporate-sponsored research: RvM-Amgen, Roche (unrestricted research grant) AS-Amgen, Novartis. Other substantive relationships: RvM-Amgen, GSK, Roche (speaker homoraria) AS-Amgen, GSK (honoraria) KF-Amgen (speaker honoraria) CC-Amgen (consultant) JEB-Amgen (consultant) HW-Amgen (employee, stockholder) AB-Amgen (employee, stockholder).*

1305 POSTER DISCUSSION

Risk factors for developing osteonecrosis of the jaw (ONJ) in patients receiving denosumab or zoledronic acid for bone metastases: Results from three phase 3 trials

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Background: The use of antiresorptive therapies such as denosumab or zoledronic acid (ZA) for patients with metastatic bone disease reduces the risk of skeletal-related events (SREs) but is associated with a risk of osteonecrosis of the jaw (ONJ). Here we report risk factors for the development of ONJ for the blinded treatment phase of three phase 3 clinical trials comparing the two agents for reduction of SREs.
Materials and Methods: Patients (n = 5677) with bone metastases from solid tumors or multiple myeloma received either SC denosumab 120 mg and IV placebo or IV ZA 4 mg (adjusted for renal function) and SC placebo Q4W in the double-blinded treatment phase of each trial. Study exclusion criteria included prior/current ONJ or osteomyelitis of the jaw, planned invasive dental procedure, or non-healed oral or dental surgery. Patients who received $\geqslant$1 active dose during the blinded treatment phase were included in this analysis for up to 44.5 months of denosumab exposure and 41.3 months of ZA exposure. Oral assessments were conducted at baseline and every 6 months thereafter by the investigator or other qualified examiner. Potential ONJ events were independently adjudicated by a blinded committee of experts.
Results: In combined data from three phase 3 trials, 63 patients in the denosumab group and 44 patients in the zoledronic acid group had adjudicated positive events of ONJ. Most patients who developed ONJ had recognized oral risk factors, among which tooth extractions were the most frequent (Table). Additional risk factors for ONJ, including concurrent chemotherapy, anti-angiogenesis medications, corticosteroid treatment, and their impact on clinical outcome, will be presented.

Table: Risk factors for developing adjudicated positive events of ONJ in three phase 3 trials

Risk factor	n (%)	
	Denosumab (n = 63)	ZA (n = 44)
History of tooth extraction, poor oral hygiene, and/or use of dental appliance	54 (85.7)	38 (86.4)
Tooth extraction	37 (58.7)	30 (68.2)

Conclusions: In combined data from three trials comparing denosumab with zoledronic acid, development of ONJ was associated with recognized risk factors encompassing oral factors. Trials sponsored by Amgen, Inc. ClinicalTrials.gov registration numbers NCT00321464, NCT00321620, NCT00330759.
Conflict of interest: *Advisory board: JEB-Amgen, Novartis, Bristol, Myers SquibbFS-Amgen, Novartis AS-Amgen KF-Amgen DH-Amgen RDB-*

Novartis, Amgen. Corporate-sponsored research: FS-Amgen, Novartis AS-Amgen, Novartis DH-Amgen RDB-Novartis, Amgen. Other substantive relationships: JEB-Amgen (consultant)FS-Amgen,Novartis (honoraria) AS-Amgen, GSK (honoraria) KF-Amgen (speaker honoraria)ID-Amgen, Novartis, GSK, Medtronic (consulting and honoraria), Roche, TEVA, Riemser (honoraria) HW-Amgen(employee and stockholder) AB-Amgen(employee and stockholder).

1306 POSTER DISCUSSION

Effect of skeletal-related events on pain interference in patients with solid tumors and bone metastases

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Background: Patients with metastatic bone disease who experience a skeletal-related event (SRE; including pathological fracture [PF], surgery [SB] or radiotherapy to bone [RB], or spinal cord compression [SCC]) often have increased pain. Using data from patients with solid tumors enrolled in three identically designed phase 3 trials, we evaluated how pain interferes with the emotional well-being (affect) and physical function (activity) of these patients.
Materials and Methods: In these completed phase 3, double-blind, double-dummy, placebo-controlled trials, patients were randomized (1:1) to receive denosumab (120 mg SC) or zoledronic acid (4 mg IV, adjusted for renal function) every 4 weeks (ClinicalTrials.gov NCT00321464, NCT00321620, and NCT00330759; sponsor Amgen Inc.). Data from patients with solid tumors in the 2 treatment arms (n = 5543) were pooled for this post-hoc analysis. Pain interference (overall, emotional, and physical) was assessed using the Brief Pain Inventory (BPI)-Short Form (0: no interference to 10: interferes completely) at baseline and each study visit. The impact of 1st on-study SREs, starting 28 days before the SRE, was assessed using a stratified Cox proportional hazards model adjusting for SREs as time-dependent covariates.
Results: On-study SREs were reported for 1925 patients (923 PF; 829 RB, 119 SCC, 54 SB). PF, RB, and SCC were associated with significantly greater risk of pain interference overall; the impact of SB was also greater, but not significantly so (Table). Results were similar for pain interference with emotional well-being. All SRE types were associated with significantly greater risk of pain interference with physical function.

Table: Effect of 1st on-study SREs on time to ⩾2-point increase from baseline in pain interference score*

Pain interference	PF	RB	SCC	SB
Overall (n = 4911)				
HR (95% CI)	1.30 (1.13, 1.51)	2.29 (1.98, 2.66)	2.60 (1.84, 3.68)	1.70 (0.97, 2.98)
P-value	0.0004	<0.0001	<0.0001	0.06
Emotional well-being (n = 4819)				
HR (95% CI)	1.27 (1.10, 1.46)	2.44 (2.12, 2.80)	2.02 (1.41, 2.91)	1.28 (0.73, 2.23)
P-value	0.0012	<0.0001	0.0001	0.39
Physical function (n = 4535)				
HR (95% CI)	1.40 (1.21, 1.62)	2.29 (1.96, 2.67)	2.42 (1.69, 3.46)	2.14 (1.20, 3.81)
P-value	<0.0001	<0.0001	<0.0001	0.01

*Includes patients with baseline BPI score ⩽8.

Conclusions: In general, pain interference was increased in patients who experienced on-study SREs. Effective treatments that prevent SREs may reduce pain interference with patients' emotional well-being and physical function.
Conflict of interest: *Ownership: none. Advisory board: R. von Moos − Amgen Inc., Novartis, Roche, Merck Sharp Dome (MSD), and BMS. C. Cleeland − Genentech, BMS, Exilixis, Merck, J & J, and Amgen Inc. J.J. Body − Amgen Inc. J.E. Brown − Amgen Inc., Novartis, and Bristol, Myers, Squibb. G Marx − Amgen Inc., AstraZeneca, and Sanofi. Board of directors: none. Corporate-sponsored research: R. von Moos − Amgen Inc. and Roche. C. Cleeland − Genentech. Other substantive relationships: R. von Moos − speaker honararia from Amgen Inc., GSK, and Roche. J. J. Body − lecture fees from Amgen Inc. and Novartis. J.E. Brown − consultancy for Amgen Inc. Y. Zhou, A. Balakumaran, and Y. Qian − employees of and hold stock/stock options in Amgen Inc.*

1307 POSTER DISCUSSION

Difficulties in meeting accrual and obtaining adequate follow up in palliative trials: Results from an intergroup randomized trial of single vs multiple fractions (Fx) for re-irradiation (RE-RT) of painful bone metastases (PBM): NCIC CTG SC.20

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Background: The optimal RE-RT dose and fractionation schedule for PBM is uncertain.
Methods: Patients (pts) with PBM after previous radiation (RT) to the same site were stratified by previous Fx schedule and pain response and randomized to 8 Gy in 1 Fx or 20 Gy in 5 Fx. The primary endpoint was overall response rate (RR) at 2 months using the International Consensus schema (Chow 2002) which combines Brief Pain Inventory worst pain score and opioid analgesic use. We tested if 8 Gy was non-inferior (NI), analyzed by intention to treat (ITT) and a per-protocol (PP) sensitivity analysis excluding those who were ineligible, inevaluable or received non-allocated therapy. Sample size was calculated using an expected RR of 70% with 20 Gy and a NI margin of 10% (i.e. upper boundary of 1-sided 95% CI for the RR difference). Patients reported adverse events (AEs) by questionnaire on Day 14.
Results: Between 01/2004 and 06/2012, we enrolled 850 pts from 9 countries. The median age was 65 years old, 59% were male, The Karnofsky performance status was 50 or more in all pts and 17% had no response to prior RT. The median follow-up was 12.2 months (range 0.03 to 15.6 months). The median survival was 8 months with no differences detected between arms (HR = 0.96; P = 0.67). Most common cancers were prostate (27%), breast (26%) and lung (22%). Before the 2 month assessment, 98 (11%) pts died. By ITT, the 2-month RR was available in 66% (557/850) and was 119/425 (28%) with 8 Gy and 136/425 (32%) with 20 Gy (P = 0.2); the upper boundary of the 95% CI for RR difference = 9.2% and is less than the pre-specified NI margin. By PP analysis, 2-month RR was available in 521 and was 117/258 (45.3%) with 8 Gy and 135/263 (51.3%) with 20 Gy (P = 0.17); the upper boundary of the 95% CI for RR difference = 13.2%, which exceeds 10% non-inferiority boundary. Among the 263 pts with a response at month two, 36 had pain progression (16 with 8 Gy and 20 with 20 Gy); the HR for freedom from progression (20 Gy vs. 8 Gy) among these pts was 1.05 (95% CI: 0.55 to 2.04). Day 14 AEs differing by treatment were: lack of appetite (P = 0.01), vomiting (P = 0.001), diarrhea (P = 0.02) and skin reddening (P = 0.002); all were worse with 20 Gy.
Conclusions: In pts with PBM receiving RE-RT, the 2-month RR obtained with 8 Gy is non-inferior to 20 Gy when assessed by ITT but findings were not robust to a PP sensitivity analysis. When choosing between options tested, trade-offs exist between pain response and acute toxicity.
No conflict of interest.

1308 POSTER DISCUSSION

Efficacy and safety of anamorelin HCl in NSCLC patients: Results from a randomized, double-blind, placebo-controlled, multicenter phase II study

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Background: Cancer cachexia is a multifactorial syndrome, characterized by decreased body weight (BW) and muscle mass/strength, and associated with worsened morbidity/survival. Anamorelin HCl (ANA), an orally active

ghrelin receptor agonist with orexigenic and anabolic activity, is in development for treating non-small cell lung cancer (NSCLC)-associated anorexia/cachexia.
Materials and Methods: We conducted an international, randomized, double-blind, multicenter Phase II trial to evaluate the effect of ANA on BW and handgrip strength (HGS) (NCT00622193; sponsored by Helsinn). Safety endpoints included adverse event (AE) profile and overall survival; secondary endpoints included IGFBP-3 and Quality of Life (MDASI). Patients with Stage IIIB or IV NSCLC, ECOG performance score $\leqslant 1$, and candidates for treatment with carboplatin/paclitaxel ($\pm$ bevacizumab) were eligible. Patients were randomized 1:1:1 to receive either 50 mg ANA, 100 mg ANA, or placebo (PL) once daily for 12 weeks.
Results: 226 patients were randomized and treated (N = 76 for 50 mg ANA; N = 73 for 100 mg ANA; N = 77 for PL), and 215 patients with at least 1 post-baseline efficacy assessment comprised the Modified Intent-To-Treat (MITT) population for efficacy analysis. A beneficial effect on weight was observed as early as 1 week after ANA treatment. Over 12 weeks, the 100 mg ANA group gained an average of 0.14 kg in BW from baseline, compared to mean losses of 0.3 kg and 1.32 kg for the 50 mg and PL groups, respectively (mean treatment difference between 100 mg ANA and PL was 1.47 kg; $p = 0.0005$). For HGS, the mean treatment difference between 100 mg ANA and PL was 0.58 kg, but was not statistically significant. ANA was safe and well-tolerated in this study, and AEs of anorexia, nausea, and fatigue were reported in fewer ANA-treated than PL-treated patients. There was no statistically significant effect on long-term overall survival in the 50 mg or 100 mg ANA groups compared with PL. ANA also increased IGFBP-3, a marker of drug activity ($p < 0.0001$ for both treatments vs placebo). MDASI total and domain scores improved in the 100 mg ANA group, but were not statistically significant.
Conclusions: ANA significantly increased BW, had a neutral effect on survival, and showed an overall favorable safety/tolerability profile. Directional improvements in HGS and QoL were also observed. These data support further investigation of ANA for treating NSCLC anorexia/cachexia.
Conflict of interest: *Corporate-sponsored research: J. Temel, S. Bondarde, and M. Jain received funding from Helsinn to conduct this study. Other substantive relationships: S. Allen and W. Mann are employees of Helsinn, which funded this study.*

1309 POSTER DISCUSSION

Do methylprednisolone 32 mg provide pain relief, improve fatigue and appetite in cancer patients using opioids? A randomized, controlled trial

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Background: Corticosteroids (CS) are used as co-analgesics in cancer pain patients. In a systematic review we concluded that CS may have a moderate analgesic effect in cancer pain, but with 'very low' level of evidence. Therefore, this randomized study was conducted to compare the analgesic efficacy of CS to placebo. Secondary aims were to evaluate the effects in analgesic consumption, patient satisfaction, fatigue and appetite.
Material and Methods: Adult cancer patients with average pain last 24 hours $\geqslant 4$ (NRS 0−10) despite ongoing opioid treatment were recruited from five palliative centres in Norway. After randomization, the patients received methylprednisolone 16 mg twice daily or placebo for seven days in a double-blind design. Primary outcome was average pain intensity on day seven (NRS 0−10); secondary outcomes were analgesic consumption (oral morphine equivalents (OME)), overall satisfaction (NRS 0−10), fatigue, and appetite (EORTC QLQ-C30 (0–100)). Sample size estimation required 22 patients in each group to show a clinical significant difference of 1.5 (NRS 0−10) with a $p < 0.05$ and a power of 0.90.
Results: A total of 592 patients were screened from April 2008 to January 2012. Fifty patients were recruited, 47 completed the study. Forty seven patients had metastatic cancer disease; mean Karnofsky index was 66 (0–100), and mean analgesic consumption 218 mg OME. On day seven there were no differences in average pain intensity (CS: 3.6, (CI: 2.8−4.4); placebo 3.7 (3.0−4.4)) or in change in opioid consumption (day seven versus baseline: CS 1.19 (1.00–1.38); placebo 1.20 (0.90–1.51)). CS improved both fatigue (CS: −17 (−27 − −6); placebo 3 (−5−11) ($p < 0.01$)), and appetite (CS: − 24 (−38 − −11); placebo 2 (−8−11) ($p < 0.01$)). Overall satisfaction was 5.4 (4.1−6.7) in the CS versus 2.0 (0.7−3.3) in the placebo group ($p < 0.01$).
Conclusions: Methylprednisolone 32 mg daily did not improve pain or decrease analgesic consumption in cancer patients with advanced disease using opioids. The patients treated with CS reported better treatment satisfaction and clinically significant improvement in fatigue and appetite compared to the placebo group.
Funding: Telemark Hospital Trust
No conflict of interest.

1310 POSTER DISCUSSION

Health-related quality of life in small-cell lung cancer: a systematic review on reporting of methodological and clinical issues in randomized controlled trials

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Purpose Small-cell lung cancer (SCLC) represents approximately 15% of all lung cancers and increasingly health-related quality of life (HRQOL) of SCLC is evaluated in randomized controlled trials (RCTs). The objective was to evaluate the adequacy of HRQOL methodology reporting in SCLC RCTs and its possible impact on clinical decision making.
Material and Methods: A MEDLINE systematic review was performed in RCTs. Eligible RCTs implemented patient-reported HRQOL assessments and oncology treatments for adult SCLC patients. Included studies were published in English between January 1991 and December 2012, with sample size $\geqslant 100$ and patient age $\geqslant 18$.
Results: Thirty RCTs out of seventy-nine studies were classified as eligible, involving over 10,000 patients. HRQOL was a secondary endpoint in 29 RCTs of which 53% reported no significant difference in overall survival (OS). A benefit of HRQOL was reported in 85% of the positive-outcome trials, and in 44% of the negative-outcome trials. Significant improvements in HRQOL were seen when standard platinum-based regimens were compared with: a) irinotecan and carboblatin, b) ifosfamine, carboplatin, etoposide and vincristine. A priori hypothesis on the expected overall HRQOL outcome was defined in 27% of the RCTs. Baseline HRQOL assessment was stated as mandatory in 14% of the RCTs. Tests of statistical significance were applied in 90% of the RCTs and missing data were discussed in detail in 30% of the trials.
Conclusions: While the overall reporting of HRQOL was of acceptable standards, some improvement in reporting RCTs could be encouraged. HRQOL assessment in SCLC RCTs clearly provides major added information in studies where no OS difference is found, but more importantly provides valuable information for these treatments where better HRQOL was associated with OS benefit.
No conflict of interest.

1311 POSTER DISCUSSION

Understanding quality of dying in a hospital

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Background: Quality of dying (QOD) is a multi-dimensional concept that includes physical, psychosocial and existential experiences, life closure, death preparation, circumstances of death, and experiences of care. We studied how relatives of cancer patients who had died in a hospital experienced QOD regarding these dimensions.
Material and Methods: Between June 2009 and February 2011 each adult cancer death at non-IC units in a university hospital was followed by an invitation to relatives, sent 10−13 weeks later, to answer a questionnaire. Relatives were asked to rate QOD overall on a 0−10 numeric rating scale; furthermore, they valuated their experiences in the different domains of QOD on verbal scales, which were merged into 2 answer categories before analysis. Data were analyzed with students' t-tests and linear regression analysis.
Results: In the study period, 259 cancer patients died; of 246 patients (95%) relatives could be traced, and 123 participated (50%). Of patients, 60% was male, mean age was 65 years (sd 13), and mean duration of final hospital stay was 12 days (sd 14). The mean score for QOD 6.3 (sd 2.8). QOD scores were higher when the final stay had been longer and relatives were younger. Adjusted for these characteristics, QOD scores were associated with almost all domains, but most strongly with experiences of care. In this domain the most important variables were

those related to participation in decision making (R^2 0.17) and to outcomes of care (R^2 0.21). QOD scores were higher when relatives were satisfied about patient's and relative's involvement in decisions on medical treatment (Mean QOD 6.8 vs 5.4 and 6.8 vs 5.2); when relatives had been informed of the imminence of death (6.7 vs 5.3); when they judged physician's efforts to relieve suffering, and nursing care as sufficient (6.8 vs 4.0 and 6.7 vs 4.0); and when the patient had primarily been approached as a person (7.0 vs 4.9).
Conclusions: On average, relatives rate QOD in hospital was moderately good. Experiences of care are more important in explaining variance in QOD scores than physical, psychosocial, or existential experiences, or circumstances of death. Therefore, improvements in end of life care can be achieved by clinical staff through being present, providing information, listening, and shared decision making.
No conflict of interest.

Poster Session (Mon, 30 Sep)
Symptom Science

1312 POSTER

Haemoglobin outcomes with biosimilar epoetin alfa in the management of chemotherapy-induced anaemia in cancer patients: first results from OnCoBOS, a French observational study

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Background: OnCoBOS is an ongoing, national, prospective, multicentre, observational study of the use of biosimilar epoetin alfa (Binocrit®) for the treatment of chemotherapy-induced anaemia (CIA). Here we present an evaluation of haemoglobin (Hb) outcomes in 444 patients treated (2000 scheduled) in France.
Patients and Methods: We analysed 444 cancer patients with CIA treated with biosimilar epoetin alfa in 75 centres in France. Data were collected at treatment start and 3−4 weeks (W3−4) and 12 weeks (W12) later. Hb outcomes assessed included the proportion of patients achieving a Hb increase of $\geqslant$1 and $\geqslant$2 g/dL, and the mean Hb change from baseline in the subsets of patients with a baseline Hb $\leqslant$9 and 9.1−10 g/dL.
Results: Median (range) age was 67 (18−93) years and 59.3% of subjects were male. Mean±SD Karnofsky score was 77.9±13.2. 77.5% of patients had solid tumours, including lung (22.1%), breast (13.4%), and colorectal cancer (12.8%), mainly stage 3 or 4 (59.3%); and 22.5% had haematological malignancies including non-Hodgkins lymphoma (55.0%) and multiple myeloma (24.0%), again mainly stage 3 or 4 (77.7%). Overall, 64% of patients had metastatic disease. Mean±SD Hb at biosimilar epoetin alfa initiation was 9.6±0.9 g/dL. Mean±SD biosimilar epoetin alfa starting dose was 32793±5147IU/week; the dose at W12 was 32605±6359IU/week. At W3−4 11.9% of patients received intravenous iron. Mean±SD Hb increased to 10.6±1.4 g/dL at W3−4 and 11.2±1.5 g/dL at W12 ($p < 0.001$ vs baseline). In patients with a baseline Hb $\leqslant$9 and 9.1−10 g/dL, the mean Hb change from baseline at W12 was 2.1 and 1.4 g/dL, respectively. 74.8% of patients achieved a Hb increase $\geqslant$1 g/dL during the study, and 47.8% achieved a Hb increase $\geqslant$2 g/dL. 81.3% of patients were able to continue their chemotherapy without delays or dose reduction. Only three patients experienced an adverse drug reaction, none of which were serious.
Conclusions: These data indicate the real-life clinical effectiveness and safety of managing CIA with biosimilar epoetin alfa (Binocrit®). The results reflect the ability to safely correct anaemia and maintain Hb, in line with current recommendations, using a weekly dose regimen. In agreement with European recommendations, treatment with an erythropoiesis-stimulating agent was not restricted to patients with metastatic disease.
Conflict of interest: *Corporate-sponsored research: J.D, O.S, JC.L, A.T, C.D, JC.I, H.O and G.B − This study is sponsored by Sandoz Biopharmaceuticals. Other substantive relationships: C.A-O and RF.S are employees of Sandoz Biopharmaceuticals*

1313 POSTER

Prospective observational study on chemotherapy-induced nausea and vomiting (CINV) for cancer patients who were to receive moderately and highly emetogenic chemotherapy (MEC and HEC) and primary care medical staffs' perception on CINV by the CINV Study Group of Japan

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Background: There has been no nationwide survey on CINV and validation of the guideline made in Japan after introduction of NK-1 receptor antagonist to the market. The aim of the study is to investigate occurrence of CINV in cancer patients (pts) who are to receive chemotherapy for the first time, and primary care medical staffs' perception on CINV for their pts.
Methods: A nationwide survey on CINV was conducted by the CINV Study Group of Japan. 108 institutions participated in the study which was approved by the review board. The written consent was obtained from the pts. A 7-day diary for CINV was provided to the pts prior to MEC and HEC to record daily occurrence and severity of CINV and an amount of food intake. Acute and delayed CINV was defined as nausea and vomiting which developed within or after 24 hours after the start of chemotherapy, respectively. The medical staffs also filled out questionnaires to estimate their pts' CINV.
Results: A total of 2068 pts were registered from April 2011 to December 2012. The number of pts' diary paired with their staffs' report was 1925 after pts received HEC or MEC. Underlying diseases were gastrointestinal (651 pts), lung (429 pts) and breast cancer (433 pts), and gynecological (215 pts) and hematological malignancy (197 pts). There were 883 males with a median age of 65 (range: 19−87) and 1042 females with a median age of 59 (range: 21−87). MEC was given to 710 pts as was HEC to 1215 pts. Acute vomiting was noted in 13 pts with MEC as was in 66 pts with HEC, while delayed vomiting was experienced in 88 pts with MEC and 101 pts with HEC, respectively. Acute nausea was experienced in 49 pts with MEC and in 250 pts with HEC, while was noted delayed nausea in 278 pts with MEC and in 542 pts with HEC, respectively. Combination of 3 antiemetics was given along the guideline to 81% of the pts with CDDP-based regimen and 64% of those with non-CDDP regimen of HEC. The staff estimated that the incidence of acute CINV was 58% and that of delayed CINV was 80% of the pts when pts of HEC and MEC were combined.
Conclusions: Chemotherapy-induced vomiting was well controlled, but delayed nausea remained to be high in both HEC and MEC, and needs further investigation. Non-CDDP regimen of HEC had a high incidence of developing acute CINV, indicating that other treatment modality should be studied. Surprisingly medical staffs overestimated the incidence of CINV suggesting that antiemetic treatment for CINV given to the pts was quite appropriate by the fact that 2/3 to 4/5 of the pts with HEC had received a combination of 3 antiemetics recommended by the Japanese guideline.
No conflict of interest.

	HEC(n = 1215)		MEC(n = 710)
	CDDP-based regimen (n = 666)	non-CDDP based regimen (n = 549)	
2 antiemetics*	129 (19%)	195 (36%)	509 (72%)
3 antiemetics**	537 (81%)	354 (64%)	201 (28%)
Acute			
nausea	55 (8%)	195 (36%)	49 (7%)
vomiting	14 (2%)	52 (9%)	13% (2%)
Delayed			
nausea	283 (42%)	259 (47%)	278 (39%)
vomiting	61 (9%)	40 (7%)	88 (12%)
Medical staff's estimation			
acute CINV	352 (53%)	436 (79%)	325 (46%)
delayed CINV	581 (87%)	411 (75%)	546 (77%)

*: 5HT3 receptor antagonist + dexamethasone. **: * + aprepitant.

1314 POSTER

The efficacy of palonosetron compared with granisetron in preventing chemotherapy-induced nausea and vomiting: A randomized study

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Background: Chemotherapy-induced nausea and vomiting (CINV) are among the most problematic symptoms for patients (pts) with cancer. 5-HT_3 receptor antagonists such as palonosetron (PAL) and granisetron (GRA) are the current standard of care for the prevention of acute CINV. This study aims to assess the efficacy and safety of PAL versus GRA in pts treated with chemotherapy.
Material and Methods: Eligible pts were randomized to receive iv PAL 0.25 mg (GrpA) or GRA 3 mg (GrpB), 30 minutes before theinitiation of chemotherapy on day 1. All pts were also given dexamethasone iv 8 mg before PAL or GRA. Aprepitant was used for pts who received highly emetogenic chemotherapy. The primary efficacy endpoint was to determine the complete response rate (CRR) for acute and delayed emesis. The secondary endpoints were to identify the safety of both medicine and the rescue medication rate. This study was approved by the institutional Research Ethics Board.
Results: A total of 177 pts were assessed in this study.Eightysix pts (45% female) were in the GrpA and 91 pts (41% female) were in the GrpB study arms. The mean age was similar between the two groups (54±12 vs. 56±11; p = 0.245). Gender distribution, the use of cisplatin and the use of a highly emetogenic protocol were indifferent between the groups. For acute emesis, CRR was 73% in the GrpA and 74% in the GrpBgroup (p = 0.492). The rate of rescue medication was similar between the two groups (22% for GrpA and 19% for GrpB; p = 0.706). The most common side effects were diarrhea, constipation, dizziness, headache and fatigue. The frequencies of these side effects were similar between the two groups (p < 0.05 for each variable). In the sub-group analyses that were performed for patients who received cisplatinum and a highly emetogenic chemotherapy regimen, the rates of acute and delayed emesis as well as the rescue medication rates were not different between the two groups (p = 0.136 and p = 0.341, respectively).
Conclusion: Both PAL and GRA have provided similar CRR for patients with CINV in acute and delayed emesis. Moreover, both PAL and GRA could safely be used and they demonstrated similar side-effect profiles, either.
No conflict of interest.

1315 POSTER

Risk factors for severe complications during febrile neutropenic episodes in patients with solid tumors

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Background: Febrile neutropenia (FN) is a frequent complication during chemotherapy in solid tumors, and to identify those patients (pts) with higher risk of developing severe complications during FN episodes is important. Here we aimed to characterize those risk factors for severe complications during FN episodes in pts with solid tumors, admitted for intravenous antibiotics.
Material and Methods: It is a retrospective study of all consecutive pts admitted with FN between May/2008 and May/2012. Eligibility criteria included: age ⩾16 y, the diagnosis of FN (documented axillary temperature greater than 37.8ºC, and neutrophil count <500/mm^3 or expected to fall below 500/mm^3) as an adverse event of chemotherapy for a solid tumor. Potentially life-threatening complications during FN episodes were collected and univariate and multivariate logistic regression analyses were performed to assess the relationships between risk factors and these complications.
Results: 333 FN episodes in 295 pts with solid tumors were studied. Median age was 57 y (16−88), 150 female (51%). Most frequent primary sites included: breast (15%), lung (14%), bone/soft tissues (13%), colorectal (10%), stomach (9%), head & neck (8%) and testis (5%). 31 pts (10%) presented more than 1 FN episode. No GCSF primary prophylaxis was prescribed in 282 pts (85%). At admission, median neutrophil count was 690/mm^3, and the median MASCC score was 19 (7−26). Infection sites were identified as pulmonary (19%), urinary tract (11%), abdominal (10%), bloodstream (8%) and soft tissues (8%), and regarding etiology, Gram-negative bacilli could be isolated in 56 (16%) and Gram-positive cocci in 26 FN episodes (8%). All pts were admitted with a median duration of hospital stay of 10 d (0–106 d). Overall, a severe complication as a consequence of FN was detected in 248 episodes (74%), being hypotension (47%), ICU admission (35%), renal failure (30%), respiratory failure (19%) and altered mental state (17%) the most common (>10%), and 46 pts died (14%). A univariate analysis revealed age ⩾60 y (OR 3.1, 95% CI 1.8−5.5, p 0.0001), controlled cancer (OR 0.5, 95% CI 0.3−0.9, p 0.01), previous COPD (OR 4.5, 95% CI 1.7–11.5, p 0.0016), presence of symptoms (OR 2.2, 95% CI 1.3−3.7, p 0.0063) or dehydration (OR 4.6, 95% CI 2.6−8.3, p < 0.0001) and regular or bad general condition (OR 3.3, 95% CI 1.9−5.7, p < 0.0001) as risk factors for complications. On multivariate analysis, only dehydration (OR 4.1, 95% CI 2.2−7.5, p < 0.0001), previous COPD (OR 3.7, 95% CI 1.3–11.0, p 0.0171) and age ⩾60 y (OR 2.5, 95% CI 1.4−4.6, p 0.0027) were associated with severe complications. The multivariate model correctly classified 75% of all FN episodes as complicated.
Conclusions: Severe complications were common during febrile neutropenic episodes in pts with solid tumors. COPD, age ⩾60 yo and dehydration represent clinically significant risk factors for severe complications in FN pts.
No conflict of interest.

1316 POSTER

Anamorelin's effect on bone mass: beyond the expected outcomes in non-small cell lung (NSCLC) cancer cachexia

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Background: Cachexia is a serious complication of cancer, characterized by decreased body weight and muscle mass/strength, and associated with poor survival. Another consequence of cancer is bone loss (a form of secondary osteoporosis), a major risk factor for fractures. Previously, we reported a Phase II study where anamorelin (ANA), an investigational ghrelin receptor agonist with orexigenic and anabolic activity, significantly increased lean body mass (LBM) and functional performance over 12 weeks. Beyond these cachexia-focused endpoints, we present here post-hoc analysis on bone mass (BM), which support additional benefits of ANA in cancer patients.
Materials and Methods: 82 patients with advanced cancers, ECOG performance score ⩽2 and weight loss ⩾5% within 6 months were enrolled in a Phase II trial (NCT00219817/NCT00267358) sponsored by Helsinn. Patients received placebo (PL, N = 36) or 50 mg anamorelin (ANA, N = 38) once daily for 12 weeks. Body composition (BM, LBM, fat mass and total body mass) by dual-energy X-ray absorptiometry and plasma inflammatory cytokines (CRP, IL-6 and TNF-α) were measured at baseline, 4, 8 and 12 weeks.
Results: At baseline, BM was comparable between groups. Over 12 weeks of treatment, BM continuously decreased in PL patients, while BM loss stopped at Week 4 in ANA patients and then stabilized. At Week 12, mean BM loss from baseline was statistically significantly greater in the PL vs. ANA patients (−0.05±0.103 kg vs. −0.02±0.09 kg, respectively; p = 0.0176). Inflammatory cytokines are important in bone remodeling and cachexia, and ghrelin has been shown to have anti-inflammatory properties. Accordingly, temporal decreases (~45% for CRP and IL-6 and ~25% for TNF-α at Week 12) were noted in ANA- compared to PL-treated patients. While not statistically significant, this generally uniform pattern of a directional decrease in ANA-treated patients was not noted in PL-treated patients.
Conclusions: Decreased BM with bone fractures are major medical concerns for cancer patients. In this study, 50 mg ANA treatment for 12 weeks significantly prevented the loss of BM compared to PL patients; trending decreases in pro-inflammatory cytokines after ANA administration may also be related to maintaining BM and improving cachexia-related endpoints. Since interventions to increase bone content typically require 1 year of exposure, a longer treatment duration may be needed to confirm these observations and could result in bigger increases in BM.
Conflict of interest: *Corporate-sponsored research: J.M. Garcia received funding from Helsinn to conduct this study and is a consultant for Helsinn and Aeterna Zentaris Inc. Other substantive relationships: S.M. Zabbatino is an employee of Medpace. M. Lu, E.M. Duus, and J. Friend are employees of Helsinn, which funded this study.*

1317 POSTER

Short post-infusion scalp cooling time still prevents docetaxel-induced alopecia

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Background: Chemotherapy-induced alopecia (CIA) is a common side effect and its pathobiology remains unravelled (Paus et al. Lancet Oncol 2013). Scalp cooling is practiced to reduce CIA, but optimum post-infusion cooling times (PICT) are unknown. Our previous study showed an unchanged and excellent prevention of CIA during 3-weekly docetaxel chemotherapy after a reduction of PICT from 90 to 45 minutes (vd Hurk et al. Support Care Cancer 2012).
Material and Methods: The objective of this prospective multicentre trial (SCALP3, number NTR1856) was to compare the proportion of hair loss between patients who were randomised between PICTs of 45 or 20 minutes and who received docetaxel (75 or 100 mg/m^2). Secondary outcomes were wig and head cover use, pattern of hair loss, quality of the remaining hairs and tolerance of scalp cooling. Multivariate analyses will be performed to identify patient and chemotherapy characteristics associated with the result, including age, type of hair, liver function, infusion times and previous chemotherapy. Scalp cooling was performed using the Paxman system.
Results: Patient inclusion has been finalised (n = 130), 57% of the patients were men, 11 patients will be excluded from analyses. Proportions of hair loss are not known yet, but preliminary results show that a 45 minutes PICT resulted in 79% of the patients (n = 53) not requiring a wig or head cover, versus 73% in the 20 minutes PICT group (n = 44). Data collection is ongoing for 22 patients in both treatment arms. During the conference final results will be presented, including associated characteristics.
Conclusion: Scalp cooling prevents docetaxel-induced hair loss, even when the PICT is shortened to 20 minutes. Therefore the PICT seems not to be determined by the half life time of the cytotoxic agent. It cannot be excluded that shorter PICTs positively contribute to the efflux of cytotoxic agents from the hair follicle cells into the blood stream, thereby questioning any PICT. The shorter PICT is a major advantage in time investment for patients but also for logistics at day care units. Scalp cooling should be offered on a routine basis when docetaxel monotherapy is given, also in men. The proportion wig use after 45 minutes PICT is in accordance with our previous trial.
Knowledge on the influence of scalp cooling time and the reached temperature on the results will possibly be obtained more accurate and quicker with a new research model in which the patient is her or his own control. This work was supported by Sanofi Aventis.
No conflict of interest.

1318 POSTER

Impact of a two-drug combination regimen for cancer-related cachexia on nutritional, anabolic/metabolic, physical activity, anti-infammatory and quality of life variables

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Background: Cancer progression is characterised by loss of lean body mass (LBM), inflammatory status, metabolic derangements and poor quality of life (QL) which result in cancer-related anorexia/cachexia syndrome (CACS). The aim of the present study was to test the safety and efficacy of a combination treatment (including nutraceuticals, i.e. quercetin, alpha lipoic acid and curcumin) with carnitine + celecoxib for the treatment of CACS. Primary efficacy endpoints were: increase of LBM, resting energy expenditure (REE) and improvement of QL, particularly fatigue. The following were assessed as secondary endpoints: physical performance (tested by grip strength and 6-min walk test, 6MWT), appetite, chronic inflammatory variables (IL-6 and CRP), Performance Status (PS) and Glasgow prognostic score (GPS).
Patients and Methods: Outpatients with advanced cancer at different sites with CACS (i.e. loss of body weight >5% of the pre-illness (or ideal) weight in the last 3 months) received L-carnitine 4 g/day plus Celecoxib 300 mg/day plus nutraceuticals /antioxidants, i.e., quercetin 300 mg/day, lipoic acid 300 mg/day, carbocysteine 2.7 g/day, curcumin 2 g/day (i.e.400 mg/day of active curcuminoids extract (Meriva, Indena, Milan, Italy). Treatment duration was 4 months.
Results: From June 2011 to October 2012, 80 patients with advanced cancer (all stage IV) at different sites were enrolled: 70 completed the treatment and were evaluable (mean age 65±9.6, range 32−82 years). Ten patients did not complete the treatment for death due to disease progression. Results showed a significant increase of LBM and a significant improvement of QL (by EORTC-QLQ-C30), and particularly fatigue (by MFSI-SF). Moreover, an improvement of physical performance assessed by 6MWT as well as a decrease of inflammatory parameters (IL-6 and CRP), ECOG PS and GPS was observed. The treatment was very well tolerated (no grade 3−4 toxicities occurred) and no patient discontinued the treatment due to severe adverse events.
Conclusions: The results of the present study showed that a combined treatment with anti-inflammatory, anabolic/metabolic agents plus antioxidants was able to improve the main nutritional, metabolic and physical activity variables as well as QL of cachectic cancer patients with an optimal safety and cost-benefit profile, so that it may be suggested in the clinical practice as treatment for CACS.
No conflict of interest.

1319 POSTER

A phase II/III, randomized, double-blind, placebo controlled study to investigate the efficacy of a probiotic VSL#3®, on chemotherapy-induced diarrhoea in cancer patients receiving fluoropyrimidines and or irinotecan (interim analysis)

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Background: Chemotherapy induced diarrhoea (CID) is a common side-effect and its treatment is non-specific. VSL#3 a safe supplemental product has shown to be effective in prevention of radiation-induced diarrhoea. Primary end point of this study is to see effect of study medication (VSL#3) or placebo on grade III or IV diarrhoea.
Material and Methods: This Phase II/III double blind placebo controlled study was approved by Institute Ethics Committee and all subjects signed informed consent prior to enrolment. 121 evaluable patients in each arm are needed to demonstrate reduction in grade III or IV CID from 30% in placebo to 15% in study group with 80% power and α value of 0.05. The trial medications were in the form of a sachet for oral use.
VSL#3: It contains 900 billion viable, lyophilized bacteria, 4 strains of *Lactobacillus*, 3 strains of *Bifidobacterium* and 1 strain of *Streptococcus thermophilus.*
Placebo: Identical sachets containing cornstarch. The daily dose is 1 sachet bid. Eligible subjects received either sachets for atleast 14 days before starting chemotherapy and continued till two weeks after the end of cycle 3. CTCAE 3.0 was used for assessment of diarrhoea. We also randomly collected serum (for VEGF, clusterin) and stool (for calprotectin) sample before and at the end of study from subset of study subjects.
Results: Interim results for primary end point are presented after evaluation of 202 subjects without unblinding. Ten patients (10.42%) in group1 and 4 (3.8%) in group 2 developed grade III or IV diarrhoea (p = 0.07). Biomarkers data given in table 1 clearly shows that levels either increased or remained same in group 2 compared to significant reductions in group 1 at the end of treatment.
Conclusion: Interim analysis suggests that incidence of grade III and IV diarrhoea in two groups is significantly lesser than expected and possibly because of this difference between the 2 groups is not evident. However, the biomarkers analysis suggests that levels of biomarkers reduced significantly during treatment in one of the groups (group =1).
Clinical Trial Registry number: CTRI/2009/091/001042
No conflict of interest.

	Mean value		
	Group 1	Group 2	P value
VEGF (pg/ml)			
baseline, N = 15&14	1253±335	1203±273	0.60
end of study, N = 15&14	899±408	1465±285	<0.001
Calprotectin (mg/kg)			
baseline, N = 11&12	395±328	610±114	0.11
end of study, N = 11&12	293±245	605±123	0.001
Clusterin (μ/ml)			
baseline, N = 10&6	156±15	153±18	.83
end of study, N = 10&6	63±17	166±15	0.001

1320 POSTER

Use of erythropoiesis-stimulating agents and comparison of different products for the treatment of chemotherapy-induced anaemia (CIA)

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Background: It is unclear if the most recent recommendations for more conservative use of ESAs to treat CIA are reflected in real-world clinical practice. In addition, there is a paucity of data on the relative effectiveness of biosimilar ESAs and other available ESAs in this setting.
Patients and Methods: This was a retrospective, single-centre audit of the treatment of CIA with ESAs at the largest oncology centre in Spain, and included patients treated by multiple physicians. It included a total of 284 patients with mostly solid tumours, treated with Binocrit® 40,000 IU QW (n = 116) or 30,000 IU QW (n = 14), darbepoetin alfa 500 μg Q3W (n = 99), darbepoetin alfa 300 μg Q2W (n = 2), darbepoetin alfa 150 μg QW (n = 45), or epoetin beta 30,000 IU QW (n = 8).
Results: Overall, the most common tumour types were NSCLC (30%), breast (12%), ovarian (6%) and bladder (5%). 27% of patients received ESA treatment for 4 weeks, and 42% received >4 weeks' treatment. The mean overall haemoglobin (Hb) at start of ESA treatment was 9.3 g/dL; 19% of patients had Hb <8.5 g/dL at the start of treatment, and 42% had Hb <9 g/dL. The mean overall Hb level at the end of treatment was 10.8 g/dL; 54% of patients had Hb in the range 10−12 g/dL, and 68% achieved a Hb >10 g/dL. Comparisons were performed of Hb outcomes according to the different ESA treatments given to patients. There were no significant differences ($p > 0.05$) between the groups in terms of Hb levels at the start of ESA treatment, Hb levels achieved at the end of ESA treatment, and the highest Hb level achieved on ESA treatment. No drug-related adverse events were recorded.

ESA	Mean treatment duration (weeks)	Mean Hb at start of treatment (g/dL)	Mean maximum Hb achieved (g/dL)
Darbepoetin 150 μg QW	4.98	9.0	11.2
Darbepoetin 300 μg Q2W	4.50	9.4	10.6
Darbepoetin 500 μg Q3W	4.68	9.3	10.7
Epoetin beta	6.50	9.6	11.3
Binocrit 30,000 IU	4.57	9.1	10.4
Binocrit 40,000 IU	4.20	9.3	10.7

Conclusions: The use of ESAs in our centre could be described as conservative and safe, and reflects the most recent ESA label change and recommendations for more moderated ESA use in patients with CIA (i.e. use the lowest possible dose and duration of treatment necessary to avoid transfusions). Our data indicate that Hb outcomes are similar for the different ESA products in a real-world clinical practice setting.
No conflict of interest.

1321 POSTER

Nutritional assessment: Who should report and how?

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Background: Malnutrition is common but likely under-diagnosed and adversely affects quality of life and survival. Recognition will improve care and facilitate timely and appropriate nutritional interventions. Local and national diagnostic criteria have been imprecise. Standardized guidelines have been developed to standardize a reliable nutrition assessment tool to identify and grade severity of malnutrition. We aimed 1) to identify prevalence and severity of malnutrition among inpatients who consulted a Registered Dietitian (RD) 2) to compare Nutrition Therapy assessments (NTA) and physician Electronic Medical Records (EMR) notes for diagnosis and severity of malnutrition.
Material and Methods: This study was a quality improvement project. Data included consecutive nutrition therapy assessments in 2009 made by a Registered Dietitian. RD used a standard assessment tool with 6 criteria to assess nutritional status; unintentional weight loss, BMI, visual muscle wasting, nutrient intake, wounds, and laboratory values e.g., pre-albumin, albumin, and transferrin. ⩾2 criteria had to be present for malnutrition. Weight loss (WL) was: moderate if 1−2% WL in 1 week, 5% in 1 month, 7.5% in 3 months, or 10% in 6 months; severe if >2% WL in 1 week, >5% in 1 month, >7.5% in 3 months, or >10% in 6 months. Physician notes were reviewed as to whether malnutrition was reported and/or graded.
Results: 213 NTA were reviewed for 116 patients. Median age 65 years (range 19−94); 57% male; 84% cancer diagnosis. Most common cancers were gastrointestinal 26%, genitourinary 26%, and respiratory 16%. 78% had metastatic disease. 147 (69%) NTA were eligible for RD assessment. Most often requested by physician/physician assistant (51%), dietetic technician (21%), or nurse (18%). Of the 147 NTA, most 99 (67%) identified malnutrition per RD; 55% of them had moderate/severe malnutrition. Malnutrition was usually noted by unintentional WL (59%), low nutrient intake (58%), and low serum albumin (54%). WL was severe in 68%. 60% of physician notes did not document nutritional status; 28% reported moderate/severe malnutrition.
Conclusions:
1. Clinically important (moderate/severe) malnutrition was highly prevalent (42%).
2. Weight loss, albumin level, nutrient intake were the most common malnutrition criteria used by RD.
3. Most physician notes did not formally document malnutrition.

No conflict of interest.

1322 POSTER

Oral nutritional support can shorten the duration of parenteral hydration in end-of-life cancer patients: A randomized exploratory trial

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Background: Varied symptoms such as anorexia and vomiting reduce oral intake in end-of-life cancer patients. Thus, nutritional support or artificial hydration needs to be initiated in clinical practice. However, these procedures jeopardize patients' quality of life (QoL). The amino acid jelly Inner Power® (IP) does not contain any fat and is easy to drink owing to its taste and semisolid form, proving useful for exhausted people. We conducted a randomised trial to compare the efficacies of IP and a liquid enteral nutrient Ensure Liquid® (EL) in terminally ill cancer patients.
Material and Methods: We randomly assigned patients to 3 arms. We started nutritional support for patients when their oral intake decreased to less than 10% of the normal amount. Patients received nutritional support in 3 arms as per their intakes: EL, supported by EL; IP, supported by IP; and EL+IP, support with EL followed by IP. When the amount of oral intake decreased despite nutritional support, patients received parenteral hydration (500–1000 ml/day). This regimen was continued until patients' death. We recorded the duration of nutritional support, the duration of parenteral hydration, and the amount of oral intake. Primary endpoint was drip infusion in vein (DIV)-free survival, which is defined as the duration from the initiation of nutritional support to the administration of parenteral hydration. Secondary endpoints included overall survival, the duration of parenteral hydration, the duration of nutritional support, adverse events, and QoL.
Results: Twenty-seven patients were enrolled in the study, of which 21 were included in intention-to-treat analysis (EL:IP:EL+IP, 8:5:8). Median age of the subjects was 69 years. Primary tumor sites were the lung (n = 6), hepatobiliary (n = 5), breast (n = 4), colorectal (n = 3), head and neck (n = 2), and cervix (n = 1). There was a significant difference between the 3 arms with regard to DIV-free survival (EL, IP, and EL+IP time was 0.5, 6.0, and 4.5 days, respectively; $P = 0.050$). Overall survival was 7, 9, and 8 days in the EL, IP, and EL+IP arms, respectively. The IP arm showed better QoL scores for fatigue and global health and low occurrence rates of adverse event in nausea.
Conclusions: IP can shorten the duration of parenteral hydration in terminally ill cancer patients and does not affect their survival.
No conflict of interest.

1323 POSTER

Transdermal fentanyl vs oral oxycodone+naloxone prolonged release: Efficacy in pain control for stage IV cancer patients

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Background: The Breakthrough cancer Pain (BTcP) is a transitory exacerbation of pain that occurs on a background of otherwise stable pain in an oncologic patient receiving chronic opioid therapy. The management of BTcP episodes require on-demand opioid (e.g., immediate-release morphine or rapid-onset fentanyl) in addition to the baseline (regular) opioid therapy. The aim of this study is to assess the pain control in metastatic cancer patients receiving either transdermal fentanyl or oxycodone+naloxone prolonged release tablets, through the evaluation of the frequency of BTcP episodes in these patients.
Methods: An observational study to assess the frequency of BTcP episodes in patients with metastatic disease receiving either transdermal fentanyl (25 mcg/h every 72 hours) or oral oxycodone+naloxone prolonged release tablets (30 mg/die). Rapid onset fentanyl (buccal or nasal at equidoses) were provided as rescue medication for Breakthrough pain. Patients were asked to record when they took additional medication for incident pain.
Results: Baseline characteristics = N patients: 132; men: 60%; mean age: 61.05 years; ECOG 1: 70%. Main location of the primary tumor: breast (44%), lung (25%), colon/rectum (17%), head and neck (14%). 60 patients were treated with transdermal fentanyl 25 mcg/h (45%), 72 with oxycodone+naloxone prolonged release tablets 30 mcg/die (55%). In the fentanyl group, patients used more on-demand opioid than the oxycodone+naloxone group (54% vs 42%; $p = 0.0005$) and a sizeable proportion of patients required upward titration of opioids (47% required at least one fentanyl dose change and 27% at least one oxycodone+naloxone dose change; $p < 0.05$).
Conclusions: Clinical practice confirms significant improvement in preventing BTcP episodes in metastatic disease with oral oxycodone+naloxone compared to trandermal fentanyl. Furthermore, we observed that the efficacy of the patch is shorter than 72 hours (in a range between 4 h − 60 h) and that in some clinical occurrences (e.g., hyperhidrosis, altered thermoregulation) transdermal opioids are less effective than oral formulations.
No conflict of interest.

1324 POSTER

Current emesis patterns of chemotherapy and antiemetic treatments in Western Europe

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Background: Cytotoxic treatments and supportive care to prevent chemotherapy-induced nausea and vomiting (CINV) vary across key cancers and countries in Western Europe, which may influence patient outcomes. This study describes the latest use of chemotherapies and antiemetic regimens in clinical practice across five countries.
Methods: For this study IMS Oncology Analyzer™ (OA) was used, a patient database collected through a quarterly physician panel survey. OA reports on patient case history information related to the treatment of patients across all cancer types. The most recent data (January-December 2012) in France, Germany, Italy, Spain and UK was used.
Results: From a total sample of 58,619, 37,444 patients were identified as being treated with chemotherapy. Among them, 22,145 (59.1%) received an antiemetic. 73.5% of patients were ⩾56 y. Overall, patients were mainly diagnosed with Colorectal (14.9%), Breast (11.7%) and Non-hogdkin lymphoma (NHL) (11.6%). 36.7% of patients received high-emetogenic chemotherapy (HEC), 41.2% moderate-emetogenic chemotherapy (MEC) and 21.5% low/minimal-emetogenic chemotherapy (LEC) −0.6% not specified-.
In HEC, 71% of chemotherapies contained a platinum compound. The antiemetic treatment used the most was Ondan±Dex (30.9%) and 25% of HEC patients received Aprepitant. HEC were mainly used in Non-small cell lung cancer (22.1%) and Breast (17.3%). In MEC, 19% of chemotherapies contained an anthracycline and cyclophosphamide. The main antiemetic treatment was still Ondan±Dex (28.6%) and only 5.4% of patients received Aprepitant. MEC were mainly used in Colorectal (30%) and NHL (23.9%). In LEC, 19% of chemotherapies contained a taxane. Only 1.3% of patients received Aprepitant. The anti-emetic treatment used the most was Dex (14.9%). LEC were mainly used in Multiple Myeloma (20.8%) and Pancreas (17.5%).
Focusing on Breast cancer, HEC and MEC were mainly used in Stage II and III (75.1% and 59.6% respectively), whereas LEC were mainly used in Stage IV (61.9%).
Conclusions: Nearly 60% patients treated with chemotherapy received a treatment for CINV in Western Europe. Over 75% of patients received a HEC or MEC. Emetogenic level of chemotherapies used depend on the tumor types but also on the stage of the disease; aggressive chemotherapies can be used mainly in early stages as in Breast cancer. Usage of setrons and aprepitant differ also across countries, key cancers and types of chemotherapy.
Ondan: ondansetron, Dex: dexamethasone
No conflict of interest.

1325 POSTER

Comparison of CKD-EPI, MDRD and Cockcroft-Gault to estimate baseline renal function in patients with head & neck and thoracic cancers

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Background: Estimation of renal function is essential in patients (pts) treated with cisplatin (DDP). We aimed to compare the estimated glomerular filtration rate (eGFR) according to different methods and also to verify the incidence of acute renal failure (ARF) after DDP in pts diagnosed with head & neck and thoracic cancers.
Materials and Methods: Uniinstitutional, retrospective and exploratory study. All pts were >18 y, diagnosed with head & neck or thoracic cancer, and were treated with DDP, at least for one cycle. DDP was administered in NS 500 mL in 60 min, following NS 1000 mL, KCl 25 mEq, $MgSO_4$ 100 mg and manitol 20 g. Baseline eGFR was calculated using Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. ARF was defined as elevation of serum creatinine ⩾0.3 md/dL, or more than 50%.
Results: 157 pts were included: median age 58 y (26−78), 68% male. Lung (61%), larynx (13%), oral cavity (8%) and oropharynx (8%) were the most common primary sites. Median weight was 60 kg (36–107), and BMI 22.7 kg/m^2 (13.2–39.3). First DDP cycle was administered to all 157 pts, the second to 154 pts, the third to 127 pts and 91 pts received the fourth one. Median DDP dose was 80 mg/m^2 for all cycles. Median baseline serum creatinine was 0.75 mg/dL (0.46–1.76) and it increased to 0.78 (0.40–1.51, p = 0.044, t-test), 0.80 (0.42–1.68; p = 0.005) and 0.77 (0.41–1.69, p = 0.075) after each DDP dose. At baseline, median eGFR (ml/min/1.73 m^2) was 86 (34–175)(CG), 107 (44–226)(MDRD) and 100 (45–145)(CKD-EPI). Considering normal eGFR as >90(CG), >125 (MDRD) and >100 (CKD-EPI), according to ROC analysis, the agreement between MDRD and CG was fair (k=0.291) and between MDRD and CKD-EPI was moderate (k=0.553). Bland-Altman analysis revealed that CG overestimates eGFR in comparison to MDRD (+19) and to CKD-EPI (+11). Overall, the incidence of ARF was low: 9 pts (6%), 11 pts (8%) and 1 pt (1%) after first, second and third cycles, respectively.
Conclusions: In comparison to MDRD, both CG and CKD-EPI overestimate eGFR, and the agreement between these equations is not good. CG should be used with caution as the reference eGFR in these pts.
No conflict of interest.

1326 POSTER

A probability/risk matrix of febrile neutropenia to select patients for G-CSF primary prophylaxis

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Introduction: Current guidelines suggest a risk threshold of 20% for G-CSF primary prophylaxis (PP) in patients with solid tumors. Individual factors that increase the risk of FN are considered when chemotherapy

yields a medium rate of FN (10−20%). This recommendation does not address the qualitative impact of FN. A probability/risk matrix could improve the prevention of severe episodes.
Patients: An ambispective study of cases with solid cancer and FN was carried out in 15 Spanish hospitals between 2006–2013. The risk factors (RF) that were available before chemotherapy initiation were used to create a vulnerability index (VI): low risk (0 RF), medium risk (1−2 RF) and high risk (⩾3 RF). We classified the cases on a 3x3 table, according to the probability of FN (<10%, 10−20%, >20%) and the VI.
Results: We reviewed 734 cases of FN. We found 207 complications (28.5%) and 27 deaths (3.7%). The independent baseline predictors of complications were COPD (OR 2), cardiovascular disease (OR 2.4), ECOG PS ⩾2 (OR 2.6) and palliative setting (OR 1.4). Rates of complications and deaths according to the VI and the predicted myelotoxicity are shown in Table 1. Of note, the use of G-CSF PP increased in parallel to the expected myelotoxicity of the regimen: 6%, 21% and 61% ($p < 0.0001$), but not in relation to the VI.
Conclusions: This study identified risk factors for severe neutropenic complications and mortality before chemotherapy initiation, with potential implications for prevention. It remains a priority to classify the patients on basis of the likelihood of FN and their individual vulnerabilities.
No conflict of interest.

Table 1. Complications and deaths

VI % FN	Low risk	Medium risk	High risk	n	p-value
Complications					
<10%	4/20 (20%)	35/97 (36%)	13/17 (76%)	134	<0.0001
10−20%	27/165 (16%)	65/239 (27%)	34/50 (68%)	454	<0.0001
>20%	14/84 (17%)	13/53 (25%)	2/3 (67%)	140	0.1
Deaths					
<10%	0/20	4/97 (4%)	6/17 (35%)	134	0.006
10−20%	1/165 (1%)	10/239 (4%)	3/50 (6%)	454	0.01
>20%	0/84	1/53 (2%)	2/3 (67%)	140	0.08

1327 POSTER

Effect of food and age on the pharmacokinetics of NEPA (a fixed-dose combination of netupitant and palonosetron) in healthy volunteers

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Background: The incidence of cancer is highest among the elderly and chemotherapy-induced nausea and vomiting has an adverse impact on their quality of life and compliance with therapy. NEPA, an oral fixed-dose combination of netupitant, a new NK_1 receptor antagonist (RA), and palonosetron, a pharmacologically distinct 5-HT_3RA, targets dual antiemetic pathways with a single-dose administration. This trial assessed the effect of food and age on the pharmacokinetics (PK) of NEPA.
Material and Methods: This was an open-label, randomized, two-way, cross-over trial (EudraCT 2010–020436−20). NEPA (300 mg netupitant + 0.5 mg palonosetron) was administered to healthy volunteers (⩽45 years) after an overnight fast of ⩾10 hours (fasted condition), or following a high-fat breakfast (fed condition). Cross-over took place after 28 days. A parallel group of healthy volunteers (⩾65 years) received NEPA in the fasted state. PK parameters of netupitant and palonosetron were calculated in the fasted and fed condition, as well as in elderly and younger volunteers in the fasted condition. Safety and tolerability were evaluated.
Results: 24 adult (22−45 years) and 12 elderly (66−79 years) volunteers were enrolled in the study. Food had no effect on the PK of palonosetron. In the fed state, netupitant maximum plasma concentration and overall plasma exposure increased by 18% and 16%, respectively, compared with the fasted condition (C_{max}: 635.0 vs. 539.3 μg/L and AUC_{inf}: 21271 vs. 18344 h*μg/L, respectively). This increase in netupitant exposure is not considered clinically relevant. Compared to younger, elderly volunteers showed an increased exposure to netupitant [C_{max}: 36% (735.4 vs. 539.3 μg/L); AUC_{inf}: 25% (22913 vs. 18344 h*μg/L)] and to palonosetron [C_{max}: 10% (839.5 vs. 760.1ng/L); AUC_{inf}: 37% (44414 vs. 32445 h*ng/L)], which was not considered clinically relevant. All reported treatment-emergent adverse events (TEAEs) were of mild and moderate intensity. TEAEs were more frequent in elderly volunteers (91.7%), as well as in the fed vs. fasted condition (47.8% vs. 34.8%). Overall, most common TEAEs were constipation (47%) and headache (33%). No deaths and no serious adverse events occurred during the study.
Conclusion: The changes in the PK profile of NEPA are not considered clinically relevant and, consequently, dose adjustments are not expected with regards to food and age. Overall, NEPA was safe and well tolerated.
This completed study was sponsored by Helsinn Healthcare SA.
Conflict of interest: *Other substantive relationships: SC, CL, MG: Helsinn Healthcare SA employees KK: The trial was sponsored by Helsinn and conducted at the Clinical Research Organization which received payment for the conduct of the trial. The co-author was an employee of the respective CRO at the time of the conduct of the trial.*

1328 POSTER

Prevalence of iron deficiency in severe anemia for cancer patients visiting the emergency unit for acute onset symptoms

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Background: Anemia in cancer is a common biological abnormality and etiological research is often not conducted. Iron deficiency is rarely explored. The objective is to determine the prevalence of absolute (A) or functional (F) iron deficiency (ID) in severe anemia for cancer patients.
Materiel and Methods: All cancer patients who visited the emergency unit for acute symptoms were included prospectively during 3 months if hemoglobine level was <10 g/dl. Three groups were defined using serum ferritin (SF (ng/ml) and transferrin saturation (TSAT): Absolute ID: TSAT<20% and SF<30; status unknown for ID (UID) (functional ID): TSAT<20% and SF>30 ng/ml and a group without ID (WID): TSAT >20%.
Results: Severe anemia was observed for 85 patients; 5% of the patients had AID. Asthenia and dyspnea were reported in 76% and 62% of the patients, respectively. A gastrointestinal localization was found for only 50% of the patients with AID. Anemia had another cause than ID in 19%. ID status was difficult to define for 76.5% of the patients (CS<20%, SF>30). For 66% of the patients in UID, inflammatory syndrome (C reactive protein >60 mg/l) might explain the functional ID, but for the remaining patients (34%), there are probably other explanations for the TSAT less <20%.
Conclusions: Absolute ID was rarely observed in cancer patients visiting for acute onset symptoms. There are more than 3/4 of the patients with an ID status difficult to determine and the reticulocytosis response after an iron supplementation could be of help for diagnosis and understanding the underlying mechanisms.
No conflict of interest.

1329 POSTER

Factors associated with mortality rates of patients with advanced cancer admitted to a specialized intensive care unit

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Background: Cancer patients often need intensive care to recover from drug- related toxicities, infections or other clinical complications. Proper selection of cancer patients who could benefit from such care would spare resources and avoid overtreatment of patients with poor prognosis. We aim to evaluate the prognostic factors associated with mortality rates of these patients during hospitalization in an Intensive Care Unit (ICU) and within 30 days after hospital discharge.
Material and Methods: All cancer patients with advanced solid tumors admitted in an ICU of a comprehensive cancer center were retrospectively evaluated in a 6-month period. We extracted the following data from the last medical visit before hospital admission: tumor type, performance status (PS), body mass index (BMI), renal and liver function, hemoglobin, cancer treatment, and number of days from the last cancer therapy. Results of laboratory tests at ICU admission were also collected. A multivariable logistic regression model was performed to evaluate potential predictors of mortality during hospitalization and after 30 days from hospital discharge. Variables were considered statistically significant if two-sided *P* values <0.05.
Results: From May 2012 to Oct 2012, 627 patients were admitted to ICU and 338 were eligible. Median age was 60.0 years; 257 (76.0%) were metastatic. Gastrointestinal was the most common primary site (30.2%). Half of patients had a poor PS (ECOG⩾2) and 16.6% were malnourished (BMI⩽18.5). Any degree of renal and liver impairment was found in 33.8% and 7.1% of patients, respectively. One-fourth (20.4%) had received two or more lines of therapies against cancer. The mortality rate was 38.8% during ICU period and 59.2% when considering the whole hospitalization. Of the 132 discharged patients, the 30-day mortality rate was 21.2%.

The multivariable analysis demonstrated that hypoalbuminemia (OR=3.93; 95%IC: 1.58–9.79) and high levels of arterial lactate (OR=1.84; 95%IC: 1.03–3.30) collected at ICU admission were significantly related to death during hospitalization.
Conclusions: In this large retrospective analysis, the mortality rate of patients with advanced cancer admitted to a specialized ICU is high, what shows the poor prognosis of these patients. Hypoalbuminemia and high level of arterial lactate at ICU admission were associated with death during hospital recovery. The indication of ICU to cancer patients should be carefully discussed.
No conflict of interest.

1330 POSTER

Chemotherapy-induced neutropenia and febrile neutropenia during chemotherapy for gynaecologic malignancy

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Background: Chemotherapy-induced neutropenia seemed to be a relevant problem in clinical practice. Febrile neutropenia (FN) seemed to be one of medical emergency in cancer treatment. In this study, we investigated chemotherapy-induced neutropenia recently performed in patients with gynaecologic malignancy.
Methods: Between January 2009 and December 2011, we examined our reported chemotherapy-induced neutropenia using the Common Terminology Criteria for Adverse Events (CTCAE) v.4.0. We analysed the incidence and clinical features of chemotherapy-induced neutropenia (grade4: absolute neutrophil count <500 /μL) and febrile neutropenia in patients with gynaecologic malignancy.
Results: During this period we administered over 1614 infusions (29 regimens) to 291 patients with gynecologic malignancy. Median age was 60 years (24−84). Neutropenia occurred in 147 (50.5%) patients over 378 (23.4%) chemotherapy cycles. Febrile neutropenia occurred in 20 (6.9%) patients over 25 (1.5%) cycles. FN occurred after cycle 1 in 14 (56%) cycles. Mean duration of neutropenia and fever was 3.6 (1−12) and 3.4 (1−9) days respectively. The source of fever was unexplained by exam or cultures in 15 (60.0%) cycles. 5 patients (25%) had bowel resection history. There were two neutropenic-related death cases. Neutropenia was associated with elderly age (over age 70) ($p < 0.0001$), less than five previous chemotherapy cycle ($p = 0.02$), disseminated disease ($p = 0.03$), platinum-based regimens ($p < 0.0001$), taxan-containing regimens ($p < 0.0001$) and combined therapy ($p < 0.0001$). Febrile neutropenia was associated with poor performance status ($p < 0.0001$), no previous chemotherapy ($p < 0.05$), disseminated disease ($p < 0.0001$) and distant metastatic disease ($p = 0.03$). Both neutropenia and febrile neutropenia were not related with bone marrow metastases or previous radiotherapy.
Conclusions: By estimating risk factor of febrile neutropenia such as performance status and progression of disease, safe management of chemotherapy-induced neutropenia may be possible in patients with gynaecologic malignancy.
No conflict of interest.

1331 POSTER

Evaluating the impact of a new oncology consultant ward round

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Background: Regular consultant ward rounds have been shown to reduce length of stay and improve discharge planning for patients. The Royal College of Physicians advises regular morning consultant led ward rounds for medical patients. Balancing the competing demands of outpatient activity and inpatient oncology specialist care has been difficult in our hospital. Previously there was no timetabled consultant ward round for oncology inpatients at our hospital. Inpatients were managed primarily by oncology specialist trainees, qualified in internal medicine, with ad-hoc review by their named consultant. A regular consultant ward round was introduced for the first time on the 7/1/13. Each consultant was timetabled to give a twice weekly morning ward round, on a rolling rota. Whilst based on the ward they provided cover for all inpatients, regardless of tumour site.
Materials and Methods: To evaluate this intervention, a retrospective case note analysis was undertaken. This included all patients admitted under oncology for the two months preceding and succeeding the new ward round. For each patient the admission date, time to first consultant review, number of consultant reviews, time to discharge after consultant review and discharge date was identified. A staff survey also took place before and after the new consultant ward round. Statistical analysis was performed using Mann-Whitney U or Chi-Squared tests.
Results: 85 patient episodes meeting the inclusion criteria were under the care of oncology between 7/11/12 and 7/3/13. Case notes were available for 63 episodes (74%). The average length of stay significantly decreased from 11 days to 3.5 days ($p < 0.05$). The time to discharge after first consultant review also significantly decreased from 6 days to 2 days ($p < 0.05$). The number of consultant reviews and time to first consultant review remained unchanged (p= not significant). The percentage of patients receiving a consultant review increased, from 54.3% to 71.4%, though this was not statistically significant. However it is likely such a large increase is clinically significant. Medical and nursing staff satisfaction assessed by an online questionnaire and free text survey also improved following the new ward round.
Conclusion: This study suggests that a regular consultant ward round improves length of stay for patients, possibly through more patients having a consultant review and by expediting treatment and discharge decisions after such a review. Further robust work is needed to establish exactly how this is achieved and how best to make the new ward round a permanent feature logistically.
No conflict of interest.

1332 POSTER

Randomized trial to explore indisetron tablets for preventing chemotherapy-induced nausea and vomiting (CINV)/acute-onset diarrhea induced by IRIS/FOLFIRI: An exploratory trial -HGCSG0704-

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Background: Indisetron is a serotonin (5-hydroxytryptamine type 3: $5\text{-}HT_3$) receptor antagonist that also antagonizes 5-hydroxytryptamine type 4 receptors. Indisetron tablets showed the non-inferiority to ondansetron tablets in terms of efficacy for preventing chemotherapy-induced nausea and vomiting (CINV). Preclinical data administered with irinotecan showed indisetron significantly reduced the stool frequency in mice and inhibited the colonic peristalsis in dogs. We designed a pilot study compared the efficacy and tolerability of indisetron for irinotecan-induced diarrhea, nausea, and vomiting with granisetron.
Material and Method: This study was a pilot, multicenter, randomized, open-label, comparative trial (HGCSG0704). Advanced colorectal cancer patients treated with FOLFIRI or IRIS (Irinotecan + S-1) with or without bevacizumab were enrolled in this study. Treatment: Arm A: indisetron tablets po day1. Arm B: granisetron iv day 1. The primary endpoints were the incidence of acute-onset diarrhea and complete protection from vomiting. Secondary endpoints were complete protection from nausea, rate of no rescue therapy and tolerability. Nausea, vomiting and other adverse events (AE) were evaluated using Common Terminology Criteria for Adverse Events, version 3.0.
Results: Between May 2008 and July 2012, 33 patients (pts) were randomized. The study was closed prematurely due to poor accrual. Arm A: 16 pts, arm B 17 pts. Median age A: 68 yrs (55−76), B: 66 yrs (47−78): ECOG PS 0/1: A: 12/4, B: 14/ 3pts. There was no significant difference of the incidence of acute-onset diarrhea between both groups (18.8% [95% CI −0.2–39.5] in A vs 35.3% [95% CI 10.7–59.9] in B, $p = 0.44$). The proportion of pts with complete protection from vomiting was 87.5% in A and 88.2% in B (p=1.00). Similarly, complete protection from nausea and rate of no rescue therapy did not have a significant difference (50.0% in A and 41.2% in B, $p = 0.73$). Severe AE as nausea and vomiting were also similar between two groups. No severe AE induced by 5-HT3 receptor antagonist were observed in both groups.
Conclusions: Although indisetron showed effective and feasible results for preventing CINV induced by regimen containing irinotecan, the proportion of acute-onset diarrhea induced by irinotecan had not improved in indisetron group. Because there were small numbers in this study, the significant difference was not recognized. However, Group A tends to be a low incidence of the diarrhea. Therefore, it may be necessary to prove it in the clinical trial with the larger number.
Conflict of interest: *Advisory board: Yakult Honsha Co., Ltd. Taiho Pharmaceutical Co., Ltd. Chugai Pharmaceutical Co., Ltd. Merck Serono Pharmaceutical Co., Ltd. Pfizer. Novartis. Sawai. Ono Pharmaceutical. Daiichi Sankyo Co., Ltd. Takeda Pharmaceutical Co., Ltd. Otsuka Pharmaceutical Co., Ltd. Bristol-Myers Squibb Co. Corporate-sponsored research: Taiho Pharmaceutical Co., Ltd. Lilly. Novartis. Yakult Honsha Co., Ltd. Daiichi Sankyo Co., Ltd. Merck Serono Pharmaceutical Co., Ltd. Takeda Pharmaceutical Co., Ltd. Kureha. Other substantive relationships: Synergy International, Inc.*

1333 POSTER

Results of ultrasonic stimulation in oncological patients with leucopenia

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The purpose of the study: Studying the role and assessment of efficiency of ultrasonic stimulation of spleen in oncological patients during chemotherapy complicated with leucopenia.
Objectives: Evaluate the effectiveness of ultrasonic stimulation of spleen in patients with leucopenia receiving polychemotherapy. Suggest indications and terms of ultrasonic stimulation of spleen in leucopenia.
Materials and Methods: The study included 113 patients with a diagnosis of a malignant tumor, complicated by leucopenia receiving specialized treatment in chemotherapy department of National Cancer Research Center in 2010–2011. From the total enrolled patients 24 (21%)- patients were diagnosed breast cancer, 26 (23%)- ovarian cancer, 13 (11.5%)- cervical cancer, 8 (7%)- brain tumors, 7 (6%) − non-Hodgkin's lymphoma. All patients before the beginning of next course or during chemotherapy had complication in the form of reduction in the total number of leukocytes. Indicators of leucocytes ranged from 1.8 to 3.2 and averaged $2.4 \pm 0.03 \times 10?/l$. To activate the production of white blood cells used ultrasonic stimulation of the spleen (USS) 1−2 times per day, 3−6 procedures without the use of corrective and immunostimulatory drugs. Ultrasonic stimulation of the spleen was prescribed in case of reducing the number of leucocytes below $3.0 \times 10?/l$. The effectiveness of treatment was assessed after the third procedure.
Results: In the study of peripheral blood on the third day after the start of the USS were detected increase in the number of white blood cells from 4.5 to $6.0 \times 10?/l$ and up to $11 \times 10?/l$ after treatment ($p < 0.05$). After the stimulation of the spleen in the period from 1 to 6 days 113 (100%) patients had recovery of white blood cells that allowed for a special anti-cancer treatment.
Conclusions: The study shows the usefulness and actuality of the method for further study of the ultrasonic stimulation at leucopenia in oncological patients receiving chemotherapy.
This method can be recommended as an effective and economically unhindered method for stimulating leucopoiesis.
No conflict of interest.

1334 POSTER

Qualitative assessment of dysgeusia in early breast cancer patients undergoing anthracycline or taxane-based standard chemotherapies

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Background: Taste alternations (TAs) during chemotherapy are common and significant complaints of cancer patients potentially leading to poor compliance, malnutrition and decreased quality of life. Although chemotherapy regimens with different toxicity profiles may vary in their impact on TAs, research on this topic has not extensively examined. Therefore, the standard prevention and treatment have not been established. Here we conduct a prospective study to assess not only the prevalence but also the quality of TAs in chemotherapy-naive patients receiving 2 distinct worldwide standard therapies.
Patients and Methods: Japanese female patients with early breast cancer who undergo 4 cycles of FEC (5-fluorouracil, epirubicin and cyclophosphamide) or TC (docetaxel and cyclophosphamide) regimen as adjuvant setting were prospectively enrolled. Patients completed a daily questionnaire, which measures dysgeusia and related adverse effects by using CTCAE criteria. In addition, alternations of 5 basic tastes were qualitatively monitored with 5-category scales consisting of very strong (+2), strong (+1), normal (0), weak (−1) and very weak (−2). These data were collected from 24 and 18 patients who received FEC and TC, respectively.
Results: (1) To assess the sequential alteration of dysgeusia, 21 days of single cycle were separated to 5 short periods; day 1−2, 3−5, 6−8, 9−14 and 15 or later. The frequency of grade 2 dysgeusia in each period was 29%, 25%, 29%, 21%, 8% and 38%, 44%, 56%, 56%, 25% in 1st and 4th cycle of FEC, respectively. On the other hand, TC showed 0%, 44%, 50%, 33%, 0% and 17%, 50%, 58%, 42%, 8% in the same setting. These results suggest that the onset of TAs induced by FEC is prompt and the symptoms prolong with accumulation. In contrast, the adverse symptoms caused by TC may appear more frequent but transient.
(2) The character of TAs developed clearly until 2nd cycle of both regimens. The average score of 5 basic tastes (salty/sweet/umami/sour/bitter) in the 2nd cycle of FEC and TC were 0.48/0.38/−0.43/0.29/0.33 and −0.41/−0.35/−0.59/−0.35/−0.12, respectively. These results support the idea that each taste sensations except umami are emphasized during FEC, while they are potentially diminishing during TC.
Conclusions: This study demonstrates that the features of TAs may differ definitively between anthracyclin-based FEC and taxane-based TC regimen. Further research has yet to be required focusing on individualized management strategies for TAs depending on the type of chemotherapy.
No conflict of interest.

1335 POSTER

Palonosetron plus dexamethasone for the prevention of nausea and vomiting in patients with locally advanced head-neck squamous cell cancer (HNSCC) treated with radiotherapy plus concomitant administration of cisplatin

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Background: Nausea and vomiting still represent relevant issues in patients treated with cisplatin (>50 mg/mq): an unsatisfactory emetic control could negatively affect the patients' compliance with potential detrimental effects in terms of treatment's efficacy. In this study we have evaluated the efficacy of single dose IV palonosetron (0.25 mg) plus dexamethasone (8 mg IV day 1 plus 8 mg PO dd.2−3) in patients with locally-advanced HNSCC treated with standard fractionation radiotherapy (total dose from 66 to 70 Gy) plus concomitant administration of cisplatin 100 mg/m^2 every 21 days.
Material and Methods: From March 2010 to January 2013 we consecutively recruited 43 pts with locally advanced HNSCC. Main characteristics of pts were as follows: M:F 34:9, median age 62.3 yrs (range 54−73 yrs), median ECOG PS 1 (range 0−1). 10 pts (23%) were still alcohol consumers. Primary end points were: Complete Response (CR: no vomiting and no rescue therapy) and Complete Control (CC: CR and no more than mild nausea). These endpoints were evaluated during the acute (0−24 h), the delayed (25–168 h) and overall (0–168 h) phases. A safety evaluation of this antiemetic protocol was also planned.
Results: All recruited patients were evaluated for efficacy and safety. During the acute phase, CR and CC were reported in 84% and 81% of patients, respectively; 86% and 81% of patients achieved CR and CC during the delayed phase; in the overall phase, 81% and 79% of patients experienced CR and CC, respectively. This antiemetic regimen was really well tolerated: 12 pts (28%) experienced G1−2 constipation easily managed by the administration of common laxatives; in 3 pts (7%) was reported G1 headache.
Conclusions: In this study the combination of palonosetron and dexamethasone has proven to be effective and safe to prevent chemotherapy-induced nausea and vomiting in patients with locally-advanced HNSCC treated with radiotherapy plus concomitant cisplatin 100 mg/m^2 administered every three weeks.
No conflict of interest.

1336 POSTER

The efficacy of low dose transdermal fentanyl in opioid-naive cancer patients with moderate and severe pain

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Background: Little was known about the efficacy of low doses of transdermal fentanyl patch(TDF) in opioid naive patients with moderate to severe cancer pain.
Methods: The study was conducted open-label, prospective design from April 2007 to February 2009 in seven tertiary cancer hospitals and 98 patients were enrolled. TDF was started with low dose formulation(12.5 μg/h) and adjusted according to clinical situation. Pain intensity, used TDF doses, adverse events were monitoring over 4 weeks. Data were analyzed by intent-to-treatment(ITT) principle.
Results: Of 98 enrolled patients, sixty-four(65%) patients completed the study. The median pain intensity decreased from 6.0 to 3.0

(p<0.0001) at the follow-up visit. The efficacy of low dose TDF on pain relief was consistently maintained after considering various variables such as sex(p<0.0001), age(p<0.0001), metastasis(p=0003), previous treatment(p<0.0001) and regardless of baseline pain intensity(p<0.0001). The decrease of pain intensity were significantly larger in severe group than in moderate group(mean±SD; 5.10±2.48 vs 2.48±1.56, p<0.0001). There were no differences between two intensity groups in the TDF dose (27.8 μg/h vs 24.8 μg/h, p = 0.423) and mean time (7.5 days vs 7.9 days, p = 0.740) for pain control.
Conclusion: Low dose TDF was effective treatment in cancer pain patients with moderate to severe intensity. Future randomized trials on efficacy of TDF in severe pain and/or optimal starting dose are warranted.
No conflict of interest.

1337 POSTER

Suppression of bone resorption by denosumab is better measured with CTX than with alkaline phosphatase

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Aim: Study design. This was a single centre study to determine the efficacy of a single injection of denosumab in 20 patients with solid tumors, to expand this experience to serum CTX and in other common metastatic tumors. The study ran from 1.6. 2012 to 1.11.2012.
Patients: Patients with radiological evidence of lytic or mixed bone metastases were enrolled into the study as well as patients with a bone isotope scan confirming diffuse bone metastases (N = 20).
Methods: On the morning of dosing, patients received a s.c. injections of 120 mg denosumab. Chemotherapy within 21 days following denosumab treatment, was allowed.
Patients were followed for at least 7 to 28 days; blood samples for alkaline phosphatase and serum CTX were scheduled on days 1, 2, 3, and 4, and then weeks 1, 2, 3. The data were recorded whenever the patient accepted this exam.
Results: The results from this study show that denosumab, a monoclonal antibody with high affinity and specificity to inhibit osteoclasts, was effective in decreasing bone resorption rapidly and for a sustained period of time in patients with all tumor types metastatic to bone. Bone resorption suppression was extensive based on changes from baseline in the measured biochemical markers, notably serum CTX. Normalisation of this bone resorption markers occurred within 4 to 21 days following single s.c. dose of denosumab in 18 out of 19 patients. Bone-specific alkaline phosphatase levels were only mildly and lately suppressed, confirming that denosumab does not have a direct effect to inhibit osteoblasts.
Conclusions:
1. The effect of denosumab can be easily followed by measuring serum CTX and the use of alkaline phosphatase can no longer be uphold.
2. CTX decreased with denosumab even in zoledronic acid pretreated patients.

No conflict of interest.

1338 POSTER

Predicting adverse outcomes after cisplatin administration in head & neck and thoracic cancer

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Background: Renal failure after cisplatin(DDP)-based chemotherapy is a harmful adverse event. Although different equations are used to estimate glomerular filtration rate (eGFR), there is no evidence supporting superiority of one over another in cancer patients (pts). We compared three equations: Cockroft-Gault (CG), Modification of diet in renal disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) in a population of cancer pts, after one cycle of DDP. We aimed to investigate if a decrease in eGFR using these equations could predict negative endpoints: death, dialysis or acute kidney failure (ARF).
Materials and Methods: Uniinstitutional, retrospective and exploratory study. All pts were >18 y, diagnosed with head & neck or thoracic cancers, and were treated with DDP, at least for one cycle. Acute renal failure (ARF) was defined as elevation of serum creatinine ⩾0.3 mg/dL, or more than 50%. We defined treatment-related deaths if they occurred in the first 28 days after DDP exposure.
Results: 157 pts were analysed. First DDP cycle was administered to all pts, the second to 154 pts, the third to 127 pts, and 91 pts received the fourth one. Median DDP dose was 80 mg/m^2 for all cycles. At baseline, median eGFR (ml/min/1.73m^2) was 86 (34–175)(CG), 107 (44–226)(MDRD) and 100 (45–145)(CKD-EPI). After first DDP administration, according to ROC analysis, cutoff values of eGFR reductions were calculated as follows (ml/min/1.73m^2): 10 (CG; sensitivity 78%, specificity 72%, AUC 0.816, 95% CI 0.74–0.88, p = 0.0001), 8 (CKD-EPI; 72%, 76%, 0.75, 0.67–0.82, 0.0003)and 20 (78%, 89%, 0.83, 0.76–0.90, 0.0001), respectively. No differences regarding ROC curves in detecting the studied outcomes were seen: CG vs. CKD-EPI (p = 0.971), CG vs. MDRD (p = 0.529) and CKD-EPI vs. MDRD (p = 0.556). Overall, 4 treatment-related deaths were observed and 14 pts developed ARF. Decreased renal function estimated by any of the studied equations was able to predict negative outcomes, with OR 9.0(CG, 95% CI 2.8–29.2, p < 0.0001), OR 33.0(MDRD, 95% CI 9.3–116.3, p < 0.0001) and OR 8.45 (CKD-EPI, 95% CI 2.8–25.6, p < 0.0001).
Conclusions: Decrease in renal function estimated by all three equations (CG, CKD-EPI and MDRD) seemed to predict negative outcomes (ARF, dialysis and 28-day mortality) after one cycle of DDP-based chemotherapy in patients with head & neck and thoracic cancers.
No conflict of interest.

1339 POSTER

Pneumocystis jiverocii infection during chemotherapy in solid cancer patients

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Background: Pneumocystis pneumonia (PCP) is an opportunistic infection caused by the ascomycetous fungus Pneumocystis jiroveci. Pneumocystis pneumonia (PCP) is common in patients with HIV infection but may also occur in patients with other causes of immunodeficiency, including hematologic and solid malignancies. Although solid tumors carry a lower risk of PCP than do hematologic malignancies, the incidence of PCP in solid tumor patients is increasing. Many studies on the risk factors of PCP in this patient group have been conducted. However, few data have been reported exploring PCP during systemic chemotherapy in solid cancer patients.
Material and Methods: We retrospectively analyzed 24 HIV-negative patients diagnosed with PCP between April 2005 and February 2013 during systemic chemotherapy for solid tumors.
Results: The median age at the time of diagnosis of PCP was 63.7 years (range 46−82) and the majority of patients (81.9%) did not have comorbidities. In terms of purpose of chemotherapy, 23 patients (95.8%) received chemotherapy for locally advanced or metastatic/recurrent disease and 1 (4.2%) had adjuvant chemotherapy after curative resection. Of 23 patients, 7 (29.2%) had non-small cell lung cancer and 4 (16.7%) breast cancer. All patients had a median of 2 metastatic lesions (range 0−4). The most common site of metastasis was brain. At the time of diagnosis, 6 patients (25.0%) were receiving first-line chemotherapy and 11 (45.8%) more than third-line chemotherapy. The proloned use of steroids was found on 17 patients(70.8%). The mean white blood cell, platelet and albumin level was 5,736, 1,205×10^3/uL and 3.1 mg/dl, respectively. The mean pressure of O_2 measured by arterial blood gas analysis was 51.3 mmHg. On analysis for disease evaluation at the time nearest to diagnosis of PCP infection, only 5 patients (20.8%) revealed tumor response to chemotherapy. After the diagnosis of PCP infection, most patients(21/24, 87.5) were treated with sulfamethoxazole and trimethoprim accompanied with steroid. Almost half of the patients (13/24, 54.2) experienced failure of therapy for PCP leading to death.
Conclusion: We reported PCP infection during systemic chemotherapy in solid cancer patients. Patients may have a relatively poor tumor response to chemotherapy at the time nearest to diagnosis of PCP infection. A prospective or matched controlled trial is needed to confirm this finding.
No conflict of interest.

1340 POSTER

Hair preservation results of scalp cooling in >3000 patients − The Dutch Scalp Cooling Registry

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Background: Chemotherapy-induced alopecia (CIA) is a frequently occurring side effect of cancer treatment. Scalp cooling can prevent CIA

and is practiced in more than 70 out of 100 Dutch hospitals. Here the results of the Dutch Scalp Cooling Registry from 2006 and onwards are presented. The main objective is to study the proportion of patients with satisfactory hair preservation after scalp cooling for each currently used chemotherapy schedule. Wetting the hair before scalp cooling is of particular interest, because it is applied in several Dutch hospitals. It has been shown to lower the scalp skin temperature, but it might be an extra burden for the patient and it is unknown whether it affects the result.
Methods: Patients who received scalp cooling with Paxman cooling devices in 55 Dutch hospitals participated in our prospective registry. Scalp cooling was performed from 30 minutes before the chemotherapy infusion until 90 minutes after stopping the infusion. Nurses registered information on type, dose and infusion time of chemotherapy. Patients completed questionnaires on the result of scalp cooling and reported age, gender, type of hair, hair length and quantity, chemical manipulation (dyeing, waving, colouring), wetting the hair before scalp cooling, and previous chemotherapy. Logistic regression analysis will be used to examine all above mentioned factors that might be associated with the scalp cooling result. The main outcome is whether a patient wore a wig or head cover.
Results: From 2006, more than 3000 scalp cooled patients have been included in the Registry. During the conference scalp cooling results will be presented for the currently used main types and doses of chemotherapy. Furthermore, associated factors will be discussed.
Conclusions: This study is by far the largest in the literature about scalp cooling. Our Registry is invaluable for informing patients and medical professionals about the scalp cooling result they may expect for a particular type and dose of chemotherapy. Besides, studying associated factors will ultimately lead to a more patient-tailored approach. The Registry also adds to improving the results by evaluating scalp cooling methods between hospitals.
No conflict of interest.

1341 POSTER

Familiarity, opinions, experiences and knowledge about scalp cooling − a Dutch survey among breast cancer patients and oncological professionals

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Background: Scalp cooling is applied to reduce chemotherapy-induced alopecia (CIA). The aim of this study was to investigate patients' familiarity and opinions and oncological professionals' attitude and knowledge about scalp cooling in the Netherlands.
Methods: (Ex)breast cancer patients, nurses and medical oncologists (MDs) from scalp cooling and non-scalp cooling hospitals were asked to fill out questionnaires.
Results: The questionnaires of 177 breast cancer patients, 49 nurses and 100 MDs were eligible for analysis. In scalp cooling hospitals, the majority of MDs (80%, n = 52) and nurses (81%, n = 30) were satisfied with the results, as were patients who had scalp cooling (61%, n = 52). In these hospitals, 41% of the MDs and 63% of the nurses perceived their level of knowledge insufficient to inform patients about the effectiveness and safety of scalp cooling. The most important reason of MDs to not apply scalp cooling was doubt about effectiveness and safety. Severe problems in implementing scalp cooling were reported by three professionals. Scalp cooling had been offered to a minority of eligible patients, especially men had been excluded. Patients were often unfamiliar with scalp cooling before breast cancer diagnosis. Twenty out of 51 scalp cooled patients (39%) reported an insufficient result and most of them (72%) reported CIA to be moderately or very bothersome. With an expected chance for hair preservation of 35%, 36% of the patients would like to use scalp cooling in case of future chemotherapy treatment, which was 54% at a success rate of 50%.
Conclusions: Much room for improvement has been shown for both patients' familiarity and oncological professionals' knowledge about scalp cooling, which will apply for other countries too. Sharing knowledge about results and safety of scalp cooling and patients' experiences with CIA will improve patient counseling and availability of scalp cooling. The results of this survey have led to the development of a national standard on chemotherapy-induced alopecia and scalp cooling.
This study was supported by the Dutch Pink Ribbon Foundation.
No conflict of interest.

1342 POSTER

Feasibility and acceptability of an interactive mobile phone application for early detection of patient reported symptom distress in prostate cancer

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Introduction: For immediate and continuous dialogue between patients and caregivers new approaches in modern technology are encouraged today. In cooperation with a Swedish health management company, we developed an interactive mobile phone application for the assessment of symptom distress, evidence-based self-care advice and an alerting function of severe symptoms with instant access to professionals in real time. By using this technique patients can communicate symptoms with instant support while cared for out-side hospital but at the same time reassured that their condition is monitored by the professionals. The objective of this study was to evaluate the feasibility and acceptability of the application for patients with prostate cancer during radiotherapy and for the involved health care staff.
Material and Methods: Evidence-based symptoms and related self-care advices were implemented in the application after literature review and interviews with patients and health care professionals. Nine patients diagnosed with prostate cancer undergoing radiotherapy treatment were recruited to test the application for two weeks. The patients reported in the electronic symptom questionnaire daily. After the two weeks they were interviewed about their experience. Nurses directly involved in the care and treatment of the participating patients were interviewed at the end of study.
Results: Overall, patients and nurses reported positive experiences of using the mobile phone system. The patients considered the application helpful and easy to use although there were some suggestions for further development of the electronic questionnaire. Most of the patients had read the self-care advice and found them useful. The alerting system was activated in several cases; the nurses found it useful to identify and manage problematic symptoms early and the patients felt safe and well cared for. Some of the nurses considered the monitoring system time-consuming and made suggestions for improvement.
Conclusions: Both patients and nurses could see the potential for using the mobile application in clinical practice. The system enables the involvement of the patients and the alerts showed problematic symptoms promoting timely interventions. The results support further development and testing of the system in full-scale.
No conflict of interest.

1343 POSTER

Complementary and integrative medicine improved quality of life, depression, anxiety and fatigue levels in cancer patients on active oncology treatment

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Background: Complementary/integrative medicine (CIM) is being integrated more frequently in conventional clinical and academic institutions as part of the treatment of cancer patients. The influence of those therapies on quality of life (QoL), depression, anxiety and fatigue on the short- and long-term was tested prospectively in this study.
Patients and Methods: CIM treatments are given as part of the service at Rambam Health Care Campus, Haifa, Israel, for oncology patients. According to a 'waiting list', patients were referred to six weekly treatments of one of the therapies: art therapy, music therapy, Reiki, Shiatsu, guided imagery, healing, cranio-sacral therapy or oil anointing. Hospital Anxiety and Depression Scale (HADS) and the Brief Fatigue Inventory (BFI) were completed every two weeks and QoL-EORTC-Q30 questionnaire every six weeks during the treatment sessions and six weeks after the end of treatment.
Results: Over a two-year period, 162 patients entered the study and 135 completed therapies sessions. There were 86% women, 60% on chemotherapy and 24% on radiotherapy treatments. Global QoL ($p < 0.001$) and parameters on the functioning scale and symptoms scale of EORTC-Q30 questionnaire showed significant improvement, which was also seen in median scores of BFI from 4.57 to 3.76 ($p < 0.001$), HADS-Anxiety from 8.27 to 6.83 ($p < 0.001$), and HADS-Depression from 7.12 to 6.16 ($p < 0.001$) after 12 weeks. Main improvement was during the treatments and for six weeks after. No significant difference was seen in use of opioids, anxiety-depression medication, steroids or epo-treatment, and haemoglobin levels were maintained without significant change.
Conclusion: A short intervention of CIM therapies improved QoL of cancer patients. The improvement lasted even six weeks following treatments.
No conflict of interest.

1344 POSTER

Polyprenol in cancer cachexia management

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Background: Many patients with advanced cancer undergo a wasting syndrome associated with cancer cachexia. Patients with cachexia generally have a short survival time, respond poorly to palliative chemotherapy and immunotherapy. A number of cytokines (TNF-apha, IL-1,IL-6, interferon-gamma) and proteolysis-inducing factor (PIF) have been proposed as mediators of the cachectic process. Dolichyl Phosphate (Dol-P) plays an essential role in cytokine synthesis and protein processing. The present study was carried out to present evidence that Dol-P is rate limiting factor in pathogenesis of cachexia using a 'urinary dolichol test' and polyprenol (PP) supplement in nutrition.
Methods: Urinary dolichol (Dol) concentrations were studied in 64 patients with cachexia with good performance status and life expectancy >1 to 2 months, treated with megestrol acetate >480 mg/day (group 1), 48 patients with poor performance status and short life expectancy, treated with prednisolone 50 mg/day (group 2). Nutrition of 30 and 25 patients in both groups contained PP 2 mg/day. Samples were taken from fresh urine and assayed by HPLC. Radioimmunoassay was used to measure cytokins and PIF.
Results: Mean urinary Dol concentration in patients from group 1 (45.9±4.8 mkg/mmol) as well as in group 2 (62.3±9.5erbfts;mkg/mmol) was significantly higher ($p < 0.001$) than that observed in patients without weight loss (19.8±2.2 mkg/mmol). Urinary Dol increase was shown to be correlated with weight loss in cachexia: 2 mkg/mmol = 1 kg. PP in nutrition caused a significant fall in production of IL-1in group 1 (from mean 74.8 pg/ml to 26.3 pg/ml, $p < 0.001$) and a fall in the proportion of patients excreting PIF (from 90% to 27%, $p < 0.001$).
Conclusions: Dol concentration in urine of cancer patients with cachexia is dictated by performance status and life expectancy. Dol-P dependent disorders in cachexia are established as a possible step in mechanism of weight loss in advanced cancer and as a new target for therapy and nutrition. Modulating effect of Dol-P substitute polyprenol on IL-1and PIF opens up possibilities for cachexia management.
No conflict of interest.

1345 POSTER

Does wetting hair during scalp cooling decrease scalp skin temperature?

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Background: Hair is frequently wetted before scalp cooling in order to improve its effect. Whether this actually improves the prevention of chemotherapy-induced alopecia (CIA) remains to be seen. If wetting improves scalp cooling results a lower scalp skin temperature may be expected. We have investigated whether scalp skin temperature was reduced by wetting the hair.
Material and Methods: Experiments were performed on 29 healthy subjects with a Paxman cooling device. Scalp skin temperatures were measured with thermocouples. To document tolerance a graded scale has been used; zero in case of no discomfort at all and 10 for feeling very uncomfortable. As theoretically wetting of one side may cause a lower temperature of the other side, scalp cooling was also performed without wetting. The scalp was first cooled with dry hair. After a warming up period of 30 minutes to normalize the scalp skin temperature, the cooling procedure was repeated, whereby only one half of the scalp was wetted.
Results: Lower scalp skin temperatures were observed on the wetted side compared to the dry side. The initial great differences between the scalp skin temperature of the dry and the wet side decreased. After 30 minutes this difference was 4.5ºC,(95% C.I. 3.8−5.1, $p < 0.001$). The inter-individual differences in scalp skin temperatures after 30 minutes were considerable, both in dry (range 13.9 −26.9ºC) as in wet hair (range 0.3 −20.8ºC). Only for a short period, till about 15 minutes after the start of scalp cooling, the feeling of discomfort was considerable higher when the hair was wetted.
Conclusions: This is the first study, as far as we know, that demonstrates that wetting of hair before scalp cooling actually results in lower scalp skin temperatures. This substantial lower scalp skin temperature may be relevant to the prevention of CIA as there are indications that a number of scalp cooled patients do not obtain the required scalp skin temperature to prevent CIA. The initial increased feeling of discomfort caused by wetting will be no problem if hair wetting leads to less CIA. Scalp skin temperature is substantially decreased if hair is wetted before scalp cooling. Wetting hair lowers scalp skin temperatures more rapidly which may lead to shorter pre-infusion cooling times. Further research is needed to determine if wetting leads to less CIA.
No conflict of interest.

1346 POSTER

Skeletal muscle density predicts prognosis in metastatic uterine sarcomas: an observational pilot study

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Background: Several studies have shown that skeletal muscle mass, skeletal muscle density and adipose tissue are linked to progression free survival (PFS) and overall survival (OS) in lung and gastro intestinal cancer. Because prognostic factors are not well defined in metastatic uterine sarcomas (MUS), another approach is required. Our aim was to analyze whether body composition parameters have a prognostic role in MUS.
Materials and Methods: Adipose tissue, skeletal muscle mass and skeletal muscle density (SMD) were assessed with computed tomography imaging by measuring cross-sectional areas of the tissues and mean muscle Hounsfield Units (HU). High level of HU reflects high SMD and high quality of muscle. As there is no defined threshold for SMD, we chose the one that had the best sensitivity: 29.5 HU. The population was dichotomized in two groups according to this value. OS and PFS were estimated using Kaplan–Meier method and compared with the log-rank test.
Results: In the 79 patients, median age was 56 years (range: 32−74). Histology: leiomyosarcoma (n = 53), undiffenciated sarcoma (n = 16), endometrial stromal sarcoma (n = 4), unknown (n = 6). OS was correlated with SMD: the median OS in the 24 patients with high SMD (5 years) was twice that in the 55 patients with low SMD (2.4 years) ($p = 0.04$). At 2 years OS was 58% (95% CI: 45%-70%) versus 81% (95% CI: 61%-93%) respectively for low and high SMD. Despite a trend for a better PFS for the group with a high level of SMD, the difference was not statistically significant. No associations between OS or PFS and adipose tissue or muscle mass were found.
Conclusion: High muscle density is associated with improved outcome in metastatic uterine sarcoma and could be part of a prognosis scores based on body composition parameters enhancing metastatic uterine sarcomas management. This hypothesis has to be confirmed in a prospective way.
No conflict of interest.

1347 POSTER

Palliative chemotherapy in patients with malignant bowel obstruction

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Background: Malignant bowel obstruction (MBO) is a frequent complication of gastrointestinal (GI) and gynecological cancers. It is often associated with advanced stages of disease and high tumor burden, and hence a marker of poor prognosis. It is unknown whether palliative chemotherapy (CT) can alter this unfavorable scenario.
Material and Methods: Retrospective, single institution analysis of 36 patients' chart data, hospitalized at the Instituto do Cancer do Estado de São Paulo (ICESP) from 2009 to 2013 due to MBO. All patients (pts) were not candidates for surgical intervention and were submitted to palliative CT. Primary objective was median survival after CT, estimated by Kaplan–Meier method. Secondary objectives were relief and failure rates of obstruction after CT, and toxicities associated with CT. Possible prognostic factors were analyzed by log-rank.
Results: Pts median age was 53 years (22−71). They were mostly females (67%), ECOG-PS >2 (94%), with gastrointestinal primaries (69%) and without previous systemic treatment (64%). Anemia was frequent (median hemoglobin 10.8 mg/dL, 5.3–15.6) as well as malnutrition (mean albumin 3 g/dL, 1.8−4.3). Normal bowel function after CT was achieved in 23 pts (64%) (median 13 days, 3−39), however 30% recurred MBO within one month (mo). Median overall survival was 1.97 months. About one third (36%) of pts experienced grades 3 or 4 toxicity. On univariate analysis the only factor associated with better outcome was reversal of MBO (5.8 vs. 1.2 mo, p 0.001). There was no correlation of serum albumin, creatinine, hemoglobin or ECOG with prognosis.
Conclusions: In this mostly treatment naive population no apparent clinically significant survival benefit was associated with CT. Its role in the reversal of MBO is unclear, as pts may have recovered normal bowel function with clinical measures alone. When considering palliative CT for pts with MBO, which is associated with other known poor prognostic

markers (such as anemia and malnutrition), patient selection is critical to avoid exposure to unnecessary toxicity and provide better quality of life.
No conflict of interest.

1348 POSTER

Complementary and integrative medicine therapy for chemotherapy induced peripheral neuropathy in breast cancer patients

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Background: Peripheral neuropathy is a known side effect of several chemotherapeutic drugs, mainly paclitaxel. When severe it can lead to significant disability and discontinuation of therapy with the offending drug. Neuropathy can be long lasting and recovery can take months and is often not complete. As patients feel that 'Western' medicine has little to offer as remedy, many turn to different types of complementary and integrative medicine (CIM) thrapies. In an attempt to evaluate CIM's efficacy we assessed the improvement in patients' neuropathy after treatment at our institution's CIM centre.
Methods: Thirty consecutive breast cancer patients files were retrospectively evaluated. Neuropathy was graded before and after treatment and was categorized to grade 1−2 and grade 3−4 according to the treating physician's follow up. Type of neuropathy was also recorded. Additional data gathered included patients' age, disease stage, presence and location of metastasis and chemotherapy used with total doses. All patients were treated with acupuncture on a weekly basis according to the CIM's protocol for neuropathy.
Results: Two patient's records were lacking sufficient information and not included in the analysis. The remaining patients had stage Ia-IV breast cancer with 4 patients (14%) having metastatic disease. Patients were treated with various agents including paclitaxel, docetaxel, doxorubicin, cyclophosphamide, trastuzumab and tamoxifen. Twenty one (75%) patients had grade 1−2 neuropathy and 7 (25%) had grade 3−4 neuropathy. Types of neuropathy included sensory neuropathy in 21 (75%) patients, proprioceptive neuropathy in 7 (25%) patients and unknown in 2 (7%) patients. Symptomatic improvemnt was recorded in 11 (39%) patients, with 82% improving after 3 months and 18% after 6 months from starting acupunture therapy.
Conclusions: Thirty nine percent of the cohort patients demonstrated symptomatic improvement, for either sensory or proprioceptive complaints, after acupuncture therapy. Most responders improved 3 months from starting therapy and while on continued oncological therapy. Shortcomings of this study include its retrospective nature and the heterogeneity of the oncological treatment patients were receiving. However, this high response rate indicate that acupuncture might offer a relief to patients and justifies further studies.
No conflict of interest.

1349 POSTER

Early treatment discontinuation and switching in 1st line metastatic breast cancer: Impact of symptom burden in a real world sample

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Background: Treatment options in 1st line metastatic breast cancer (MBC) vary and may include chemotherapy, targeted and hormone therapy. Some patients (pts) discontinue or switch therapies, perhaps due to treatment related toxicities. The current study examined the association of symptom burden with early treatment discontinuation or switching (EDS) of 1st line therapy of MBC in real-world settings.
Materials and Methods: Data were abstracted from medical records of pts at 9 community oncology practices. Eligible pts had stage IV MBC with start of 1st line therapy 1/2004 to 6/2012, were $\geqslant$18 years old, and had $\geqslant$1 Patient Care Monitor (PCM) survey, an 86-item survey of cancer-related symptoms, during 1st line. Age, race, HER2 and hormone status, oral and infused agents, dates of diagnosis, treatment, progression, and death were recorded. EDS was defined by direct indication of early stopping in the record, and by treatment duration $\leqslant$6 weeks or regimen change without evidence of disease progression. Cox regression of EDS with time varying covariates was used to examine the impact of 23 separate symptoms, a multivariate (MV) model with multiple symptoms, and an overall composite symptom burden score based on individual symptoms.
Results: 797 pts were included, with mean age of 58.4 years, 62.1% White; with 340 on Chemotherapy (CT), 349 on CT + Targeted therapy (T) and 108 on Hormone therapy only (H). Overall, EDS occurred in 197 (24.7%) pts, with rates highest among CT (27.9%), followed by T (26.1%) and H (11.5%). Cox regression showed that 22 of 23 symptoms each increased the risk of EDS. Across several MV models, 4−6 symptoms were retained, each reflecting distinct symptom clusters (e.g., hair loss, fatigue, numbness/tingling, mouth sores/ulcers, diarrhea). In the composite symptom burden score (median 6; range 0−22) analysis, overall symptom burden was found to be significant (HR = 1.13, $p < 0.0001$), indicating a 13% increased risk of EDS with each additional symptom. Pts with 10+ symptom score had a significantly increased risk of EDS (HR = 4.03, $p < 0.0001$) compared to pts with <5 symptom score. Pts with 15+ symptom score had the highest risk of EDS (HR = 5.12, $p < 0.0001$).
Conclusions: Pts treated with CT or T for 1st line MBC had higher rates of EDS than pts on H. The likelihood of EDS increased as the number of symptoms increased. Additional research is needed to evaluate impact of symptoms on other pt outcomes, including overall survival.
Conflict of interest: *Other substantive relationships: The study was sponsored by Genentech, Inc. A. S. Masaquel, D. Lalla, and O. D. Abidoye are employed by Genentech/Roche and have stock or stock options at Genentech/Roche. L.S. Schwartzberg has received honoraria from Genentech/Roche.*

1350 POSTER

Patient-proxy agreement of health-related quality of life (HRQOL) measurements in low-grade glioma patients

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Background: HRQOL has become an important outcome measure in clinical trials of glioma patients. Utilizing patient-by-proxy HRQOL assessment might increase data availability, provided that patient and patient-by-proxy ratings show high levels of concordance, which we aim to investigate in this study. Based on Sneeuw et al (1997), we hypothesized that 1) concordance levels are relatively high in cognitively intact patients, and 2) decrease in cognitively impaired patients, with proxies being more negative on patients' HRQOL.
Material and Methods: We analyzed cross-sectional data on HRQOL of 281 Dutch low-grade glioma (LGG) patients with stable disease who participated in a study comparing cognition and HRQOL after radiotherapy versus no radiotherapy. Proxy rating was assessed via the SF36 and EORTC BN20 in 246 patients. Data on the cognition were collected on a subgroup of 195 patients. The Bland-Altman limit of agreement (LA), mean difference (MD) [proxy minus patient], concordance correlation coefficient (CCC) and the percentage difference (PD, +/− 0, 5, or 10 points) were used to assess patient-proxy agreement. To investigate the effect of cognitive function on agreement we defined patients to be cognitively impaired (n = 66) or cognitively intact (n = 129) based on their neuropsychological performance and investigated the level of agreement via LA and MD.
Results: Patients were more negative in rating their HRQOL than their proxies in general except for the SF36 scale role emotional and social functioning and the BN20 scale future uncertainty, motor dysfunction, headaches, seizures and drowsiness. We found no statistically significant difference in MD except for the SF36 scale general health ($p = 0.03$) and BN20 scale visual disorder ($p = 0.04$). Results from the LA revealed a fairly high agreement between the patient and proxy rating in all HRQOL domains. However, a slightly poorer agreement was observed for the physical component summary (PCS) [LA; −13.63–11.03). The CCC was fairly high overall in all HRQOL domains (ranging from 0.37 to 0.80). The CCC for PCS was (r = 0.69) and mental component summary (MCS) was [r = 0.55]. The percentage of perfect agreement (PD +/− 0 point) ranged from 8.54% (general health) to 76.83% (hair loss). The PD for the PCS and MCS for a +/− 5 points and +/- 10 points was 76.42% and 65.85%, and 93.0% and 87.40%, respectively. The magnitude of the MD in the cognitively intact patient group was overall smaller and agreement by LA higher in the cognitive intact group.
Conclusion: Preliminary results suggest that there was overall a high level of agreement between patient and proxy rating of patient's HRQOL but a larger mean difference was observed in the cognitively impaired patients. Contrary to our hypothesis, patients tended to rate their HRQOL more negatively. Future research will focus on the validity of using complementary HRQOL assessments provided by proxies for patients with declining cognitive functioning.
No conflict of interest.

1351 POSTER

Internal and external validation of a fatigue prognostic score for overall survival (OS)

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Background: Fatigue is an adverse reaction related both to disease and treatment in cancer patients (pts). We previously found (ESMO2012, lecture 15450) that the F score was able to discriminate pts in "Good", "Intermediate" and "Poor" risk groups. We present the results of an internal and external validation of our model.
Methods: Patients included in the PROCHE program between 2008 and 2011 at the HEGP hospital (Paris, France). Pts were contacted before each chemotherapy (CT), and F experienced since the last cycle was collected (patient's reported outcome from CTC-NCI grading: 0=none, 1=mild, 2=moderate, 3=severe, 4=3+long-term condition). Scores were calculated as weighted means of F over the whole CT period and during the 2 and 4 first cycles. OS was calculated from CT initiation to death or censured at last contact. Cox regression covariates: age, tumor localization, disease setting and continuous or categorized Fatigue score. Patients with localized (M0) or metastatic (M+) disease during the study period led to two distinct cohorts: C1 and C0 according to M1 or M0 period considered. A bootstrap internal validation was performed on 1000 samples, followed by an external validation using Kaplan–Meier curves for risks groups.
Results: 1279 pts entered the program, 662 had at least 1 assessment of fatigue. Excluded pts (617) due to lack of survival status did not differ (log-Rank=0.98). Median age=64.9 y, sex-ratio=1.1, more frequent localization (%): lung: 25, breast: 21, urogenital: 21, gynecological: 13, ENT: 12. OS (m, 95% CI) was 27.8 (26.2–29.4). Median follow-up was 26.7m (25.5–27.9). F score was still strongly associated with prognosis after updating data. Model obtained from the initial dataset was internally validated (C-index=0.733, shrinkage=0.957). To evaluate prognostic value, 197 patients among 617 previously excluded were used for external validation. Concordance was very good (Kendall t=0.816, $p < 0.0001$). Predicted F score HR (95% CI): 0.17 (0.09–0.35) and 0.34 (0.21–0.56) for the "Good" and "Intermediate" prognosis groups, respectively; actual HR for the same groups (validation cohort): 0.19 (0.11–0.37) and 0.31 (0.19–0.52).
Conclusion: This study confirmed through internal and external validation the prognostic value of our fatigue score based on patients reported outcomes.
No conflict of interest.

1352 POSTER

Quality of life improves after placement of percutaneous tunneled drainage catheter for refractory ascites in prospective study of patients with end stage cancer

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Background: Refractory ascites in the terminal cancer patient causes debilitating symptoms often managed by serial paracenteses. Percutaneous tunneled drainage catheters (PTD) offer the advantage of home management but the impact on quality of life (QoL) has not been described.
Materials and Methods: Adult patients with stage IV or end-stage cancer undergoing PTD placement for refractory ascites were eligible for this prospective study. Subjects completed the EORTC QLQ-30 (EORTC) and McGill Quality of Life (MQoL) instruments prior to the procedure, immediately post procedure (PP) (2−7 days), and 3 weeks PP (+/− 7 days). Patients were interviewed for catheter function at the time of the instrument assessments as well as 2, 4, 6, 8 weeks (wks) and 4 and 6 months PP. Completion rates were defined as total number of completed instruments out of number of subjects surviving. QoL data were scored according to instrument manuals and analyzed using a pattern-mixture model to adjust for informative drop-out.
Results: 50 patients enrolled, 48 were evaluable of which 3 withdrew during follow up. All evaluable patients had a Tenckhoff catheter placed (Cook Inc., Bloomington, IN). Median survival post catheter placement was 1.2 months (95% CI: 1−1.8 months). For all time points, median completion rate among survivors was 88% (range: 65%-100%). Three PTD were removed for infection (26, 101, 170 days PP). Analysis of EORTC demonstrated an improvement in global QoL ($p = 0.04$), functional role ($p = 0.01$), emotional ($p < 0.01$), and cognitive ($p = 0.02$) scales at 1 wk PP. At the same time point significant symptom improvement was seen in reported fatigue ($p = 0.005$), nausea/vomiting ($p = 0.002$), pain ($p = 0.005$), dyspnea ($p = 0.001$), insomnia ($p = 0.001$) and appetite loss ($p = 0.009$). This improvement was sustained at 3 wks for dyspnea ($p = 0.006$), insomnia ($p = 0.002$), and appetite loss ($p = 0.03$). Baseline scores did not effect survival. The MQoL offered similar results with a significant overall QoL improvement at 1 wk ($p < 0.001$) and 3 wks ($p = 0.016$).
Conclusions: QoL and symptoms improved for end-stage patients after placement of a tunneled catheter to relieve refractory ascites. The benefits of placement diminish over time, likely due to progression of disease. This study supports the use of a PTD to palliate the debilitating symptoms associated with refractory ascites.
No conflict of interest.

1353 POSTER

What do patients really mean when they complain of fatigue after treatment? Reliable identification of post cancer fatigue

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Introduction: Fatigue is a ubiquitous symptom. Self-reported fatigue symptoms following cancer diagnosis and treatment are common. A definitive diagnosis of post cancer-related fatigue (PCF) is challenging due to lack of consensus on diagnostic criteria and the diversity of measurement tools currently used to identify the syndrome(predominantly self-report). A semi-structured clinical interview to reliably identify PCF and differentiate co-morbid symptoms (like insomnia or mood disturbance) has been developed.
Methods: Using both qualitative and quantitative methods, a semi-structured clinical interview to identify PCF was developed. Analogous to clinical interview schedules used in sleep medicine or psychiatry, it incorporates published diagnostic criteria for the syndromes of cancer related fatigue (CRF); chronic fatigue syndrome (CFS) and major depression. For validation, the interview was trialled in patients with clinician identified fatigue syndromes: multiple sclerosis ($n = 9$), post infectious and chronic fatigue ($n = 108$) and post cancer fatigue ($n = 30$).
Results: In the interview seven symptom domains were assessed: fatigue; fatigability; neurocognitive difficulties; mood disturbance; sleep problems; pain and other symptoms (e.g. night sweats). Symptom severity − both frequency and intensity − were identified. A diagnostic algorithm was developed to classify the symptom complexes. Sensitivity (sens) and specificity (spec) was determined against specialist-clinician diagnosis and syndrome diagnostic criteria ($n = 128$): CFS (sens 100, spec 83); major depression (100, 72) and PCF (72, 58).
Conclusion: While the interview schedule facilitates the diagnosis of clinically significant fatigue and co-morbid symptoms the lower sensitivity and specificity in those patients reporting fatigue post cancer, in comparison to those in groups diagnosed with CFS or major depression, suggests need for the further refinement of the diagnostic criteria for post cancer fatigue. Potential uses of the interview are in aetiopathological studies (identification of homogenous cases), for clinical management, and monitoring of patients participating in clinical intervention trials.
No conflict of interest.

1354 POSTER

A prospective investigation of nutritional status of ambulatory Irish oncology patients undergoing chemotherapy: prevalence of malnutrition, cachexia, sarcopenia and impact on quality of life

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Background: Malnutrition is a significant factor in predicting quality of life (QoL) in oncology patients. Our study describes the general health of Irish cancer patients (pts), including QoL, and determines the prevalence of cachexia, malnutrition, and sarcopenia in ambulatory pts undergoing chemotherapy in a regional cancer centre.
Methods: A prospective cross sectional study of ambulatory adult cancer pts, undergoing chemotherapy at a university teaching hospital was conducted. The risk of malnutrition was examined using the Malnutrition Universal Screening Tool (MUST). QoL was measured using the European Organisation for Research and Treatment of Cancer QoL Questionnaire (EORTC QLQ-C30). In addition, CRP (C-reactive protein) levels were recorded. Cancer cachexia was defined as weight loss (WL) >5% over the

past 6 months or WL >2% in combination with a Body Mass Index (BMI) <20 kg/m^2 or sarcopenia. Skeletal muscle cross-sectional area at L3 was measured on baseline CT scan. Sarcopenia was defined using published cut offs.
Results: 150 pts (96 male) with solid tumours, mean age 64 yrs, were included. 52.7% were overweight/obese (BMI>25 kg/m^2). Frequency of cancer subtypes was recorded: colorectal cancer(35%), upper gastro-intestinal cancer (GI, 31%), and lung cancer (15%) were the most common. Sarcopenia was present in 54% (38% of normal BMI, 40% of overweight groups) with fatigue significantly associated with sarcopenia in males ($p < 0.05$). Sarcopenia and reduced adipose tissue index were significantly associated with adverse QoL ($p < 0.05$) in females but not in males. Overall 73% met the criteria for cachexia, with the highest prevalence of cachexia observed in hepatobillary (86%) and upper GI cancers (79%). Using MUST, 42% of pts were classified as at risk of malnutrition (n = 63) and appetite loss scores were worse in malnourished patients than in non malnourished ($p < 0.05$). WL ($\geqslant$5%) in the previous 6 months was reported by 34%, 45% and 50% of pts with colorectal, lung and upper GI tumours, respectively. Furthermore, WL ($\geqslant$5%) in the previous 6 months was significantly associated with loss of appetite ($p < 0.05$), low fat free mass ($p < 0.05$) and advancing age (65 yrs vs. 61 yrs $p < 0.05$). 55% had a CRP >10 mg/L which was significantly associated with adverse QoL ($p < 0.05$). There was a significant association between cancer type and poor QoL ($p < 0.05$) with upper GI cancers reporting the worst global QoL scores.
Conclusion: Cancer patients undergoing chemotherapy experience weight loss, sarcopenia and a high percentage are cachectic in an inflammatory milieu. Site of primary tumour appears to be associated, at preliminary analysis, with significant weight loss, nutritional risk and QoL. Early nutritional screening is warranted in cancer patients.
No conflict of interest.

1355 POSTER

A prospective analysis of the association between skeletal-related events and quality of life in patients with advanced lung cancer (CSP-HOR13)

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Background: Bone metastasis (BM) is a frequent complication in patients with advanced lung cancer. We had reported incidence of BM and **skeletal-related events** (SREs) in patients with advanced lung cancer as a prospective study in the previous meeting, while there have still been few reports on the association between SREs and quality of life (QOL). The aim of this study was to investigate prospectively how QOL of patients with advanced lung cancer was affected by SREs.
Material and Methods: The eligibility criteria are newly-diagnosed patients with stage III B or IV lung cancer, whose ages were over 20-years old, and those who had a written informed consent. The patients were closely followed up in every four weeks to see if they developed SREs. QOL questionnaires were conducted at the time of the enrollment, three- and twelve-months later, and one month after the onset of SREs, using QOL scores including EQ-5D, FACT-G and Barthel Index. Then each QOL score was analyzed. Treatment for lung cancer and use of zoledronate were done at the discretion of the investigator. We evaluated QOL of the patients who developed SREs. SREs are defined as pathologic fracture, radiation or surgery to bone lesion, spinal cord compression or hypercalcemia.
Results: 274 patients were enrolled in this study from April 2007 through December 2009 (median age was 68-years old). Small/non small cell = 77/197. Stage IIIB/IV = 73/124, Male/female = 193/81. The median follow-up period was 13.8 months. 78 patients already had BM at the enrollment. Among them, 24 had accompanying SREs and another 12 developed SREs during the follow-up. Among 196 patients without initial BM, 34 developed BM. 16 patients of these 34 developed SREs during the follow-up.
A chronological analysis did not show statistically significant difference in QOL of all patients in whom the QOL evaluation was performed. QOL data were collected in nine patients out of 28 who had SRE during the follow-up. For those nine patients, QOL scores fell by 0.05 in EQ5D, by 9.4 in FACT-G, and by 6.9 in Barthel Index. Statistically, these declines in QOL scores subsequent to SREs were not significant. Analysis on FACT-G by four factors (physical, social/family, emotional, functional), however, showed that the emotional factor decreased by 4.76, which was statistically significant.
Conclusion: QOL of the patients with advanced lung cancer was not proven to have been affected by SREs when measured by EQ5D, FACT-G and Barthel Index. However, the evaluation by each four factor of FACT-G revealed that the statistically-significant decline in the emotional factor after SREs.
Conflict of interest: *Ownership: Yuko Saito is Clinical Trial Head at Novartis pharma K.K.*

1356 POSTER

Be positive, it can help you! The role of positivity on quality of life of cancer patients

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Background: Positive beliefs and emotions among people experiencing adversities are crucial to manage the emotional costs associated with negative events. Self-esteem, life satisfaction, and optimism to well-being, all traced to the common factor of Positivity, correspond to pervasive views of experience, highly correlated to each other and commonly associated with various domains of functioning and across the life-span.
The Authors examined the function of positivity and proper management of positive emotions in buffering the progress of illness, the recovery and the quality of life (QoL) in cancer patients.
Material and Methods: 110 patients with pulmonary, colorectal and breast cancer, aged 40−70, have been prospectively enrolled between 2012 and 2013, at the S. Andrea Hospital in Rome. Patients with previous diagnosis of other malignancies and psychiatric disorders were excluded from the analysis. Positivity was assessed by the Positivity scale (Caprara et al., 2012), EORTC QLQ-C30 was used for the QoL and physical functioning evaluation and the Mini-mental adjustment to cancer scale for the patients coping style assessment.
Results: Level of positivity is significantly associated to better QoL ($r=-.26$, $p < 0.01$) and less reported symptoms of worse physical ($r=-.26$, $p < 0.01$), cognitive ($r=-.30$, $p < 0.01$) and emotional functioning ($r=-.25$, $p < 0.01$). Moreover, positivity is related to the implementation of coping strategies focused on good illness management. In particular, patients with medium-high level of positivity tend to cope actively the illness($r=-.32$, $p < 0.01$) and to report less feelings of hopelessness($r=-.36$, $p < 0.01$). Furthermore, hierarchical regression analysis showed the role of positivity in predicting the impairment in physical functioning, after controlling for sex, type of diagnosis and coping strategies. Finally, a significant interaction between sex and positivity was found, showing that males tend to report less impairment in physical functioning than females.
Conclusions: According to previous studies, our results support the hypothesis that psychological characteristics may influence the QoL of cancer patients. In particular, in our sample, the level of positivity is associated to better QoL, less impairment in physical, cognitive and emotional functioning.
Positivity can predict QoL in cancer patients, and can support physicians to promptly identify patients that could need psychological support.
No conflict of interest.

1357 POSTER

Ocular disorders in long-term survivors

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Background: To describe the frequency of ocular disorders in long-term cancer survivors ($\geqslant$3 years) observed in Interdisciplinary Units and evaluate the relationship with comorbidities.
Patients and Methods: In 118 consecutive long-term survivors with different cancer type we identified 18 patients, surviving $\geqslant$3 years after primary diagnosis, who reported ocular sequelae. All patients received multimodality treatments, including surgery (n = 17, 14%), conventional chemotherapy (n = 12, 10%), radiotherapy (n = 6, 5%), hormonal therapy (n = 9, 7.6%) and biological treatment (n = 2, 1.69%).
Results: Of 18 patients, 22% (n = 4) were males and 77% (n = 14) females, median age 62.5 (range 35−84 years); of them, 11 were affected of breast cancer, 3 of colorectal cancer, 1 of prostate cancer, 1 of kidney cancer, 1 of testicular cancer, 1 of GIST. 16.6% (n = 3) patients reported diabetes non-insulin-dependent, 33.3% (n = 6) hypertension in medical treatment. In particular, we have seen that 6 patients (33.3%) reported cataract, 7 (38.8%) visual deficit, 2 (11%) myopia, 1 (5.5%) keratoconjunctivitis, 1 (5.5%) retinal detachment and 1 (5.5%) periorbital edema.

Conclusion: The ocular complications may affect the quality of life of these patients and need more attention in clinical practice, especially in consideration of the use of new biological drugs.
No conflict of interest.

1358 POSTER

Efficacy of tapentadol for managing severe pruritus related biological cancer treatments: Multicentric experience

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Background: Severe pruritis affects a large proportion of the cancer patients treated with anti-EGFR antibodies and tyrosine-kinase inhibitors. Tapentadol is a centrally acting analgesic with 2 mechanisms of action, μ-opioid receptor agonism and norepinephrine reuptake inhibition. We designed a multicentric study to asses of Tapentadol for the management pruritis induced by biological treatments.
Material and Methods: In this multicentric study we enrolled 30 patients with metastatic solid tumours treated with biological drugs between November 2012 at February 2013. Intensity of itch was evaluated by Visual Analogue Scale (VAS) score. The primary endpoint was change in median VAS score during treatment with biological drugs. All patients were enrolled in the failure of standard therapies for itch. All patients received tapentadol 50 mg cpr bid.
Results: Median VAS was 9.00 at baseline and 1.00 after 3 days of treatment. 25 patients responded to Tapentadol. the only side event was experienced nausea G1 resolved in a week.
Conclusions: Tapentadol showed excellent efficacy in the control of pruritus associated with the use of biological drugs. is not a minor reduction on the quality of life of pain associated with hand–foot syndrome typical of TKI inhibitors.
No conflict of interest.

1359 POSTER

To assess the effect of regular exercise, involvement with art (origami) and group therapy on quality of life, anxiety, depression, patient satisfaction and hope levels in patients with remission who had a variety of cancer diagnoses "quality of life support program (QoLSP)" was developed

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Informed consents and demographic information was compiled prior to the onset of study, HOPE, HADS (Hospital anxiety and depression) and EORTC QLQ-C30 (30th question assessing the quality of life) quality of life scales were used at the beginning and at the end of the study. Apart from these scales, program satisfaction and scales evaluating the arm use in breast cancer patients were also used. QoLSP consisted of excercise on Mondays, art therapy (origami) or group therapy on Wednesdays (rotating once a week) and home excercise on Fridays.
A total of 26 patients took part in this study, 23 of whom were female and the remaining 3 were male patients. Twenty patients were diagnosed as breast cancer, 2 patients had cancer of the overy and the remaining four had endometrium, colon, larynx cancer and soft tissue sarcoma.
When the analyses were completed, average hope scores of the patients were 25.6±3.9 and EORTC QLQ-C30 scale 30th question average score was found as 5.1±1.5 before QoLSP. After the QoLSP, average score for the HOPE scale was raised to 29.7±1.9 and EORTC QLQ-C30 scale's 30th question score average was increased to 6.6±0.82. Before QoLSP, HADS anxiety scale score was 8.2±4.7 on the average and HADS depression scale average score was found as 6.4±4.4. After QoLSP HADS anxiety scale score average was decreased to 3.5±3.3 and HADS depression scale score average was down to 2.5±3.2. All differences in scale scores between before and after QoLSP program were considered as statistically significant ($P < 0.001$).
After QoLSP, factors which might have been involved with quality of life were also evaluated. In multivariate analysis, hope score differences were statistically significant when compared with preliminary hope scale ($\beta = -0.89$, $t = 11.21$, $p < 0.001$), which is also true for anxiety score differences when compared with HADS preliminary anxiety scores; and global QoI preliminary scores ($\beta = -0.55$, $t = -3.65$, $p = 0.001$ and $\beta = 0.33$, $t = 2.2$, $p = 0.038$ respectively), depression score differences according to HADS depression scale preliminary results ($\beta = -0.74$, $t = -5.39$, $p < 0.001$), as well as Global QoI score differences according to Global QoI preliminary results ($\beta = -0.88$, $t = -9.22$, $p < 0.001$) were also statistically significant.
In the study population 6 patients had breast sparing surgery + lymph node dissection out of 20 patients who were operated for breast cancer. When assessed arm movement of 3 patients (out of the 6) have been defined as 'much beter', 2 patients' arm movements were 'better' and 1 patient reported 'no change'. Out of 14 patients who had modified radical mastectomy 13 had rated their arm movements as 'much beter' and 1 reported arm movement as 'beter'.
When program satisfaction was assessed via a questionnaire, all of the 26 participants rated themselves as 'highly satisfied'.
QoLSP a multidisciplinary activity, if established in other centers, can have very positive influence on cancer patients' quality of life, anxiety, depression, hope and patient satisfaction by having an active part in diagnosis and treatment.
No conflict of interest.

1360 POSTER

The experience of partners of cancer patients' participation in a phase I study after the patients' death: A retrospective study

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Background: Research data on the impact of patients (pts)' participation in a phase I study on the well-being of their partners after death is scarce. Yet, partners' well-being is essential as it can affect their mental and physical health. This study aimed to explore the experience of partners of deceased pts who participated in a phase I study and investigate their well-being after pts' death.
Patient and Methods: 63/74 (85%) eligible partners of deceased pts participating in a phase I study between 2007 and 2010 agreed to participate. Between 0.5 and 2 years after bereavement, they completed 5 self-assessment questionnaires, the RAND-36 Health Survey (RAND-36), Shortened Fatigue Questionnaire (SFQ), Beck Depression Inventory for Primary Care (BPI-PC), Inventory of Traumatic Grief (ITG) and the Hospital Anxiety and Depression Scale (HADS). Furthermore, they completed a general questionnaire (GQ) about their experience on the participation in a phase I study.
Results: Participants had a mean age of 58 years (range 37−82), 67% was female. 58/63 partners returned the questionnaires. Retrospectively partners reported negative effects on the pts' QoL (14/58), burdensome side effects (21/58) and burdensome increase of visits to the outpatient clinic (16/58). In contrast, 55/58 partners did not regret participation of the pts in a phase I study, where 24/58 partners even reported a positive effect on the pts' QoL. Regarding the impact that the disease and it's treatment have on partners after pts' bereavement, the GQ reports that 8/58 partners experienced a decline in their general health after bereavement compared to the time before the diagnosis. In addition, the RAND-36 showed significantly lower average scores on 2 subscales compared to normative data, namely limitations in social functioning ($p < 0.011$) and mental functioning ($p < 0.004$). Assessment of severe fatigue, depression, complicated grief and distress showed no abnormalities on the SFQ, BPI-PC, ITG and HADS respectively.
Conclusion: Even though one third of the partners reported burdensome consequences and negative effects on the QoL of pts during participation in a phase I study, retrospectively most of the partners did not regret pts' participation in phase I studies. Assessment of the well-being of the partners after pts' death shows, aside from significant differences compared to normative data on social and mental functioning, no abnormalities.
No conflict of interest.

1361 POSTER

Chemobrain in patients participating in clinical trials

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Background: The observation that cytotoxic drugs given systemically for non-CNS tumors might have neurotoxic effects on cognitive functioning was made decades ago.The term chemobrain or chemotherapy-induced cognitive impairment is recognized as a common adverse effect of chemotherapy. In the past years, the number of clinical trials has increased rapidly in Croatia and the actual degree of understanding or perceptions of

clinical trial participating is unknown. Aim of this study is to evaluate does patients which signed informed consent to participate in clinical trials (both, academic and sponsored) have different chemobrain status than other.
Methods: Adult cancer patients receiving chemotherapy in General Hospital Pula between January 2010 and December 2012 were included. In experimental arm were 32 adult patients with advanced cancer, ECOG PS 0−3, without CNS involvement which signed clinical trial ICF. In control arm were 92 patients matched for same conditions as experimental arm patients (matched for location, age, stage, gender, ECOG PS, fatigue, anemia, and chemotherapy line.Cognitive impairment was detected using cognitive tests HVLT-R, TMT, and COWA after signed Informed consent form (ICF). After approval of sponsors and conductors of clinical trials, for using some data from trials, patients in both arms were evaluated.
Results: Median age was 63.5 years, 39% were female, and 11% had poor ECOG PS ($\geqslant$2). Patients had advanced solid tumors (Lung: 32%; colorectal: 27%; breast: 15%; other solid tumors: 26%). Average time of follow up and chemotherapy were 14.5 and 6.7 months, respectively. Patients were well balanced between arm in age, gender, overall survival (8.7 months), performance status, locations of tumors, stage, anemia, number of chemotherapy lines, and fatigue (FACIT-F test result). There were less cognitive impairment in term of chemobrain (detected with HVLT-R, TMT, and COWA tests) in experimental arm than in control arm 21.9% and 39.1% of patients, respectively ($p < 0.05$). Also, patients in control arm had trend to be more anemic (21.9% vs 31.5%) but not statistically significant ($p = 0.07$).
Conclusion: This is, to our knowledge, the first evaluation of chemobrain in patients inside and outside of clinical trials. Cognitive impairment could significantlly influence willing to participate in clinical trials independently of clinical trial eligibility criteria. This data provides more light on importance of psycho-oncological estimation of patients affected by cancer.
No conflict of interest.

1362 POSTER

Alternative and complementary treatments in cancer patients

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Background: Alternative and Complementaries therapies (CT) are used widely, especially among cancer patients. Insufficient clinical research data exist to indicate whether CT is safe and efficacious. The purpose of this study was to determine the prevalence and the impact of sociodemographic and clinical factors on CT use among cancer patients.
Methods: A cross-sectional survey was conducted in a sample of adults with cancer seeking care at Instituto Oncologico Nacional within a 4-month period. Univariate and multivariate analyses were performed to detect differences between CT users and nonusers.
Results: 421 pts were interviewed. Median age was 57 years. 69% female, 31% male. Breast (34%) and gastrointestinal cancer (14%) were the most frequent tumors. 47% of pts were within the first year of diagnosis. 128 pts (30%) used CT. The most common was herbal remedies (38%). Median use time was 4 months. Factors associated with CT use were female gender ($p = 0.04$), recent diagnosis ($p = 0.001$), higher education level ($p = 0.001$) and income ($p = 0.08$). The main reason for CT use was to assist conventional treatments (45%). 50% of CT users did not informed to their physician about its use. In 43% of cases the use of CT was recommend by friends and family. Multivariate analysis showed that higher education (OR 2.01 95% CI 1.18–3.43) and recent diagnosis (OR 2.03 95% CI 1.26–3.27) were predictive for CT use.
Conclusion: Educational level and recent diagnosis are associated with CT use. Patient and physician communication regarding potential risk and benefit is highly recommended.
No conflict of interest.

1363 POSTER

Decreasing anxiety with welcoming workshops in a radiation oncology unit

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Background: In recent years the treatment of cancer has changed as more and more people are treated and the greater number survive the disease. The main goal of cancer treatment is survival, but also the treatment of symptoms and quality of life of patients and their family members also become important. Cancer treatment by radiotherapy is an opportunity to control, improvement or cure of the disease, but also involves facing major challenges that can affect their quality of life and emotional state. In order to improve the emotional care, we have begun work on workshops within the welcoming plan of our unit, where through group techniques and information received as well as the opportunity to resolve doubts and feel heard, we improve the management of anxiety in patients and their families, and therefore, dealing with the treatment and disease.
Material and Method: We choose patients who will receive radiation treatment in our unit as long as they are not palliative, and invite their main caregiver too. After signing the informed consent of the study, are given a first HADS questionnaire (Hospital Anxiety and Depression Scale) and then they assist to the workshop. The first day of radiotherapy answer a second test and the third is answered at the end of radiation therapy. The three test results are compared.
Results: Data shows that at baseline levels of anxiety and depression are very high, and decrease at the beginning and as the treatment goes on. This study demonstrated that the welcoming workshop decreased symptoms of anxiety and depression during radiation therapy, In addition there was a high prevalence of anxiety and depressive symptoms in family caregivers groups, and also was improved.
Conclusions: The emotional state, in particular anxiety, is a variable that should be assessed throughout the entire radiotherapy process as it affects the welfare status of patients and caregivers. The welcoming workshops, as support groups, are affective in treatment of the oncological disease.
No conflict of interest.

1364 POSTER

Symptom clusters and demographic characteristics in advanced cancer

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Background: Little is known about demographic variations in cancer symptom clusters (SC). Our objective was to determine whether SC are associated with age, gender, race, performance status (PS), or primary cancer site.
Material and Methods: Symptoms from 1000 advanced cancer patients referred to a palliative medicine program were recorded prospectively. Among 922 patients with complete symptom data, hierarchical cluster analysis identified 7 SC. A SC was considered present if the patient had $\geqslant$50% of the symptoms in the cluster. Comparisons were made between patients with and without each cluster using the chi-square test (age <65 vs. $\geqslant$65 years; gender female (F) vs. male (M); race Caucasian (C) vs. African American (AA); 10 primary site groups (PSG), or Wilcoxon rank sum test (ECOG PS 0−4). A p value <0.05 indicated statistical significance.
Results: 83% of patients were C, 52% $\geqslant$65 years, 56% M, and 55% ECOG PS 3−4 Most common PSG were lung (25%), genitourinary (18%), and gastrointestinal (GI) (11%). Fatigue/anorexia-cachexia cluster was associated with race (58% AA vs. 68% C, $p = 0.032$) and PSG (range 47% melanoma to 83% pancreas, $p = 0.012$); Neuropsychological cluster was associated with older age (29% $\geqslant$65 vs. 39% <65, $p < 0.001$) and race (22% AA vs. 36% C, $p = 0.001$). Upper GI cluster was associated with female gender (16% M vs. 22% F, $p = 0.035$) and PSG (range 8% Head & Neck to 32% pancreas, $p = 0.035$). Nausea/vomiting cluster was associated with younger age (35% $\geqslant$65 vs. 43% <65, $p = 0.010$) and female gender (33% M vs. 47% F, $p < 0.001$). Aerodigestive cluster was associated with male gender (36% F vs. 44% M, $p = 0.010$) and PSG (range 24% pancreas to 58% Head & Neck, $p < 0.001$). Debility cluster was associated with race (33% AA vs. 44% C, $p = 0.016$) and poor PS (range 17% PS0 to 54% PS4, $p < 0.001$). Pain cluster was associated with younger age (88% $\geqslant$65 vs. 92% <65, $p = 0.028$).
The table briefly summarizes the associations that are significant at P<0.05.

Cluster	Age	Gender	Race	Performance status	Primary Site
Fatigue/anorexia-Cachexia					+
Neuropsychological	+		+		
Upper GI		+			+
Nausea/vomiting	+	+			
Aerodigestive		+			+
Debility			+	+	
Pain	+				

Conclusions: We identified 7 SC whose prevalence were influenced by age, gender, race, PS, or primary cancer site. This supports the clinical relevance of the cluster concept in palliative and supportive care. Demographic characteristics may warrant different clinical approaches to patient care. Identification of these differences may help develop more effective cancer treatment and management strategies.
No conflict of interest.

1365 POSTER

Denosumab treatment of hypercalcemia of malignancy (HCM) in patients not responding to bisphosphonate therapy

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Background: HCM, resulting primarily from tumor-induced bone resorption, is commonly treated with intravenous (IV) bisphosphonate therapy but patients may relapse or not respond. We present results evaluating denosumab for treatment of HCM in patients who remained hypercalcemic despite IV bisphosphonate by bone metastases (BM) or PTHrP status at study baseline (ClinicalTrials.gov NCT00896454; May, 2009; sponsor Amgen Inc.).
Material and Methods: In this single-arm, open-label study, patients with HCM (corrected serum calcium [CSC] >12.5 mg/dL; CTCAE grade $\geqslant$3) despite IV bisphosphonate treatment $\geqslant$7 and $\leqslant$30 days before screening, received subcutaneous denosumab 120 mg on days 1, 8, 15, and 28, then every 4 weeks. The primary endpoint was the proportion of patients with CSC $\leqslant$11.5 mg/dL (CTCAE grade $\leqslant$1) within 10 days of denosumab initiation.
Results: The study enrolled 33 patients (64% men; mean age 60 years; 39% with BM; median PTHrP 4.2 pmol/L). Median baseline CSC was 13.7 mg/dL and median time from last bisphosphonate treatment to enrollment was 17 days. By day 10, 21 patients (64%) reached CSC $\leqslant$11.5 mg/dL, with a total of 23 patients (70%) over the course of the study; estimated median response duration was 104 days. Of patients without BM, 14 of 20 (70%) reached CSC $\leqslant$11.5 mg/dL by day 10 compared with 7 of 13 (54%) with BM. The estimated median time to reach CSC $\leqslant$11.5 mg/dL was 8 days for patients without BM and 11 days for patients with BM. Of patients with PTHrP $\leqslant$4 pmol/L, 10 of 12 (83%) achieved CSC $\leqslant$11.5 mg/dL by day 10 compared with 6 of 12 (50%) with PTHrP >4 pmol/L. The estimated median time to CSC $\leqslant$11.5 mg/dL was 8 days for both the $\leqslant$4 pmol/L and the >4 pmol/L PTHrP groups. The most frequently reported serious adverse event was hypercalcemia (5 patients, 15%). Two patients had isolated episodes of CSC levels $\leqslant$8.0 mg/dL and no patients had CSC <7.0 mg/dL.
Conclusions: Denosumab lowered CSC to CTCAE grade $\leqslant$1 in 64% of patients within 10 days and induced durable responses in patients with HCM not responding to IV bisphosphonate treatment. Denosumab decreased CSC levels in patients independent of BM or PTHrP status. These results suggest that denosumab may offer a new treatment option for HCM in this challenging patient population.
Conflict of interest: *Ownership: none. Advisory board: none. Board of directors: none. Corporate-sponsored research: Amgen Inc. Other substantive relationships: W.Ying, A.Braun, and R.Jain are employed by and own Amgen Inc. stock.*

1366 POSTER

Palliative effect of MR guided focused ultrasound (MRgFUS) on patients with bone metastases previously receiving sham treatment

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Background: Radiation therapy (RT) is the primary treatment for most patients with painful bone metastases. Many patients have persistent or recurrent pain after RT or are not candidates for RT. Magnetic resonance guided focused ultrasound (MRgFUS) combines non-invasive focused ultrasound with MR guidance. We showed in a multi-center phase III trial significantly greater pain relief for patients treated with MRgFUS (67% response) compared to controls receiving sham treatment (21% response). Non responders on the placebo arm were unblinded and offered MRgFUS. We analyse here the response to MRgFUS in these patients who acted as self-control.
Materials and Methods: Patients with a painful bone metastasis amenable to MRgFUS treatment and NRS pain score >4 for whom RT was not considered appropriate (e.g. prior RT to target site) were randomized 3:1 to MRgFUS or sham. Subjects were followed for 3 months. Sham subjects without pain relief after 2 weeks were offered crossover to MRgFUS treatment. Significant pain response was defined as decrease in worst pain NRS score >2 from baseline without increase in pain medication. Quality of life (QOL) including BPI-QOL, and safety were also evaluated.
Results: Blinding of sham subjects was excellent; 94% of MRgFUS and 88% of sham subjects believed they had received MRgFUS treatment. 18 of 28 non-responders on the placebo arm elected to receive MRgFUS therapy. One patient did not complete MRgFUS therapy due to transient pain exacerbation during treatment. There were no other side effects. 13 of 17 treated patients (76%) had significant pain reduction. 15 patients were followed for the 3 month study period, follow up was discontinued for 2 patients after 2 months. One died of disease progression at other sites and one received additional RT. Mean NRS pain score of all 17 patients improved from 7.6 after sham treatment and before MRgFUS treatment to 3.1 at 2 months after treatment and 2.7 three months after treatment. BPI-QOL score improved from a mean of 5.8 before MRgFUS to 3.4 at 2 months and remained stable.
Conclusions: MRgFUS is well tolerated and results in significant durable pain relief, improvement in QOL, and function for patients with metastatic bone pain who are not candidates for RT. This analysis of patients who did not respond to prior sham treatment confirms that pain relief after MRgFUS is not due to placebo effect. MRgFUS should be considered for patients with painful bone metastases when RT is contraindicated.
Conflict of interest: *Corporate-sponsored research: This phase III study was sponsored by Insightec Ltd.*

1367 POSTER

Weight loss in solid tumors: Clinical features and prognostic importance

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Background: Large cancer databases provide valuable information on weight change and its impact on different clinical parameters. Body weight change in adults with solid tumors examined in outpatients. Objective was to determine if demographics, clinical and biochemical indices predicted weight loss (WL). Examine if WL and related parameters were prognostic for survival.
Material and Methods: Electronic medical records (EMR) for outpatient visits from a tertiary cancer center retrospectively reviewed. Body weight and other clinical parameters on first visit (V1) − within a year post diagnosis − last visit (V2) $\geqslant$3 weeks after V1. WL at V2 from V1 categorized as: $\leqslant$5%, 5.01−10%, >10%. Logistic regression and Cox proportional hazards analysis identified risk factors for WL and prognosis.
Results: N = 5901; Mean age ($\pm$SD): 61$\pm$12 years; 82% were Caucasians; 16% African Americans. Common cancers were genitourinary (GU) 31%; gastrointestinal (GI) 16%; breast 15%; lung 15%; head and neck 6%; brain 5% and others 12%. Metastatic disease in 18%. Bone, brain, lymph nodes − common. 45% had radiotherapy and

41% chemotherapy. Median (min, max) weight, kgs: V1 = 81 (32.0, 223), V2 = 79 (34, 221). Median duration (min, max), days V1→V2: 195 (22, 1080).
Weight loss V1→V2: ⩽5% (73%), 5.01−10% (13%) and >10% (14%). Median change in BMI V1→V2: −0.2 (−19, 13). Median change systolic/diastolic blood pressure (BP) V1→V2: −3 (−99, 80)/−1 (−57, 47). Change in REE V1→V2: −13 (−890, 365). Median survival for 5.01–10.0% WL = 9.4 months, >10.0% = 5.3 months and not observed for ⩽5%.
Conclusions:
1. Majority lost ⩽5% of body weight by V2
2. Head & Neck and GI cancers (primary) − the greatest risk of WL; breast − lowest
3. High BMI predicted greater WL compared to normal or underweight
4. ⩽5% WL had a survival advantage 5.01−10% and >10%
5. WL remained prognostic for survival after adjusting for other prognostic factors

No conflict of interest.

1368 POSTER

Malignancy-related hypercalcemia in the bisphosphonate era

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Background: Malignancy-related hypercalcemia (MRH) is associated with a poor prognosis. With advances in supportive care and cancer treatment, the impact of such occurrence might have diminished. Bisphosphonates (BP) play a role in symptom control but survival gain is unknown. In order to assess the prognostic implication of MRH in a present population, this retrospective analysis was performed.
Methods: Charts of 310 consecutive patients (pts) hospitalized due to symptomatic hypercalcemia in a single tertiary institution were retrospectively reviewed, from 2009 to 2012. All patients had solid tumors and serum ionized calcium (iCa) >5.5 mg/dL or total Ca >10.5 mg/dL. The Kaplan–Meier survival curves, long-rank test and the Cox regression model were used for analysis.
Results: 310 pts were included with a median age 58 y.o., most with diagnosed squamous cell carcinoma (61%) and ECOG-PS >1 (96%). 141 pts (45%) had no previous chemotherapy (no CT) and mean iCa 6.8±0.9 mg/dL. Most frequent primary sites were head and neck (27%), lung (15%), esophagus (10%) and breast (10%). At presentation, 171 pts (55%) had altered mental status (AMS); median Hb 9.7 g/dL (3.9–15.4), C-reactive protein (CRP) 129 mg/L (3–448, NV <5), albumin (alb) 2.9 g/dL (1.6−4.5) and creatinine clearance 66 mL/min (9.7–199). Hypercalcemia episodes ranged from 1−5 (median 1). 245 pts (79%) were treated with pamidronate and 11 (4%) with zoledronic acid. No difference in overall survival was seen between those pts treated or not treated with BP (HR 0.65, p 0.7). Subsequent CT was administered to 99 pts (32%). Median OS was 40 d (95% CI 33−47 d). Pts with ECOG-PS >1, AMS, CRP >30, alb <2.5 or body mass index <18 kg/m^2 had significantly poorer survival. Longer OS was related to treatment-naive pts, subsequent CT and breast primary. On multivariate analysis, subsequent CT led to a survival improvement (HR 0.23, 95% CI 0.14–0.38, p < 0.0001).
Conclusions: MRH implicates a dismal prognosis in pts with solid tumors, despite administration of BP. Even so, pts treated with CT had a better survival, suggesting that appropriate treatment of selected pts can alter the course of this syndrome.
No conflict of interest.

1369 POSTER

Should patients with advanced colorectal cancer and ECOG 3/4 be treated with chemotherapy?

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Background: Patients (pts) with metastatic colorectal cancer (mCRC) and ECOG 0/1 benefit from chemotherapy (CT). However, the impact of CT on the overall survival (OS) of ECOG 3/4 pts remains uncertain, since they are generally excluded from clinical trials.
Material and Methods: We retrospectively analyzed all consecutive mCRC pts who started first line CT at our institution in a 4-year period. The objectives were to compare the OS of pts with ECOG 3/4 who underwent CT with those receiving best supportive care (BSC) only and to compare the outcomes of these pts with those with ECOG ⩽2. Multivariable Cox regression model was used to verify prognostic factors and logistic regression, to identify predictive factors for grade 3/4 toxicity.
Results: From Jun/2008 to Jun/2012, 240 consecutive pts were included: 72 pts had ECOG 2, 54 ECOG 3 and 11 ECOG 4. Among pts treated with CT, the median OS was: 18.4 months for ECOG 0/1, 10.8 months for ECOG 2 and 6.8 months for 3/4. For pts with ECOG 3/4, CT led to a non-significant OS gain (median: 6.8 vs 2.3 months for BSC; p = 0.13). Factors significantly associated with worse OS were right-sided tumors (HR: 2.97; p = 0.005), ECOG 2 (vs ECOG 0/1, HR: 1.67; p = 0.025) and ECOG 3/4 (HR: 2.67; p < 0.0001). The rate of grade ⩾3 toxicities during first cycle did not differ significantly whether ECOG 3/4 pts received or not CT; likely because 40% of them received upfront dose-reduced CT. This was confirmed by multivariable analyses. While 70% of ECOG 3/4 pts were hospitalized during the first cycle, only 19% were admitted due to CT toxicity. This rate was similar across other ECOG groups.
Conclusions: Despite the small sample size, it seems that palliative CT may benefit selected pts with mCRC and poor performance status, without resulting in an increase in the risk of major adverse events when a reduced CT dose is used and pts are closely followed for toxicity. Further research may help to better identify the best candidates for CT.
No conflict of interest.

1370 POSTER

Impact of race on cancer symptom profiles and survival in advanced cancer

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Background: Racial differences have not been investigated in detail in advanced cancer. In this study, we first examined whether race had an influence on cancer symptom prevalence and severity. Secondly, we investigated whether survival differed by race.
Material and Methods: 38 symptoms were assessed in 1000 consecutive advanced cancer patients referred to a palliative medicine program. Moderate/severe symptoms may be more clinically relevant than overall prevalence; hence, they were grouped together and referred to as 'clinically important'. Race was unknown in 30 patients. Age was compared between 167 African-Americans (AA) and 803 Caucasians (C) with the t-test. Gender and primary site groups (PSG) were compared with the Chi-square test. Performance status (PS) and symptom severity was compared with the Wilcoxon rank sum test. Survival after referral was estimated using the Kaplan–Meier method and compared with the log-rank test. A p value ⩽0.05 indicated statistical significance.
Results: Age, gender, PS, and PSG did not differ between AA and C (p⩾0.36). AA had less edema (21% vs. 30%, p = 0.02), depression (27% vs. 44%, p < 0.001), anxiety (14% vs. 26%, p = 0.001), tremors (1% vs. 6%, p = 0.018), anorexia (57% vs. 67%, p = 0.022), and dry mouth (48% vs. 59%, p = 0.01) than C, but more headache (17% vs. 11%, p = 0.035). Severity of 5 symptoms was lower in AA relative to C: edema (p = 0.038), depression (p < 0.001), anxiety (p < 0.001), tremors (p = 0.048), and dry mouth (p = 0.021). AA had more clinically important weight loss (24% vs. 16%, p = 0.024), but less depression (12% vs. 23%, p = 0.002) and anxiety (3% vs. 13%, p = 0.001) than C. AA had longer survival than C (median 2.4 vs. 1.6 months, p = 0.006).
Conclusions: We identified 7 symptoms whose prevalence and/or severity was associated with race. Caucasians had more common and severe edema, depression, depression, anxiety, tremors, and dry mouth. AA had less moderate/severe weight loss. Survival after referral was better among AA than C. Our study demonstrated that it is important and clinically relevant to examine race as a variable of cancer symptom research. This may stimulate research to evaluate racial variability in the provision of supportive and palliative care services for individuals with advanced cancer.
No conflict of interest.

1371 POSTER

The use of catumaxomab for treatment of malignant ascites in clinical practice: Results of an observational trial

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Background: The trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3) is approved in the EU for intraperitoneal (IP) treatment of

malignant ascites (MA) in patients (pts) with EpCAM positive carcinomas. Catumaxomab (CATU) has been investigated in two randomized phase III and several phase I/II trials but there are no data on routine use of CATU. Therefore, a prospective observational study (CARMA, DRKS00000458) started in 2010 investigating the administration of CATU in a total of 160 pts with MA under routine conditions. Participating centers were hospitals and oncologic practices in Germany and Austria. Results of the pre-planned 2nd interim CARMA analysis are reported here.

Patients and Methods: This analysis included 103 pts with MA due to EpCAM positive carcinomas: ovarian, n = 37; gastric, n = 13; breast, n = 13; pancreatic, n = 10; colorectal, n = 6, other, n = 24. Pts were treated with CATU according to the approved posology schedule with 4 increasing IP dosages over up to 20 days. Primary endpoint was puncture-free interval (PFI). Secondary endpoints included safety and overall survival (OS).

Results: The study population was more advanced compared to the phase III studies with regard to distant metastases, performance status, time since first diagnosis of tumor and of MA. Patients were treated in 24 hospitals (73%) and in 9 outpatient facilities (27%). Before treatment, pts suffered from typical MA-related symptoms such as abdominal swelling (77%), pain (56%), dyspnea (27%), anorexia (31%), constipation (14%). 67 pts (65%), received the planned CATU schedule, 36 pts (35%) received <4 infusions. Median PFI was 57 days (d), median OS was 100 d. For the subgroups ovar/non-ovar, a median PFI of 93/41 d and a median OS of 115/72 d was observed. Preliminary results show improved quality of life after CATU therapy. Most frequent adverse events related to CATU were fever (20%), abdominal pain (17%), nausea (14%), vomiting (9%) and diarrhea (6%).

Conclusions: CARMA represents the first systematic evaluation of CATU therapy given for MA under routine conditions. Despite the fact that pts were more advanced than in prior interventional trials, the data demonstrate a clinically relevant benefit of CATU which was particularly pronounced in ovarian cancer pts. Overall, the data confirm the position of CATU treatment in clinical practice including outpatient setting for adequately selected pts with MA. The final CARMA analysis will be performed after including 160 pts.

Conflict of interest: *Advisory board: Fresenius Biotech GmbH. Corporate-sponsored research: Fresenius Biotech GmbH. Other substantive relationships: Employee Fresenius Biotech GmbH*

1372 POSTER

A phase II study of cediranib as palliative treatment in patients with symptomatic malignant ascites or pleural effusion

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Background: Malignant ascites and pleural effusion are challenging clinical problems with a major impact on quality of life. Because malignant effusions are associated with high levels of VEGF we wanted to determine the palliative effect of the oral VEGF TKI cediranib.

Materials and Methods: We conducted an open label, randomized phase II trial (ClinicalTrials.gov nr. NCT01262612). After a baseline paracentesis or thoracentesis (day 0), pts with symptomatic malignant ascites and/or pleural effusion were randomized between immediate treatment with cediranib (ITC) or delayed treatment with cediranib (DTC) on day 29, or after a new puncture was needed. The starting dose of cediranib was 30 mg orally once daily. The primary objective of the study was the puncture free survival (PuncFS), defined as the time from study start (day 1) to the first need for paracentesis or thoracentesis, or time to death, which event occurred first. Secondary objectives of the study were the change in puncture free interval (PuncFI) (defined as the difference between the PuncFS and the PuncFI before start of the study in days) and the tolerability of cediranib. Cediranib was provided by AstraZeneca for this investigator-initiated study.

Results: Twelve pts were enrolled. The median PuncFS was 45 days (range 10–368 days) in the ITC pts and 7 days (range 4−13) in the DTC pts ($P = 0.011$). The PuncFI increased with a median of 31 days in the ITC pts and shortened with a median of 3 days in the DTC pts ($P = 0.015$). Cediranib was well tolerated; the most common observed AEs were fatigue and anorexia. Dose reductions took place in 25% of pts. No bowel perforations were observed, contrary to other studies with intravenous anti-VEGF treatments for ascites. We planned to enroll 32 pts but the study was prematurely discontinued, due to slow accrual and the withdrawal of cediranib for further clinical development by AstraZeneca.

Conclusions: Cediranib increased the PuncFS and PuncFI in pts with malignant ascites or pleural effusion with an acceptable toxicity. This is the first study in which an oral VEGFR TKI showed beneficial palliative effects in pts with malignant ascites or pleural effusion. Although confirmation of these results by a larger prospective placebo-controlled trial would be preferred, the feasibility in terms of patient accrual rate appears low. Our data suggest that an oral anti-VEGFR treatment may be considered as palliative treatment in pts with malignant effusions.

No conflict of interest.

1373 POSTER

Can megesterol acetate induce thrombosis in oncology patients?

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Background: Megestrol acetate (MA) is a white, crystalline solid steroid origin medicine often use for cachexia in oncologic palliative care. Thrombosis is a common problem in oncology patients. There is a question can MA induce thrombosis in oncologic care setting? So, this trial is a retrospective, registry-based analysis to assess the thrombotic process in oncology patients using MA.

Materials and Methods: Data on oncology patients at metastatic stage using MA were obtained from the archives of our center. Outcomes of patients were evaluated if they developed any thrombotic process during the treatment.

Results: Fifty five oncology patients with metastatic disease using MA were analyzed if they developed any venous thrombosis. The median age of the patients was 64 (40−84) years. Most of the patient's diagnostic histopathology consists of lung cancer, gastric cancer, colorectal cancer, and pancreatic cancer. During the mean follow-up of 31.80 months, 34 (61.81%) patients died and 21 (38.18%) patients are still alive. The median time of overall survival (OS) was 19 months (6–180). All patients had been treated with chemotherapy. Cisplatin, taxanes, gemcitabine and 5-fluorouracil had been commonly used agent. Three patients received bevacizumab, one received cetuximab and one patients received everolismus as targeted therapy. Twenty six patients (47.27%) received radiotherapy. MA had been initiated if patient's weight loss was more than 10% on palliative care settings. The mean time of MA use was 8.69 months (±3.53). Seven thrombotic process have detected after MA use. Three of seven patient's diagnosis were pancreatic cancer. The other four patient's diagnosis were gastric cancer (2), lung cancer and endometrial cancer. Five of seven patients had received platin based chemotherapy. The patients with thrombosis non-significantly had worse OS, comparing without thrombosis ($P = 0.106$).

Conclusion: As a conclusion, in this trial it is revealed that the patients on MA treatment have rarely developed thrombosis. As a general rule, cisplatin based chemotherapy and pancreatic cancer seems to be more related with thrombosis than MS use. More detailed and prospective randomized studies should be planned on MA use in oncologic care.

No conflict of interest.

1374 POSTER

Symptomatic palliation of musculoskeletal metastasis: Efficacy and safety of intra-arterial chemoembolization

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Background: Metastasis to musculoskeletal tissues is commonly associated with severe or sometimes intractable pain. If it is not sufficiently controlled with standard therapies including chemotherapy, radiotherapy, and opioid analgesics, patients' quality of life (QoL) might be seriously compromised. This study evaluated the efficacy and safety of intraarterial chemoembolization in the palliation of the musculoskeletal metastasis resistant to standard therapies.

Materials and Methods: From 2008 June to 2012 December, intraarterial chemoembolization was tried in total 51 patients with musculoskeletal metastasis. All patients had intractable pain or discomfort unresponsive to medication and/or radiotherapy, compromising patients with serious deterioration of QoL. Correlating clinical symptoms with findings on CT or PET-CT, arterial suppliers to problematic lesions were selected. Cocktail of chemoagents (gemcitabine 200 mg, oxaliplatine 50 mg, adriamycin 10 mg mixed in glycirrhyzinic acid 40 ml) was infused, followed by 15 mg pamidronate in bone metastasis. Embolization with imipenem-induced micro-particles (40−50um) was performed when the lesions were highly vascular and safe for embolotherapy. This treatment was repeated per 4 to

8 weeks according to the patients' response and clinical condition. Changes of bone pain, analgesics consumption, and quality of life were evaluated after treatment.
Results: Technical success was achieved in 45 patients. Six patients underwent just angiography, showing no definite tumour staining in them. In 45 patients, improvement of pain (reduction of numerical pain rating scales, reduction of analgesics consumption) was demonstrated in 31 patients (clinical success 68.9%). Neurological improvement was demonstrated in 2 patients. The pain was not changed in 9 patients, and aggravated in 5 patients. Improvement of quality of life occurred in 28 patients. The time reaching maximum clinical improvement ranged from 1 day to 8 days. Clinical improvement maintained for 2 to 8 weeks. The procedure was well tolerated without significant complications.
Conclusion: Intraarterial chemoembolization is a useful, sometimes like a saviour, modality for the symptomatic palliation of musculoskeletal metastasis uncontrolled with standard therapies. However, personal difference exists in the commencement of palliation and duration of effectiveness. To maintain therapeutic effectiveness, decision for interval of procedure repetition is important.
No conflict of interest.

1375 POSTER

Initial symptom management of cancer patients, as an outcome of care, in a home palliative care unit in Greece

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Background: 'Galilee' palliative care service provides home care for adult cancer patients, in a large suburban area of Athens since March 2010. The purpose of the study was to explore symptom management of patients for the first two months of home palliative care.
Material and Methods: A total number of 162 cancer patients received home palliative care from March 2010 to December 2012. Retrospective data collection included: demographic and clinical characteristics and patients' ESAS-r (Edmond Symptom Assessment System Revised) self evaluation of symptoms (Likert type 0−10 scale) at the time of referral to the service (T_0), one month (T_1) and two months later (T_2).
Results: Most of patients were female (53.1%). Their mean age was 67.1 years and ECOG performance status 2.7. The most frequent diagnosis was lung cancer (18.5%) followed by breast (17.3%) and gastrointestinal cancer (17.3%). Most of the patients (46.9%) did not receive any antineoplasmatic treatment and 56.2% were not hospitalized during home care. Health status deterioration (27.5%), unrelieved breathlessness (19.6%) and persisted infection/ fever (15.7%) were the most prevalent reasons for hospital admission. Patients' median length of care was 54.5 days. The majority of patients (70.8%) died and 36.8% and 20.2% of them within the first and the second month respectively after referral to service. Anxiety (T_0 6.2±3.6, T_1 4.9±3.8, T_2 5.3±3.8), depression (T_0 5.9±3.3, T_1 5.2 ±3.4, T_2 5.3±3.5), tiredness (T_0 5.1±3.6, T_1 4.7±3.3, T_2 4.5±3.8), and pain (T_0 4.8±3.4, T_0 4.1±3.2, T_2 4.3±3.7) were the most self reported symptoms at referral to the service and the two subsequent months. On the contrary nausea (T_0 1.1±1.0, T_1 0.8±0.6, T_2 0.4±0.1), breathlessness (T_0 1.5±1.0, T_1 1.0±0.2, T_2 1.2±0.5) and drowsiness (T_0 1.8±1.0, T_1 1.9±0.3, T_2 2.4±0.6) were less frequently reported. Overall during the study period, patients' well being improved. Moreover all of their symptoms were alleviated except for drowsiness, but only anxiety and depression at a statistical significant level ($p < 0.050$).
Conclusions: Study results highlight that regardless of the deterioration of patients' health and high rates of death among them, they reported improved well being in a home palliative care program. Further research is needed to specify the relevant involved factors.
No conflict of interest.

1376 POSTER

Four years experience of treatment painful bone metastases with magnetic resonance guided focused ultrasound

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Background: Magnetic Resonance guided Focused Ultrasound Surgery (MRgFUS) is an innovative technology combining non-invasive deposition of high intensity focused ultrasound energy into a specified target inside the body, with high resolution Magnetic Resonance Imaging (MRI) guidance and real-time thermal feedback. Starting from 2009 up to 2012 our Institute participated in the clinical randomized multi-site study to evaluate safety and efficacy of MRgFUS for palliative treatment of Bone Metastasis. This study was approved by FDA at 18 October 2012.
Material and Methods: 37 patients with painful bone metastases were treated with the ExAblate® system (*InSightec*, Haifa, Israel) at Petrov Research Institute of Oncology, St. Petersburg, Russia. Immediately after procedure patients were examined for any adverse events and after a brief recovery discharged. Patients were followed up on 1 and 3 days, 1 and 2 weeks, 1, 2 and 3 months post treatment. During each visit, treatment safety was evaluated by recording and assessment of device or procedure related adverse events. Effectiveness of palliation was evaluated using the standard pain scale (0-no pain/10-worst pain imaginable) and by monitoring changes in the intake of pain-relieving medications. A reduction of 2 points or more on pain scale was considered a significant response to treatment. 8 patients were male and 29 female. Mean age was 58 years old (19−76). The primary cancers were: 25 breast, 4 stomach, 2 bronchus, 2 bladder, 4 other. Targeted lesions were 14 osteolytic, 8 osteoblastic and 15 mixed. 27 were pelvis metastases, 4 were located in the humerus bone and 6 were located in the ribs.
Results: No significant device or procedure related adverse events were recorded. 4 patients died during the follow-up period due to disease progression, thus 3 months follow-up data includes only results of 33 patients. All patients reported significant improvement in pain with no change in their medication intake. Mean worst pain score at baseline, 1 day, 3 days, 1 week, 2 weeks, 1, 2 and 3 months post-treatment was 6.9, 6.1, 5.1, 3.5, 2.6, 1.8, 1.2 and 0.9.
Conclusions: MRgFUS can provide effective, safe and noninvasive palliative therapy for patients suffering from painful bone metastases. The ability to achieve rapid pain relief after only one treatment session, combined with the high safety profile of the procedure implies that MRgFUS has a significant potential for patients suffering from painful bone metastases.
No conflict of interest.

1377 POSTER

Attitudes and referral patterns of lung cancer specialists in europe to specialized palliative care (SPC) and the practice of early palliative care (EPC)

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Purpose: To examine availability of Palliative Care (PC) services as well as referral patterns of European Lung cancer specialists to PC and in particular the timing of this referral.
Methods: All members of the EORTC Lung Cancer Group (LCG) were asked via email to participate in an on-line survey. Three (3) emails were sent.
Results: 50 out of 170 (29.4%) EORTC LCG members replied. Among the respondents there were 24 (48%) medical oncologists, 14 (28%) radiation/clinical oncologists, 11 (22%) pulmonologists and 1 (2%) thoracic surgeon. All but one of respondents (98%) reported that either most of their practice (30%) or a substantial proportion of their practice (68%) involved the care of patients with metastatic/incurable cancer. All but two (2) of respondents (96%) had access to at least one component of PC services. Twenty seven (54%) had access to comprehensive PC services, including hospital based teams and outpatient/community based PC teams and inpatient Hospice services. In terms of referral of metastatic lung cancer patients to PC almost 75% of participants would refer almost all or most of their patients when they were close to death, while 22% or less would refer their patients at earlier stages of disease. Regarding barriers for referral to PC, negative attitudes of patients to PC was cited by 26% of participants, lack of availability of PC services by 20%, lack of expertise of PC physicians by 18%, and only 8% of participants felt that referral to PC signifies abandoning their patients, and that PC specialists interfere or discourage active oncological therapy. Although most of the respondents expressed positive attitudes towards PC, 12−22% had overtly negative attitudes towards PC. Seventy-eight (78%) of participants expressed an interest to participate in a trial of early PC (EPC).
Conclusion: There is good availability of PC services at institutions of members of the EORTC LCG. Most respondents expressed positive attitudes towards PC, however the majority of them referred patients to PC late in the disease trajectory, hence lung cancer specialists in Europe have not adopted the practice of EPC concurrent with active oncological care. Negative attitudes of patients to PC and lack of expertise of PC physicians remain the major barriers, according to Lung Cancer specialists, for referral to PC. The majority of EORTC LCG members would like to participate in a trial of EPC.
No conflict of interest.

1378 POSTER

Availability of informal caregivers for palliative care patients with cancer: Is there a difference between higher- and lower-income settings?

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Background: The modern palliative care movement started in higher-income countries where palliative care models were developed to meet the needs of patients and their families. Although the principles of palliative care are the same, the ideal palliative care models in lower-income countries may be different from that in higher-income ones due to many variables related to culture and resources. Among the factors that need to be considered when developing suitable palliative care models in lower-income settings are the availability of informal caregivers and the degree to which they are involved in the care of palliative care patients. This study was conducted to compare the availability of informal caregivers between a higher-income setting and a lower-income one.
Patients and Methods: We investigated the availability of informal caregivers for 190 palliative care patients with advanced cancer from a higher-income setting in the United Kingdom (UK) and 115 patients from a lower-income setting in Egypt.
Results: Patients in Egypt were significantly younger than patients in UK (mean age 52 vs. 71, respectively; $p < 0.001$) and were more likely to be married (84% vs. 48%, respectively; $p < 0.001$). An informal caregiver was available in 92% of the cases from Egypt compared to 76% of the cases from the UK ($p < 0.001$). While 100% of Egyptian informal caregivers were family caregivers, 10% of those from the UK were non-family caregivers. In the Egyptian setting, 100% of informal caregivers were living with the patient compared to 63% in the UK setting ($p < 0.001$). In total, 92% of Egyptian palliative care patients with cancer had an informal caregiver living with them. In comparison, 48% of palliative care patients with cancer from the UK had an informal caregiver living with them ($p < 0.001$).
Conclusions: The results of this study suggest that there are significant differences between higher-income and lower-income settings regarding the availability and characteristics of informal caregivers for palliative care patients with cancer. The palliative care models developed in higher-income countries may not be 'universally' suitable for lower-income countries. Future research is essential to develop palliative care delivery models suitable for the culture and resources in Egypt and other lower-income settings.
No conflict of interest.

1379 POSTER

Hyponatremia severity among cancer patients and its association with type of cancer and mortality

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Background: Hyponatremia is common in patients with cancer, and results in clinical symptoms that might increase the burden of the disease. It has been associated with increased mortality and length of hospital stay. The objectives of this study are to investigate the severity distribution of hyponatremia among different malignancies and study its association with mortality at a tertiary cancer center in Qatar.
Material and Methods: This is a retrospective study of all cancer patients admitted or seen at the National Center for Cancer Care and Research in Doha, Qatar between 2008 and 2012. We reviewed electronic medical records for patients and analyzed demographics and clinicopathological reports. Descriptive statistical analysis was used to determine hyponatremia severity distribution among malignancy groups. A model was built through multivariate analyses to investigate the role of hyponatremia in mortality.
Results: A total of 2048 patients were included in this study. Prostate (57.1%), pancreatic (50%), liver (49%), and lung (40.2%) cancers showed the highest frequency of severe hyponatremia, while breast cancer showed the lowest frequency at 23.5%. In the multivariate analyses, patients with moderate-severe hyponatremia (Na <130 mmol/L) were 4.28 times more likely to die than those with normal sodium levels ($p < 0.05$).
Conclusion: The present study shows that hyponatremia is a common electrolyte disturbance among hospitalized patients with cancer diagnosis. The severity of hyponatremia was a statistically significant independent factor associated with higher in-hospital mortality. This is in accordance with the reported literature and emphasizes the importance of early diagnosis and correction of hyponatremia.
No conflict of interest.

1380 POSTER

Palliative care in the last days of cancer patients admitted to Torrecardenas Hospital

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Introduction: The cancer is still an incurable disease, and in the last moments of life aims primarily to avoid the pain and consequently suffering of cancer patients. The objective of this study is to describe the characteristics of patients who died in the ward of Medical Oncology during 2012.
Material and Methods: We retrospectively reviewed all patients who died in our service between January and December 2012, and performed a descriptive analysis of the following variables: sex, age, primary tumor, symptoms that led to the hospitalization, length of hospital stay, presence of refractory symptoms, and if received palliative sedation.
Results: We analyzed 90 patients: 52 were male (57.7%) and 38 women (42.2%) with a median age of 59 years (range 23−89). According to the primary tumor: lung cancer 38.8%, non-colorectal gastrointestinal cancer 21.1%, breast cancer 10%, colorectal cancer and cervical cancer 8.8%, genitourinary 6.6% and soft tissue sarcoma and 5.5%. All patients were in an advanced or terminal stage of their condition, and 49.5% in an agonal phase. The most common symptoms that prompted admission were dyspnea (22.6%), followed by fatigue (18.6%) and uncontrolled pain (15.4%). The median stay was 11 days, but 19 patients (21.1%) died in the first day of admission. Symptoms leading to sedation were: dyspnea (42.7%), pain (26.6%) and delirium (21.8%). Informed consent (IC) was explicit in 9% of the cases, and given prior to the appearance of refractory symptoms and/or agonal phase. In 82% of sedation cases, the IC was given by a representative. Midazolam was the most used drug of choice in 91% of the cases. Up to 75% of sedations required drug changes or combinations.
Conclusions: The tumor with increased mortality was lung cancer followed by gastrointestinal cancer (esophagus, gastric and pancreas). Dyspnea as a symptom and liver failure secondary to tumor progression were the main reasons for admission. The most common refractory symptoms were: dyspnea, pain and dyspnea association with pain or delirium. The therapeutic approach to these patients is complex, since most suffer a variety of symptoms and any complications during hospitalization. In 100% of cases was agreed sedation, being in most cases the conformity with the patient.
No conflict of interest.

1381 POSTER

Exploring knowledge and experience of cancer patients in pain relief − a Greek study

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Background: The purpose of this study, funded by the University of Athens, was to explore Greek patients' knowledge and experience of cancer pain relief.
Material and Methods: A descriptive correlation research design was used. A sample of 100 cancer patients with pain was interviewed, in a central oncology hospital in Attica area. A demographic and clinical form and the Patient Pain Questionnaire (PPQ-Ferrell BR, 2000, City of Hope) were used for data collection. The PPQ consists of 16 items divided between two subscales. The 'Experience' subscale consists of seven items measuring current pain and pain relief, pain over the last week, expectation of future pain, sense of control over pain and distress related pain. The 'Knowledge' subscale consists of nine items to evaluate knowledge of, and attitudes to, cancer pain and medications. Patients rate their agreement or disagreement on 0−10 rating scales. Higher scores indicate poorer experience of pain, and poorer knowledge and attitudes.
Results: Most patients were female (55.5%), with a mean age 65.0 years. The prevalent diagnosis was lung cancer (20.0%). Median time since cancer diagnosis was 17.5 months and pain onset 6.5 months. More patients were prescribed with mild (45.0%), or strong (41.0%) opioids and only 14% non opioids analgesics. Patients reported a median knowledge about cancer pain management (5.3 ± 1.4) and a median experience of pain (5.3 ± 1.7). Although patients experienced almost severe pain over the past week (6.8 ± 2.3), they reported receiving a great deal of pain relief (3.1 ± 2.8). Patients' knowledge about cancer pain management was positively related with their pain experience (rho=0.22, $p = 0.028$). None of patients' demographic and clinical characteristics was related with patients knowledge and experience about cancer pain ($p > 0.050$). Patients'

analgesic treatment was associated with their pain experience ($z = -2.3$, $p = 0.021$). Patients prescribed with opioids described a poorer pain experience (6.4±2.0), than those prescribed with non-opioids (5.2±1.3). Additionally patients receiving opioids reported more pain over the past week (7.0±2.3 vs 5.5±2.2), present pain (4.0±2.8 vs 2.2±2.2) and more distress (7.6±2.6 vs 6.8±1.6), than those receiving non-opioids.
Conclusions: Although patients were undertreated they described a great deal of pain relief. Most patients held limited knowledge about pain and pain management, and those with poorer knowledge reported worse pain experience.
No conflict of interest.

1382 POSTER

Knowledge and experience in pain management of Greek cancer patients − the family caregivers perceptions

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Background: The aim of this study, funded by the University of Athens, was to investigate knowledge and experience of family caregivers in patient's cancer pain management.
Material and Methods: Patients with a cancer diagnosis who reported pain, in 'Ag. Anargyroi' Cancer Hospital, were approached for a three month period (January-March 2013) and accepted to name their primary family caregiver. A total of 100 caregivers consented to participate to the study. A demographic form and the Family Pain Questionnaire (FPQ-Ferrell BR, 2000, City of Hope) were administrated. The FPQ is a 16 item ordinal scale that measures the Knowledge and Experience of a family caregiver in managing chronic cancer pain. The FPQ includes 9 items that measure knowledge about pain and 7 items that measure the caregivers experience with patient's pain. Caregivers rate their agreement or disagreement on 0−10 rating scales (0= the most positive outcome, 10= the most negative outcome).
Results: Most caregivers (59.0%) were female with a mean age 52.9 years. The majority of them was patients' wife/ husband (48.0%) with no previous caregiving experience (66.0%), assisted by others (76.0%). Most of caregivers were married (76.0%) with two children (54.0%) having the same residence with the patient (66.0%). The median time of care provision was 13 months. Caregivers reported a median knowledge about cancer pain management (5.7±1.4) and a median patient's experience of pain (6.4±1.3), which were not correlated each other ($rho = -0.15$, $p = 0.146$). Caregivers reported almost severe patient's pain over the past week (6.7±2.4), mild current pain (4.2±2.9) and an inadequate pain relief (7.0±2.4). Caregivers' knowledge in pain management was associated only with patient's age ($r = 0.26$, $p = 0.006$). The caregivers of younger patients had higher score in knowledge subscale. Similarly, higher score in knowledge had those that did not receive assistance from others ($t=2.2$, $p = 0.030$). Caregivers experience with patients' pain was worse for patients receiving opioids analgesic ($z = -2.3$, $p = 0.019$) particularly strong opioids ($z = -2.7$, $p = 0.006$), than those receiving non opioids. Moreover, caregivers reported that patients receiving strong opioids had worse pain experience over the past week ($p < 0.006$) and current pain ($p < 0.018$), than those receiving non opioids or mild opioids.
Conclusions: Family caregivers had a moderate knowledge about cancer pain management and experienced undertreated their patient's pain.
No conflict of interest.

1383 POSTER

Optimal timing of influenza virus vaccination during chemotherapy treatment in adult patients with solid tumours

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Background: Higher rates of hospitalisation and mortality are described in oncology patients with influenza virus infection compared to the general population. Yearly influenza vaccination is strongly recommended for patients treated with chemotherapy or other immunosuppressive drugs. The optimal moment to administer the vaccine during a treatment cycle has not been studied extensively.
Patients and Methods: During the influenza season 2011–2012 we conducted a multicenter randomised controlled trial (OFLUVAC, NTR2858, no sponsoring) in the Netherlands. Patients receiving adjuvant chemotherapy for breast or colorectal cancer were randomized between early (day 5 after chemotherapy) and late (day 16 after chemotherapy) vaccination with the influenza virus vaccine (Influvac® 2011/2012, Abbott Biologicals B.V, Weesp, the Netherlands and Vaxigrip® 2011/2012, Sanofi Pasteur MSD, Brussels, Belgium). Influenza virus-specific antibody titres were determined before and 3 and 12 weeks after vaccination by haemagglutination inhibition.
Results: Thirty-eight breast cancer patients (early = 21; late = 17) and 18 colorectal cancer patients (early =8; late = 10) were analysed. In breast cancer patients overall serologic responses were adequate. A statistically significant higher response in patients who received early compared to late vaccination in the chemotherapy cycle was observed. Geometric mean titres post vaccination day 5 versus day 16 were 69.3 versus 27.4 (H3N2), 76.4 versus 17.5 (H1N1) and 34.4 versus 26.0 (B/Brisbane), respectively. In colorectal cancer patients overall serologic responses were adequate, no significant difference was found between early and late vaccination with the influenza virus vaccine. Geometric mean titres post vaccination day 5 versus day 16 were 170.1 versus 192.4 (H3N2), 233.0 versus 280.8 (H1N1) and 62.6 versus 75.9 (B/Brisbane), respectively.
Conclusion: The overall serologic response to the influenza vaccine in patients treated with chemotherapy for breast or colorectal cancer patients is adequate. The optimal timing of vaccination in breast cancer patients is early after receiving chemotherapy (⩽ day 5). No difference was found between early and late vaccination in colorectal cancer patients.
No conflict of interest.

1384 POSTER

Impact of chemotherapy on activated protein C-dependent thrombin generation − association with venous thromboembolism occurrence

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Background: Cancer patients have an increased risk of venous thromboembolism (VTE), which differs among patients, or even in the same patient over the course of cancer natural history. The high VTE rates observed during the first year after diagnosis have been possibly related to the administration of combined anti-cancer therapies. However, the prevalence of coagulation abnormalities or VTE occurrence as a result of different anticancer agents or treatment schemes is largely uncharacterized. Thus, we aimed at analyzing the impact of different anticancer drugs on the pro-thrombotic status of cancer out-patients scheduled for chemotherapy.
Material and Methods: Activated protein C (APC) function (by HemosIL ThromboPath) was prospectively analyzed in 505 cancer out-patients with primary or relapsing solid cancer at the start of a new chemotherapy regimen (6% neoadjuvant, 31% adjuvant and 63% metastatic treatments). Blood was withdrawn in all 505 patients prior to chemotherapy start and before the second cycle. Additional samples were obtained from 51 patients before the start of the third and sixth cycle (elapsed time between cycles: 28d).
Results: APC function was impaired in roughly 27% of all cases at baseline and significantly decreased after one cycle of chemotherapy (mean ThromboPath value: 78.0±11.8 PICI%, $p = 0.0001$). Subgroup analysis of the 51 patients who consented to repeated blood withdrawal showed that APC functionality was progressively impaired during the first 3 months of chemotherapy (82.3±8.9 PICI% at T0 vs. 78.4±12.2 PICI% at T1 vs. 78.7±11.5 PICI% at T3), but reverted to baseline levels by the sixth month (84.9±8.6 PICI% at T6; Friedman's ANOVA among the four study points: $p = 0.008$). Advanced age (>65 y, $p = 0.01$), ECOG-PS ($p = 0.01$), platinum-based ($p = 0.035$) and fluoropyrimidine-based regimens ($p = 0.008$) were independent predictors of impaired APC function during chemotherapy. Multivariate Cox proportional hazards analysis demonstrated that a decline in APC function ($HR = 2.4$; $p = 0.013$) and platinum-based regimens ($HR = 2.2$; $p = 0.042$) were both capable of predicting the occurrence of a first VTE episode during chemotherapy. Indeed, 14% of patients with platinum-associated APC impairment had VTE over a 1 yr follow-up, compared to 3% of patients treated with other regimens and in whom APC function remained stable ($HR = 1.5$; $p = 0.003$).
Conclusions: Use of platinum-based regimens is responsible for induction of an acquired thrombophilic condition in the first three months of therapy and represents a predictor for VTE even after adjustment for other risk

Table 1 (abstract 1386). Congruence scores

	All Pts	Oncology Pts	Haematology Pts	p*
Pt Risk Score 0−11 (mean±SD)	2.82±1.94	2.81±1.88	2.87±2.15	0.6544
Correct type of prophylaxis given	48.8%	49.0%	47.8%	0.7501
Biosimilar filgrastim initiated 24−72 hrs after chemo	52.9%	58.1%	33.1%	<0.0001
Biosimilar filgrastim persisted as recommended	91.6%	92.5%	87.8%	0.4304
Overall Congruence Score 0−3 (mean±SD)	2.26±0.61	2.31±0.61	2.09±0.59	<0.0001

*Significance of difference between oncology and haematology pts.

factors. Monitoring coagulation changes during the first cycle, more than the determination of a single point measurement at baseline, could provide a valid estimate of the associated pro-thrombotic risk and might help to identify patients susceptible of developing VTE during treatment.
This work has been performed within the PhD Programs XXVI and XXVII Ciclo and was partially supported by the Italian Ministry of Health Grant MERIT RBNE08NKH7.
No conflict of interest.

1385 POSTER

Complications and mortality associated with febrile neutropenia in a Spanish multicenter cohort of patients with cancer

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Background: Febrile Neutropenia (FN) is associated with complications, mortality, severe infections and long hospital stays, in a significant number of adult cancer patients. Nevertheless, FN is a heterogeneous disease, and patients are not exposed to the same risk factors.
Patients and Method: An ambispective registry of adult cancer patients with FN from 15 Spanish hospitals was carried out from 2006 to 2013. Cases were clustered according to the primary tumor and the source of infection. Primary outcomes included mortality, complications and hospital stays. Univariate and multivariate analyses were performed to fit these variables to other well-known risk factors.

Table 1. Multivariate Logistic regression analysis: severe complications

Category	OR	95% CI	p-value
Pneumonia	3.4	1.92−6.2	<0.0001
Lower respiratory infection	2	1.21–3.58	0.007
Stomatitis ⩾2	1.9	1.25–3.05	0.003
ECOG PS ⩾2	2.3	1.31–3.56	<0.0001
Cardiovascular disease	2.08	1.22–3.56	0.007
Palliative chemotherapy	1.5	1.03–2.41	0.036
Bacteremia	2	1.23–3.36	0.005
MASCC <21	4.8	2.81–8.31	<0.0001

Results: 734 events were included with 28.5% complications, 3.8% deaths and a mean hospital stay of 7.1±5.8 days. The most frequent tumors were breast (30.3%) and lung (25.2%) cancers, while other tumors occurred at a frequency below 10%. Complications were significantly higher in patients with pancreatobiliar (68.8%) and lung (40.4%) tumors*, while breast cancer had the lowest rate (17.9%)*. Mortality was higher in patients with lung (6.6%), colorectal (10.8%) and ovarian cancer (11.1%)*. Otherwise, the most common infections were fever of unknown origin (FUO) (28.7%), stomatitis (15.2%), enteritis (11.9%) and pneumonia (10.3%). A higher rate of complications was associated with pneumonia (61.8%) and bronchitis (42%)*, whereas coughs and FUO had the lowest rates. Mortality showed a similar trend, highlighting the role of lower respiratory infections, specially pneumonia. These disparities persisted even after adjustment for comorbidities and other risk factors, as shown on Table 1. Otherwise, the rate of bloodstream infections was significantly higher in patients with catheter-related infections (42.9%), urinary infections (31.4%) and cellulitis (25%)*.
*$p < 0.05$ (z-test of proportions with Bonferroni method for multiple comparisons).

Conclusions: This study revealed several risk factors for complications, mortality and longer hospital stay, with potential implications for prevention and treatment, especially in lung cancer and respiratory pneumonia.
No conflict of interest.

1386 POSTER

Congruence to EORTC guidelines of biosimilar filgrastim treatment in patients enrolled in MONITOR-GCSF

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Background: The MONITOR-GCSF study is a prospective observational study of practice patterns and outcomes of biosimilar filgrastim (Zarzio®, Sandoz) for prophylaxis of chemo-induced febrile neutropenia (CIN/FN). One study objective is to describe the congruence of individual patients' (pts) biosimilar filgrastim treatment with the 2010 EORTC GCSF guidelines. The goal of this interim analysis is to assess the internal validity of a proposed congruence scoring methodology.
Methods: A methodology for scoring pt-level congruence of actual biosimilar filgrastim treatment with EORTC guidelines was developed (possible range 0−3). Scoring focuses on 4 aspects of therapy: 1) pt risks; 2) primary vs. secondary prophylaxis, 3) day of treatment initiation, 4) persistence. We conducted this interim analysis on 1168 pts enrolled to date (target 1500) from 139 centers in 12 European countries.
Results: In this mainly female (61%) and older (61.6±11.7 y) sample with predominately (79%) solid tumours, 11% of pts were on chemo regimens with <10%, 54% on regimens with 10−20%, and 35% on regimens with >20% FN risk. Table 1 summarizes biosimilar filgrastim congruence scores by tumour type. About half of pts were treated with biosimilar filgrastim (either primary or secondary prophylaxis) as recommended by the guidelines; just over a quarter of pts on chemo with FN risk >20%, or 10−20% FN risk in combination with other pt-related risk factors, did not receive primary prophylaxis as recommended. Half were initiated 24−72 hours after chemo and with few exceptions pts persisted as recommended.
Conclusions: Variability in biosimilar filgrstim (Zarzio®) treatment is captured in the congruence scoring indicating internal validity. Proportionately more oncology pts were initiated within the 24−72 hr time window, explaining also the higher overall congruence scores in these pts. Initiation time for haematology pts may need to be reconsidered. The relationship between EORTC guideline congruence and clinical outcomes will be evaluated at study end.
Conflict of interest: *Advisory board: HL, MB, MA, PG, CB are part of the Monitor GCSF advisory board for Sandoz. Corporate-sponsored research: HL, MB, MA, PG, CB, KD, IA, KM are all involved in Sandoz-sponsored research (Monitor GCSF study). Other substantive relationships: MT and MM are employees of Sandoz Biopharmaceuticals*

1387 POSTER

The use of probiotics in people with cancer: A systematic review

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Background: Probiotics are living microorganisms that are generally thought of as being beneficial to the recipient. They may be consumed

as dairy drinks and have been shown to be effective in people with acute infectious diarrhoea. Probiotics may have a role in people with cancer, as various cancer treatments often lead to diarrhoea; however, guidance for their use is yet to be determined. People with cancer are often immunocompromised, so it is important to assess for adverse events such as infection, which could potentially be a consequence of deliberate ingestion of living microorganisms.
Materials and Methods: A systematic review was performed to collect, analyse and synthesise all available data on the efficacy and safety of probiotics in people with cancer (PROSPERO registration: CRD42012003454). Randomised control trials (RCTs), identified through screening multiple databases and grey literature, were included. Primary outcomes were the reduction in duration, severity and incidence of antibiotic-associated diarrhoea and chemotherapy-associated diarrhoea, and adverse events, especially probiotic-associated infection. All included studies and data extraction forms were independently reviewed. Where possible, data was combined for meta-analysis by a random effects model, assessing causes of heterogeneity, including differences in strains, dosage and patient characteristics.
Results: Data was extracted from 10 RCTs totalling 1431 participants. There was little strong evidence of difference in mean number of average daily bowel movements between probiotic and control groups of −3.98 stools per day [95% confidence interval (CI)-14.99 to 7.04; p = 0.48]. Liquid stools tended to be less common in the probiotic group [odds ratio (OR)=0.37; 95% CI 0.06 to 2.43; p = 0.30], whereas soft/semi-solid stools possibly occurred more often (OR=2.12; 95% CI 0.49 to 9.18; p = 0.31). The change in enterobacteriaceae count was lower in the probiotic group; mean difference of −1.98 (log10 CFU/g of faeces) (95% CI −2.56 to −1.39; p < 0.00001) compared to the control group. Probiotics seemed to decrease the use of rescue (anti-diarrhoeal) medication (OR=0.63; 95% CI 0.27 to 1.45; p = 0.28). No probiotic-associated infection was identified in any of 1311 patients assessed for adverse events.
Conclusions: There is a potential that probiotics may reduce the severity and frequency of diarrhoea in patients with cancer and may reduce the requirement for anti-diarrhoeal medication, though there is currently insufficient evidence with which to draw conclusions. More studies are needed to assess the true efficacy and safety of probiotics in people with cancer.
No conflict of interest.

1388 POSTER

Neutropenia score as an overall survival (OS) prognosis factor

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Background: Chemotherapy (CT) is a common cause of neutropenia (N). N is a prognostic factor in several studies. Prognostic value of N can be evaluated with global scores calculated over the whole CT period.
Methods: Patients included in the PROCHE program between 2008 and 2011 at the HEGP hospital (Paris, France). PNN levels were assessed at least once before each CT and graded using the CTC-NCI scale: 0: >2000, 1: [2000–1500[, 2: [1500–1000[, 3: [1000–500[, 4: <500. Scores were calculated as weighted mean of grades from 1st cycle to end CT (mean grade per cycle when multiple values). OS was calculated from CT initiation to death or censured at last contact. Cox regression covariates: age, tumor localization, disease setting, neutropenia after 1st cycle, and continuous or categorized PNN score. Patients with localized (M0) or metastatic (M+) disease during the study period led to two distinct cohorts: C1 and C0 according to M1 or M0 period considered. Bootstrap validation was performed using 1000 samples.
Results: Among the 1279 pts who entered the program, data were available for 657 who had at least 1 assessment of PNN. Excluded pts (622) due to lacking or dubious PNN results did not differ (log-Rank=0.98). Median age=63 y, sex-ratio=1, more frequent localization: lung (25%), breast (21%), urogenital (21%), ovary (13%), ENT (12%). 269/388 and 191/466 pts had localized/metastatic disease respectively for C0 and C+. Median cycles received: 4 (IQR=5). Median follow-up time=26.9m. Median PNN (/μl) was 4400 at baseline and 3820 thereafter. Score: 308 pts (47%) and 349 had a score>0 (S+: at least 1 neutropenia) and a score=0 (S0), respectively. 148 (22%) were S+ as soon as the 1st cycle. OS (m, 95% CI) was S+=35.2 (28.5-NR) and S0=17.0 (14.5–21.5). PNN continuous score was an independent predictor of OS: HR(C0)=0.70 (0.56–0.88), HR(C1)=0.72 (0.56–0.91). Categorical PNN score (S+/S0): HR(C0)=0.58 (0.45–0.73), HR(C1)=0.55 (0.43–0.69). The deeper the neutropenia after 1st cycle, the longer the OS. Other independent factors were age, disease setting, ENT and lung tumors. The model was internally validated (C-index=0.732, shrinkage=0.956).
Conclusion: Neutropenia scored over the whole CT period is a strong prognostic factor of OS.
No conflict of interest.

1389 POSTER

Epidemiology, resistance profile and origin of bacteremia in non-neutropenic patients with solid tumor

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Background: In oncology, and especially for solid tumors, the principal studies describe bacteriema in patients with neutropenia, Few microbiological data are available without neutropenia. Furthermore, bacteria resistance in immucompromised patients is increasingly reported.
The aim of this study is to describe the bacteremia occurring in solid tumors and to identify a potential association with other sites of infection.
Materials and Methods: This retrospective study was conducted in patients visiting an emergency oncology department for acute onset symptoms. Urinary, skin and/or sputum samples were analyzed according to clinical symptoms. Central venous catheter infection (CVC) was defined with the differential time to positivity between hub-blood and peripheral-blood cultures.
Results: We reported 290 bacteremia, Gram-positive represented 58%: coagulase-negative staphylococci (CNS) (n = 105), *Staphylococcus aureus* (SA) (n = 38) (one methicillin-resistant), *Streptococcus* sp (n = 36). The majority of gram-negative were Enterobacteria (E) (n = 97) and most of them were *E coli* (n = 62). Only 2 (*E coli*) developed extended spectrum beta-lactamases resistance. Two ore more microorganisms were found in 41 bacteremia.
When CNS, SA and *Candida* sp were isolated it was most often related to CVC, and, 25/98 of E bacteremia were also attributed to a catheter infection.
It is important to highlight that all the bacteremia were associated with another site of infection.
Conclusion: In patients with solid tumors without the context of neutropenia, all the bacteremias are associated with documented sites of infection. Bacteria resistance is the exception (1%), 58% of the bacteremia are Gram-positive microorganisms and catheter infection could be with Enterobacteria microorganisms.
No conflict of interest.

1390 POSTER

Real-world hypertension (HTN) adverse events (AEs), interventions and outcomes in bevacizumab (BV) − treated patients

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Background: HTN is one of the most common AE associated with BV. In clinical trials, the incidence of NCI CTCAE Grade 3 or 4 HTN ranged from 5−18%. Utilizing US clinical practice data, this study aimed to understand the occurrence of HTN AE, interventions and outcomes in cancer patients receiving BV.
Methods: Adult patients with select cancer types including mCRC, NSCLC, mBC, OC and other indications who initiated BV between 2007 and 2011 were identified from the IMS US Oncology Electronic Medical Record database. Patients were followed for 12 months post initial administration of BV for the occurrence of HTN events, defined as either elevated/suboptimal blood pressure (BP) readings, HTN related diagnosis, or use of antihypertensive medications. Post AE interventions, including BV treatment modification and/or HTN treatments, as well as HTN outcomes were evaluated.
Results: Final sample consisted of 3,850 adult cancer patients; median age was 61 years and 62.9% were female. The top four cancer types–mCRC (43.8%), non-squamous NSCLC (26.4%), mBC (23.6%), OC (4.6%)-- accounted for almost all study patients, and most were stage III or IV. Baseline HTN were observed in 37.7% (n = 1,453) of patients; most of them (90.3%, n = 1312) did not have an exacerbation during the 12-month follow-up. Overall, 21.1% (n = 811) of patients had either newly developed HTN (n = 670; 28.0% of patients without baseline HTN) or exacerbated HTN (n = 141; 9.7% of patients with baseline HTN). 54.4% (365/670) of patients with newly developed HTN and 34.8% (n = 49/141) of patients with exacerbated HTN were grade 3 or 4. The majority continued BV post AE; only 9.6% had a BV dose reduction, 3.3% had a dose withheld and 18.9% discontinued BV. HTN medications (new or addition to existing) were prescribed for 58.8% of the AE patients (n = 477); diuretics or ACEI were most commonly prescribed. BV modification and HTN treatments mostly occurred within 2 weeks of each other (92.5%). Among approximately half

of the HTN AE patients (n = 406) with known HTN status before the end of follow-up, 92.3% (n = 375) had HTN resolved within 45 days; only 7.6% (31 patients) were confirmed to have persistent HTN.
Conclusions: Results of this real-world study suggest that after BV treatment, HTN AE occurred in 9.7% of patients with baseline HTN and 28.0% of patients with normal baseline blood pressure. In most cases, the AE is manageable without causing significant interruptions to BV treatment in patients.
Conflict of interest: *Ownership: E.Yu owns stock/options of Roche. Corporate-sponsored research: Genentech Inc. sponsored this research. Other substantive relationships: E.Yu, A.Ravelo, and K.Look are employees of Genentech Inc.*

1391 POSTER

Osteonecrosis of the jaw (ONJ) in patients with renal cell cancer (RCC) treated with bisphosphonates and sunitinib or other biological agents: Characteristics of 39 cases in a multicenter survey

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Background: Up to 35% of advanced renal cell cancer (RCC) develop bone metasteses and are often treated with Bisphosphonates (BPs), mainly Zoledronic Acid. Osteonecrosis of Jaw (ONJ) is a complication of BP treatment, but it was rarely reported in RCC patients in first years of ONJ observation (2003–2006). Recent studies reported that angiogenesis suppression may play a role in increasing risk of BP-related ONJ. On the other hand, treatment and prognosis of most metastatic RCC patients have improved thanks to availability of 7 novel agents: one anti-VEGF monoclonal antibody (Bevacizumab), 4 Tyrosine Kinase Inhibitors (TKIs), i.e.Sorafenib, Sunitinib, Pazopanib and Axitinib, and 2 mTOR inhibitors, i.e.Temsirolimus and Everolimus. Recently sporadic ONJ cases have been reported in RCC patients receiving both BPs and biological agents, and even Sunitinb alone. An interaction between BPs and biological agents towards an increased ONJ risk has been suggested, underlined by EMA (European Medical Agency) alerts.
Materials and Methods: Three Medical Oncology units and 5 referral Oral Medicine and Surgery centres in Italy were asked to look for cases of ONJ in RCC patients in their database. Collected characteristics: age; sex; BP treatment (type and duration at ONJ diagnosis time); biological agent treatment ongoing at ONJ diagnosis time, its duration, and eventual other targeted therapy administered in the past; basic data about ONJ (site, ONJ risk factors or triggers).
Results: Charts of 39 ONJ patients have been found, treated with BPs (34 receiving Zoledronic Acid only, 1 Ibandronate, 2 Pamidronate, 2 switching from Pamidronate to Zoledronic Acid) and biological agents (27 Sunitinib, 3 Sorafenib, 1 Bevacizumab, 1 Deforolimus, 7 two or more of these agents in sequence) at time of ONJ diagnosis. Patients' characteristics: 32 males/7 females; median age 62 years (range 45−85). BP treatment duration at ONJ onset: median 12 months (range 1−48). Latest biological treatement was Sunitinib on 34/39 cases (87%). Treatment duration of latest biological agent at ONJ onset: median 8 months (range 1−26). Site of ONJ: 20 in mandible, 14 in maxilla, 4 in both (1 unspecified). Possible risk factors or precipitating events (teeth extraction, oral surgery, dental implants, ill-fitting denture, infections, etc.) have been reported on 28/39 cases (72%).
Conclusions: At our best knowledge, only 16 similar ONJ cases in RCC patients have been reported in recent medical literature as case reports worldwide. Furtherly, 5 ONJ cases among 21 patients were recorded by Bozas et al (ASCO 2011) and 5 out of 49 patients by Beuselinck et al (BJC 2012), with high ONJ cumulative hazard after 24 months of treatment with BPs and TKIs. The unexpectedly high number of cases collected in our survey (39 ONJ cases in 8 Italian centers) suggests that ONJ incidence in RCC patients could be largely underestimated in literature and seems to confirm a potential role of antiangiogenic agents and other biological agents in increasing ONJ risk in RCC patients treated with Bisphosphonates.
Conflict of interest: *Ownership: NO. Advisory board: C. Porta (GSK, Pfizer, Bayer-Schering, Novartis, Astellas, Aveo, Boehringer-Ingelheim). Board of directors: NO. Corporate-sponsored research: C. Porta (GSK, Pfizer, Bayer-Schering, Novartis, Astellas, Aveo). Other substantive relationships: C. Porta (GSK, Pfizer, Bayer-Schering, Novartis, Astellas)*

1392 POSTER

First comparison of biosimilar epoetin alfa and darbepoetin alfa for the treatment of chemotherapy-induced anaemia

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Background: A biosimilar epoetin alfa (Binocrit®) was approved in 2007 for the treatment of chemotherapy-induced anaemia (CIA). There is currently little, if any, comparative data on the relative effectiveness of this biosimilar epoetin alfa and darbepoetin alfa in this setting.
Patients and Methods: This was a retrospective, matched-cohort analysis of patients from a single centre with solid tumours and CIA. Patients were treated with biosimilar epoetin alfa 40,000 IU once weekly (n = 95) or darbepoetin alfa 500 μg once every 3 weeks (n = 50), with the aim of achieving a haemoglobin (Hb) level of 12 g/dL. For this analysis, both treatments were assessed against several parameters, including Hb outcomes and red blood cell (RBC) transfusion requirements.
Results: The two cohorts were well matched in terms of tumour type (85% and 81% in the biosimilar epoetin alfa and darbepoetin group, respectively, were patients with breast cancer) and chemotherapy received (>50% in each group received one of the following regimens; docetaxel/adriamycin/cyclophosphamide [TAC] and 5-fluorouracil/doxorubicin/cyclophosphamide [FEC] with or without docetaxel). All patients received concomitant oral iron. Mean (SD) Hb level before erythropoiesis-stimulating agent (ESA) treatment was 9.85 (0.57) g/dL in the biosimilar epoetin alfa group and 9.92 (0.62) g/dL in the darbepoetin group. The mean maximum Hb achieved was 11.91 and 11.93 g/dL in the biosimilar epoetin alfa and darbepoetin group, respectively. The median time to achieve a Hb increase >1 g/dL was 2 weeks in both groups, and median time to achieve an increase >2 g/dL was 4 weeks in both groups. Four patients (4.2%) in the biosimilar epoetin alfa group and 3 (6%) in the darbepoetin group underwent RBC transfusion during their period of ESA therapy. No adverse events were recorded in either group.
Conclusions: The ability of biosimilar ESAs to effectively and safely treat CIA is the subject of debate. These data indicate the real-life clinical effectiveness and safety of managing CIA with biosimilar epoetin alfa (Binocrit®). Once-weekly treatment with this agent appears to be as effective as darbepoetin once every 3 weeks for raising Hb levels and transfusion avoidance in patients with solid tumours and CIA.
No conflict of interest.

1393 POSTER

Utility of 2D-speckle tracking echocardiography in diagnosis of left ventricular dysfunction in anti-ErbB2 therapy

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Background: ErbB2 is overexpressed in about 25% of breast cancers; in the heart, it modulates myocardial development and function. Trastuzumab (T), an anti-ErbB2 inhibitor, has improved the prognosis of patients with breast cancer, but is related to an increased risk of asymptomatic left ventricular (LV) dysfunction (3−34%) and heart failure (2−4%). Conventional measures of ventricular function, such as fractional shortening (FS) and ejection fraction (FE) are insensitive in detecting early cardiomyopathy induced by antineoplastic therapy.
Here, we aim at assessing whether myocardial strain by 2D-speckle tracking (ST) is able to identify early LV dysfunction in mice treated with doxorubicin (D) and T, alone or in combination (D+T) and to relate data of cardiac function with tissue alterations.
Material and Methods: Cardiac function was measured with FS, by M-mode echocardiography, and with radial myocardial strain with ST in sedated C57BL/6 mice (8−10 wk old) at time 0, 2 and 6 days of daily administration of D (2.17 mg/kg/day), T (2.25 mg/kg/day), D+T (2.17 mg/kg/day + 2.25 mg/kg/day, respectively) and in a control group. In excised hearts, we evaluated TNFα and CD68 by immunohistochemistry; interstizial fibrosis was analyzed with picrosirius red staining.
Results: FS was reduced in group D and D+T at 2 days (52±0.2% and 49±2% respectively), both $p < 0.001$ vs 60±0.4% (sham), while in group T it decreased only at 6 days (49±1.5% vs 60±0.5%, $p = 0.002$). In contrast, after 2 days, myocardial strain was already reduced not only in D and D+T, but also in T alone: 43±3%, 49±1%, and 44±7%, respectively, all $p < 0.05$ vs sham (66±0.6%). Cardiotoxicity was associated with significant alterations in extracellular matrix remodeling as confirmed by an increase of interstizial collagen with D (4.56%), T (2.17%) and D+T (3.77%) at 6

days $p < 0.05$ vs sham (1.17%) and by increased cardiac inflammation, in fact the myocytes were positive for TNFα and CD68 cells/mm^2 at 6 days in group D (16.46% and 155 respectively), in group T+D (12.35% and 74.16) and in group T (5.65% and 72.32) $p < 0.01$ vs sham (0.56% and 2.3).
Conclusions: Myocardial strain identifies LV systolic dysfunction earlier than conventional echocardiography and can be a useful tool to predict cardiotoxicity in this setting.
No conflict of interest.

1394 POSTER

Is there any role of intravenous iron for the treatment of anemia in patients with cancer?

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Background: Anemia is a major cause of morbidity in cancer patients resulting in poor physical performance, prognosis and therapy outcome. Anemia causing clinical symptoms is characterized as a decline of haemoglobin (Hb) below 12 g/dL. The incidence of anemia in initial diagnosis of cancer is about 50% or more, depending on the type and stage of disease. Iron deficiency as a cause of anemia in oncology can be considered under two main headings, those of reduced iron intake and those of increased iron loss. The aim of this study is to evaluate the effect of intravenous (IV) iron treatment on anemia, blood transfusion rates and survival.
Material and Methods: Anemia is defined by World Health Organization criteria; Hb <12 g/dL in women and <13 g/dL in men, and Iron deficiency was defined as serum ferritin <20 μg/dL. The medical records of 34 patients who had IV iron treatment between January 2008 and October 2012 were reviewed. Of these patients, 11 had metastatic disease receiving palliative treatment while 23 had localized disease receiving adjuvant treatment. Patients with haemoglobin levels between 9 and 10 mg/dL, without anemia symptoms undergoing treatment either with chemotherapy, radiotherapy or both were administered IV iron infusion (100 mg IV iron sucrose in 100 mL of saline solution, in 30 minutes, 5 times in 10 days). All patients were followed regularly by physical examination, complete blood count, serum ferritin and serum iron levels one month after the end of IV iron administration and at every 3 months subsequently. The profile of anemia over 24 months of follow-up was analyzed.
Results: Median age was 57 (range 24−81), 58.8% were female and 25.6% had gastrointestinal cancers. Median follow-up period was 19.5 months. Initial median serum hemoglobin, serum ferritin and serum iron levels were 9.2 g/dL, 63 μg/ml and 30 ng/dL respectively. All patients received IV iron as described above during their planned treatment. Median serum hemoglobin, serum ferritin and serum iron levels 1 to 3 months after intravenous iron treatment were 10.7 g/dL, 259 μg/mL and 63 ng/dL, respectively. During 6 to 12 months of follow up, median serum hemoglobin was 11.4 g/dL, serum ferritin was 138 μg/ml and serum iron was 52 ng/dL. At the end of the follow-up period, only 9 patients needed blood transfusion. In this study, IV iron increased serum hemoglobin, serum ferritin and serum iron levels with decreased necessity of blood transfusion.
Conclusions: IV iron is efficacious and effective for the treatment of anemia in cancer patients under treatment either with chemotherapy, radiotherapy or both. Increase of hemoglobin by IV iron administration is cheap and safe and it may prevent blood transfusion and associated complications.
No conflict of interest.

1395 POSTER

Comparison of the effect of filgrastim vs. lenograstim started during febrile neutropenia attack in patients with solid tumors

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Background: Chemotherapy induced Febrile neutropenia (FN) in solid tumors causes mortality and morbidity at a significant rate. In this study, we aimed to compare the effects of filgrastim or lenograstim started with the first dose of antibiotics in patients diagnosed with FN.
Patients and Methods: Between february 2009 and May 2012, one hundred and fifty one patients diagnosed with FN were evaluated retrospectively. Patient's characteristics and other data were collected from patient files. Febrile neutropenia was defined as the number of a single body temperature equal or greater to 38.3ºC measured from mouth or a constant body temperature with equal or greater to 38.0ºC in one hour period in patient with neutropenia which has an absolute neutrofil count less than 500/mm^3. Whenever febrile neutropenia was defined antibiotics to gether with granocyte colony stimulating factors(GCSF) either filgrastim or lenograstim started in 30 minutes.
Results: In this study 175 febrile neutropenia attacks in 151 patients were examined. Seventy three of the patients were male and 78 of them were women. The median age was 53.6 and 53.6 in male and females respectively. The most common solid tumor was breast carcinoma in 38 (25%) patients. One hundres and five FN patients (58%) were patients who received GCSF as primary propylaxis. Demographic characteristics and laboratory findings of patients given Filgrastim and Lenogastrim are represented in Table 1.

Table 1. The characteristics of cases according to the type of GSCF given during febrile neutropenia attack

	Filgrastim use (n = 131)	Lenogastrim use n = 44	P value
Number of cases (M/F)	65/66	21/23	0.82
Age	52.45±15.54	53.13± 17.05	0.76
Days with fever (Mean±SD)	2.27±1.63	2.73±2.40	0.44
Comorbid illness (Y/V)	75/56	23/21	0.56
Number of days in hospital (Mean)	7.79±5.93	7.75±4.63	0.42
Recovery day from neutropenia (Mean)	3.31±1.55	4.00 ±1.79	**0.02**
Number of days given GSCF in hospitalization (Mean)	4.31±2.02	5.46±2.36	**0.002**
Duration of antibiotic treatment (Mean)	7.36 ±5.10	7.66±4.85	0.45

Conclusion: Compared to lenograstim filgrastim shortens the duration of hospitalization time during FN attack by correcting neutropenia faster in solid tumors.
No conflict of interest.

1396 POSTER

Evaluation of sleep disorders in cancer patients with Pittsburgh Sleep Quality Index

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Background: The estimated prevalence of insomnia in cancer patients varies between 23% and 61%. Insomnia, poor sleep quality, short sleep durations are the most common problems seen in cancer patients. In Turkey, there are not enough studies about sleep disorders of cancer patients and more studies are needed. In our study we aimed to investigate the frequency of sleep disorders and the effects of these problems to the quality of life in cancer patients.
Material and Methods: 314 patients receiving chemotherapy for cancer are involved in our study. After getting informed consent from the patients Pittsburgh Sleep Quality Index (PSQI) was administered as a self-report instrument.
Results: The median age for women was 54.8 while for men the median age was 61.8. 53.2% of patients were female, and 46.8% were male. 33.8% of patients had gastrointestinal system malignancies 22.6% had breast cancer, 21.7% had lung cancer, 8.3% had gynecological and 13.7% had other type of malignancies. From the Psychometric evaluation of the Turkish version of the Pittsburgh Sleep Quality Index (PSQI) in cancer patients, 127 (40.4%) had patients had global PSQI scores >5, indicating poor sleep quality. There were no statistically significant difference in PSQI scores between patients according to sexuality, marital status, stage and chemotherapy type ($p > 0.05$). when PSQI was evaluated according to metastatasis regions the patients with bone and visseral metastasis had more lower PSQI scores (p:0.006). In patients with ECOG scores 3 or more had lower PSQI scores (p: 0.02).
Conclusion: PSQI is short,easy to administer, and has well established validity and reliability. This questionnaire may be used to evaluate the sleep disorders in cancer patients. It is important to determine the sleep disorders in cancer patients and the effect of insomnia to patients'quality of life.
No conflict of interest.

1397 POSTER

Epidemiological characteristics of febrile neutropenia in medical oncology unit: Torrecardenas Hospital experience

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Background: Febrile neutropenia (FN) in cancer patients is among the most serious complications related to chemotherapy. In patients with febrile neutropenia are treated with empirical antibiotics until the absolute neutrophil count (ANC) has recovered and the fever has abated. The aim of this study was to analyze the characteristics and infectious complications of neutropenic patients in a referral hospital.
Material and Methods: We retrospectively reviewed 51 patients (pts) diagnosed with febrile neutropenia of Torrecardenas Hospital between January and December 2012. We analyzed: age, sex, neutrophil count, daily living abilities of patients with ECOG performance status (Eastern Cooperative Oncology Group), type of neoplasic, chemotherapy and evolution. We also analyzed the microbiological variables.
Results: The following variables were studied: age 58±13 years, sex 19 males (34%) and 36 females (66%). ECOG performance status: 0 (24 patients: 43.6%), 1 (17 patients: 30.9%), 2 (8 patients: 14.5%), 3 (6 patients: 10.9%). We analyzed the neoplasia type: breast in 25 patients (45.5%), lung in 13 patients (23.6%), gastric in 6 patients (10.9%) and other (ovarian, prostate and pancreas cancer) in 9 patients (16%). Nine (17.5%) had an ANC less 100 cells/mm(3), 12 (23%) one between 100–300 cells/mm(3) and 30 (58%) an ANC greater than 300 cells/mm(3). Thirty five (70%) patients showed ANC recovery in 1−3 days, and 16 (30%) within 4−7 days. The overall mortality was 2 (3.6%) patients. 69.1% (38 patients) received adjuvant chemotherapy. 90% received granulocyte colony stimulating factors (GCSF). Blood cultures were performed in all patients. Only two blood cultures were postive (with methicillin-susceptible staphylococcus aureus). The source of the feber were found in 8 patients: urinary in 2 patients, lung in 3 and abdominal in 3. The most used antimicrobial agents were cefepime and amikacine (65%). 47.3% of patients received antibiotics at discharge (combinations with: fluoroquinolones + amoxicillin clavulanate).
Conclusions: The use of intravenous antibiotics, in addition to GCSF, in treatment of patients with chemotherapy induced febrile neutropenia accelerates neutrophil recovery, and shortens antibiotic therapy and hospitalization.
No conflict of interest.

1398 POSTER

Dutch multidisciplinary evidence-based guideline 'Malnutrition in patients with cancer'

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Background: Malnutrition is highly prevalent in patients with cancer, however, a multidisciplinary evidence-based guideline was not available in the Netherlands. Therefore, the aim of this project was to formulate evidence based recommendations for daily practice to support optimal nutritional care.
Material and Methods: With an inventory among patients and professional care givers, the most important bottlenecks concerning malnutrition and cancer were selected. A multidisciplinary expert group was initiated consisting of an oncologist, surgeon, radiation oncologist, general practitioner, nursing home physician, patient, oncology nurses, oncology dieticians, epidemiologist and with process management of IKNL (Cancer Center the Netherlands). Seven research questions were formulated using the PICO method (P=population; I=intervention; C=control group; O=outcome). Thereafter, a systematic literature search was performed in Pubmed, Embase and Cinahl for the period 1995 till 2010. All articles were evaluated on methodological quality and summarized in evidence tables. The recommendations where formulated using the EBGD method.
Results: From the 5250 articles found in the literature search, 320 were used to answer the research questions. For the following topics the multidisciplinairy guideline describes recommendations for daily practice based on the conclusions from the literature and considerations of the expert group.

1. The definition of malnutrition in patients with cancer and the determination of malnutrition.
2. The consequences of malnutrition.
3. The value of early screening of malnutrition and the preferential screeninginstrument.
4. The increase of requirements for energy, protein and other nutrients in cancer?
5. The effect of nutritional counseling and oral nutritional supplements on malnutrition.
6. The effect of enteral and parenteral nutrition on malnutrition during surgery, radiotherapy, chemotherapy and in the palliative stage of disease.
7. The effect of pharmacological interventions on malnutrition in patients with cancer.

Conclusion: The Dutch multidisciplinary evidence-based guideline 'Malnutrition in patients with cancer' describes the definition, prevalence and consequences of malnutrition in patients with cancer and provides recommendations for the diagnosis, early detection (nutritional screening) and interventions which can be used to treat malnutrition. This guideline is being implemented in clinical practice of all professionals dealing with malnutrition in cancer patients.
No conflict of interest.

1399 POSTER

Safe omission of blood test prior to day 8 dose of oral vinorelbine

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Background: Vinorelbine is a standard treatment for non small cell lung cancer (NSCLC) and advanced breast cancer (ABC). The main dose limiting toxicity is neutropenia and a blood test is recommended prior to each administration. However the incidence of neutropenia detected following a blood test is not known. In addition, the test requires resource on the day ward and incurs laboratory and transport costs, as well as putting pressure on peripheral veins. As most of the patients receiving vinorelbine are being treated in the palliative setting, it is important to minimise any impact on quality of life. We perceived a low incidence of dose delays and haematological toxicity with vinorelbine so initiated an audit of day 8 blood tests and their consequences, with a view to omission.
Methods: Retrospective review of chemotherapy charts/laboratory results of all patients receiving vinorelbine at the hospital between January 2012 to February 2013. Further patients number until August 2013 will be evaluated and included at the time of presentation.
Results: Twenty-five were identified who received vinorelbine in the selected time frame. Seven patients were male and 18 were female with an age range 39 to 82 years. Thirteen patients were receiving vinorelbine for NSCLC and 12 for advanced breast cancer; 13 received vinorelbine as a 1st line treatment, 5 as 2nd line and 7 as 3rd line. 16 patients received vinorelbine in the oral form and 9 as iv.
Vinorelbine was scheduled 121 times and was deferred on 10 (8.3%) occasions, with only 3 delays due to neutropenia. The absolute neutrophil count was found to be below 1 000/mm (grade 3)on five occasions; however vinorelbine was given on time for two of these following careful clinical assessment.
Conclusion: Our data shows that in 121 cycles of treatment, deferment due to neutropenia was only required on three occasions. On the basis of a low incidence of dose delays and haematological toxicity we plan to initiate a local policy of omitting the day 8 blood test in selected patients. There are five published audits looking at the value of a day 8 blood test with vinorelbine. They have generally concluded that the blood test can be omitted provided that patients are reviewed by an oncologist before a day 1 dose, and undergo a telephone assessment by the Oncology nurse specialist before a day 8 dose. We believe our results will support this recommendation.
No conflict of interest.