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The metabolic landscape browser

Leeuwenburgh, V.; Bhattacharya, A.; Urzua, C.; Walvoort, M.; Jalving, H.; de Jong, S.; Fehrmann, R.

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Wednesday, 14 November 2018 12:50–13:30

POSTERS IN THE SPOTLIGHT SESSION

Poster in the Spotlight I

19 (PB-199) Poster Spotlight

Discovery and functional characterization of small molecule inhibitors of SWI/SNF ATPase activity in BRG1/SMARCA4-Deficient non small cell lung cancers

Z. Jagani[1], G. Chenail[1], K. Xiang[1], G. Bushold[1], C. Bhang[1], A. Li[1], A. Vattay[1], T. Gilbert[1], J. Zhu[2], R. Terranova[3], V. Dubost[3], F. Huet[4], D. Farley[5], J. Cantwell[6], D. Ruddy[1], D. Rakiec[1], M. Shirley[1], G. Elliott[1], K. Nakajima[7], J. Papillon[7]. *[1]Novartis Institutes for BioMedical Research, Oncology, Cambridge, USA; [2]Novartis Institutes for BioMedical Research, Scientific Data Analytics, Cambridge, USA; [3]Novartis Institutes for BioMedical Research, Preclinical Safety, Basel, Switzerland; [4]Novartis Institutes for BioMedical Research, PreClinical Safety, Cambridge, USA; [5]Novartis Institutes for BioMedical Research, Chemical Biology and Therapeutics, Cambridge, USA; [6]Novartis Institutes for BioMedical Research, Protein Sciences, Emeryville, USA; [7]Novartis Institutes for BioMedical Research, Global Discovery Chemistry, Cambridge, USA*

Members of the ATP-dependent SWI/SNF chromatin remodeling complexes are among the most frequently mutated genes across various cancers informing critical roles of their dysregulation toward the malignant state. While elucidating the mechanisms of SWI/SNF mutations remains a significant area of investigation, recent studies have also revealed an important role for the remaining SWI/SNF activity in supporting the growth of SWI/SNF-mutant cancers. In particular, the discovery of synthetic lethality between BRM/SMARCA2 and BRG1/SMARCA4, the highly homologous and mutually exclusive catalytic subunits of SWI/SNF complexes, has driven great interest in pursuing the therapeutic targeting of BRM in *BRG1*-mutant/deficient cancers. We report for the first time the discovery and functional characterization of allosteric small molecule dual inhibitors of BRM and BRG1 ATPase activity. BRM011 and its structurally related analogs display cellular activity in modulating BRM-dependent gene expression and inducing growth inhibition in *BRG1*-mutant lung cancer models. Genome wide assessments show that BRM011 treatment induces specific changes in chromatin accessibility and gene expression profiles similar to genetic depletion of BRM. Overall, these studies not only elucidate the previously unexplored feasibility of chemically modulating the enzymatic activity of such a complex and unprecedented target, but also provide fundamental tools for further dissecting SWI/SNF function in cancers, normal tissues and other disease contexts.

No conflict of interest

20 (PB-200) Poster Spotlight

Phase I dose expansion results from a multicenter, open-label study of the MET inhibitor capmatinib (INC280) in adult patients with MET-dysregulated advanced NSCLC

M. Schuler[1], R. Barardi[2], W.T. Lim[3], J.H.M. Schellens[4], M. de Jonge[5], T.M. Bauer[6], A. Azaro[7], M. Gottfried[8], J.Y. Han[9], D.H. Lee[10], D. Hong[11], A. Vogel[12], A. Delmonte[13], C. Bensaid[14], S. Ghebremariam[15], N. Nwana[15], M. Giovannini[15], Y.J. Bang[16]. *[1]West German Cancer Center University Hospital Essen, Department of Medical Oncology, Essen, Germany; [2]Università Politecnica delle Marche, Department of Clinical Oncology, Ancona, Italy; [3]National Cancer Centre Singapore, Division of Medical Oncology, Singapore, Singapore; [4]Netherlands Cancer Institute, Division of Pharmacology, Amsterdam, Netherlands; [5]Erasmus MC Cancer Institute, Department of Medical Oncology, Rotterdam, Netherlands; [6]Sarah Cannon Research Institute and Tennessee Oncology PLCC, Hematology/Oncology, Nashville, USA; [7]Vall d'Hebron University Hospital, Institute of Oncology, Barcelona, Spain; [8]Meir Medical Center, Institute of Oncology, Tel Aviv, Israel; [9]National Cancer Center, Lung Cancer Branch, Goyang, South Korea; [10]Asan Medical Center, University of Ulsan College of Medicine, Department of Oncology, Seoul, South Korea; [11]MD Anderson Cancer Center, Department of Investigational Cancer Therapeutics/Division of Cancer Medicine, Houston, USA; [12]Hannover Medical School, Department of Gastroenterology, Hepatology and Endocrinology, Hannover, Germany; [13]Istituto Romagnolo per lo Studio e la Cura dei Tumori – IRCCS, Thoracic Oncology, Meldola, Italy; [14]Novartis Pharma AG, Novartis Oncology, Basel, Switzerland; [15]Novartis Pharmaceuticals Corporation, Novartis Oncology, East Hanover, USA; [16]Seoul National University Hospital, Biomedical Research Institute, Seoul, South Korea*

Background: MET dysregulation (exon 14 skipping mutation or gene amplification) occurs in 3–4% of NSCLC; concurrent *MET* amplification (median gene copy number [GCN] 10) has been reported in 15% of cases with *MET* mutation. MET dysregulation has been described as a negative prognostic factor and predictor of poor response to standard systemic therapies. In the dose escalation part (NCT01324479), capmatinib, a potent and selective oral MET inhibitor, was well tolerated with a manageable safety profile; RP2D 400 mg BID tablets or 600 mg BID capsules. We present primary data from combined expansion groups of patients (pts) with advanced MET-dysregulated NSCLC.

Materials and Methods: Eligible pts (aged ≥18 years; ECOG PS ≤2) with MET-dysregulated advanced NSCLC, defined as either, (1) MET IHC 2+ or 3+ or H-score ≥150, or *MET*/centromere ratio ≥2.0 or GCN ≥5, or (2) documented *EGFR*wt and centrally assessed MET IHC 3+, received capmatinib at the RP2D until disease progression or excessive toxicity. Additional retrospective MET status assessment (*MET* gene amplification by FISH and NGS) was also performed. The primary objective was to determine the safety and tolerability of capmatinib; key secondary objective was to explore the antitumor activity of capmatinib at the RP2D.

Results: Of 55 pts with advanced MET-dysregulated NSCLC, 40/55 (72.7%) had received ≥2 prior systemic therapies. All pts discontinued treatment, primarily due to disease progression (38/55, 69.1%). Median treatment duration was 10.4 weeks. The most frequent study treatment-related AEs of any grade were nausea (41.8%), peripheral edema (32.7%), vomiting (30.9%), fatigue (20.0%), diarrhea (18.2%), and decreased appetite (16.4%). Drug-related Grade ¾ AEs were reported in 21/55 (38.2%) pts. Among the 55 pts, ORR (BIRC per RECIST 1.1) and disease control rate (DCR), irrespective of MET status, were 23.6% and 50.9%, respectively. In pts with MET IHC 3+ (n = 37) there was 1 CR and 10 PRs (ORR 29.7%; DCR 62.2%). In pts with MET GCN ≥6 (n = 15) there was 1 CR and 7 PRs (ORR 53.3%; DCR 80.0%). A response to capmatinib (CR or PR) was reported in ¾ pts with retrospectively identified *MET* exon 14 skipping mutations. The estimated median PFS (BIRC per RECIST 1.1) was 3.7 months irrespective of MET status, 7.4 months for pts with MET IHC 3+, and 11.0 months for pts with MET GCN ≥6. PFS was 3.0, 3.8, 3.9, and 18.6 months (all censored) for the 4 pts with *MET*-mutated NSCLC.

Conclusions: Capmatinib showed promising antitumor activity in pts with heavily pretreated, MET-dysregulated advanced NSCLC. Clinical benefit was particularly prominent if *MET* amplification and/or *MET* exon 14 skipping mutations were present, highlighting the key role of precise biomarker selection. A pivotal Phase II study (NCT02414139) is ongoing to confirm the efficacy of capmatinib in *MET*-mutated and *MET*-amplified advanced NSCLC.

Conflict of interest: Ownership: Hong: Ownership (founder) – Oncoresponse. Advisory Board: Bauer: Consulting or advisory role – Guardant Health Ignyta (Inst) Loxo Moderna Therapeutics (Inst) Pfizer. Hong: Consulting or advisory role – Bayer, Baxter, Guidepoint Global, Takeda, Janssen, Molecular Match. Corporate-sponsored Research: Bauer: Research funding – Abbvie (Inst) Aileron Therapeutics (Inst) Amgen (Inst) Astellas Pharma (Inst) AstraZeneca (Inst) Boehringer Ingelheim (Inst) Bristol-Myers Squibb (Inst) Calithera Biosciences (Inst) Daiichi Sankyo (Inst) Deciphera (Inst) Five Prime Therapeutics (Inst) Genentech/Roche (Inst) GlaxoSmithKline (Inst) Ignyta (Inst) Immunocore (Inst) Immunogen (Inst) Incyte (Inst) Kolltan Pharmaceuticals (Inst) Leap Therapeutics (Inst) Lilly (Inst) MabVax (Inst) MedImmune (Inst) Medpacto, Inc. (Inst) Merck (Inst) Merrimack (Inst) Millennium (Inst) Mirati Therapeutics (Inst) Moderna Therapeutics (Inst) Novartis (Inst) Peleton (Inst) Pfizer (Inst) Principa Biopharma (Inst) Principa Biopharma (Inst) Roche (Inst) Sanofi (Inst) Stemline Therapeutics (Inst). Other Substantive Relationships: Schuler: personal fees from AstraZeneca, grants and personal fees from Boehringer Ingelheim, grants and personal fees from Bristol-Myers Squibb, grants and personal fees from Novartis, personal fees from Roche, personal fees from Abbvie, personal fees from Alexion, personal fees from Celgene, personal fees from Lilly, personal fees from MSD, personal fees from Pierre Fabre, outside the submitted work. Lee: personal fees from AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, CJ Healthcare, Eli Lilly, Janssen, Merck, MSD, Mundipharma, Novartis, Ono, Pfizer, Roche, Samyang Biopharm and ST Cube, outside the submitted work. Vogel: grants and personal fees from Novartis, outside the submitted work. Bang: grants from Novartis Pharma, during the conduct of the study (to the institution). Hong: grants from Bayer, grants from Lilly, grants from Genentech, grants from LOXO, grants from Pfizer, grants from Amgen, grants from Mirati, grants from Ignyta, grants from Merck, grants from Daichii-Sanko, grants from Eisai, personal fees from Mirna, personal fees from LOXO, grants from Abbvie, grants from Astra-Zeneca, grants from BMS, grants from Genmab, grants from Infinity, grants from Kite, grants from Kyowa, grants from Medimmune, grants from Molecular Template, grants

from Novartis. Ben Said: employee of Novartis. Ghebremariam: employee of Novartis, stocks from Novartis. Nwana: employee of Novartis. Giovannini: employee of Novartis.

21 (PB-201) Poster Spotlight

Utility of a selective SHP2-inhibitor in KRAS-mutant cancer

H. Hao[1], H. Wang[1], C. Liu[1], S. Kovats[1], R. Velazquez[1], H. Lu[1], B. Pant[1], J. Lim[1], M. Fleming[1], M. Shirley[1], M. Lamarche[2], S. Moody[1], S. Silver-Brown[1], G. Caponigro[1], T. Abrams[1], P. Hammerman[1], J. Williams[1], J. Engelman[1], S. Goldoni[1], M. Mohseni[1]. *[1]Novartis Institute of Biomedical Research, Oncology, Cambridge, USA; [2]Novartis Institute of Biomedical Research, Global Discovery Chemistry, Cambridge, USA*

Background: RTK-feedback reactivation of the MAPK pathway by MEK inhibition in Kras-mutant cancers can be effectively blocked by targeting SHP2, suggesting that SHP2 inhibition alone can alter activity of mutant Kras. The in vivo utility of the selective allosteric SHP2 inhibitor, SHP099, has made it possible to further interrogate single agent activity of SHP2 within the mutant Kras context.

Methods: Efficacy of Kras mutant models to SHP099 and RTK pathway inhibitors (Lapatanib, BGJ398, INC280) was evaluated in vitro using Cell-Titer-Glo, under both monolayer conditions and in matrigel or ultra-low attachment plates. RNAseq was performed in vitro from SHP099 or Trametinib treated for 19 hours in the Kras-mutant, MiaPaca-2 cell line. In vivo efficacy of SHP099 in Kras-mutant xenografts implanted subcutaneously or orthotopically was performed at 50–100 mg/kg, daily for >10 days, during which caliper measurements and bodyweights were collected twice weekly. At the end of treatment, tumors were collected and evaluated for DUSP6 and phospho-ERK. Genetic depletion of SHP2 by Crispr or by shRNA was evaluated in vitro in 2D, 3D and in vivo in the KRAS-mutant cell line, MiaPaCa-2. Phenotypic rescue of SHP099 efficacy was performed by over-expression of an inducible SHP099 binding-deficient mutant of SHP2 ($SHP2^{T253-Q257L}$) and by overexpression of constitutive active SOS1 (SOS1-F).

Results: Inhibition of SHP2 in KRAS mutant cancers is efficacious across variants (G12C, G12D, G12V, G13D, and Q61H). Sensitivity of KRAS-driven cancers towards the allosteric SHP2-inhibitor, SHP099, is only revealed under anchorage-independent growth conditions. RNAseq analysis on SHP099-treated tumor spheroids demonstrated significant gene expression changes in contrast to the monolayer setting, suggesting that activity of SHP2 in the presence of KRAS mutations is dependent on cellular context. 3D *in vitro* activity correlated with *in vivo* efficacy, moreover, we find that *in vitro* 3D sensitivity and in vivo efficacy with SHP099 is similar to that of RTK-pathway inhibitors, revealing a dependency for mutant KRAS on upstream signaling. We further demonstrate that efficacy is tumor cell autonomous as genetic depletion of SHP2 recapitulated SHP099 activity. Mechanistically, overexpression of constitutive active SOS1 rescues SHP2 inhibition, highlighting the dependency of mutant KRAS, and the downstream signaling, on SHP2. Interestingly, cell lines harboring Kras mutations with low intrinsic GTP hydrolysis, i.e., $KRAS^{Q61H}$, also display *in vivo* efficacy upon SHP099 treatment with concomitant downregulation of many growth factors and suppression of pERK in vitro.

Conclusions: Taken together, these data support that mutant KRAS depends on upstream signaling from SHP2 and support a potential opportunity for treatment of Kras-mutant cancers.

No conflict of interest

22 (PB-202) Poster Spotlight

PLX8394 selectively disrupts BRAF-dimers and RAS-independent BRAF mutant-driven signaling

Z. Yao[1], Y. Gao[1], W. Su[1], R. Yaeger[2], N. Na[1], Y. Zhang[3], C. Zhang[3], A. Rymar[3], A. Tao[4], N. Torres[1], R. Mcgriskin[1], H. Zhao[1], Q. Chang[1], B. Qeriqi[1], E. de Stanchina[1], M. Barbacid[5], G. Bollag[3], N. Rosen[1]. *[1]Memorial Sloan Kettering Cancer Center, Molecular Pharmacology, New York, USA; [2]Memorial Sloan Kettering Cancer Center, Department of Medicine, New York, USA; [3]Plexxikon Inc., Berkeley, USA; [4]New York University, NYU Langone Medical Center, New York, USA; [5]Centro Nacional de Investigaciones Oncológicas CNIO, Molecular Oncology Programme, Madrid, Spain*

Background: BRAF mutants and fusions are expressed in ~8% of human tumors. Almost all known activating BRAF mutants and fusions signal as RAS-independent, constitutive, active dimers with the single exception of BRAF V600 alleles which function as active monomers in cells with low levels of RAS activation. Current RAF inhibitors potently inhibit BRAF V600 mutant monomers but their ability to inhibit RAF dimers is limited by their induction of negative cooperativity when bound to one of the protomers in the dimer.

Material and Methods: We tested the activity of PLX8394 and six other RAF inhibitors in four cell lines, in which the ERK signaling is driven by mutant BRAF monomer, mutant BRAF dimers, or WT RAF dimers activated by WT or mutant RAS. We determined the activity of PLX8394 against the second protomer of the BRAF dimers of which the other protomer was occupied by LGX818. The effects of PLX8394 on the formation of different RAS-dependent WT RAF homo- or heterodimers and RAS-independent mutant RAF homo- or heterodimers were deteremiend by Co-IP assays. We also studied the mechanism of the different properties of PLX8394 against BRAF or CRAF dimers based on the structural data. Last, we tested the activity of PLX8394 in a panel of tumor cell lines and PDX models driven by RAS-dependent or -independent BRAF mutants.

Results: We show here that PLX8394 inhibits signaling driven by mutant BRAF dimers by specifically disrupting BRAF-containing dimers, including BRAF-BRAF homodimer and BRAF-CRAF heterodimers, but not CRAF homodimers or ARAF-containing dimers. The differences in the amino acid residues situated at the N-terminus of the kinase domain between the RAF isofroms appear to be responsible for this differential venerability. This feature of PLX8394 allows it to inhibit both activating BRAF monomers (V600 mutant alleles) and dimers, but paradoxically activate CRAF homodimers by binding to one of the two CRAF protomers. Thus, this drug has minimal effects on ERK signaling in wild type RAF cells, in which it disrupts and inhibits BRAF dimers, but activates CRAF homodimers. In contrast, in tumors driven by activated mutant BRAF dimers or fusion BRAF dimers, CRAF homodimer levels are vanishingly low because of feedback inhibition of RAS. In these tumors, ERK signaling is dominantly driven by mutant BRAF dimers which are disrupted by this drug; thus, the signaling and tumor growth can be effectively inhibited by the drug.

Conclusions: PLX8394 is the first drug that selectively disrupts and therefore inhibits BRAF-containing dimers as well as BRAF V600 monomers in tumors at doses that have almost no effect on WT dimers in normal cells.

Conflict of interest: Ownership: N.R. owns stocks in Beigene, Wellspring and Kura. Advisory Board: N.R. is on the SAB of Chugai, Beigene, Wellspring and Kura. N.R. is also on the SAB of Daiichi-Sankyo, Astra-Zeneca and Takeda, and is a consultant to Novartis. Corporate-sponsored Research: N.R. receives research funding from Chugai. Other Substantive Relationships: Y.Z., C.Z., A. R. and G. B. are employees of Plexxikon Inc.

Thursday, 15 November 2018 13:00–13:30

POSTERS IN THE SPOTLIGHT SESSION

Poster in the Spotlight II

23 (PB-203) Poster Spotlight

Analysis of cell-free plasma DNA to identify tumors with microsatellite instability and exceptionally high tumor mutation burden in patients treated with PD-1 blockade

A. Georgiadis[1], J.N. Durham[2,3,4], L. Keefer[1], B.R. Bartlett[5], M. Zielonka[1], D. Murphy[1], J.R. White[1], S. Lu[3], E. Verner[1], F. Ruan[1], D. Riley[1], R.A. Anders[2,3,6], S.V. Angiuoli[1], S. Jones[1], V.E. Velculescu[3], D.T. Le[2,3,4], L.A. Diaz, Jr.[7], M. Sausen[1]. *[1]Personal Genome Diagnostics, Research and Development, Baltimore, USA; [2]Johns Hopkins, Bloomberg-Kimmel Institute for Cancer Immunotherapy, Baltimore, USA; [3]Johns Hopkins, Sidney Kimmel Comprehensive Cancer Center, Baltimore, USA; [4]Johns Hopkins, Swim Across America Laboratory, Baltimore, USA; [5]Johns Hopkins, Ludwig Center and Howard Hughes Medical Institute, Baltimore, USA; [6]Johns Hopkins, Department of Pathology, Baltimore, USA; [7]Memorial Sloan-Kettering Cancer Center, Division of Solid Tumor Oncology, New York, USA*

Background: Microsatellite instability (MSI) and tumor mutation burden (TMB) are promising pan-tumor biomarkers used to select patients for treatment with immune checkpoint blockade; however, real-time sequencing of unresectable or metastatic solid tumors is often challenging. Here we report a plasma-based next generation sequencing (NGS) approach using a 98 kb targeted panel of 58 genes for the detection of MSI and exceptionally high TMB in cancer patients with advanced disease.

Materials and Methods: Our approach utilized a hybrid-capture based, targeted NGS analysis of a pan-cancer gene panel, designed at Personal Genome Diagnostics (PGDx), including targeted mononucleotide repeat loci for MSI detection. We developed a multifactorial error correction method to detect sequence alterations in plasma, and used a novel peak finding

algorithm to identify rare MSI frameshift alleles in cell-free DNA (cfDNA). To demonstrate its capacity to predict response to immune checkpoint blockade using baseline plasma, 16 samples from patients treated with PD-1 blockade therapy, both with or without mismatch repair deficiency, were analyzed for the presence of MSI and exceptionally high TMB. In addition, serially collected plasma was used to assess molecular remission and circulating tumor DNA (ctDNA) dynamics in patients during PD-1 blockade treatment.

Results: Using the error correction and peak finding approaches developed by PGDx to detect rare mutations in plasma derived cfDNA, we demonstrate a per-patient specificity of 99.4% (162/163) and 100% (163/163), respectively for MSI and exceptionally high TMB. In the PD-1 blockade treated patient cohort, MSI and TMB status demonstrated the capacity to predict progression-free survival using baseline plasma samples ($n = 16$, $p = 0.01$ and 0.004, respectively). Additionally, we analyzed longitudinal plasma samples for reduction in protein biomarkers, and analyzed cfDNA for MSI mutant allele fraction, and mutational burden to develop a prognostic signature for patients who achieved a durable response to PD-1 blockade ($p = 0.01$, $p = 0.032$, $p = 0.013$, respectively).

Conclusions: Here we have described the development of a method for simultaneous detection of MSI and exceptionally high TMB directly from cfDNA. These data provide feasibility for pan-cancer screening and monitoring of patients who exhibit these biomarkers and may respond to immune checkpoint blockade.

Conflict of interest: Board of Directors: Luis Diaz and Victor Velculescu are on the Board of Directors for Personal Genome Diagnostics. Other Substantive Relationships: All authors affiliated with Personal Genome Diagnostics are employees of Personal Genome Diagnostics.

24 (PB-204) Poster Spotlight

Chromatin destabilization by CBL0137 and panobinostat leads to complete tumour regression of childhood neuroblastoma in immunocompetent transgenic mice

L. Xiao[1], J. Murray[1], A. Ehteda[2], C. Somers[3], C. Mayoh[3], L. Gamble[3], R. Pandher[1], M. Tsoli[2], G. Eden[3], S. Allan[3], S. Sarraf[3], A. Kosciolek[3], D. Ziegler[2], M. Henderson[3], N. Issaeva[4], K. Gurova[5], A. Gudkov[5], M. Haber[3], M. Norris[1]. [1]*Children's Cancer Institute, Molecular Diagnostics, Sydney, Australia;* [2]*Children's Cancer Institute, Targeted Therapy, Sydney, Australia;* [3]*Children's Cancer Institute, Experimental Therapeutics, Sydney, Australia;* [4]*Yale Cancer Centre, Yale School of Medicine, Division of Otolaryngology, Surgery Department, New Haven, Australia;* [5]*Roswell Park Cancer Institute, Department of Cell Stress Biology, Buffalo, USA*

Background: Neuroblastoma is the most common extracranial solid tumour in children. High-risk neuroblastomas, which have less than 50% survival rates, are frequently characterised by amplification of the oncogene *MYCN*. Using the highly clinically relevant *Th-MYCN*transgenic neuroblastoma mouse model representative of high-risk neuroblastoma, we have previously found that CBL0137, which is a safe, non-DNA damaging drug currently in phase I trials for adult cancers, significantly extended survival rates of these mice in a dose-dependent manner. CBL0137 belongs to a novel class of anti-cancer drugs, curaxins, which can induce chromatin damage leading to tumour cell death. Here, we identify the FDA-approved histone deacetylase (HDAC) inhibitor, panobinostat, as a strong potentiator of CBL0137 efficacy and have delineated the mechanistic pathway underlying their synergy.

Material and Methods: Drug synergy *in vitro*was determined by colony and cytotoxic assays. Animal models included the *Th-MYCN*transgenic neuroblastoma model, and xenograft models for *mixed lineage leukaemia (MLL)*-rearranged leukaemia and diffuse intrinsic pontine glioma (DIPG). Histone eviction was visualised in cells transduced with fluorescence-tagged histone 1 protein. DNA damage repair inhibition was quantified by pulsed-field gel electrophoresis. RNA-seq was used to determine gene expression in *Th-MYCN*tumours after CBL/panobinostat treatment.

Results: Combination of CBL0137 and panobinostat produced strong synergy to reduce cell viability and clonogenicity in neuroblastoma cells *in vitro*. More strikingly, the combination eradicated established neuroblastoma in 100% *Th-MYCN* mice tested, which is the most significant result we have ever obtained in this aggressive neuroblastoma model. Mechanistic studies show that panobinostat markedly enhanced chromatin destabilization induced by CBL0137, resulting in histone eviction and DNA repair suppression. Furthermore, the combination elicited a rapid and robust interferon response in tumours, increasing expression of interferon-induced genes, such as *Ifit1*, *Ifit3*, and *Ifit3b,* by more than 200 fold, strongly indicating that CBL0137/panobinostat may activate anti-tumour immunity. Using preclinical xenograft models of two other aggressive paediatric tumours, DIPG and *MLL*-rearranged leukaemia, we also demonstrated that CBL0137 and panobinostat synergised strongly to slow tumour progression and significantly extend survival.

Conclusions: Our studies have identified CBL0137 and panobinostat as a highly effective drug combination for neuroblastoma and other aggressive paediatric malignancies. This combination likely halts tumour growth through a two-pronged attack: chromatin destabilisation and activation of interferon pathways. Our results will greatly facilitate clinical development of effective and non-toxic therapies for childhood cancer.

Conflict of interest: Ownership: Cleveland BioLabs stock ownership.

25 (PB-205) Poster Spotlight

MAPKAPK5 inhibition suppresses YAP-driven tumorigenesis

M.H. Kim[1], J. Seo[2], H. Hong[2], S.K. Kim[3], J. Kim[2]. [1]*Yonsei University College of Medicine, Division of Medical Oncology, Department of Internal Medicine, Seoul, Korea;* [2]*KAIST, Graduate School of Medical Science and Engineering, Daejeon, Korea;* [3]*Yonsei University College of Medicine, Department of Pathology, Seoul, Korea*

Background: Recent evidence suggests that the Hippo pathway effector, YAP critically involves tumorigenesis in human malignancies. The oncogenic YAP activation is induced by various molecular mechanisms, including loss of LATS1/2, the negative regulators of YAP. We aimed to find therapeutic targets for tumors with LATS1/2 loss by RNA interference (RNAi) screening.

Materials and Methods: We deleted LATS1/2 alleles in human RPE1 cells by CRISPR-Cas9, and the cells were subjected to an image-based kinome RNAi library screening for identifying YAP downregulating hits. The influence of target kinase activity on nuclear localization, protein stability, and tumorigenic potential of YAP was investigated.

Results: The LATS1/2 knockout in RPE1 cells caused nuclear localization and transcriptional activation of YAP. In contrast to wild-type, LATS1/2-null RPE1 cells showed robust cell proliferation on matrigel culture and tumor formation on xenograft model. Our RNAi screening firstly found that inhibition of MAPKAPK5 (MK5) provokes YAP degradation and cytoplasmic retention. MK5 physically interacted with YAP and prevented its proteasomal degradation. The MK5 particularly involved $CK1_{\delta/\varepsilon}$–dependent YAP degradation pathway that appears independent from LATS1/2 activity. The MK5-dependent gene signature, derived by RNA-seq of MK5 knockdown experiment, correlated with poor prognosis of YAP-driven cancers, malignant mesothelioma (MM) and uveal melanoma (UVM), in TCGA database. The MK5 depletion significantly suppressed growth of LATS1/2-null RPE1 tumor xenograft, as well as proliferation of MM and UVM cell lines.

Conclusions: These results propose MK5 as a novel component of YAP protein stability regulation. We suggest that MK5 inhibition is a promising therapeutic strategy for YAP-driven tumors.

No conflict of interest

Tuesday, 13 November 2018

POSTER SESSION

DNA Repair Modulation

50 (PB-001) Poster

SY-1365, a selective CDK7 inhibitor, enhances carboplatin activity in ovarian cancer cell lines and xenografts, and transcriptionally inhibits homologous recombination repair (HRR) genes

L. Johannessen[1], S. Hu[2], N. Ke[1], N. Rajagopal[3], D. Orlando[3], S. Alnemy[2], J. Carulli[2], G. Hodgson[1], E. di Tomaso[1]. [1]*Syros Pharmaceuticals, Translational Medicine, Cambridge, USA;* [2]*Syros Pharmaceuticals, Discovery, Cambridge, USA;* [3]*Syros Pharmaceuticals, Computational Biology, Cambridge, USA*

Introduction: Recurrent platinum-resistant ovarian cancers are marked by resistance to chemotherapies and account for 70% to 80% of ovarian cancer deaths. Previous studies have shown that SY-1365, a selective CDK7 inhibitor in phase 1 clinical development, induces cytotoxicity in OC cell lines in vitro and induces tumor growth inhibition, including regressions, in OC patient-derived xenograft models. Here we report on the activity of SY-1365 in combination with carboplatin (CP) and show that the underlying mechanism of action may be anchored in the effects of SY-1365 on transcriptional DNA damage responses in OC preclinical models.

Methods: Dose-response curves were generated in OC cell lines (TOV21G, OVCAR3, A2780) in vitro with SY-1365 in combination with CP; isobolograms and synergy were calculated using the Chou-Talalay method. RNA expression of HRR genes was analyzed by NanoString. In vivo activity of SY-1365 and CP as single agents and in combination were evaluated in

CP sensitive OC xenografts. CP was dosed 50 mg/kg IP once weekly (QW). SY-1365 was dosed 30 mg/kg IV QW, 8–24 h after CP. Tumor growth inhibition (TGI) was determined by comparing the average change in tumor volume in drug- versus vehicle-treated mice on the last dosing day.

Results: Treatment of OC cell lines in vitro with varying concentrations of SY-1365 and CP revealed synergistic interactions in all 3 cell lines. In vivo, SY-1365 and CP each induced measurable TGI in OC xenografts as single agents at sub-maximal therapeutic doses, whereas regressions were observed when the same doses of both agents were used in combination (Table).

	Tumor Growth Inhibition (%)		
Xenograft	CP	SY-1365	CP+SY-1365
TOV21G	79	84	120
OVCAR3	88	37	105

To explore a potential mechanistic basis for synergy, RNA expression levels of HRR genes BRCA1/2, ATM, ATR, and RAD51, important for sensing and repairing double strand breaks created by CP, were assessed at 0, 6, and 16 hours (h) after treatment with 50 nM SY-1365. Relative to 0 h, lower RNA expression was evident for most genes by 6 h; by 16 h all genes were down regulated in all cell lines (average 2.2-fold; range 1.1–4.6). Similar mechanistic analyses in OC xenograft models are ongoing.

Conclusions: SY-1365 is synergistic with CP in OC cell lines in vitro and enhances CP activity in OC xenografts in vivo. SY-1365 transcriptionally downregulates key mediators of the HRR pathway, including BRCA1/2, in OC cell lines in vitro. Taken together these results suggest that SY-1365 may impede DNA damage responses and DNA repair in OC patients, and support the potential for combination strategies aimed at exploiting this mechanism of action. SY-1365 is currently being assessed in a phase 1 trial in adult patients with advanced solid tumors (NCT03134638) with a planned expansion cohort in OC to explore SY-1365 in combination with carboplatin.

Conflict of interest: Ownership: All authors are employees and shareholders in Syros Pharmaceuticals.

51 (PB-002) Poster

Phase II trial of pembrolizumab in patients with solid tumors functionally competent or deficient for the Fanconi Anemia repair pathway

M. Villalona[1], J.P. Diaz[1], W. Duan[2], Z. Diaz[1], E.D. Schroeder[1], S. Aparo[1], F. Vargas Madueno[1], A. Alonso[1], S. Cuitiva[1], F. Albrecht[1], S. Venkatappa[1], V. Guardiola[1], M. Troner[1], G. Walker[3], G. Narasimhan[4]. [1]*Baptist Health South Florida, Miami Cancer Institute, Miami, USA;* [2]*Florida International University, Molecular Genetics, Miami, USA;* [3]*Baptist Health SouthFlorida, Research and Grants, Miami, USA;* [4]*Florida International University, School of Computing and Information Sciences, Miami, USA*

Background: Based on the activity of immune checkpoint inhibitors (ICI) in mismatch repair deficient tumors we are evaluating the interplay between homologous recombination (HR) repair deficiency, and solid tumor response to ICI using an all-inclusive functional triple stain (FANCD2/DAPI/Ki67) immunofluorescence assay of the Fanconi Anemia pathway (FATSI).

Material and Methods: We are conducting a phase 2 trial (NCT03274661) of pembrolizumab (PEM) in patients with metastatic solid tumors progressing on standard of care therapy and for whom PEM does not have an FDA approved indication. FATSI is performed in all patients to evaluate if it is a useful patient selection biomarker. Patients with microsatellite instability (MSI) high tumors are not eligible. The primary objective is to evaluate objective response rate (iORR, CR+PR) by Immune Response Criteria in patients with FA Repair Pathway functionally competent and functionally deficient (FATSI negative) tumors, with the exploratory hypothesis that patients with FATSI negative tumors will have better clinical outcome. Secondary objectives are 20-week progression free survival and overall survival. Exploratory objectives include evaluation of mutation load, stool analyses for microbiome composition (before and after treatment), and alterations in HR repair genes. We utilized a two-stage phase II trial design to detect an iORR ≥20% in the whole population tested vs. the null hypothesis that the true iORR ≤5%. At least 2 of the first 20 evaluable patients should have an objective response in order to proceed to full accrual of 39 evaluable patients. Better outcomes in the FATSI negative group would support a biomarker selected population approach.

Results: Starting Nov 2017 29 patients (23F, 6M; median age 60[36–83] have enrolled). The median number of prior regimens was 3 [0–7]. Primary Dx include ovarian/fallopian(11), endometrial(4), colorectal(3), cervix(2), pancreatic(2), and breast, esophagus, small-cell lung, oral cavity, GI-neuroendocrine, small bowel and thymic carcinoma (1 each). No unexpected toxicities have occurred. Response evaluation for the 1st stage was completed with 2PR, 8SD, 10PD among 20 patients. FATSI analysis in this first batch of patients showed 11 positive, 4 negative, 5 additional tissue required (no ki67) tumors. 1PR, 5SD, 5PD occurred among the 11 FATSI(+) and 1PR, 2SD, 1PD among the 4 FATSI(-) tumors. Given the rapid pace of accrual, complete clinical data and the rest of correlatives will be available at the meeting.

Conclusions: Meaningful antitumor activity was observed with PEM in non MSI-high malignancies with no current FDA approved indications. Evaluation of FATSI as a predictive biomarker needs full accrual to the trial. If suggestive, further testing of the combination of ICI plus a PARP inhibitor versus individual agents in FATSI (-) selected population is planned.

Conflict of interest: Corporate-sponsored Research: We receive a grant to support clinical trials costs and free pembrolizumab from Merck (manufacturer of pembrolizumab).

52 (PB-003) Poster

PARP inhibitors activate cancer cell-intrinsic immunity via cGAS/STING in ERCC1- and BRCA1-defective contexts

R. Chabanon[1], G. Muirhead[2], D. Krastev[3], J. Adam[1], M. Garrido[1], D. Morel[1], N. Dorvault[1], T. Eychenne[1], C. Hénon[1], R. Marlow[4], C. Massard[5], A. Ashworth[6], S. Pettitt[3], S. Haider[2], A. Tutt[2], A. Marabelle[5], J.C. Soria[5], C. Lord[3], S. Postel-Vinay[1]. [1]*Gustave Roussy, ATIP-Avenir Group – Inserm Unit U981, Villejuif, France;* [2]*The Institute of Cancer Research, The Breast Cancer Now Toby Robins Breast Cancer Research Centre, London, United Kingdom;* [3]*The Institute of Cancer Research, CRUK Gene Function Laboratory, London, United Kingdom;* [4]*King's College London, The Breast Cancer Now Research Unit, London, United Kingdom;* [5]*Gustave Roussy, DITEP Département d'Innovations Thérapeutiques et Essais Précoces, Villejuif, France;* [6]*UCSF, Helen Diller Family Comprehensive Cancer Centre, San Francisco, USA*

Background: The cyclic GMP-AMP synthase–stimulator of interferon genes (cGAS/STING) pathway is an essential regulator of innate immune responses and a potent catalyst of anti-tumour immunity. This pathway is activated upon detection of cytosolic DNA and can be triggered therapeutically by the use of specific STING agonists, radiotherapy or some S-phase-specific chemotherapies. Poly(ADP-ribose) polymerase inhibitors (PARPi) have shown clinical efficacy in *BRCA*-deficient tumours, as well as preclinical efficacy in other DNA repair-deficient contexts such as *ERCC1*-deficient non-small cell lung cancer (NSCLC). Here, we evaluated the potential of PARPi to elicit cell-autonomous immune responses in DNA repair-deficient cancer cells.

Material and Methods: Using several isogenic models of *ERCC1*-deficient NSCLC and *BRCA1*-mutated triple-negative breast cancer (TNBC), we performed high-content immunofluorescent screening to assess the presence of cytosolic DNA in cells exposed to the clinical PARPi olaparib and rucaparib. cGAS/STING activation was monitored through the detection of cGAS cytoplasmic re-localization, TBK1 phosphorylation, and activation of other downstream signalling effectors. We assessed the modulation of immune signatures in *ERCC1*-deficient NSCLC cells using RNA-sequencing and Gene Set Enrichment Analysis, and evaluated lymphocytic infiltrates in a series of human NSCLC samples.

Results: We observed that *ERCC1*-defective NSCLC cells harbour an enhanced STING expression and type I interferon transcriptomic signature, and that low ERCC1 expression associates with increased lymphocytic infiltration in human NSCLC samples. We further found that PARPi induce cell cycle-dependent formation of cytosolic chromatin fragments (CCF) with micronuclei characteristics in *ERCC1*-defective NSCLC and *BRCA1*-mutant TNBC cells. These CCF are detected by cGAS and associate with the activation of cGAS/STING downstream effectors – including pTBK1, pIRF3 and pNFκB –, which stimulates type I interferon signalling and promotes the secretion of lympho-attractant chemokines, such as CCL5, in a cGAS/STING-dependent manner. These effects are suppressed in *BRCA1*-revertant and *PARP1*-null TNBC cells, supporting that this phenotype results from a *BRCA1*-dependent and on-target effect of PARPi, respectively.

Conclusion: Clinical PARPi trigger cGAS/STING activation in a cell-autonomous fashion in *ERCC1*-deficient NSCLC and *BRCA1*-mutant TNBC cells. These data provide the preclinical rationale for exploiting PARPi as immunomodulatory agents in combination with immunotherapies in appropriately molecularly-selected populations.

No conflict of interest

53 (PB-004) Poster

Investigating the interaction of the ATR inhibitor, AZD6738, with platinum chemotherapy

S. Hall[1], A. Lau[2], E. Dean[3], E. Martin[4], C. Huins[1], C. Ottley[5], G. Veal[1], Y. Drew[1]. *[1]Northern Institute for Cancer Research, Paul O'Gorman Building, Newcastle-upon-Tyne, United Kingdom; [2]Bioscience, Oncology, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom; [3]Early Clinical Development, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom; [4]Drug Safety and Metabolisn, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom; [5]Department of Earth Sciences, Durham University, Durham, United Kingdom*

Background: Drug combinations involving conventional chemotherapy with inhibitors of the DNA damage response may be therapeutically challenging due to potential overlapping toxicities, such as myelosuppression, and determination of the optimal sequence and schedule. AZD6738, an oral ATR inhibitor, has entered early phase clinical trials in combination with anti-cancer drugs, including carboplatin (NCT02264678). An understanding of the mechanism of interaction of AZD6738 with platinum (Pt) chemotherapy at a molecular level may be beneficial to guide future trial design and clinical use, in terms of maximising efficacy whilst minimising toxicity. In particular, quantification of Pt-DNA adducts formed in the presence of AZD6738 monotherapy would provide a novel insight into the repair and recovery of DNA following combination treatment.

Material and Methods: Sulphorhodamine B (SRB) colorimetric assays were performed to test the growth inhibition of AZD6738 alone and in combination with cisplatin and carboplatin in a panel of non-small cell lung (H460, H23) and breast (MCF7, HCC1806, MDA-MB-436, MDA-MB-468) cancer cell lines. Data were evaluated by median effect analysis using CalcuSyn software (Biosoft, Cambridge, UK). Time-dependent analyses of Pt-DNA adduct formation in cells treated with cisplatin (5 μM) and AZD6738 (1.5 μM) were measured over varying incubation times using inductively-coupled plasma mass spectrometry (ICP-MS).

Results: AZD6738 in combination with cisplatin was synergistic in all the cell lines investigated. Synergy was also observed with AZD6738 and carboplatin in H23, HCC1806 and MDA-MB-468 cell lines. Co-incubation of H460 cells with AZD6738 and cisplatin (4–24 hours), compared to cisplatin alone, did not identify any significant differences in Pt-DNA adduct formation. Varying the sequencing of AZD6738 in relation to cisplatin and maintaining exposure to AZD6738 following cisplatin administration did not affect the total number or longevity of Pt-DNA adducts formed in H460 cells.

Conclusion: Preliminary data suggests that the *in vitro* synergy observed between AZD6738 and cisplatin does not involve an interaction at the level of Pt-DNA adduct formation. Work is ongoing and we will present further time-courses of Pt-DNA adduct formation in additional cell lines using a lower dose range of cisplatin to investigate if this is a consistent effect.

Conflict of interest: Advisory Board: Y.D. sits on an advisory board for AstraZeneca and has received research grant funding from AstraZeneca for projects unrelated to AZD6738. Corporate-sponsored Research: S.H. is funded by a joint Cancer Research UK and AstraZeneca grant. Other Substantive Relationships: A.L., E.D., E.M., are employees of AstraZeneca as stated in author affiliations.

54 (PB-005) Poster

Targeting DNA-PK along with irradiation in head and neck cancer preclinical models suggests an HPV/p53 status-related pattern of response

S.M. Roth[1], D.M. Aebersold[1], M. Medová[1], Y. Zmner[1]. *[1]Department for BioMedical Research, Radiation Oncology, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland*

Background: In this study, we are assessing potential sensitization of head and neck cancer (HNC) to ionizing radiation (IR) by limiting the repair of DNA-double strand breaks (DSB) through inhibition of DNA-PK, a kinase of the non-homologous end-joining pathway, critical for the resolution of DNA damage. Moreover, we are investigating whether these treatments lead to different outcomes between human papillomavirus positive (HPV+) and negative (HPV–) cancers.

Material and Methods: In order to sensitize HNC cell lines to IR, a novel selective DNA-PK inhibitor currently being investigated in a PhI clinical study was used. Specifically, the impact of irradiation, DNA-PK inhibition and the combination of these two treatments on proliferation (XTT proliferation assay, cell cycle and CFSE proliferation tracing via flow cytometry, β-Gal activity assay for senescence), survival and cell death (Caspase 3 activity, LIVE/DEAD, crystal violet viability assays) in a panel of HNC cell lines was examined. Furthermore, to study whether differences in treatment outcome are due to the genetic background, the cell lines are divided into HPV+/p53wt, HPV–/p53wt, HPV–/p53mutated groups.

Results: Our results show that the drug alone does not cause any cytotoxic effects, even at high dosages, but when combined with IR, an effect on viability, proliferation and survival can be observed. A significant dose enhancement can be attained by anticipating the DSB repair through DNA-PK inhibition in all three cell groups, although the underlying mechanisms differ depending on the genetic background. Interestingly, in HPV+/p53wt and HPV–/p53mutated cell lines, an accumulation of G2 phase arrested cells over a course of 48 hours post DNA-PK inhibition plus IR treatment is observed, eventually leading to induction of cell death through apoptosis. In HPV–/p53wt cell lines on the other hand, none of the perturbations leads to cell cycle phases redistribution over the same time-course; nevertheless, when exposed to the combined treatment, these cell lines seem to abrogate proliferation and undergo a change of phenotype, resulting in DNA-damage-induced senescence.

Conclusions: We have corroborated the radio-enhancing effect of DNA-PK inhibition on proliferation, survival and viability of HNC cell lines, independently of their HPV or p53 status. Ultimately, apoptosis is induced in HPV+/p53wt and HPV–/p53mutated cells, and senescence related growth arrest is observed in HPV–/p53wt cells. In conclusion, more pronounced effects of the use of the DNA-PK inhibitor in combination with IR are observed in cancer cells exhibiting dysfunctional p53, either via inactivation through HPV infection or mutation in the p53 gene, thus indicating the use of DNA-PK inhibition preferentially in malignancies displaying these phenotypes.

No conflict of interest

55 (PB-006) Poster

Inhibition of dUTPase induces a state of nucleotide pool and DNA repair imbalance that sensitises triple negative breast cancer cells to standard of care chemotherapies

C. Davison[1], C. Knowlson[1], K. Savage[1], R. Wilson[1], K. Mulligan[2], P. Wilson[2], P. Johnston[1], R. Ladner[1], M. LaBonte Wilson[1]. *[1]Queen's University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom; [2]CV6 Therapeutics, Belfast, United Kingdom*

Background: Triple negative breast cancer (TNBC) makes up 15% of all breast cancer cases and is associated with a poor prognosis due to a high rate of lymph node involvement and early visceral metastasis at the time of diagnosis. TNBC treatment is also limited by a lack of effective therapeutic options. Advances in treatment that have translated to significant improvements in outcome have been painstakingly incremental, despite advances in molecular profiling and subsequent TNBC subtyping.

We previously identified the enzyme deoxyuridine triphosphate nucleotidohydrolase (dUTPase) as a critical gatekeeper that protects tumour DNA from the misincorporation of uracil following exposure to standard of care therapies that target thymidylate synthase (TS) such as 5-FU and pemetrexed.

We hypothesised that targeting dUTPase and inducing uracil misincorporation during the repair of DNA damage induced by both TS-targeted therapies such as 5-fluorouracil and non-TS-targeted therapies such as the anthracyclines, represents a future clinical strategy to significantly improve clinical outcome in TNBC.

Materials and Methods: Inhibition of dUTPase (DUTi) was carried out using SMARTpool siRNA or small molecule inhibition (CV6-530). Drug response was assessed by growth inhibition and clonogenic assays. DNA damage was assessed by Western blot, foci detection, and flow cytometry.

Results: DUTi by both siRNA and treatment with CV6-530 significantly enhanced cancer cell death in TNBC cell lines treated with FUdR (5-FU active metabolite) and epirubicin as shown by the colony formation assay. This sensitisation to FUdR correlated with a significant increase in growth inhibition and an increase in magnitude and persistence of DNA damage in combination-treated cells. DUTi plus epirubicin treatment did not increase absolute DNA damage above that of epirubicin alone, but significantly increased the persistence of unrepaired DNA double-strand breaks. These data suggest that imbalanced nucleotide pools (TTP/dUTP) following DUTi interferes with the repair of DNA damage induced by FUdR and epirubicin. Consistent with this, silencing of uracil DNA glycosylase (UNG), the primary enzyme involved in excising misincorporated uracil, resulted in enhanced sensitisation to FUdR and epirubicin when combined with DUTi, indicating that uracil-DNA misincorporation is a contributory mechanism.

Conclusion: These results provide clear rationale for the future application of dUTPase inhibitors in combination with fluoropyrimidines for TNBC. In addition, these results indicate that repair of anthracycline-induced DNA damage requires dUTPase to prevent uracil misincorporation at the site of repair and provides compelling evidence that dUTPase inhibitors represent a future clinical strategy to significantly enhance the anticancer activity of anthracycline-based combinations in TNBC.

No conflict of interest

56 (PB-007) Poster

Talazoparib and decitabine: a promising combination for BRCA-mutated cancers treatment

R. Pacaud[1], S. Thomas[1], E. Roche[1], N. Pawlowska[1], M. Dhawan[1], P. Munster[1]. [1]*UCSF, Hematology & Oncology, San Francisco, USA*

Background: *BRCA* genes (e.g. *BRCA1* and *BRCA2*) are known to play a major role in tumorigenesis. These genes are key mediators of DNA damage repair response including the homologous recombination repair (HRR) pathway. To leverage impaired DNA repair in tumors, a new class of drugs, the poly (ADP-ribose) polymerase inhibitors (PARPi), were developed and olaparib, niraparib, rucaparib have already been approved as single agents. Efforts to combine these agents with cytotoxic agents showed increased efficacy but overlapping toxicities renders combination therapy difficult to tolerate. Furthermore, the short duration of responses in diseases like breast cancer has further inspired the search for combinations with other agents including epigenetic modifiers.

To overcome those hurdles, we explored synergistic interactions between PARPi and DNA Methyltransferases inhibitors (DNMTi) such as decitabine (a global DNA methyltransferase inhibitor). This cytidine analog, when incorporated in newly synthesized DNA strands (during DNA replication phase (S phase), will trigger a covalent protein-DNA complex formation thereby causing cell cycle arrest and ultimately causing cell death.

Material and Methods: Breast and Ovarian cell lines with/without *BRCA* mutations are used for *in vitro* analysis: SUM149PT (Breast, *BRCA1* mutated), JHOS-2 & COV362 (Ovary, *BRCA1* mutated), KURAMOCHI (Ovary, *BRCA2* mutated), MDA-MB-231 (Breast, *BRCA* Wild-Type), MCF10A (Breast non-tumorigenic cells, *BRCA* Wild-Type). Combinational effect of talazoparib (PARPi) and decitabine (DNMTi) analyzed by Combenefit® software. Colony assays, cell death analysis, as well as pH2AX level are evaluated. *In vivo* toxicity analysis has also been done on CD-1 IGS mice.

Results: Our preclinical data in *BRCA* deficient breast and ovarian cancer cell lines, demonstrated a synergistic inhibition of cell growth and cell death improvement at concentrations of talazoparib and decitabine where each agent individually had minimal efficacy. In cells with intact HRR pathways, the drug combination impact is not significant on the cell death ratio (compared to *BRCA* deficient cell lines) and only causes a cell growth arrest. The drug combination efficacy is related to a better accumulation of DNA breaks (pH2AX level is higher in the combination group than drug alone groups).

Conclusion: The low drug doses used in these preliminary experiments present a promising therapeutic window for patients with tumors carrying HRR pathway mutations. Ongoing experimentations are also being done in pancreatic cell lines carrying a *BRCA* mutation as well as CRISPR/Cas9 DNA editing for *BRCA1* & *2* genes in MDA-MB-231 cells. Translational *in-vivo* studies are currently underway to determine whether synergistic *in vitro* cytotoxicity translates to anti-tumor efficacy.

No conflict of interest

57 (PB-008) Poster

BRCA reversion mutations in circulating cell-free tumour DNA predict primary and acquired resistance to the PARP inhibitor rucaparib in high-grade ovarian carcinoma (HGOC)

I.A. Mcneish[1], K.K. Lin[2], M.R. Radke[3], A.M. Oza[4], A. Oaknin[5], I. Ray-Coquard[6], A.V. Tinker[7], E. Helman[8], J. Isaacson[2], L. Maloney[2], D.M. O'malley[9], S.K. Chambers[10], S.H. Kaufmann[11], C. Scott[12], G.E. Konecny[13], R.L. Coleman[14], H. Giordano[2], J.D. Brenton[15], T.C. Harding[2], E.M. Swisher[3]. [1]*Imperial College London, London, UK;* [2]*Clovis Oncology, Boulder, CO, USA;* [3]*University of Washington, Seattle, WA, USA;* [4]*Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada;* [5]*Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology, Barcelona, Spain;* [6]*GINECO, Centre Léon Bérard and University Claude Bernard, Lyon, France;* [7]*BC Cancer, Vancouver, BC, Canada;* [8]*Guardant Health, Redwood City, CA, USA;* [9]*The Ohio State University, Columbus, OH, USA;* [10]*University of Arizona, Tucson, Arizona;* [11]*Mayo Clinic, Rochester, MN, USA;* [12]*Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia;* [13]*University of California Los Angeles, Santa Monica, CA, USA;* [14]*University of Texas MD Anderson Cancer Center, Houston, TX, USA;* [15]*University of Cambridge, Cambridge, UK*

Background: In cancers with *BRCA1* and *BRCA2* (*BRCA*) mutations, the acquisition of *BRCA* reversion mutations that restore protein function is a key resistance mechanism to platinum-based chemotherapies and PARP inhibitors. To determine the prevalence of *BRCA* reversion mutations after prior platinum-based chemotherapies and assess their relationship with clinical efficacy of PARP inhibitor treatment, we performed targeted next-generation sequencing (NGS) on pre-rucaparib treatment and postprogression circulating cell-free DNA (cfDNA) from patients (pts) with tumours harbouring deleterious *BRCA* mutations who enrolled in a phase 2 study (ARIEL2; NCT01891344) of rucaparib in HGOC.

Materials and Methods: cfDNA was extracted from plasma samples and targeted NGS was performed on a panel of cancer-related genes, including all exons of *BRCA1*, *BRCA2*, and *TP53*. *BRCA* reversion mutations were defined as those mutations predicted to restore the *BRCA* open reading frame and were confirmed by NGS of available matched pretreatment tumour biopsies. Platinum status was classified based on time to progression of the most recent platinum-based treatment. Progression-free survival (PFS) was defined as the time from the first dose of rucaparib to investigator-assessed disease progression or death from any cause.

Results: We sequenced 112 pretreatment cfDNA samples and detected somatic *TP53* mutations in 96% (107/112) of samples, indicating that shedding of neoplastic DNA is frequent in pts with relapsed HGOC. Of these cases, primary deleterious *BRCA* mutations (germline or somatic) were detected in 97 cfDNA samples. *BRCA* reversion mutations were identified in pretreatment cfDNA from 18% (2/11) of platinum-refractory and 13% (5/38) of platinum-resistant cancers compared with only 2% (1/48) of platinum-sensitive cancers ($P = 0.049$). Pts without *BRCA* reversion mutations detected in pretreatment cfDNA had significantly longer PFS with rucaparib than those with reversion mutations (hazard ratio [HR], 0.12; 95% confidence interval [CI], 0.05–0.26; $P < 0.0001$), with a median PFS of 9.0 and 1.8 months, respectively. Furthermore, within the platinum-resistant/-refractory subgroup, pts without *BRCA* reversion mutations had significantly longer PFS with rucaparib than those with reversion mutations (HR, 0.16; 95% CI, 0.07–0.42; $P < 0.0001$), with a median PFS of 7.3 and 1.7 months, respectively. To study acquired resistance, we sequenced 78 postprogression cfDNA samples and identified 8 additional pts with *BRCA* reversion mutations not found in pretreatment cfDNA. Sequencing of cfDNA also detected multiple *BRCA* reversion mutations not detected in tumour biopsies, highlighting the ability to capture multiclonal heterogeneity.

Conclusions: *BRCA* reversion mutations are detected in cfDNA from platinum-resistant/-refractory HGOC and are associated with decreased clinical benefit from rucaparib treatment.

Conflict of interest: Advisory Board: Iain A. McNeish has served on advisory boards for Clovis Oncology, Tesaro, Takeda and AstraZeneca. Amit M. Oza has served on steering committees for AstraZeneca, Clovis Oncology, and Tesaro. Ana Oaknin has served on advisory boards for Roche, AstraZeneca, PharmaMar, Clovis Oncology, and Tesaro. Isabelle Ray-Coquard has served on an advisory board for AstraZeneca, Pharmamar, and Roche. Anna V. Tinker has served on an advisory board for AstraZeneca. David M. O'Malley has served on an advisory board for Clovis Oncology, AstraZeneca, Janssen, Gynecologic Oncology Group, Myriad, and Tesaro has served on steering committees for Clovis Oncology, Amgen, and Immunogen has served as a consultant to AbbVie, AstraZeneca, Tesaro, Health Analytics, and Ambry. Clare L. Scott is a consultant/advisory board member for Clovis Oncology and AstraZeneca. Gottfried E. Konecny has served on speakers bureaus for AstraZeneca and Clovis Oncology. Robert L. Coleman reports serving as an advisor to AstraZeneca, Roche/Genentech, Janssen, OncoMed, Millennium, Merck, Clovis Oncology, Esperance, Tesaro, GamaMabs, Pfizer, Genmab, Gradalis, Bayer, and AbbVie. James D. Brenton has been advisor for Invivata, and has served on a speakers' bureau for AstraZeneca. Corporate-sponsored Research: Anna V. Tinker has received grants from AstraZeneca. David M. O'Malley's institution has received research support from Agenus, Ajinomoto, Array BioPharma, AstraZeneca, Bristol-Myers Squibb, Clovis Oncology, ERGOMED Clinical Research, Exelixis, Genentech, GlaxoSmithKline, Gynecologic Oncology Group, ImmunoGen, INC Research, inVentiv Health Clinical, Janssen Research and Development, Ludwig Institute for Cancer Research, Novartis Pharmaceuticals, PRA International, Regeneron Pharmaceuticals, Serono, Stemcentrx, Tesaro, and TRACON Pharmaceuticals. Clare L. Scott has received speakers bureau honoraria from Prime Oncology. Gottfried E. Konecny has received research funding from Amgen and Merck. Robert L. Coleman reports grants from AstraZeneca, Roche/Genentech, Janssen, OncoMed, Millennium, Merck, Clovis Oncology, Esperance, and AbbVie. Other Substantive Relationships: Kevin K. Lin, Jeff Isaacson, Lara Maloney, Heidi Giordano, and Thomas C. Harding are employees of Clovis Oncology and may own stock or have stock options in that company. Ana Oaknin has received support for travel or accommodation from Roche, AstraZeneca, and PharmaMar. Elena Helman is an employee of Guardant Health and may own stock or have stock options in that company. Gottfried E. Konecny has received honorarium from Novartis. James D. Brenton has received nonfinancial support from Clovis Oncology and Aprea AB, and has a pending patent for a diagnostic method of relevance to the current work.

58 (PB-009) Poster

Finding determinants of PARP inhibitor resistance using genome-wide and focused CRISPR screens

S. Pettitt[1], D. Krastev[1], F. Song[1], J. Frankum[1], R. Brough[1], I. Brandsma[1], J.M. Lee[2], E. Swisher[3], A. Ashworth[4], C. Lord[1]. [1]*Institute of Cancer Research, Division of Breast Cancer, London, United Kingdom;* [2]*National Cancer Institute, Center for Cancer Research, Bethesda, MD, USA;* [3]*University of Washington, School of Medicine, Seattle, WA, USA;* [4]*University of California San Francisco, Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA*

Background: We are using genomic approaches to study PARP inhibitor resistance mechanisms. Experimental study of potential resistance mechanisms can inform the ongoing clinical development of these drugs, as well as reveal new aspects of PARP function in normal and homologous recombination deficient cells.

Methods: To investigate mechanisms of PARP inhibitor resistance, we carried out genome wide CRISPR screens for mutants resistant to the potent PARP inhibitor talazoparib (BMN 673) in *BRCA1* mutant human breast cancer cell lines. We also use a high density CRISPR tiling mutagenesis approach to specifically study mutations in *PARP1* that confer PARP inhibitor resistance.

Results: Many PARP inhibitor resistant clones had loss of function *PARP1* mutations, demonstrating that PARP trapping drives cytotoxicity of PARP inhibitors in HR-deficient cells. We showed that survival of PARP1 mutants in these cells depends on some residual BRCA1 function, thus *PARP1* loss could be tolerated despite the expected synthetic lethal relationship between these genes. PARP1 loss could not be tolerated in tumour cells with mutations in the *BRCA1* BRCT domain, nor in cells with large engineered deletions of *BRCA1*. As well as *PARP1* mutations we identified *TP53BP1* mutants, and novel components of the 53BP1 pathway that confer resistance when mutated via a lack of protection from end resection at DNA double strand breaks and consequent restoration of homologous recombination activity.

To further investigate the role of PARP1 mutations in *BRCA1* mutant cells, we used a high-density focused sgRNA library targeting only *PARP1*. We developed a reporter cell line that allows us to selectively isolate in-frame mutations that preserve PARP1 protein expression. By deep sequencing mutagenised and appropriately selected cells we identified a series of subtle mutations in *PARP1* that result in PARP inhibitor resistance in *BRCA1* mutant cells, giving us a detailed insight into structure-function relationships in PARP1. Among these, we found mutants that display trapping despite conferring PARP inhibitor resistance, suggesting that PARP trapping is not sufficient for cytotoxicity. Mutations that confer resistance are clustered in DNA binding domains and a network of residues in the WGR and helical domains of PARP1 that may be involved in intramolecular activation of PARP1 upon DNA binding and thus affect trapping. We found a missense mutation in the WGR domain cluster in a patient that showed *de novo* resistance to PARP inhibitors and verified that this mutation also caused loss of normal PARP1 DNA binding.

Conclusions: PARP1 mutation is a potential cause of drug resistance in patients with *BRCA1* exon 11 mutations. Other potential resistance genes in the 53BP1 pathway have also been identified. We are now looking to see whether these are mutated in patients that progress after PARP inhibitor treatment.

No conflict of interest

61 (PB-012) Poster

DNA repair and its guradian angel protein: computational/experimental study of RAD51/ss-DNA interaction

D. Marson[1], M. Fermeglia[1], E. Laurini[1], S. Pricl[1]. [1]*University of Trieste, MolBNL@UniTS – DEA, Trieste, Italy*

Background: NA replication, repair, and recombination proteins form complex and agile networks. These networks organize the participating proteins into molecular machines that act on different substrates and channel them to different outcomes. Some of these machines display the capacity to accurately repair DNA damage or reestablish damage DNA replication forks without the loss of genetic information. Under other circumstances, action of the same molecular machines destabilizes the genome, leading to cancer or to the accumulation of toxic repair intermediates resulting in cell death. The central player in homologous recombination (HR) is the RAD51 DNA strand exchange protein (aka recombinase); its inactive conformation is tightly controlled by a double-heptameric doughnut-like assembly while its active form is a nucleoprotein filament assembled on the single-strand DNA generated at the site of DNA damage.

Here we will present the results of a study based on the combination of performance computing-based molecular simulations, high resolution imaging techniques and biophysical methods taimed at getting a comprehensive structural and thermodynamic perspective of RAD51/ssDNA interaction.

Materials and Methods: Experimental structural studies of the RAD51/ssDNA were performed with a high resolution transmission electron microscopy (HRTEM) and by far-UV circular dichroism spectroscopy (far-UV CD). Massively parallel molecular simulations of the protein/nucelic acid interactions were performed using both in house and external European High Performance Computing facilities. RAD51/ssDNA interaction thermodynamics in solution was fully characterized by isothermal titration calorimetry (ITC).

Results: The combination of different experimental and HPC-based simulation techniques has allowed us for the first time to (i) dissect the initial mode of RAD51/ssDNA interaction, (ii) derive the energetics of the interface in the protein/nucleic acid interface, (iii) uncover the role of DNA and protein flexibility in their assembly formation, and (iv) determining the mechanism and binding thermodynamics of RAD51 to ssDNA.

Conclusions: Among other fundamental evidences, through the application of HRTEM, HPC-based simulations, far-UV CD and ITC measurements this study for the first time allowed us to evidence that, under important physiological conditions, RAD51 is able to bind ssRNA also in its double-heptameric doughnut-like assembly, corresponding to its inactive form, and that this interaction might have a specific biological role.

No conflict of interest

62 (PB-013) Poster

DNA-PK regulates the radiosensitivity of MET-addicted cancer cell lines via a novel MET phosphosite

J.P. Koch[1], S.M. Roth[1], A. Quintin[1], J. Gavini[2], E. Orlando[1], M. Medo[1], R. Aebersold[3], D.M. Stroka[2], D.M. Aebersold[1], Y. Zimmer[1], M. Medová[1]. [1]*Department for BioMedical Research, Radiation Oncology, Inselspital, Bern University Hospital, and University of Bern, Bern, Switzerland;* [2]*Department for BioMedical Research, Visceral Surgery, Inselspital, Bern University Hospital, and University of Bern, Bern, Switzerland;* [3]*Faculty of Science, University of Zürich, Department of Biology, Institute of Molecular Systems Biology, ETH Zürich, Zürich, Switzerland*

Background: While the use of receptor tyrosine kinase (RTK) inhibitors is being investigated as a radio-sensitization strategy, a better understanding of the molecular crosstalk linking RTK signaling to the DNA damage response remains necessary to optimize treatment options. The present study focuses on the link between DNA-PK and the RTK MET, an oncogene mainly expressed in epithelial cells and involved in development and tissue regeneration but deregulated in numerous cancer and thus an attractive candidate for targeted therapies.

Methods: Using western blotting, DNA damage measurement, cell cycle and viability/toxicity assays, we tested the radiosensitivity of human cancer cell lines overexpressing constitutively active MET and transformed mouse fibroblasts ectopically expressing wild type or activating MET-mutated variants combined with a phosphodeficient Ser to Ala mutation. Assays include comet assay, γH2AX foci formation and α-/γ-Tubulin staining for DNA damage; PI staining and CFSE dye dilution assay for cell cycle analysis; and crystal violet staining, resazurin fluorescence, EthD/calcein staining and Annexin V/PI staining for cell proliferation, viability and toxicity. The radiosensitization effect of the Ser to Ala mutation was confirmed in vivo in a mouse xenograft setup.

Results: A study of post-translational changes in a MET-addicted cancer cell line upon MET inhibition and ionizing radiation revealed a yet unreported phosphorylation site on MET that is part of a consensus motif recognized by master DNA-damage response (DDR) kinases. Our results show that this site is phosphorylated by DNA-PK and that its phosphorylation fluctuates in response to MET inhibition and DNA-PK activity. MET-addicted transformed cells expressing the phosphodeficient (Ser to Ala mutation) form of active MET prematurely evade from irradiation-induced cell cycle arrest, leading to abnormal mitosis and lower proliferation, rendering them more radiosensitive than their nonmutated counterpart. A phosphoproteomics analysis revealed cell cycle proteins potentially involved in the downstream function of this phosphosite and we confirmed the radiosensitization effect of the Ser to Ala mutation in vivo by performing a mouse xenograft experiment.

Conclusions: In the present study we show that DNA-PK directly phosphorylates MET and that preventing this phosphorylation negatively affects viability after irradiation, providing new insights into the crosstalk linking MET and the DDR.

No conflict of interest

63 (PB-014) Poster
Development of tumor-targeted PARP inhibitors for the treatment of solid cancers

R. Aiello[1], B. Ranjit[2], D. Marshall[3], J. Csengery[3], P. Bourassa[1], Q. Zhang[1], B. Robinson[1], L. Lopresti-Morrow[1], J. Bechtold[1], L. Tylaska[1], R. Sundaram[2], P. Hellsund[1], P. Glazer[2], V. Paralkar[1]. [1]*Cybrexa Therapeutics, Biology, New Haven, USA;* [2]*Yale School of Medicine, Therapeutic Radiology, New Haven, USA;* [3]*Cybrexa Therapeutics, Chemistry, New Haven, USA*

Background: Poly (ADP-ribose) polymerase inhibitors (PARPi's) are a new class of DNA repair inhibitors which have revolutionized the field of oncology. These agents are most effective against tumors with defects in key DNA repair pathways, such as homologous recombination deficient (HRD) cancers. Monotherapy PARPi regimens have demonstrated efficacy against metastatic solid tumors, with the greatest activity observed in HRD cancers. However, resistance occurs rapidly, and there is limited activity against non-HRD cancers. Thus, these drugs are not curative for the vast majority of cancer patients. As such, there is great interest in combining PARPi's with other systemic therapies, including chemotherapy. Pre-clinical studies indicate the potential for exquisite synergy between PARPi's and DNA-damaging chemotherapies. Furthermore, PARPi combinations with TMZ have the potential to greatly enhance anti-tumor activity against non-HRD cancers. However, bone marrow suppression is a major barrier to treatment efficacy when PARPi's are combined with chemotherapy. As such, there is a great unmet need to enable safer and more effective means to combine PARPis with conventional chemotherapy.

Materials and Methods: We sought to develop a new class of tumor-targeted PARPi's which will (a) address the issues of off-target toxicity, and (b) increase treatment efficacy against both HRD and non-HRD cancers, when used in combination with chemotherapy. These tumor-specific drugs (TSDs) thus will have a greatly enhanced therapeutic index. Tumor targeting is achieved by a novel peptide triggered by low pH to insert its C-terminus across the cell membrane into the cytosol. Cargoes attached to the C-terminus of pHLIP can be targeted and delivered into cancer cells based on the acidity in the tumor microenvironment.

Results: Using our novel TSD platform, we have successfully conjugated a diverse range of structurally unique PARPi's to pHLIPs and demonstrated the following: (1) pH-dependent delivery of functionally active drug into tumor cells *in vitro*; (2) sustained and selective *in vivo* tumor localization, without free drug detection in systemic circulation; (3) target engagement by the drug specifically in tumor tissue, at levels similar to that observed with free drug; (4) prevention of bone marrow toxicity when combined with chemotherapy, again compared with free drug; and (5) selective tumor cell killing in both HRD and non-HRD xenografts, including patient-derived xenografts (PDXs).

Conclusions: Our approach will greatly increase the safety and efficacy of PARPi's in combination with chemotherapy and will expand their use into a wider range of HRD and non-HRD solid tumor types. Our TSD platform can be applied to other DNA repair inhibitors in the future, and it will allow safe and effective combinations with other systemic therapies, including immunotherapy.

No conflict of interest

64 (PB-015) Poster
A Phase I study of the poly-adp ribose polymerase (PARP) inhibitor, niraparib (NIR), in combination with irinotecan (IRN) in patients with advanced ewing sarcoma: results of sarc025 arm 2

K. Zaki[1], K.V. Ballman[2], L.J. Hellman[3], B.C. Widemann[4], D.S. Hawkins[5], L. Mascarenhas[3], J.W. Glod[4], J. Ji[11], Y. Zhang[11], B. Geoerger[6], J. Whelan[7], D. Reinke[8], S.R. Patel[9], R. Chugh[10], S.J. Strauss[1,7],
SARC (Sarcoma Alliance for Research through Collaboration). [1]*University College London Hospitals NHS Foundation Trust, NIHR/UCLH Clinical Research Facility, London, United Kingdom;* [2]*Weill Cornell Medical College, Department of Healthcare Policy and Research, New York, USA;* [3]*Keck School of Medicine of USC, Children's Center for Cancer and Blood Diseases, Los Angeles, USA;* [4]*National Cancer Institute Centre for Cancer Research, Pediatric Oncology, Bethesda, USA;* [5]*Seattle Children's Hospital, Hematology/Oncology Division, Seattle, United Kingdom;* [6]*Gustave Roussy Cancer Center, Department of Pediatric and Adolescent Oncology, Villejuif, France;* [7]*University College London Hospitals NHS Foundation Trust, The London Sarcoma Service, London, United Kingdom;* [8]*SARC Sarcoma Alliance for Research through Collaboration, SARC Sarcoma Alliance for Research through Collaboration, Ann Arbor, USA;* [9]*M.D. Anderson Cancer Centre, Department of Sarcoma Medical Oncology, Houston, USA;* [10]*University of Michigan, Michigan Medicine Sarcoma Clinic Rogel Cancer Center, Ann Arbor, USA;* [11]*National Clinical Target Validation Laboratory, Bethesda, MD, USA*

Background: PARP inhibitors are postulated to have potential therapeutic value in Ewing sarcoma via PARP trapping of the EWS-FLI1 complex as well as catalytic inhibition and resultant decrease in DNA damage repair. The co-administration of PARP inhibitors with the topoisomerase I inhibitor, irinotecan (IRN), exhibits notable synergistic anti-cancer activity *in vitro* and *in vivo*. In ARM 2 of a phase I study of the potent and highly selective PARP-1 and -2 inhibitor niraparib (NIR), we evaluated the combination with IRN to determine the dose-limiting toxicities (DLT) and maximum tolerated dose in patients with pre-treated incurable Ewing sarcoma.

Material and Methods: Using a 3+3 design, eligible patients >13 years with recurrent/relapsed Ewing sarcoma were assigned to cohorts evaluating NIR 100–300 mg qd D1-7 and IRN 20–50 mg/m^2 D2-6 of a 28 day cycle commencing at 100 mg NIR and 25 mg/m^2 IRN (Dose level 1). Pre-treatment tumor biopsy was mandated and for those >18 years on C2D8. RECIST 1.1 response assessment was performed every 2 cycles for first 6 cycles, then every 2–3 cycles.

Results: Between Nov 2016 and May 2018, 12 eligible patients (9 male) with confirmed EWSR1-FLI1 translocation positive Ewing sarcoma were enrolled at 2 dose levels. Median age was 27 years (range 16–50); median prior therapies 4 (range 1–9) with 2 patients having received prior IRN. At time of data cutoff, median number of cycles was 2 (range 1–17). DLTs were observed in all 3 patients at dose level 1 (1 patient each with grade 3 anorexia, grade 3 colitis, grade 3 transaminitis). No DLTs were reported in 7 evaluable patients treated at NIR 100 mg and IRN 20 mg/m^2 (dose level -1), 2 patients experienced transient grade 3 neutropenia and 1 patient grade 3 gastro-intestinal toxicity (diarrhea, abdominal pain, nausea and vomiting lasting <72 hours) and grade 3 thrombocytopenia. In 10 evaluable patients, best response was PR in 1 patient, SD in 4 patients, and PD in 5 patients. Median progression-free survival was 4.9 months (1.18-NA). Pharmacodynamic analysis of tumor samples demonstrated >80% PARP inhibition across all doses of NIR.

Conclusions: NIR 100 mg qd D1-7 in combination with IRN 20 mg/m^2 was well tolerated with preliminary evidence of efficacy that warrants further investigation. Patient biopsy was feasible and pharmacodynamic analysis supported the recommended phase 2 dose. Further cohorts incorporating temozolomide are planned and additional correlative analysis is ongoing.

No conflict of interest

65 (PB-016) Poster
Less is more: subclinical doses and choice of replication stress inducer in combination with CHK1 inhibitors in vitro and in vivo

Z.U. Oo[1], M. Proctor[1], A. Stevenson[1], D. Nazareth[1], N. Haass[2], J. Larsen[3], B. Gabrielli[1]. [1]*The University of Queensland, Mater Research Institute, Brisbane, Australia;* [2]*The University of Queensland, Diamantina Institute, Brisbane, Australia;* [3]*Queensland Institute of Medical Research Berghofer, Cancer Genomics, Brisbane, Australia*

Background: Lung cancer and melanoma are responsible for almost 25% of cancer deaths in Australia. Despite recent progress with targeted and immuno-therapies, the 5 year survival rate in late stage patients is still <20%. CHK1 inhibitors are being investigated as chemosensitising agents with agents that increase replication stress, primarily Gemcitiabine. Clinical trials of this combination have shown some good responses but high levels of normal tissue toxicity.

Materials and Methods: Here we have investigated the molecular basis of sensitivity to CHK1 inhibitors as combinations with subclinical doses of hydroxyurea (HU) in melanoma and non-small cell lung cancer (NSCLC) tumour spheres and cell lines in 2% O2 conditions and xenograft models.

Results: We report that low dose HU increased the sensitivity of >70% of both melanoma and NSCLC cell lines to CHK1 inhibitor (GDC-0575) triggered apoptosis, with complete loss of viability found with clinically achievable doses of this combination. Similar sensitivity was observed in xenograft models of both melanoma and NSCLC. We also demonstrate that a low dose of Gemcitabine combination with CHK1 inhibitor results in complete loss of proliferative potential in normal tissue, whereas normal tissue retaining proliferative potential after treatment with even high doses of hydroxyurea in combination with CHK1 inhibitor. In vivo, this translates to minimal effect on lymphocyte populations in the blood. The combination also triggers an inflammatory response involving the recruitment of macrophages, associated with increased HMGB1 nuclear staining.

Discussion: These data indicate that the combination of low dose HU and CHK1 inhibitor have strong anti-cancer activity in the setting of melanoma and NSCLC cancer, and triggers an inflammatory response, and is likely to be better tolerated than current combinations with Gemcitabine. Our data

suggest a significant proportion of melanoma and lung cancer patients could benefit from treatment with this drug combination.

No conflict of interest

66 (PB-017) Poster

Measurement of SLFN11 protein in circulating tumor cells (CTCs) as a proposed liquid biopsy biomarker to predict response to DNA repair targeted therapies

L. Fernandez[1], L. Chu[1], R. Richardson[1], R. Graf[1], Y. Wang[1], M. Landers[1], R. Dittamore[1]. [1]*Epic Sciences, Translational Research, San Diego, USA*

Background: SLFN11 regulates response to DNA damage and replication stress, and is being investigated as a potential predictive biomarker for response to platinum agents and PARP inhibitors (PARPi). In a Phase II trial in recurrent small cell lung cancer (SCLC), patients with high SLFN11 expression in tissue biopsies had better survival when PARPi used. In clinical practice, recurrent lung biopsies are not common. It has been hypothesized that SLFN11 could also identify patients who might respond to an additional round of inexpensive platinum agents. A CTC-based test could aid these indications. The Epic Sciences platform plates all nucleated cells from a blood sample onto microscope slides and stains for DNA (DAPI), blood lineage marker (CD45) and epithelial marker (CK, cytokeratins), with a fourth channel for additional biomarkers. CTCs are characterized via automated microscopy. The technology was utilized to create the first commercially available predictive test in prostate cancer to be considered for CMS reimbursement, AR-V7. We applied the technology to develop an assay for SLFN11 and tested clinical feasibility.

Material and Methods: Contrived samples consisting of healthy donor blood spiked with cell line cells were used: three PARPi sensitive (DU145, MD-MBA-436 and PC3) and two PARPi resistant (MCF-7 and MD-MBA-231). Rabbit polyclonal anti-SLFN11 antibody was used to assess SLFN11 expression in the 4th channel. Contrived samples, along with SCLC, triple negative breast cancer (TNBC) and mCRPC patient samples were processed as patient samples in a CLIA-like environment.

Results: DU145, MD-MBA-436 and PC3 PARPi sensitive cell lines express nuclear-specific localized SLFN11 signal, at times visualized in sub nuclear focal bodies. PARPi resistant MD-MBA-231 cell line showed low non-nuclear expression of SLFN11. BRAC1-wt PARPi resistant MCF-7 does not express SLFN11. The median difference in single-cell SLFN11 expression observed between the PARPi resistant and PARPi sensitive cell lines as contrived patient samples was approximately 12 fold. In patient samples, a diversity of SLFN11 expression was seen between and within samples in multiple indications.

Conclusions: The expression levels and localization of SLFN11 observed in individual cell line cells spiked into healthy honor blood and processed as patient samples is consistent with the established biology and emerging clinical biomarker associations of SLFN11. The assay is able to qualitatively assess localization, and semi-quantitatively assess the expression level of SLFN11. Use of this assay is underway in research studies with SCLC patient samples to assess correlation with PARPi and platinum agent response.

Conflict of interest: Other Substantive Relationships: We are employee of Epic Sciences.

67 (PB-018) Poster

Preclinical evaluation of the ATR inhibitor VE-821 alone and in combination with the PARP inhibitor olaparib in neuroblastoma

H. Southgate[1], L. Chen[1], N.J. Curtin[2], D.A. Tweddle[1]. [1]*Newcastle University, Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle Upon Tyne, United Kingdom;* [2]*Newcastle University, Northern Institute for Cancer Research, Newcastle Upon Tyne, United Kingdom*

Background: Neuroblastoma (NB) is the commonest extra-cranial malignant solid tumour of childhood and one of the most difficult to cure. Around 50% of high-risk NBs have amplification of the *MYCN* oncogene that promotes rapid DNA replication, leading to errors and replication stress (RS). Defects in the p53 pathway are frequently observed at NB relapse. Cancer cells with defective p53 signalling and/or increased oncogene-driven RS are acutely dependent on ataxia telangiectasia and Rad3-related (ATR) protein kinase signalling. Poly-ADP ribose polymerase (PARP) inhibition increases RS, therefore ATR inhibitors should enhance the cytotoxic effect of PARP inhibitors. This study aimed to determine which molecular features lead to sensitivity to the ATR inhibitor VE-821 alone and in combination with PARP inhibitors in NB cell lines.

Materials and Methods: XTT (Roche) and clonogenic assays were used to assess cell proliferation and survival respectively in response to 72 hours treatment with VE-821 in a panel of 14 NB cell lines of varying *MYCN* amplification and *TP53* mutation status. The XTT cell proliferation assay was also used to determine the effect of ATR inhibition on olaparib cytotoxicity.

Results: VE-821 caused significantly more growth inhibition in *MYCN* amplified cell lines by XTT assay ($p = 0.04$ Mann-Whitney U test). A similar trend has been observed by clonogenic assay. No significant difference was found in sensitivity between p53 mutant and p53 wild type cell lines in either assay. Combinational index analysis (Calcusyn) showed that ATR inhibition by VE-821 is synergistic with olaparib at sub lethal concentrations (<1 μM), although this effect is lost at higher concentrations.

Conclusion: *MYCN*-amplification but not p53 mutation is a determinant of ATRi sensitivity in NB cell lines. In addition, ATR inhibition by VE-821 is synergistic with olaparib at sub lethal concentrations (<1 μM), suggesting the degree of replication stress may predict ATR inhibitor sensitivity in NB.

Conflict of interest: Corporate-sponsored Research: NJC is the grant holder of the MRC-Merck Industrial CASE studentship.

69 (PB-020) Poster

Multiple deletions as a prognostic factor in metastatic colorectal cancer with chromothripsis

E. Skuja[1], D. Kalniete[2], M. Nakazawa-Miklasevica[2], Z. Daneberga[2], G. Purkalne[1], E. Miklasevics[2]. [1]*P.Stradins Clinical University Hospital, Clinic of Oncology, Riga, Latvia;* [2]*Riga Stradins University, Riga, Latvia*

Background: Chromothripsis is a massive chromosome shattering occurred as a single catastrophic event leading to random repair of chromosomes. Multiple deletions and amplifications are very common in cancer cells affected by chromothripsis. In our previously reported study, we found a correlation between DNA massive fragmentation and increased progression free survival (PFS) in metastatic colorectal cancer (mCRC), but not overall survival (OS). The aim of this study is to find overlapping deleted genome regions in selected mCRC patients with chromothripsis and detect possible cause of increased PFS, and find new genes or combinations, involved in colorectal cancer oncogenesis.

Material and Methods: 10 mCRC patients with chromothripsis receiving FOLFOX first-line palliative chemotherapy between August, 2011 and October, 2012 were selected for this study. *Genotyping.* Microarray analysis was performed using the Infinium HumanOmniExpress-12 v1.0 FFPE BeadChip kit (Illumina). BeadChip was scaned on HiScan (Illumina). Analysis was performed by GenomeStudio software (Illumina) and R version 3.1.2. (https://www.r-project.org/). Copy number variation and breakpoints on the chromosomes were analyzed using the DNA copy package (http://bioconductor.org/pack-ages/release/bioc/html/DNAcopy.html).

Results: Eight deleted tumor suppressor genes (ROBO2, CADM2, FAT4, PCDH10, PCDH18, CDH18, TSG1, CTNNA3) and four deleted oncogenes (CDH12, GPM6A, ADAM29, COL11A1) were identified in more than half of patients. In 70% patients' deletion in COL11A1 was detected. Deletion of MIR1269, MIR4465, MIR1261 and MIR4490 in patients with longer time to progression was observed. Four patients (40%) with PFS over 14 months, presented with NRG3 deletion (oncogene, EGFR ligand) what could possibly decrease proliferation of cancer cells via decreasing EGFR activation.

Conclusions: Multiple chromosomal deletions (MIR1269, NRG3, ADK) in mCRC patients with chromothripsis are associated with better response to first line palliative FOLFOX-type chemotherapy and increased PFS.

No conflict of interest

70 (PB-021) Poster

Characterization of small molecule inhibitors of ubiquitin specific peptidase 1 (USP1) as anti-cancer agents

E. Brenndörfer[1], D. Jönsson[1], K. Böhm[1], A. Eneroth[1], K. Acs[1], I. Henderson[1], M. Kullman-Magnusson[1], C. Rydergård[1], G. Stenberg[1], A.K. Sternbeck[1], E. Ulander[1], M. Albertella[1], R. Bethell[1]. [1]*Medivir AB, Research & Development, Huddinge, Sweden*

Background: The deubiquitinase USP1, which plays an important role in the cellular response to DNA damage and the maintenance of tumor cell stemness, is overexpressed in various cancer types. This suggests that USP1 inhibitors may have the potential to be used as anti-cancer agents in combination with DNA damaging drugs. While the previously published USP1 inhibitor ML323 is characterized by high selectivity, it has a rather low efficacy.

Material and Methods: Established biochemistry, physiochemistry, biophysical, cell biology and drug metabolism/pharmacokinetic assays

were used to profile USP1 inhibitors generated from the internal chemistry program. For primary screening, USP1/USP1 associated factor 1 (UAF1) activity was monitored using an enzymatic assay with diubiquitin as substrate. For validation, surface plasmon resonance (SPR) and a cellular ubiquityl-proliferating cell nuclear antigen (Ub-PCNA) deubiquitination assay were used. *In vivo* pharmacokinetic studies were performed in mice.

Results: We report the characterization of orally bioavailable USP1 inhibitors exhibiting highly potent activities against USP1/UAF1, excellent selectivity against other USPs, biophysical validation by SPR, and promising *in vivo* pharmacokinetics. The potencies against USP1/UAF1 were <100 nM in the enzymatic assay with a selectivity of >1000× against all other tested USPs. Compound binding was confirmed by SPR with Kd values in the sub-μM range. Inhibition of endogenous USP1 resulted in accumulation of ubiquitinated PCNA with IC50 values <200 nM in multiple cell lines and there was a strong correlation between the values in this assay and the enzymatic assay data. While the inhibition of USP1 had only a minor cytotoxic effect in the μM range, combination of USP1 inhibitors with DNA crosslinking chemotherapeutics such as cisplatin or mitomycin C resulted in strong synergy confirming the importance of USP1 in the DNA damage response. In *in vivo* pharmacokinetic studies, our compounds were characterized by a good oral bioavailability and dose linearity between the tested doses.

Compound	ML323	Compound A	Compound B
USP1/UAF1 enzymatic assay IC50 (μM)	6.3	0.07	0.05
Kinetic solubility in PBS pH7.4 (μM)	5	10	74

Conclusions: We have developed highly potent and selective USP1 inhibitors that show strong synergy with DNA damaging agents *in vitro* and have promising pharmacokinetic profiles, justifying advancement to *in vivo* efficacy studies.

Conflict of interest: Ownership: D.J., A.E., C.R., G.S., E.U., M.A., and R.B. own Medivir AB shares. Corporate-sponsored Research: All authors are employees of Medivir AB, Huddinge, Sweden.

72 (PB-023) Poster

CRISPR-mediated base editing screens to identify PARP inhibitor resistant mutations

D. Krastev[1], S. Pettit[1], C. Lord[1]. [1]*Institute of Cancer Research, Division of Breast Cancer, London, United Kingdom*

Background: Three PARP inhibitors are currently in the clinic for the treatment of advanced ovarian and breast cancers with underlying defects in the homologous recombination DNA repair pathways. Acquired and *de novo* resistances have been observed, necessitating the mechanistic understanding of the mode of action of this class of drugs. We have investigated the mechanism of PARP inhibitor resistance via CRISPR-based mutagenesis of PARP1 and other candidate genes.

Material and Methods: We used tiling sgRNA libraries to direct various base editing enzymes (BE2, BE3, BE4-Gam, AID and ABE7.10) to target genes in order to induce missense mutations in BRCA deficient cellular models. The pools of thus mutagenized cells were subsequently exposed to lethal doses of PARP inhibitors and resistant clones were selected. We devised a pipeline to identify and annotate these mutations through a deep sequencing approach. A selected set of missense mutations in PARP1 were functionally characterised by biochemical and biosensor approaches.

Results: We carried out the mutagenesis screen in BRCA1 mutant breast cancer cell line. This led to the identification of a number of missense mutations in PARP1 that confer PARP inhibitor resistance in this setting. Mutations were found in both the DNA binding Zn-finger domains, and the regulatory WGR and HD domains of PARP1. Interestingly, different base editor enzymes generated different sets of mutations around certain hotspots, showing the merit of using as many and as diverse as possible orthogonal reagents in order to saturate the mutational space. We have selected a set of mutations and validated that they impact PARP1 DNA binding and drug-induced “trapping” ability. However, other mutations showed a complex relationship between DNA interaction and enzymatic activity.

Conclusions: We have developed an experimental system, where CRISPR-mediated mutagenesis is employed for the isolation of drug resistance-inducing mutations in target proteins. In the case of PARP1, we have defined mutations, which functionally uncouple DNA binding and enzymatic activity. These observations support the notion of reverse allostery in PARP1, and suggest a mechanism of the inhibitor-induced DNA trapping.

No conflict of interest

73 (PB-024) Poster

Investigating the effect of replication stress and other phenotypic factors as determinants of sensitivity to single agent ATR inhibitor, VE-821 in ovarian cancer cell lines

A. Bradbury[1], D. King[2], H. Bryant[2], F. Zenke[3], Y. Drew[1], N. Curtin[1]. [1]*Newcastle University, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom;* [2]*University of Sheffield, Academic Unit of Molecular Oncology, Sheffield Institute for Nucleic Acids SInFoNiA, Department of Oncology and Metabolism, Sheffield, United Kingdom;* [3]*Merck KGaA, Biopharma R&D, Translational Innovation Platform Oncology, Darmstadt, Germany*

Background: ATR is a key component of the DNA damage response (DDR), responsible for signalling to S and G2/M checkpoints and facilitating repair. Cancer cells are often defective in their G1 checkpoint control due to e.g., *TP53* mutation, a common event in ovarian cancer. This, coupled with frequent activation of oncogenes that drive replication, such as cyclin E, which is reported to be highly amplified in 20% of high-grade serous ovarian cancers, make cancer cells much more likely to enter S phase with increased replication stress (RS). Therefore, cancer cells are more reliant on their G2/M checkpoint; making the S and G2/M checkpoint an attractive anti-cancer target.

Methods: The single agent activity of VE-821 has been assessed by western blot (CHK1 phosphorylation) and clonogenic survival assay in a panel of 14 ovarian cancer cell lines representing a spectrum of phenotypes. Expression of DDR proteins was assessed by western blot and levels of RS assessed by DNA fibre assay.

Results: Cells exhibited a range of sensitivities to VE-821 with LC_{50} values ranging from 0.61 ± 0.14 μM (BRCA1 mutated UWB1.289) to 11.64 ± 1.90 μM (NIH-OVCAR3). In IGROV1 cells, VE-821 significantly decreased replication fork speed, and increased the percentage of new origins and collapsed forks. However, there was no significant relationship between the expression of previously reported determinants of sensitivity to VE-821 (*TP53*, cyclin E, DNA-PKcs or ATM) and VE-821 cytotoxicity across the cell line panel.

Conclusions: VE-821 exhibits differential single agent activity in a panel of ovarian cancer cell lines; however, this does not appear to correlate with the expression of known determinants of sensitivity to ATRi. Studies are ongoing to further to assess the impact of VE-821 on RS as well as baseline levels of RS as a determinant of sensitivity to VE-821 across the panel of cancer cell lines.

Conflict of interest: Corporate-sponsored Research: Alice Bradbury is jointly funded by MRC and Merck. Other Substantive Relationships: Frank Zenke is an employee of Merck, as stated in the author affiliations.

74 (PB-025) Poster

Deacetylase activity of Sirtuin1 is required to protect the genome by preventing excess replication origin initiation

A. Baris[1], H. Fu[1], B. Thakur[1], S. Jang[1], C. Redon[1], P. Kiran[1], A. Mirit[1]. [1]*National Cancer Institute, Developmental Therapeutics Branch, Bethesda, USA*

Chromatin structure affects DNA replication patters, but the roles that individual chromatin modifiers play in the regulation of DNA replication has yet to be elucidated. Sirtuins, homologs of the yeast gene silencing modifier (Silent Information modifier 2, or Sir2), are crucial in maintaining genomic stability. The largest homolog of Sir2 in metazoans, Sirt1, has been implicated in circadian signaling, epigenetic modifications, as well as cell cycle signaling and DNA damage repair. Using molecular combing, a method in which DNA is labelled with thymidine analogs and observed via microscopy, we can analyze replication patterns in cells with wild type and enzymatically inactive Sirt1. We report that the deacetylation activity of Sirt1 is necessary for Sirt1 to modulate DNA replication. We determined that enzymatically inactive Sirt1 exhibits more active origins, as well as a decreased rate of replication and increased replication stalling. This combined data leads to the conclusion that deacetylase activity is required for Sirt1 inhibition of dormant origins, which prevents excess DNA damage.

No conflict of interest

75 (PB-026) Poster

ATM mRNA expression, somatic mutation counts, and survival in breast and gastric cancer

K.H. Lee[1], S. Koung Jin[1], K. Kwangsoo[2], C. Seongmin[2], K. Seongyeong[3], M. Ahrum[3], K. Tae-Yong[1], I. Seock-Ah[1]. [1]*Seoul National University Hospital, Department of Internal Medicine, Seoul, South Korea;* [2]*Seoul National University Hospital, Division of Clinical Bioinformatics, Seoul, South Korea;* [3]*Cancer Research Institute, Seoul National University, Seoul, South Korea*

Background: Ataxia-telangiectasia-mutated (ATM) plays an important role in cell cycle delay after double-strand breaks. Low expression, as well as loss-of-function mutation of ATM gene can contribute to mutagenesis and carcinogenesis. We investigated the relationship between mRNA and protein expression levels of ATM gene and the somatic mutation count using whole exome sequencing data derived from the Cancer Genome Atlas (TCGA) database in various cancer types.

Materials and Methods: mRNA expression, protein expression, and somatic mutation data of 22 cancer types were collected from the TCGA data portal, and their association with overall survival (OS) was analyzed. To validate the survival outcome in an independent cohort, MTCI Breast Cancer Survival Analysis Tool (http://glados.ucd.ie/BreastMark/) was used for survival analysis in breast cancer.

Results: Out of 22 cancer types from the TCGA database, mRNA expression levels of ATM were inversely correlated with somatic mutation counts in 4 cancer types (adenoid cystic carcinoma, breast cancer, stomach cancer, and thyroid carcinoma). Tumors with higher somatic mutation counts above the median value had shorter OS compared to those with lower somatic mutation count ($p = 0.001$) in these cancer types. mRNA expression and protein level of ATM showed positive correlation in breast and stomach cancer ($p < 0.001$ and $p = 0.001$, respectively).

Regarding the subgroups (luminal A, luminal B, HER2, and triple-negative) of breast cancer, low ATM mRNA expression (the bottom 25% expression level) was associated with high somatic mutation count ($p = 0.023$), and trends to shorter OS (median 114 months *vs.* 123 months, $p = 0.072$) in luminal A subtype. Likewise, OS was significantly shorter in tumors with low ATM mRNA compared to high in the luminal A tumors in MTCI validation cohort (n = 609, hazard ratio 0.54, 95% Confidence Interval 0.33–0.86, $p = 0.009$). In contrast, ATM mRNA expression was not associated with somatic mutation counts in TNBC.

Somatic mutation count in TNBC was higher than in the other subtypes. In TNBC, somatic mutation count-high tumors (above the median) was associated with longer OS (median not reached *vs.* 114 months, $p = 0.037$).

In stomach cancer, MSI-high tumors had higher somatic mutation counts compared to microsatellite stable (MSS) or MSI-low tumors. ATM mRNA was lower in MSI-high tumors, but low ATM mRNA expression was not associated with high somatic mutation count, regardless of MSI status. In MSI-high tumors, low ATM mRNA level was associated with trend to better survival (median OS 55 months *vs.* 31 months, $p = 0.128$).

Conclusions: ATM mRNA expression is associated with somatic mutation count and prognosis in subtypes of breast and stomach cancers. These associations need to be assessed in the contexts of molecular subtypes of breast cancer and MSI status of stomach cancer.

No conflict of interest

76 (PB-027) Poster

High-throughput combinatorial CRISPR-Cas9 gene knockout and novel analysis platform to identify therapeutically-relevant synthetic lethal interactions

J.P. Shen[1], B. Munson[2], P. Mali[2], T. Ideker[3]. [1]*University of Texas MD Anderson Cancer Center, Gastrointestinal Medical Oncology, Houston, USA;* [2]*University of California, San Diego, Bioengineering, La Jolla, USA;* [3]*University of California, San Diego, Medicine, La Jolla, USA*

Genetic interactions, in particular negative or “synthetic-lethal” interactions, have implications for therapeutic development as has been demonstrated by the clinical success of PARP inhibitors specifically for tumors with loss-of-function mutations in BRCA1/2. To enable systematic mapping of these genetic interaction networks, we recently developed a CRISPR-Cas9 screening methodology for knocking out single and pairs of genes in high-throughput. To robustly quantify gene fitness and genetic interactions we developed a novel computational analysis framework called Compositional and Time-course aware Genetic analysis (CTG), designed specifically for high-throughput genetic interaction screens. Unique from prior methods, CTG accounts for the compositionality of the pool screening format, and also incorporates time course data to greatly improve the precision of fitness measurements. Using gRNA abundance counts from four time points (3, 14, 21 and 28 days post-transduction) the correlation of single gene knockout fitness was highly correlated between two experimental replicates (Peason r = 0.94, p = 1.2×10^{-37}), this result was robust to down sampling to 50% of the original total read counts. However, removing the day 14 and 21 samples significantly increased the error in single gene fitness measurement and also increased the relative error of genetic interactions scores to a greater degree than down sampling to 50% across all four data points (0.58 vs. 0.42, p = 0.0007). Additionally, performing an initial log-ratio transformation and using a Bayesian model to determine genetic interaction scores the CTG model prevents spurious findings due to compositionality.

Evaluating all pairwise gene knockout combinations among a panel of 73 genes divided between tumor-suppressor genes (TSG) and cancer-relevant drug targets (DT) in a total of 5 cancer cell lines from diverse lineages (HeLa, A549, 293T, U2OS, LN229) we identified 226 synthetic lethal and 14 epistatic interactions at a Z-score cut off of –3 (FDR ~ 0.3). Of the synthetic lethal interactions 203 (89.8%) were private to a single cell line, and no interaction was seen in more than 3 of 5 five cell lines. Thus far 10 (out of 16 tested) therapeutically relevant interactions have been replicated in low throughput assays (71% precision or positive predictive value). These include synthetic lethal interactions between SMAD4 loss-of-function and CDK9 inhibition with palbociclib as well as dual inhibition of CHEK1 and MAP2K1.

In summary, we have discovered many therapeutically-relevant genetic interactions in cancer. Recognizing that there will be great diversity in genetic interaction between different tumors it will be important to perform future studies across a large number of samples, which is enabled by the high-throughput method we have developed.

Conflict of interest: Advisory Board: Ideker is a member of advisory board for Ideaya Biosciences. Corporate-sponsored Research: Shen & Ideker receive research support from Ideaya Biosciences.

77 (PB-028) Poster

Targeting the DNA damage response via chemical exhaustion of replication protein A (RPA): Development and anti-cancer activity of small molecule RPA inhibitors

J. Turchi[1], N. Gavande[2], P. Vandervere-Carozza[2], K. Pawelczak[3]. [1]*Indiana University school of Medicine, Medicine and Biochemistry & Molecular Biology, Indianapolis, USA;* [2]*Indiana University School of Medicine, Medicine, Indianapolis, USA;* [3]*NERx Biosciences, Research, Indianapolis, USA*

The DNA damage response (DDR) is a tractable pathway to target for the treatment of cancer. The inherent replication stress (RS) experienced by rapidly dividing cancer cells provides a therapeutic window for anticancer activity of agents targeting the DDR. In addition, inhibiting the DDR can enhance the activity of common chemo-and radio-therapeutic interventions that impart their clinical efficacy via the induction of DNA damage. The DDR is initiated by engagement of the of PI3 kinase-related kinases ATM, ATR, and DNA-PK. Oncogenic RS coupled with DDR blockade results in local effects at the replication fork and global effects on cell cycle and signaling which ultimately result in replication catastrophe (RC) and cell death. The human single stranded DNA (ssDNA) binding protein, replication protein A (RPA), is a critical regulator of RC with depletion of RPA or “RPA exhaustion” driving RC and cell death. RPA level and activity is tightly regulated and high levels of ssDNA can exhaust cellular RPA such that there is insufficient RPA-DNA binding capacity to engage the ssDNA generated. The lack of RPA protection renders chromosomal DNA susceptible to nuclease catalyzed degradation, ultimately leading to RC and cell death. To exploit this phenomenon we have sought to identify small molecule inhibitors of the RPA-ssDNA interaction. Synthetic medicinal chemistry along with molecular modeling and structure guided design was used to discover and develop a series of potent and selective small molecule inhibitors of RPA. Biochemical, cellular and in vivo analyses were performed to determine mechanism of action and impact on the cellular DDR and therapeutic action. The results obtained by extensive structure activity relationship analyses defined and optimized substituents for potency, selectivity and cellular bioavailability. Biochemical analyses reveal potent RPA inhibition and cellular analysis reveal target engagement and impacts on the DDR indicative of chemical exhaustion of RPA. Potent single agent anti-cancer activity was observed in numerous cancer cell models and synergistic activity is observed with a both DNA damaging chemotherapeutics other molecularly targeted DDR therapeutics. *In vivo* analyses in a series of xenograft models and patient derived spheroid model demonstrate that chemical inhibition of RPA can be exploited as single agent therapy and in combination regimens. These data demonstrate the successful identification of SMI of the RPA-DNA interaction and highlight their utility as potential anti-cancer therapeutics.

No conflict of interest

79 (PB-030) Poster

Clinical implications of Next Generation Sequencing-based somatic BRCA1/2 mutation profiling in consecutive ovarian carcinoma cases

S. Kowalik[1], K. Zalewski[2,3,4], J. Kopczyński[5], P. Macek[6], M. Lech[1], J. Kalisz[1], K. Hińcza[1], S. Zięba[1], M. Misiek[4], S. Góźdź[7,8]. *[1]Holycross Cancer Center, Molecular Diagnostics, Kielce, Poland; [2]Maria Sklodowska-Curie Institute – Oncology Center, Molecular and Translational Oncology, Warsaw, Poland; [3]Warsaw Medical University, Chair and Department of Obstetrics, Gynecology and Oncology, 2nd Faculty of Medicine, Warsaw, Poland; [4]Holycross Cancer Center, Gynaecologic Oncology, Kielce, Poland; [5]Holycross Cancer Center, Surgical Pathology, Kielce, Poland; [6]Holycross Cancer Center, Epidemiology and Cancer Control, Kielce, Poland; [7]Holycross Cancer Center, Clinical Oncology, Kielce, Poland; [8]Jan Kochanowski University, The Faculty of Medicine and Health Sciences, Kielce, Poland*

Background: Ovarian cancer (OC) is the most aggressive and lethal gynaecological malignancy. Up to 70% of patients are diagnosed when the disease has already spread contributing to only 25% five-year survival rates. The standard of care for OC patients consists of debulking surgery followed by systemic treatment with platinum compounds – regimen that remains unchanged for almost two decades.

Interestingly, with the advent of Poly (ADP-ribose) polymerase inhibitors (PARPi), a new class of anticancer drugs, an important change in standard of care is being observed. PARPi have been thoroughly studied in tumours with defects in Homologous Recombination-based DNA repair – a feature caused by mutations of *BRCA1*, *BRCA2* and a number of other genes. Molecular profiling of *BRCA* genes have become inseparable part of the standard of care in OC patients' management. Next Generation Sequencing (NGS) is a robust tool to assess mutation in BRCA genes in – germline and recently also in the somatic setting. Although with the appropriate diagnostic workflow, NGS of tumour derived DNA allows timely and accurate testing, a number of important areas still require further evidence generation.

Material and Methods: 201 consecutive ovarian carcinomas of the following histopathological subtypes: 155 (77,1%) serous, 21 (10,4%) endometrioid, 9 (4,5%) clear cell, 6 (3%) mucinous, 6 (3%) undifferentiated and 4 (2%) mixtum (serous+endometrioid) were studied. Majority (76%) of the patients were FIGO stage III-IV. FFPE tissue samples, were assessed by pathologist, DNA was isolated and assessed for BRCA1/2 mutation by using Oncomine BRCA Research Assay.

Results: In the studied population pathogenic mutations were detected in 27% (54) of cases, 8% of cases (16) were assigned as variant of uncertain significance (VUS). Out of the 27% of identified BRCA mutants, 19% were cases with BRCA1 mutations and 8% affecting BRCA2 gene. Mutations were only found in the following histopathological subtypes: 31.6% of serous, 14.3% of endometrioid and, 22.2% of clear cell. Interestingly in cases with over 50% AF of tumour-detected pathogenic variant 96% of mutations were germline, as opposed to only 36% in cases with AF below 50%.

Conclusions: In many countries BRCA-mutations status remains a critical PARPi therapy gatekeeper. Here we show that NGS is a powerful tool allowing timely detection of not only germline but also somatic mutations further expanding the eligible group to approximately 27%. VUS repeatedly remains an important challenge in result interpretation of clinical relevance. For familial risk management BRCA testing should be performed in serous but also in endometroid and clear cell OC. Although more data is needed AF seems to be correlated with the germline vs somatic status of the detected mutation.

No conflict of interest

80 (PB-031) Poster

Biomarker development in ovarian cancer using multispectral immune profiling of the DNA damage response

H. Robinson[1], L. Gentles[1], C. Kirk[1], H. Smith[1], L. Wilson[1], N. Curtin[1], R. O'donnell[2]. *[1]Northern Institute for Cancer Research, Newcastle University, Newcastle-upon-Tyne, United Kingdom; [2]Northern Gynaecological Oncology Centre, Queen Elizabeth Hospital, Gateshead, United Kingdom*

Introduction: Germline mutations in BRCA1 or BRCA2, key tumour suppressor genes within in the homologous recombination DNA repair (HRR) pathway, are seen in 15% of epithelial ovarian cancers (EOC). BRCA-mutant EOC have been shown to be responsive to poly(ADP-ribose) polymerase inhibitors (PARPi), however there may be opportunity to expand their use in the 50% of EOCs which have defects in HRR independent of BRCA. A functional HRR biomarker assay, dependent upon live cellular culture, has been shown to correlate with cisplatin sensitivity in vivo and PARPi sensitivity ex vivo but a clinically reproducible biomarker is lacking.

This pilot study aimed to quantify proteins key to the DNA damage response (DDR) in patient tumour material correlating results with the functional HRR status undertaken in corresponding live tumour subcultures.

Methods: FFPE EOC tumour specimens, alongside fresh tumour for live culture, were prospectively collected from consented patients undergoing surgery for ovarian cancer (REC 12/NW/0202). HRR status was determined in live cultures using the RAD51 immunofluorescent foci assay. Tissue microarrays (TMAs) were generated from 1 mm cores of corresponding FFPE material. An antibody panel was used to quantify (modified H-score) key proteins within the HRR and other DDR pathways (CHK1, DNA-PK$_{cs}$, PAR, PARP-1, RAD51, and 8-oxoguanine), using IHC and Vectra® multispectral immunofluorescent microscopy.

Results: 68 live ovarian cancer cultures were generated from 25 patients with advanced EOC. 36 (52.9%) were HRR deficient (HRD) with a less than 2-fold increase in Rad51 foci following induction of DNA damage. In the FFPE TMA cores assessed there was variable expression of each of the DDR antigens ranging from an H score of 0–24. There was no correlation between individual antigen expression by IHC and HRR function ($p = 0.13$). When antigens were combined using Vectra multiplexed immunofluorescent assessment there was no pattern clustering within HRD vs HRC patient samples. The median progression free survival (PFS) for HRD tumours was 12 months compared to 11 months in the HRC group, ($p = 0.075$). Median PFS of cultures homogenous for HRR function was 11 months in comparison to 14 months for patients with heterogeneous subcultures, ($p = 0.050$).

Conclusions: Protein quantification of key proteins in DDR pathways are not predictive of HRR function when assessed in isolation or combination. FFPE-based protein biomarkers cannot replace functional assessment in live culture material and future work should focus on making culture and functional HRR assessment more reproducible in a diagnostic laboratory setting.

No conflict of interest

81 (PB-032) Poster

Free-circulating tumor DNA of JAM3 promoter methylation in colorectal cancer

W. Tang[1,2], H. An[1], D. Zhou[3], L. Chen[1], M. Cai[1], F. Ye[1], X. Lv[1]. *[1]The First Affiliated Hospital of Xiamen University, Xiamen Cancer Hospital, Department of Medical Oncology, Xiamen, China; [2]Fujian Medical University, Department of Oncology, Fuzhou, China; [3]Xiang`an Hospital of Xiamen University, Oncology, Xiamen, China*

Background: ctDNA (circulating tumor DNA), compared with classic tumor markers, has higher accuracy and specificity, and can be real-time monitoring of tumorgenesis. Furthermore, there are a number of ctDNA have abnormal methylation that correlation with different clinical pathological features in colorectal cancer (CRC). Studies was carried out on the basis of bioinformatics analysis, we aims to explore the relationship between plasma JAM3 methylation and the occurrence and development of CRC, and to identify JAM3 ctDNA methylation as a new tumor biomarker in the diagnosis and real-time monitoring of CRC, in order to provide a theoretical basis for early clinical diagnosis and intervention.

Material and Methods: Genome-wide methylation data of 125 CRC tissues and 29 adjacent normal intestinal tissues were downloaded from GEO database of NCBI, combined with the screening of differential hypermethylation genes in primary literature, study were further confirmed in SW480, SW620, HCT116 and HT29 CRC cell lines, that was treated with demethylated drugs—5-aza-2-oxyeytidineine(5-AZA) for 48 hours. The expression change of JAM3 in CRC cells and mRNA level in plasma samples was detected by qPCR, and the methylation status of JAM3 was detected by qMSP in plasma samples and surgical tissues, respectively.

Results: We found that the methylation level of JAM3 was significantly higher in CRC tissues than in adjacent normal controls from Chip datas. As expected, the methylation status of CRC cell lines, which initially showed high level of JAM3 methylation, was decreased after 5-AZA treatment. The mRNA and protein expression level of JAM3 was rescued in the 5-AZA-treated group compared to that in the control group, strongly indicating that CpG island hypermethylation was responsible for loss or decreased expression of JAM3 in CRC. Meanwhile, JAM3 mRNA was markedly downregulated in CRC (50%) as paired normal plasma samples, which suggest that JAM3 can be used as a potential circulating tumor suppressor marker of CRC. We also found the methylation level of JAM3 was observably higher in CRC circulating cell-free DNA(cfDNA)(mean 73.14%) than in

normal controls(mean 19.03%). Hypermethylation was also found to be frequent in 18 of 24 primary CRC tissues analysed, and there were statistically significant differences in the methylation status of JAM3 between CRC tissues(mean 82.62%) and normal controls(mean 38.84%), suggest that methylation modification may be involved in the silence of JAM3. And the methylation status of JAM3 was significantly associate with tumor stage in CRC tissue samples.

Conclusions: These results demonstrated that DNA methylation involved in the tumorgenesis of CRC was responsible for loss of JAM3, which suggest that detection of JAM3 gene methylation in plasma can be used as a potential noninvasive biomarker for CRC diagnosis and prognosis.

No conflict of interest

Tuesday, 13 November 2018

POSTER SESSION

Molecular Targeted Agents – PART I

82 (PB-033) Poster

Four-weeks preoperative use of tocotrienol (delta-T3) from Annatto Bixa Orellana L. (Achiote tree) in breast cancer patients

C. Ferraris[1], C. Listorti[1], B. Ballestra[1], A. Gambaro[2], L. Rivoltini[3], L. Mariani[4], G. Martelli[1], E. Tagliabue[5], M. Giussani[5], I. Maugeri[1], S. Folli[1], P. Limonta[2], F.M. Celotti[2]. [1]*Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Surgery, Division of General Oncological Surgery Senology, Milan, Italy;* [2]*Università degli Studi di Milano, Department of Pharmacological and Biomolecular Sciences, Milan, Italy;* [3]*Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Research///Unit of Immunotherapy of Human Tumors, Milan, Italy;* [4]*Fondazione IRCCS Istituto Nazionale dei Tumori, Direzione Scientifica Statistica Medica, Milan, Italy;* [5]*Fondazione IRCCS Istituto Nazionale dei Tumori, Struttura Semplice Dipartimentale Bersagli Molecolari, Milan, Italy*

Background: Tocotrienols have shown strong *in vitro* and *in vivo* anti-oxidant and anti-cancer activity, promoting apoptosis and regulating oncogenic targets in breast cancer. However their clinical use is still experimental especially in preoperative setting, where there are still no data in efficacy in terms of anti-cancer, antioxidant and antinflammatory beneficial role.

Material and Methods: We conducted a prospective observational clinical study enrolling 50 patients with primary breast cancer (T1-2, N0-1, M0) who received 4-weeks oral treatment of delta-T3 (Tocotrienol FG®, EFFEGILAB, Lavis, TN, Italy) before surgery (400 mg/twice daily). We collected serum samples to evaluate oxidant (tock fast-Li Starfish) and antioxidant capacity (TAC Track, Li Starfish) as well as anti-inflammatory activity before and after four weeks of treatment with delta-T3. In 10 patients, complete profiling of adaptive and innate cell immunity was performed by 13-color cytofluorimetry on peripheral blood mononuclear cells. Clinicopathological determination of estrogen receptor, progesterone receptor, HER2 and Ki-67 was performed on preoperative tumor biopsy and on surgical specimen.

Results: Analysis on plasma soluble factors showed that concentration of VEGFA and TOC was remarkably reduced in post vs pre blood samples (231 vs 199 median, p = 0.0027 and 696 vs 614 median, p = 0.0462, respectively). Of note, differences in all these parameters were more significant in patients showing compliance >=1.00 (n = 26). Concerning the blood immune profiling, frequency of myeloid inflammatory and immunosuppressive cells of both granulocytic and monocytic lineage (CD11b+CD15+, CD14+HLA-DRneg and CD15+CD30+PDL-1+) was relevantly diminished in post with respect to pre-treatment peripheral blood mononuclear cells (PBMC), together with a decline of regulatory T cells (CD4+CD25highCD127+). This effect was paralleled by a boost of cytolytic NK cells (CD3-CD16+CD56dim) and activated Th1 CD4+ helper T lymphocytes (CD4+CD25+CXCR3+CXCR6-). No major change in the frequency of the principle PBMC populations (T cells, B cells, NK cells and monocytes) could instead be detected, indicating a specific activity of the drug on selective immune cell subsets. No side effects were observed during and after the administration of delta-T3.

Conclusions: Delta-T3 was found to have anticancer, antioxidant and anti-inflammatory activity in breast cancer patients treated preoperatively. Future clinical studies with prolonged treatment with higher dosage of delta-T3 will better clarify tocotrienol biomolecular activity in neoadjuvant and adjuvant therapy in breast cancer patients.

No conflict of interest

83 (PB-034) Poster

RENCA macrobead therapy in advance mCRC: Phase Iia and Iib preliminary multi-site tumor marker findings and comparison of patients treated with RMB vs. hospice care

S. Nazarian[1], S. Sureshbabu[1], Z.P. Andrada[1], J. Thomas[1], N. Berman[1], D.J. Wolf[2], L.H. Gazda[3], T.J. Fahey[4], A.J. Ocean[2], C.P. Comfort[5], B.H. Smith[1]. [1]*The Rogosin Institute, Weill Cornell Medicine, RENCA Macrobead Project, New York, USA;* [2]*Weill Cornell Medicine, Oncology, New York, USA;* [3]*The Rogosin Institute, Xenia Division, RENCA Macrobead Project, Xenia, USA;* [4]*Weill Cornell Medicine, Endocrine Surgery, New York, USA;* [5]*Calvary Hospital, Hospice Care, Bronx, USA*

Background: Mouse renal adenocarcinoma cells (RENCA) entrapped in agarose macrobeads (RMB) present a novel biologic systems-based therapeutic approach to late-stage metastatic colorectal cancer (mCRC). While undergoing genomic changes, the RMBs adapt to the cancerous environment through the release of numerous anti-neoplastic factors and regulation of biochemical pathways including MEF-2, mTOR, Akt, etc. Reported here are preliminary efficacy and survival results from the Phase Iia and Iib studies.

Material and Methods: A total of 76 eligible mCRC patients who have failed all FDA-approved therapies underwent laparoscopic intraperitoneal implantation of RMBs (8 RMB/kg body weight) up to 4 times in these open-label clinical trials. Physical examinations, lab profiles, and radiologic imaging were conducted pre- and post-implant to determine safety, efficacy, and overall survival. CEA and/or CA19-9 tumor marker values were assessed for correlation between Responders (R³ 20% decrease) and Non-Responders (NR). Preliminary survival comparison was performed among mCRC patients treated with RMB vs. non-RMB treated, hospice end-of-life-care patients.

Results: Two groups of patients; R (62%; n = 47) and NR (38%; n = 29) were defined by the decrease of tumor marker values from baseline to Day 14 post- RMB implant (baseline, mean R CEA = 352.80 ± 1164.85, D14 mean R 256.22 ± 801.20 vs baseline mean NR 743.41 ± 2669.83, D14 mean NR 1556.80 ± 4100.82; p < 0.000028). The R and NR groups were also statistically different with respect to their CA19-9 levels (baseline, mean R CA19-9 = 304.94 ± 858.03, D14 mean R 198.98 ± 594.00 vs baseline mean NR 888.92 ± 1994.28, D14 mean NR 2042.19 ± 4443.29; p < 0.00001) [Table 1]. In addition, preliminary survival data comparison between RMB-treated vs hospice-care patients suggests a 7.5-month difference from Date of Progression to Date of Death (hospice mean 49 days [~7 weeks], median 44 days [~6 weeks] vs RMB-treated patients mean 261 days [~37 weeks], median 226 days [~32 weeks]; p < 0.00001).

Table 1

Mean CEA & CA19-9 Values	Responder (n = 47)	Non-Responder (n = 29)	P Value
Baseline (CEA)	352.80 ± 1164.85	743.41 ± 2669.83	<0.000028
Day 14 (CEA)	256.22 ± 801.20	1556.80 ± 4100.82	
Baseline (CA19-9)	304.94 ± 858.03	888.92 ± 1994.28	<0.00001
Day 14 (CA19-9)	198.98 ± 594.00	2042.19 ± 4443.29	

Conclusion: The combined data from Phase Iia and Iib trials supports the efficacy of RMB treatment in late-stage mCRC patients. CEA and CA19-9 levels at baseline and Day 14 were predicting of positive response to RMB therapy. Comparison of hospice patient survival with that of RMB-treated patients further supports the clinical anti-tumor effect of RMBs. A Phase III randomized trial of RMB vs standard therapy to further assess RMB effectiveness is in development.

No conflict of interest

84 (PB-035) Poster

Androgen Receptor regulation of HGF/Met pathway in clear cell renal cancer

D. De Silva[1], A. Roy[1], M. Lee[1], R. Costello[1], D. Bottaro[1]. [1]*National Institute of Health/National Cancer Institute, Urology Oncology Branch, Bethesda, USA*

Hepatocyte growth factor receptor (Met) dysregulation has been implicated in multiple neoplasms including gastric, lung, and renal carcinomas. Binding by the Met ligand, hepatocyte growth factor (HGF), induces Met tyrosine kinase activation and drives motility, proliferation, survival, angiogenesis and morphogenesis. Met dysregulation is frequent in renal carcinoma (RCC)

including germline *MET*mutations in hereditary and sporadic papillary RCC, somatic mutations in head and neck carcinomas, and *MET*amplification in lung, gastric, and multiple other cancers. Previous studies have demonstrated high Met protein abundance in papillary and clear cell RCC tissues compared to normal correlating with poor prognosis and over-all survival. The Cancer Genome Atlas (TCGA) provisional ccRCC data indicates that mutation, copy number alteration, and amplification/over-expression occur in ~12% of 446 total cases. Interestingly, we found that androgen receptor (AR) over-expression occurring in 6% of 534 ccRCC TCGA cases was associated with significantly improved over-all (OS) and progression-free survival (PFS). This potential benefit of AR overexpression was not seen in papillary RCC TCGA data (280 cases). Furthermore, previous studies reported AR loss in RCC compared to normal kidney tissue. Our initial investigation of the potentially beneficial role of AR signaling in ccRCC was designed to identify AR effects on the HGF/Met pathway. We found that AR and MET expression appear to be mutually exclusive in TCGA data for ccRCC and in kidney, bladder and prostate cancer cell lines, consistent with several reports that androgen deprivation leads to increased MET expression in prostate cancer. We found that reconstitution of *AR* in ccRCC cell lines with robust *MET* expression (786-0 and Caki-2) did not significantly reduce *MET* mRNA levels. However, it did reduce Met protein content, implying that an additional negative regulatory mechanism by AR may exist. To gain a more global view of how AR expression might potentially benefit ccRCC patients and identify potentially novel therapeutic strategies, we performed RNA-Seq on ccRCC cell lines over-expressing wild-type AR or the constitutively active variant Arv7 in the presence or absence of the AR ligand dihydrotestosterone (DHT). Data analysis using Partek Flow identified significant alterations in steroid pathways (e.g. PPAR, thyroid hormone, oxytocin and estrogen receptor signaling) and RCC relevant pathways (e.g. Hippo, TGF-beta, Wnt, FOXO, oxidative phosphorylation pathways), including those of therapeutically targetable proteins. Androgen/AR suppression of Met has been demonstrated in prostate cancer and we found a similar relationship in ccRCC cell lines between AR and Met. AR over-expression could be beneficial for ccRCC patients since TCGA data showed significantly improved OS and PFS for that cohort.

No conflict of interest

85 (PB-036) Poster

Discovery of a potent androgen receptor degrader for castration resistant prostate cancer

C. Wang[1,2], X. Han[1], E. Fernandez-Salas[1], C. Qin[1], W. Xiang[1], T. Xu[1], D. Mceachern[1], S. Przybranowski[1], C. Foster[1], L. Bai[1], J. Lv[1], L. Huang[1], S. Wang[1]. *[1]University of Michigan, Internal Medicine, Pharmacology, Medicinal Chemistry, Ann Arbor, USA; [2]Peking University, School of Pharmaceutical Sciences, Beijing, China*

Background: Androgen receptor (AR) is a central therapeutic target in metastatic castration resistant prostate cancer (mCRPC). Previous successful strategies targeting AR signaling have focused on blocking the synthesis of androgen with abiraterone and inhibition of AR with AR-antagonists. These agents ultimately become ineffective against advanced prostate cancer through AR gene amplification, mutations and alternative splicing, despite the fact that continued AR signaling is retained in tumors.

Methods: Degradation of AR may achieve much better clinical efficacy than inhibition of the protein. Here we report an AR degrader, **ARD-61**, containing an AR inhibitor and a ligand for an E3 ligase utilizing PROteolysis TArgeting Chimeric (PROTAC) technology as a new therapeutic strategy for the treatment of CRPC.

Results: ARD-61 can effectively induce AR degradation in a dose- and time-dependent manner in all the cell lines tested, with observed low nanomolar 50% degradation concentrations (DC_{50}). AR degradation resulted in over 40 times more potent anti-proliferation effects compared to the inhibitor. Degradation of AR in the cytoplasm is faster than that in the nucleus. Proteomics results confirmed AR degradation by **ARD-61** and revealed no obvious off-target effects. Degradation of AR protein by **ARD-61** leads to effective inhibition of mRNA expression of PSA and TMPRSS2 but not that of AR, indicating effective inhibition of AR signaling. **ARD-61** showed potent AR degradation effects and anti-tumor activities in multiple murine xenograft models of prostate cancer at well-tolerated dosing schedules.

Conclusions: Our data provides clear evidence that targeting AR for degradation by the PROTAC methodology represents a very promising therapeutic approach for the treatment of CRPC retaining AR signaling. **ARD-61** is a highly potent AR degrader with optimal drug-like properties for advanced preclinical development and future clinical trials for the treatment of CRPC.

No conflict of interest

86 (PB-037) Poster

Combination effect of thymoquinone and extracts of Iksan526 callus in A375 human melanoma cell line

S.J. Kim[1]. *[1]Kongju National University, Biological Sciences, Gongju, Korea*

Background: Iksan526 is resveratrol-enriched transgenic rice that overexpresses the stilbene synthase gene isolated from the peanut (Arachis hypogaea var. Palkwang). Experimental data show that resveratrol-enriched transgenic rice might down-regulates melanin synthesis in UVB-induced Guinea Pigs epidermal skin tissue The aim of the present study was to investigate the effect of thymoquinone (TQ) and extracts of Iksan526 callus (IS526) treatment on A375 cells.

Materials and Methods: A375 were treated with TQ or IS526, and the effect of TQ or IS526 on cell proliferation, tyrosinase activity, reactive oxygen species (ROS) generation and Cyclooxygenase (COX)-2, pp38, pERK expression levels were respectively tested by the MTT assay, tyrosinase activity assay, ROS assay and western blot analysis.

Results: In this study, TQ has been shown to inhibit cell proliferation, ROS production and tyrosinase activity. TQ also induced COX-2, pERK, pp38 expression. IS526 reduced cell proliferation, tyrosinase activity and COX-2 expression. IS526 increase ROS production, pERK, pp38 expression. We found that compared with treatment alone, co-exposure of cells to TQ and IS526 resulted in a significant decrease in cell proliferation and tyrosinase activity, pERK expression in the A375. In addition, TQ-induced COX-2 expression, which was inhibited by IS526 and these effects were enhanced by ERK-1/-2 and p38 inhibitors. TQ inhibits IS526-induced ROS, which reversed by PD98059 (PD) and SB203580 (SB). SB enhanced the decrease in tyrosinaase activity by TQ combined with IS526 while, PD restored tyrosinase activity. The blockage of p38 kinase or ERK-1/-2 with SB and PD did not have any effect in cell proliferation by both drugs.

Conclusions: These results suggest that IS526 inhibits TQ-induced COX-2 expression and TQ suppresses IS526-induced ROS via the ERK-1/-2 and p38 kinase pathways. The ERK-1/-2 and p38 kinase pathways regulate tyrosinase activity by TQ combined with IS526.

No conflict of interest

87 (PB-038) Poster

HMBD001-10D1, a novel humanized anti-HER3 antibody with a unique mechanism of action, demonstrates superior tumor inhibition in multiple tumor models compared to other EGFR family therapies

D. Thakkar[1], M. Taguiam[1], V. Sancenon[1], S. Guan[1], K. Paszkiewicz[1], P. Ingram[1], J. Boyd-Kirkup[1]. *[1]Hummingbird Bioscience, Discovery, Singapore, Singapore*

HER3 is increasingly implicated as a critical node in oncogenic EGFR family signaling. HER3 is a potent heterodimer partner for EGFR and HER2, which signal through the MAPK pathway and, additionally, HER3 heterodimerization triggers signaling through the PI3K/AKT/mTOR pathway. HER3 activation has thus been implicated in acquired resistance to EGFR/HER2 therapies and other MAPK pathway therapies such as BRAF inhibitors, where activation of the PI3K pathway represents a possible escape route for tumors. Further, emerging clinical evidence has shown that solid tumors refractory to anti-PD1 therapy have higher HER3 expression compared to anti-PD1 responders. Despite this, existing anti-HER3 therapies such as MM-121 that inhibits the ligand-binding to HER3, and LJM-716 that tries to lock HER3 in a closed confirmation, have shown only limited efficacy in clinical trials, likely due to high levels of ligand independent dimerization in many tumors.

HMBD-001-10D1 is a novel therapeutic antibody designed to directly block the heterodimerization surface of HER3. This precise targeting of a specific functional epitope at the dimerization interface was enabled using Hummingbird Bioscience's proprietary Rational Antibody Discovery Platform. HMBD-001-10D1 is a humanized IgG1 antibody with low picomolar affinity and high specificity to cell-surface expressed HER3, with no cross-reactivity to EGFR or HER2, and additionally binds with high affinity to HER3 cyno, mouse and rat orthologs, avoiding the need for surrogate antibodies during pre-clinical screening. In addition, HMBD001-10D1 has been optimized to remove development liabilities and shows excellent manufacturability including expression titers, stability and purity.

HMBD001-10D1 has demonstrated potent tumor growth inhibition and broad efficacy across multiple tumor models that express HER3 and HER2/EGFR, both *in vitro* and *in vivo*. HMBD001-10D1 inhibits phosphorylation of HER3 and HER2/EGFR by blocking the formation of HER3 heterodimers and significantly decreases downstream signaling through the PI3K/AKT/mTOR *and* MAPK pathways. As a result of its distinct molecular mechanism of action, HMBD-001-10D1 blocks both ligand-dependent and independent activation. Notably, HMBD-001-10D1 shows over 90% efficacy in pre-clinical

models known to be refractory to existing EGFR family therapy (Cetuximab, Trastuzumab, LJM-716 and MM-121).

HMBD-001-10D1 is expected to provide significant patient benefit in multiple solid tumors, both as a monotherapy and in combination with other EGFR family targeted therapies, and may also benefit patients refractory to BRAFi who have limited treatment options. First-in-human trials of HMBD001-10D1 are expected to commence in late 2019.

No conflict of interest

88 (PB-039) Poster

Broad spectrum activity of sabutoclax in haematological and solid cancer cell lines is associated with defined biomarkers

V. Vuaroqueaux[1], H. Al Hasani[2], G. Kelter[3], H.R. Hendriks[4], H.H. Fiebig[1]. *[1]4HFBiotec GmbH, Freiburg, Germany; [2]4HFBiotec GmbH, Bioinformatics, Freiburg, Germany; [3]Charles River, Discovery Services, Cell Biology & Compound Screening, Freiburg, Germany; [4]Hendriks Pharmaceutical Consulting, Purmerend, Netherlands*

Background: The B cell lymphoma 2 gene family (BCL-2) plays a central role in regulating programmed cell death and is involved in tumors apoptosis evasion and resistance toward cytostatic agents. The selective inhibition of BCl-2 family proteins is an emerging therapeutic option, the BCl2 inhibitor Venetoclax was approved as second line therapy in CLL while several inhibitors are currently in clinical trials for other indications. Sabutoclax is a pan-Bcl-2 inhibitor targeting Bcl-xL, Bcl-2, Mcl-1 and Bfl-1 which has shown antitumor activity in various models of tumors. Here we studied Sabutoclax efficacy in a broad range of tumor models to determine possible indications and predictive biomarkers.

Material and Methods: The compound was tested *in vitro* in a monolayer assay in well characterized human tumor cell lines from hematological cancers (n = 51) and solid tumors (n = 222). The cell lines molecular data generated by whole exome sequencing, Affymetrix snp 6.0 and Affymetrix u133 plus 2.0 arrays were used to elucidate determinants of drug response.

Results: *In vitro* assays revealed high anti-tumor efficacy (IC70 < 0.2 μM) in 42 out of 273 cell lines (15%). Similar IC70 geometric means (0.7 μM) were observed in both hematological cancers and solid tumors. Overall Sabutoclax was active in a broad spectrum of tumors including lymphomas, leukemia (AML and ALL), sarcomas (soft tissues and osteosarcoma), pleuramesothelioma, adeno carcinomas of the lung, breast, head and neck, prostate, and bladder tumors. The compound was however active in a limited number of cell lines per tumor type, making the identification of predictive biomarker essential. The biomarker screening revealed BCL2L1 (Bcl-xL, probeset 212312_at) to be among the most significant transcript that correlated with Sabutoclax IC70s (Spearman r = 0.35, p adjusted = 4.5E – 05) while BCL2, Mcl-1 and Bfl-1 members were not. Based on cell line gene expression profiles, we derived a signature classifying the cell lines into clusters with response rates ranging from 0 to 59%. The analysis also revealed 133 mutated genes which were associated with sensitivity to Sabutoclax (Fisher and Wilcoxon tests p < 0.05). Notably, 5 out of 6 models mutated for BCL2 were found to be highly sensitive to Sabutoclax (IC50 < 0;2 μM, Fisher test p = 0.004). On the contrary, 48 genes including AXL, KMT2B, ADAMTS17 were associated with tumor resistance when mutated (fisher p values <0.05).

Conclusions: Sabutoclax showed efficacy in lymphoma, leukemia as well as in wide range of solid tumors without however clear indication regarding the histological type. Molecular analysis revealed multiple genomic and transcriptomic predictors of tumor response which will need to be validated. They may serve to develop a predictive model helping in tumor selection for the next phases of the drug development.

No conflict of interest

89 (PB-040) Poster

Inhibitors of p38 MAPK and MK-2 signaling pathways sensitize NCI-H69 cells to etoposide treatment

D. Alimbetov[1], B. Umbayev[1], T. Davis[2], S. Askarova[1], D. Kipling[2]. *College of Biomedical and Life Sciences Cardiff University School of Medicine [1]Nazarbayev University, National Laboratory Astana, Astana, Kazakhstan; [2]Cardiff University, College of Biomedical and Life Sciences, Cardiff, United Kingdom*

The main goal of of cancer therapy is to kill cancer cells leaving healthy cells intact in the patient – a concept known as the therapeutic window. Although a long-term goal always has been the development of agents that selectively kill cancer cells, a more recent trends have been to seek secondary agents that sensitize cancer cells to existing treatment regimens when used in combination. P53 is a tumour suppressor gene that mutated frequently in many cancer types. Furthermore, in many cancer cells have abrogated G1 checkpoint due to selection for lesions in tumour suppressor molecules such as p53. However, such cancer cells often retain a residual G2 checkpoint, in particular a chromatin-quality checkpoint in late G2 involving ATR/p38MAPK/MK2. Work using transgenic mice has shown that genetic disruption of the p38/MK2 pathway specifically sensitizes p53-null mouse cells to DNA damaging agents. The mechanism of action is thought to be that the p53-null cells in the presence of ablation of p38/MK2 have lost both G1 and G2 DNA damage checkpoint function, and enter mitosis despite the presence of DNA damage, where they die by “mitotic catastrophe”. In this study we explored the effect of p38/MK2 inhibitors on the G2 checkpoint in cancer cells (NCI-H69) in combination with known DNA damaging agents, particularly with Etoposide.

Materials and Methods: NCI-H69 Small Cell Lung Cancer (SCLC) cell line was obtained from ATCC, subjected to growth characteristic assessment and treatment with DNA damaging agents in the presence and absence of p38 and MK2 inhibitors. Cells were seeded into a 12 well plate and treated with 10, 20, 50, 100 and 150 μM Etoposide with/without p38 inhibitor SB203580 (2.5 μM) and MK2 inhibitor MK2/3 (1 μM).

Results and Conclusion: Results suggest that Etoposide produce a profound effect on the cell cycle profile of cells in a manner that is consistent with the degree of cell viability that is seen using the viable cell assay. Results of the co-treatment experiments revealed that the p38/MK2 kinase inhibitors SB203580 and MK2/3 (small molecules) both enhanced the negative effects of etoposide (DNA damaging agent) on H69 cell viability. Thus both p38 and MK2 inhibitors improved the sensitivity of H69 cells to DNA damaging agents. Our most recent ongoing data on the response to combined DNA damage and p38/MK2 inhibition will be presented.

No conflict of interest

90 (PB-041) Poster

Tumor immune modulation by the PI3-kinase (PI3K) inhibitor MEN1611 via tumor-associated macrophages polarization

S. Capano[1], A. Fiascarelli[1], A.M. Tomirotti[1], A. Paoli[1], M. Bigioni[1], A. Bressan[1], D. Bellarosa[1], M. Salerno[1], M. Binaschi[1]. *[1]Menarini Ricerche S.p.A., Experimental and Translational Oncology Department, Pomezia RM, Italy*

MEN1611 (previously PA799) is a phosphatidylinositol 3-kinase (PI3K) inhibitor, that selectively targets PI3Kα (both wild type and mutated forms) and PI3Kγ isoforms. Its inhibitory and anti-tumor activity has been mainly characterized against PI3Kα, known to be frequently mutated in various human cancers. On the contrary, its activity against PI3Kγ, that is highly expressed in leukocytes but not in cancer cells and is reported to be involved in inflammatory cell recruitment to tumors, is not well described yet. Previous studies have shown that selective targeting of PI3Kγ by IPI-549 can reshape the tumor immune microenvironment towards a less immunosuppressive phenotype and promote cytotoxic T cell-mediated tumor regression. These evidences suggest that MEN1611 might lead to anti-tumor immune response via PI3Kγ inhibition.

To determine whether PI3Kγ inhibition mediated by MEN1611 directly affects macrophage polarization we analyzed *in vitro* cell morphology, mRNA expression and cytokine release both in murine and human macrophages. Murine macrophages have been differentiated from bone marrow-derived monocytes by M-CSF stimulation, followed by M1 (lipopolysaccharides and interferon γ) or M2 stimulation (interleukin 4). M1 and M2 human macrophages were obtained from buffy coats in the same way. IPI-549 treatment was used as positive control, while BYL-719, which selectively inhibits PI3Kα, was used as negative control. In order to evaluate whether MEN1611 tumor growth inhibition *in vivo* might be mediated also by an immune modulatory activity, a syngeneic tumor model (4T1 breast cancer) has been established.

Both IPI-549 and MEN1611 treatments were able to modulate macrophage polarization towards an immune-activating phenotype *in vitro*, and as a consequence M2 cell morphology was reverted towards an M1-like round cell shape, gene expression analysis revealed a significant increase of immunostimulating factors mRNAs (such as IL-1b, IL-12b and iNOS, both in M1 and M2 macrophages), and the secretion of pro-inflammatory cytokines was enhanced both in M1 and M2 macrophages. This shift towards a more inflammatory M1-like state was not clearly evident in BYL-719-treated macrophages. 4T1 syngeneic mouse model characterization by flow cytometry revealed M1 and M2 macrophage subpopulation together with CD4+ and CD8+ T cells infiltrates. The impact of MEN1611 treatment on macrophages and T lymphocytes subsets infiltrating tumors is currently under investigation.

In conclusion, we demonstrated that targeting PI3K with MEN1611 at clinically relevant doses can switch the activation of macrophages towards a less immunosuppressive state *in vitro*. Nevertheless further studies are

necessary to investigate the ability of MEN1611 to impact on macrophages subsets *in vivo* and to promote a cytotoxic immune response by stimulating T cell recruitment into tumors.

No conflict of interest

91 (PB-042) Poster

Rational-based drug design of novel, highly potent MER inhibitors as potential treatment of cancers

A. Mikolajczyk[1], A. Yamani[2], P. Olejkowska[2], N. Piorkowska[2], M. Naitana[2], P. Maliszewski[2], K. Dubiel[2], J. Pieczykolan[1], M. Wieczorek[3], A. Stanczak[1]. [1]*Celon Pharma SA, Preclinical Research Development, Lomianki-Kielpin, Poland;* [2]*Celon Pharma SA, Medicinal Chemistry, Lomianki-Kielpin, Poland;* [3]*Celon Pharma SA, Research and Development Center, Lomianki-Kielpin, Poland*

Background: MER is a member of TAM (TYRO3, AXL, MER) family of receptor tyrosine kinases. MER is aberrantly expressed in several types of solid and hematological tumors, playing a substantial role in tumor growth and invasion. Inhibition of MER individually can be particularly prominent in solid tumors, whereas dual targeting of MER and other kinases potentially may bring higher therapeutic benefit both in solid and hematological cancers e.g. dual targeting of MER/AXL in NSCLC or MER/FLT3 in leukemias. Up to date, no such mono nor dual MER/FLT3 and MER/AXL inhibitors has been approved for therapy.

Material and Methods: Using rational-based drug design we have carried out extensive search and development of mono- and dual-inhibitors targeting mainly MERTK, but also potentially other kinases like AXL or FLT3 which could bring increased benefits in particular types of both solid and hematological cancers. *In vitro* inhibitory activity was tested in biochemical and cell-based assays. Luminescent kinase ADP-Glo™ assay was used to determine the inhibitory effect toward recombinant proteins (MERTK, AXL, TYRO3, FLT3). Intracellular downstream signaling modulation was measured by Western blotting. Cell cycle distribution was analyzed by flow cytometry using propidium iodide.

Results: Our results indicate that designed compounds have shown potent *in vitro* activity against MER and FLT3 kinases with low subnanomolar and nanomolar IC_{50} values. Most potent compound CPL-305-095 in ADP-Glo™ assay inhibits MER and FLT3 at low subnanomolar IC_{50} (0.17 nM and 0.36 nM, respectively), comparable to the reference MER/FLT3 inhibitors – UNC2025 or MRX2843 (Meryx). Another potent compound – CPL-305-061 shows very high activity on FLT3 and MER (<0.1 and 0.49 nM, respectively), with significant activity on AXL (8.8 nM). In cell-based assays, analyzed compounds demonstrated a dose-dependent activity. Treatment of cancer cell lines with new MER inhibitors evokes substantial decrease in activation of MER-mediated intracellular signaling. CPL-305-095 causes a decrease in Akt phosphorylation – significant inhibition at 300 nM and practically complete inhibition at 1 uM. Moreover, compounds designed and synthesized at Celon Pharma, induce robust G2/M arrest.

Conclusions: We have designed potent MER inhibitors, which show encouraging *in vitro* results. The presented results support further development of these compounds as drugs with potential anti-cancer properties.

No conflict of interest

92 (PB-043) Poster

Combining NAE inhibition and IAP antagonism leads to apoptosis through enhanced NF-kB inhibition in DLBCL cells and demonstrates potent anti-tumor activity in a preclinical DLBCL model

G. Ward[1], A. Miura[2], T. Smyth[1], H. Muraoka[2], J. Lyons[1], A. Hashimoto[2], R. Ferraldeschi[1], Y. Nakatsuru[2], M. Sims[1], S. Ohkubo[2], S. Jueliger[1], C. Yoshimura[2]. [1]*Astex Pharmaceuticals, Tranlsational Biology, Cambridge, United Kingdom;* [2]*Taiho Pharmaceutical Co. Ltd., Discovery and Preclinical Research Division, Tsukuba, Japan*

Background: Seeking rational drug combination options is a goal in oncology to overcome resistance to targeted agents. TAS4464 is a novel, highly potent NEDD8-activating enzyme (NAE) inhibitor which demonstrates significant anti-tumor activity in hematological cancer cells lines, especially those driven by oncogenic activation of the NF-kB signaling (including multiple myeloma and diffuse large B-cell lymphoma (DLBCL) cell lines). ASTX660 is a novel non-peptidomimetic antagonist of cellular inhibitor of apoptosis protein 1 and 2 (cIAP1/2) and X-linked IAP (XIAP) which can switch NF-kB signaling from pro-survival to apoptosis. Therefore, we hypothesized that a combination of these compounds could produce enhanced anti-tumour activity in preclinical models of DLBCL.

Materials and Methods: TAS4464 and ASTX660 activity alone and in combination was tested *in vitro* in human DLBCL cell lines, WSU-DLCL2 and TMD8. Target engagement and signaling pathway modulation was evaluated by Western blotting. Induction of cell death was evaluated by cell viability and caspase activation assays. A subcutaneous xenograft model with WSU-DLCL2 grown in nude mice was used to evaluate the anti-tumour activity of the combined treatment *in vivo*.

Results: Inhibition of cullin neddylation by TAS4464 and degradation of cIAP1 by ASTX660 were confirmed as expected target engagement in DLBCL cell lines. The accumulation of phospho-IkB and phospho-p100 by TAS4464, which results in the inhibition of canonical and non-canonical NF-kb pathway, was enhanced in combination with ASTX660. This combination treatment led to increased apoptosis as revealed with cleaved caspase-8, 9 and 3. *In vivo* treatment of nude mice bearing WSU-DLCL2 xenografts with TAS4464 (50 mg/kg intravenous injection twice a week) or ASTX660 (25 mg/kg orally for 7 consecutive days every other week) significantly reduced the tumour growth compared to the control and when combined, the tumours underwent regression (see table below). A putative mechanism for this enhanced activity was determined: NAE inhibition reduces the induction of non-canonical NF-kB-mediated resistance to IAP antagonism (e.g. cIAP2 and c-FLIP induction), and hence the combination of these two compounds effectively shuts down NF-kB signaling and leads to sustained apoptotic induction.

Treatment	Tumor Growth Inhibition (% on Day 15)	Regression (>50%)	Survival (median days for tumors to reach 400%)
Vehicle	0	0/6	12
TAS4464 alone	82	1/6	31
ASTX660 alone	76	0/6	25
ASTX660 + TAS4464 combination	>100	5/6	>50

Conclusions: Enhanced inhibition of NF-kB activity was demonstrated with the ASTX660 and TAS4464 combination in WSU-DLCL2 and TMD8 DLBCL cells *in vitro*. Data from the WSU-DLCL2 *in vivo* model demonstrated that the combination is tolerated and shows significant anti-tumor activity, providing proof-of-concept for this combination.

No conflict of interest

93 (PB-044) Poster

Combining novel STAT3 inhibitor YHO-1701 with multi-targeted tyrosine kinase inhibitor sorafenib improves anti-tumor response in solid tumor xenograft model

K. Taniguchi[1], F. Nishisaka[1], M. Tsugane[1], G. Hirata[1], A. Takagi[1], S. Ishii[2], H. Takahashi[2], T. Iijima[2], A. Asai[3], T. Matsuzaki[1], Y. Shishido[1]. [1]*Yakult Honsha Co., Ltd., Yakult Central Institute, Tokyo, Japan;* [2]*Yakult Honsha Co., Ltd., Pharmaceutical Department, Tokyo, Japan;* [3]*Graduate School of Pharmaceutical Sciences University of Shizuoka, Center for Drug Discovery, Shizuoka, Japan*

Background: Signal transducer and activator of transcription (STAT) 3 plays a key role in many cellular processes, including proliferation, invasion, and survival. Aberrant STAT3 signaling has been demonstrated in various malignant tumors. Using biochemical and virtual screening, we previously identified a novel orally active STAT3 inhibitor, YHO-1701. The aim of the present study was to investigate its anti-proliferative activity in a wide variety of cancer cell lines alone and in combination with the multi-targeted tyrosine kinase inhibitor sorafenib.

Material and Methods: First, the anti-proliferative potential of YHO-1701 was investigated in 20 human and murine cancer cell lines derived from various tumor types. Based on the results, human oral squamous cell carcinoma cell line SAS (known for IL-6 signaling) was mainly used for further analysis. The phosphorylation and dimerization levels of STAT3 and survivin were determined by immunoblotting. Anti-proliferative activity was measured by WST-8 dye-based assay after 48 hours of treatment with YHO-1701 and/or sorafenib. STAT3 nuclear translocation and DNA-binding were examined by immunocytochemistry and ELISA, respectively. The antitumor efficacy of YHO-1701 in combination with sorafenib was explored in an SAS

subcutaneous xenograft model. The test compounds were administered orally once a day for 5 consecutive weekdays followed by a 2-day rest at the weekend for 4 weeks.

Results: YHO-1701 showed cytotoxic activity against various cancer cell lines, particularly SAS, where the IC50 was ≤0.5 μM. Dimerization of STAT3 was blocked and pSTAT3 levels reduced in this cell line. In addition, YHO-1701 reduced cellular survivin levels, a STAT3-regulated downstream target. Moreover, YHO-1701 treatment reduced STAT3 nuclear translocation and blocked STAT3 DNA-binding activity in a dose-dependent manner. In an in vitro cytotoxicity assay, YHO-1701 enhanced the anti-proliferative activity of sorafenib. In the SAS xenograft mouse model, the tumor growth inhibitory rate increased to 74.6% when YHO-1701 was administered with sorafenib, whereas that for YHO-1701 and sorafenib alone was 26.5% and 56.9%, respectively. The results further revealed a close correlation between this additive effect and change in survivin levels, both in vitro and in vivo. It is also noteworthy that combination therapy with YHO-1701 and sorafenib was well tolerated in tumor-bearing mice.

Conclusions: The present study suggests that combination therapy with YHO-1701 and sorafenib is promising and worthy of further investigation in clinical trials.

No conflict of interest

94 (PB-045) Poster

Distinct relationship of antitumor activity of lenvatinib (LEN) and sorafenib (SOR) to FGF21 expression levels in preclinical hepatocellular carcinoma (HCC) models

S. Watanabe Miyano[1], H. Taisuke[1], J. Ito[1], K. Kodama[1], H. Watanabe[1], K. Takase[1], J. Matsui[2], Y. Funahashi[1], K. Nomoto[2]. *[1]Eisai.Co., Ltd., Tsukuba Research Laboratories, Tsukuba, Ibaraki, Japan; [2]Eisai Inc., Oncology Business Group, Woodcliff Lake, NJ, USA*

Background: LEN is a multitargeted tyrosine kinase inhibitor that selectively inhibits VEGFR1–3, FGFR1–4, PDGFRα, RET and KIT. In a phase 3 clinical trial in unresectable HCC (REFLECT study), LEN showed statistical non-inferiority of overall survival compared to SOR and clinically meaningful outcome in objective response rate, progression-free survival, and time to progression. We previously reported that LEN inhibited FGF signaling pathway in preclinical HCC models in vitro and in vivo. Here, we investigated expression patterns of FGF ligands and their receptors in human HCC cell lines and patients, and the role of FGF21 in tumor growth of HCC xenografts in nude mice.

Methods: Expression levels of genes in the FGF signaling pathway were analyzed using TCGA (HCC patients) and CCLE (HCC cell lines) databases. Protein expression of FGF21 and KLB, co-receptor of FGFRs, was examined by immunohistochemistry (IHC) using human HCC tissue arrays and historical tumor tissues from human HCC patient-derived xenograft (PDX) models. Antitumor and antiangiogenic activities were evaluated against human FGF21 and mock transfected human HCC PLC/PRF/5 (FGF21-TF and Mock-TF) xenografts in nude mice at LEN (10 mg/kg), SOR (30 mg/kg) or anti-VEGFR2 Ab (800 μg/head).

Results: The TCGA database analysis showed that *FGF21*, *FGFR1-4* and *KLB* mRNA were expressed in almost all HCC patients (*FGF21*, 97%; *FGFR1-4*, 100/99/100/100%; *KLB*, 100%). IHC analysis in HCC tumor tissue array confirmed that most of tumors expressed FGF21 and KLB, consistent with TCGA database analysis. However, *FGF21* mRNA expression was below detection levels in all HCC cell lines based on the CCLE data base. FGF21-TF and Mock-TF xenografts showed virtually identical in vivo tumor growth rates. Each of treatments with LEN, SOR or VEGFR2 antibody showed antitumor activity in the Mock-TF xenograft model. However, only LEN showed antitumor activity against FGF21-TF xenografts. Overexpression of FGF21 did not affect microvessel density. LEN, SOR and VEGFR2 Ab showed significant anti-angiogenic activity in the presence or absence of FGF21. IHC analysis showed that the LI0334 human HCC PDX xenograft tumors express FGF21, in which we previously reported LEN but not SOR showed antitumor activity.

Conclusion: Majority of tumors in HCC patients expressed *FGF21*, *FGFR1-4* and *KLB* mRNA. LEN showed antitumor activity in preclinical HCC PLC/PRF/5 transfectant models despite of high FGF21 expression levels, while SOR and VEGFR2 Ab only showed antitumor activity against mock-TF xenografts without high FGF21 expression. These results suggest that the LEN showed the antitumor activity against FGF21-TF PLC/PRF/5 HCC xenografts probably through inhibition of FGF21-FGFR signaling pathways.

Conflict of interest: Corporate-sponsored Research: Research funding from Eisai Co., Ltd. Other Substantive Relationships: Employees of Eisai Co., Ltd.

95 (PB-046) Poster

Towards in vitro oncology trials: drug testing in breast patient-derived organoid cultures

L. Badder[1], B. Herpers[2], E. Knight[3], R. Marlow[1], S. Pettitt[3], D. Larcombe-Young[3], E. Francesch-Domenech[1], M. Lomzik-Borowik[1], A. Rushton[3], W. Vader[4], K. Yan[2], D. Novo[3], L. Price[2], C. Lord[3], A. Tutt[1]. *[1]King's College London, Breast Cancer Now Unit, Division of Cancer Studies, London, United Kingdom; [2]OcellO, OcellO BV, Leiden, Netherlands; [3]The Institute of Cancer Research, Breast Cancer Now Toby Robins Research Centre, London, United Kingdom; [4]VitroScan, n/a, Leiden, Netherlands*

Background: It is widely recognised that Patient-derived Xenografts (PDXs) are gold-standard *in vivo* models of breast cancer, capable of faithfully recapitulating tumour biology. High throughput drug screening methods currently rely on *in* vitro clonal two-dimensional (2D) cell lines that lack such pathophysiological relevance to parental tumours, limiting their ability to predict patient responses to therapies. There is a need for *in vitro* models to bridge the gap between such model systems to reduce and refine the number of animal experiments for pre-clinical drug testing, whilst retaining accurate readouts of tumour responses.

Three-dimensional (3D) organoid models have shown promise as disease-relvant models, capable of providing a comparable genetic and phenotypic profile to parental tumours. However, robust protocols for organoid derivation are somewhat limited for breast cancer subtypes, including Triple negative breast cancers (TNBCs), which lack targeted therapies in the clinic. Furthermore, it is yet to be ascertained whether organoid treatment responses correlate with current gold standard PDX models of breast cancer.

Methods: We have generated a cohort of patient-derived organoids from both our defined panel of TNBC PDX models, as well as directly from patient tissue. To systematically evaluate whether our models can recapitulate drug responses of companion *in vivo* PDX models, we have assessed and will present standard of care drug responses of matched organoid and *in vivo* pairs. This includes characterising BRCA-1 mutant models based on their sensitivities to Poly(ADP-ribose) polymerase (PARP) inhibitors.

Results: Thus far, we have found differential sensitivities to PARP inhibition within our BRCA-mutant- derived organoid models, reflective of comparative *in vivo* studies.

Conclusions: We aim to interrogate drug sensitivities of our models further using high throughput phenotypic screening, which will enable us to rapidly test hypothesis-driven drug/target combinations, to establish whether organoid readouts can predict responses in the clinic.

Conflict of interest: Ownership: Leo Price is a shareholder of OcellO BV and VitroScan BV. Board of Directors: Leo Price is a director of OcellO BV. Willemijn Vader is a director of VitroScan BV.

96 (PB-047) Poster

An oral and selective CDK7 inhibitor demonstrates substantial anti-tumor effect in breast and ovarian cancer models

S. Hu[1], J. Marineau[1], M. Bradley[1], K. Hamman[1], S. Alnemy[1], D. Smith[1], J. Carulli[1], C. Chuaqui[1]. *[1]Syros Pharmaceuticals, Inc., Drug Discovery, Cambridge, USA*

Background: CDK7 has emerged as an attractive cancer target due to its role in transcriptional control and cell cycle regulation, and demonstration of tumor cell killing in pre-clinical models with small molecule inhibitors. SY-1365 is an IV administered CDK7 inhibitor and currently in phase I clinical study (NCT03134638). Here we profile new oral and selective CDK7 inhibitors.

Material and Methods: CDK2,7,9, and 12 inhibition assays: Each enzyme was incubated with a fluorescently-labelled peptide substrate, 2 mM ATP, and an inhibitor, with product conversion measured by Perkin Elmer LabChip EZ Reader II.

SPR assay: CDK7/cyclin H dimer was immobilized on a CM5 chip and each compound was titrated over the immobilized protein and response units used to determine K_d, off- and on-rates.

CDK7 occupancy assay: Cells were treated with compounds for 1 hr, lysed, and incubated with biotinylated small molecule probe to pull down free CDK7, and total and unoccupied CDK7 quantitated.

Cellular assays: Cell lines were incubated with compounds for 72 hrs and cell number determined using CyQUANT™ Direct Cell Proliferation Assay kit. Cells were stained for annexin V and PI and analyzed by flow cytometry to

assess apoptosis after 48 hrs of treatment. Cells were fixed and stained with FxCycle violet stain and analyzed by flow cytometry to assess cell cycle following 48 hrs of treatment.

Mouse xenograft: balb/c mice were implanted subcutaneously with HCC70 cells or patient-derived breast cancer cells and randomized for treatment with test drug or vehicle when tumors reached 150–200 mm^3. Mice were dosed BID through oral administration for 3 weeks.

Results: A series of CDK7 inhibitors were designed and profiled in biochemical assays and tumor cell lines. Analysis of 467 compounds revealed a correlation between CDK7 K_d, CDK7 occupancy (EC_{50}) and cell growth inhibition (EC_{50}). A representative member of the class, compound A, exhibited selectivity over CDK12, CDK9, and CDK2 of 236-, 1174-, and 1202-fold, respectively. In addition, compound A inhibited proliferation of triple negative breast cancer (TNBC) and ovarian (OVA)cells, with EC_{50} in the low nanomolar range. Compound A induced apoptosis in a dose-dependent manner in multiple TNBC and OVA cell lines and also induced G2/M arrest. Strong tumor growth inhibition in breast cancer CDX and PDX models was observed when Compound A was dosed orally at 4 mg/kg BID.

Conclusions: We designed and profiled orally available, CDK7 selective inhibitors with potent activity against TNBC and OVA cells and induced tumor growth inhibition in breast cancer cell and patient derived xenograft models. These data support the rationale for advancing one or more members of this class toward clinical development.

Conflict of interest: Ownership: Employees and stock holders of Syros Pharmaceuticals.

97 (PB-048) Poster

Establishment of a methodological platform for the exploration of MCL1 inhibitors

J. Ehlert[1], D. Müller[1], S. Umber[1], C. Heidemann-Dinger[1], C. Ketterer[1], A. Chakrabarti[1], D. Feger[1], O. Siedentopf[1], M. Birkle[1], C. Schächtele[1], M.H. Kubbutat[1]. [1]*ProQinase GmbH, Drug Discovery, Freiburg, Germany*

Background: Many cancer cells succeed to survive by neutralizing apoptotic triggers. A frequently found evasive mechanism is to keep proapoptotic BCL2 family proteins (e.g. BAK; BIM) in check. To avoid apoptosis, cancer cells overexpress anti-apoptotic BCL2 family proteins (e.g. BCL2, BCLXL or MCL1). Latter proteins block pro-apoptotic effectors via binding to the so called BH3 binding domain and thus act as oncogenic survival factors. Therapeutic strategies aim at the disruption of this protein-protein interaction to reactivate apoptosis. Small molecular weight compounds targeting BCL2 and BCLXL such as ABT-263 (Navitoclax®) or ABT-199 (Venetoclax®) have been successfully promoted into clinics. However, there is still an urgent need for inhibitors to MCL1, which would be especially useful for MCL1 addicted cancers as well as for cancers that turn resistant to the previously mentioned drugs.

Material and Methods: To facilitate discovery and optimization of compounds targeting MCL-1, we established a methodological platform that allows the differentiated analysis of MCL1 inhibitors applying biochemical FRET analyses, combinatorial proliferation studies with relevant cell lines, Caspase 3/7 activation apoptosis assays and protein degradation assays.

Results: We report on the establishment of FRET-based biochemical assays measuring the interaction of MCL1 as well as of BCLXL with either BAK or BIM derived peptides. Cellular phenotypic assays were set up suitable to analyze the impact of potential inhibitors on proliferation and Caspase 3/7-related apoptosis in MCL1-high expressing NCI-H929 cells and on K562 control cells. In addition, a combination treatment assay was set up to analyze the synergistic effect of MCL1 inhibitors on cell lines poorly responsive to BCL2/BCLXL-inhibitor ABT-263 such as OPM2 cells. Finally, mechanistic assays were established focusing on the impact of MCL1-inhibition on the MCL1 stability in e.g. HCT116 cells.

Conclusions: Applying these assay systems, we here report for the first time on the side-by-side comparison of benchmark MCL1-inhibitors A1210477 and S63845 in identical biochemical and cellular assay systems. Our data support the suitability of our methodological platform for the development of MCL1 inhibitors. With synergistic studies of these compounds in combination with ABT-263 (Navitoclax®) on a large panel of ∼ 100 cell lines we furthermore provide novel data on the relevance of BCLXL/MCL1 co-inhibition in several entities.

Conflict of interest: Corporate-sponsored Research: Research was performed at company ProQinase GmbH and all authors are employees of ProQinase GmbH.

98 (PB-049) Poster

Involvement of Notch signaling pathway in a panel of human cancer cell lines

L. Astorgues-Xerri[1], M. Martinet[1], E. Raymond[2], S. Faivre[3], A. Tijeras-Raballand[1]. [1]*AFR Oncology, Preclinical and Translational Department, Paris, France;* [2]*Saint-Joseph Paris Hospital, Medical Oncology, Paris, France;* [3]*Beaujon University Hospital, Medical Oncology, Clichy, France*

Background: Notch pathway has been involved in cell differentiation, cell proliferation, apoptosis, angiogenesis and drug resistance, as well as epithelial-to-mesenchymal transition. Our team has demonstrated the involvement of the PlGF/VEGFR1/Notch4 axis in the angiogenesis of hepatocellular carcinoma. What about the role of Notch in other cancer types? The aim of this work is to characterize the basal activation and the role of Notch1 and Notch4 in a panel of human cancer cell lines.

Materials and Methods: We characterized a panel of 8 pancreatic (PDAC), 5 cholangiocarcinoma (CK), 5 colorectal (CRC), and 8 head and neck (H&N) human cancer cell lines for Notch1 intracellular domain (NICD1), Notch4 intracellular domain (NICD4), Hes1 (a target gene of Notch activation), E-cadherin, and Vimentin expressions by Western Blot. In each tumor type, 2 cell lines were selected (one NICD4 high and one NICD4 low) to assess the role of Notch4 basal activation on cell proliferation and migration, using MTT and wound-healing assays, respectively. We also assessed the expression of NUMB, a protein involved in NICD proteasomal degradation, in the selected cell lines. Notch inhibition, using PF-03084014, was studied on cell signaling, proliferation, and migration.

Results: Notch4 is highly activated in 6 out of 8 (6/8) PDAC cell lines, 7/8 H&N cell lines, 3/5 CK cell lines, and 2/5 CRC cell lines, whereas Notch1 is activated in all CK cell lines, and 3/8 H&N cell lines. In PDAC cells, Notch4 activation is correlated with high expression of Vimentin. In each tumor type, 2 cell lines were selected regarding NICD4 expression, to decipher why we observed differential Notch4 activation in our cell lines as well as to investigate the role of Notch4 basal activation in proliferation and migration. NUMB is overexpressed in all low NICD4 cell lines, suggesting a correlation between low NICD4 expression and degradation through the proteasome. Interestingly, Notch4 activation is associated with an increased proliferation rate in all tumor types and an increased basal migration of PDAC and CRC cells. Furthermore, all high NICD4 selected cell lines were more sensitive to PF-03084014 than the low NICD4 cells. In addition, we are exploring the effect of Notch inhibition and Notch stimulation (using a specific ligand) on cancer cell signaling and migration. These results will be displayed at the conference.

Conclusions: In this study, we demonstrated the involvement of Notch4 basal activation on cell proliferation and migration, as well as in the sensitivity to Notch inhibition. This study could help to discriminate tumor types or tumor characteristics that are good candidate for Notch inhibition in the clinics.

No conflict of interest

99 (PB-050) Poster

Evaluation of drug–drug interaction of itraconazole and ivosidenib (AG-120), an oral, potent, targeted, small molecule inhibitor of mutant IDH1, in healthy subjects

B. Fan[1], C. Prakash[1], H. Liu[2], G. Liu[1], C. Korth[3], H. Yang[1], D. Dai[1]. [1]*Agios Pharmaceuticals Inc., DMPK/Clinical Pharmacology, Cambridge, USA;* [2]*Agios Pharmaceuticals Inc., Biostatistics Biometrics & DM, Cambridge, USA;* [3]*Agios Pharmaceuticals Inc., Clinical Operations, Cambridge, USA*

Background: Mutant isocitrate dehydrogenase 1 (mIDH1) produces the oncometabolite D-2–hydroxyglutarate (2–HG), accumulation of which results in impaired myeloid differentiation. IDH1 mutations are found in myeloid malignancies, including acute myeloid leukemia (AML). Ivosidenib (IVO; AG–120) is an oral, potent, targeted, small molecule inhibitor of mIDH1 for the treatment of mIDH1–driven hematologic malignancies, including AML. Antimicrobial agents, including antifungal medications, are commonly prescribed in the AML population, and many are moderate to strong CYP3A4 inhibitors. As IVO is metabolized predominantly by CYP3A4, this study was conducted to evaluate the effect of itraconazole, a strong CYP3A4 inhibitor, on the pharmacokinetics (PK) of a single dose of IVO in healthy subjects, and to predict the effect of itraconazole on IVO exposure under steady-state conditions.

Materials and Methods: This was a two-period, single-sequence, crossover study (Clinicaltrials.gov NCT02831972). Twenty-two subjects received a single oral dose of IVO 250 mg on Day 1 of Period 1. In Period 2, subjects received itraconazole 200 mg once daily (QD) for 18 days and a single oral dose of IVO 250 mg on Day 5. PK sampling was carried out for 21 days in each period, and parameters were determined using

noncompartmental methods. The magnitude of drug–drug interaction (DDI) was assessed by analysis of variance of log-transformed AUC and C_{max}. A physiologically based pharmacokinetic (PBPK) model was validated to further predict the DDI effects of strong CYP3A4 inhibitors under steady-state conditions. Trial status: complete; trial sponsor: Agios.

Results: Coadministration of a single dose of IVO 250 mg with itraconozole resulted in higher AUC, longer $t_{1/2}$, and lower apparent oral clearance (CL/F) than for IVO alone (Table). Itraconazole increased IVO AUC by ~169% (geometric mean ratio [GMR]: 269%) but had no effect on C_{max}. PBPK modeling suggested a smaller effect of itraconazole on steady-state IVO AUC (GMR: 144%) than on single-dose IVO AUC because the CYP3A4 induction effect of IVO resulted in a reduced DDI. IVO administered alone or with itraconazole was generally well tolerated, with similar favorable safety profiles.

Table. PK parameters following administration of IVO ± itraconazole

PK parameter	250 mg IVO alone	250 mg IVO + 200 mg QD itraconazole
AUC_{0-t} (ng·hr/mL)[a]	107,500 (40.9)	282,000 (33.5)
$AUC_{0-\infty}$ (ng·hr/mL)[a]	115,000 (38.6)	302,300 (31.4)
C_{max} (ng/mL)[a]	2240 (25.1)	2270 (25.0)
$t_{1/2}$ (hr)[b]	60.7 (22.5)	140 (65.2)
CL/F (L/hr)[b]	2.31 (0.778)	0.863 (0.247)

[a]Geometric mean (%CV).
[b]Arithmetic mean (SD).

Conclusions: Coadministration of single dose IVO with a strong CYP3A4 inhibitor, itraconazole, resulted in increased AUC but not C_{max}. PBPK modeling predicted that the magnitude of DDI with strong CYP3A4 inhibitors at steady state would be a ≤100% increase in AUC.

Conflict of interest: Corporate-sponsored Research: All authors are employees of and stockholders in Agios Pharmaceuticals Inc.

100 (PB-051) Poster

Mutant p53 as a Therapeutic Target for the Treatment of Triple-Negative Breast Cancer

N. Synnott[1], J. Crown[2], M.J. Duffy[3]. *[1]University College Dublin, School of Medicine, Dublin, Ireland; [2]Department of Medical Oncology, St Vincent's University Hospital, Dublin, Ireland; [3]School of Medicine, University College Dublin, Ireland*

Background: P53 is the most frequently mutated gene in breast cancer, being mutated in over 80% of triple-negative (TN) cases. The aim of this study was therefore to investigate the potential value of the mutant p53 reactivating compound, APR-246 for the treatment of breast cancer with particular emphasis on the TN form.

Methods: Cell viability was determined using the MTT assay. p53 protein levels were determined using Western blotting, ELISA and immunofluorescence. Knockdown of p53 protein was carried out in 3 different breast cancer cell lines using 3 independent predesigned Flexitube sequences (Qiagen). Changes in the refolding of mutant p53 were investigated using the conformation-specific anti-p53 antibodies, PAb1620 and PAb240.

Results: Using a panel of 23 breast cancer cell lines, significantly lower IC_{50} values were found for APR-246 in p53 mutant compared to p53 wild-type cells ($p = 0.014$). Of potential clinical value, we found a significant inverse correlation between IC_{50} values for APR-246 and endogenous p53 protein levels ($p = 0.0001$, $r = -0.76$), ie, the higher the endogenous p53 protein, the greater the response. Knockdown of p53 expression reduced p53 protein levels by almost 90%. Reduction of p53 protein levels resulted in a significant decrease in the growth inhibitory effects of APR-246, in all 3 cell lines investigated, suggesting that p53 was the target for APR-246. To establish if APR-246 reversed the mutant unfolded state of p53, we treated the mutant p53 expressing cell line, SKBR3 with APR-246. This resulted in a dose-dependent increase in fluorescent staining with the WT associated p53 antibody, PAb1620. Simultaneously, there was a dose-dependent decrease in fluorescence using the mutant specific p53 antibody, PAb240. In contrast, treatment of the WT-p53 cell lines, MCF7 with APR-246 failed to alter staining intensity using the PAb1620 or PAb240. This finding suggests that APR-246 alters the conformation of mutant unfolded p53 protein, converting it to a WT-like conformation. To confirm the changes in fluorescent staining were due to p53 refolding, we quantified the absolute p53 protein levels under the same conditions. No change in the absolute p53 protein levels was seen.

Conclusion: Our results suggest that the anticancer activity of APR-246 is dependent on the presence of mutant p53 and that the compound acts by altering conformation of the mutant protein.

No conflict of interest

101 (PB-052) Poster

CPL-410-005, a novel ubiquitin-activating enzyme (UAE) inhibitor in preclinical evaluation as an anticancer treatment for solid tumors

D. Kozlowska[1], A. Gornicka[1], B. Stypik[2], M. Mroczkiweicz[2], A. Mikolajczyk[1], J. Hucz-Kalitowska[1], A. Szwalbe[2], K. Mulewski[2], D. Smuga[2], K. Dubiel[2], J. Pieczykolan[1], M. Wieczorek[3], A. Stanczak[1]. *[1]Celon Pharma SA, Preclinical Research Development, Lomianki-Kielpin, Poland; [2]Celon Pharma SA, Medicinal Chemistry, Lomianki-Kielpin, Poland; [3]Celon Pharma SA, Research and Development Center, Lomianki-Kielpin, Poland*

Background: UPS (ubiquitin-proteasome system) is essential in cancer development and progress since protein degradation is disturbed in comparison to non-malignant cells. This feature provides a new opportunity to target protein homeostasis and ubiquitin signaling for the treatment of cancer. Bortezomib is the first proteasome inhibitor approved by EMA and FDA for the blood cancer treatment. However, drugs beneficial for solid tumors are still missing. The strategy based on targeting of the E1 enzyme (UAE) triggering UPS cascade may demonstrate clinical benefits in solid tumors.

Materials and Methods: CPL-410-005 compound was designed and developed as an E1 small molecule inhibitor. Cellular polyubiquitylation and ubiquitin-like modifications (neddylation, sumoylation) level were analyzed using Western blot technique. The compound's biological activity and selectivity was studied on PC-3 and HCT-116 cell lines using cell viability test and Western blot technique. Programmed cell death and unfolded protein response (UPR) was monitored. Drug-like properties (lipophilicity, metabolic stability, genotoxicity) were also determined using *in vitro* approach. Finally, PK/PD *in vivo* study of CPL-410-005 compound was performed on SCID mice bearing HCT-116 cells xenografts.

Results: CPL-410-005 at 250 nM inhibits cellular polyubiquitylation with greater potency than reference compound MLN7243 (Takeda), while the impact on neddylation and sumoylation are minor using HCT-116 cell line. The proliferation of HCT-116 and PC-3 were potently inhibited (IC_{50} 20 and 50 nM, respectively). Unfolded protein response (Ire-1, PERK, BiP) and programmed cell death (PARP, caspase-3) were induced after CPL-410-005 treatment of 250 nM dose. CPL-410-005 exhibits higher metabolic stability on mice (4,7 µ/min*mg) and human (2,8 µ/min*mg) microsomes in comparison to MLN7243 (18,6 µ/min*mg and 3,8 µ/min*mg, respectively). The logD values are: pH 1.2 = 1.7; pH 4.5 = 3.06; pH 6.8 = 3.19; pH 7.4 = 3.40 which fall under the Lipinski rules. Moreover, no mutagenicity of CPL-410-005 was observed in Ames test. Finally, PK/PD analysis of CPL-410-005 on SCID mice bearing HCT-116 xenografts showed rapid elimination of CPL-410-005 from the plasma after single i.v. dosing of 5 mg/kg with long CPL-410-005 retention in tumor (up to 24 h).

Conclusions: We have designed a potent E1 inhibitor with promising *in vitro* and *in vivo* results. Preclinical findings may lead to the development of a novel anticancer therapy for solid tumors.

No conflict of interest

102 (PB-053) Poster

Aspartate-glutamate mitochondrial carrier's contribution to the energetic balance during carcinogenesis

S. Rabinovich[1], A. Silberman[1], A. Bahat[1], S. Levin-Zaidman[2], Z. Porat[3], D. Helbling[4], D. Dimmock[4], A. Erez[1]. *[1]Weizmann Institute of Science, Biological Regulation, Rehovot, Israel; [2]Weizmann Institute of Science, Chemical Research Support, Rehovot, Israel; [3]Weizmann Institute of Science, Sorting Unit, Rehovot, Israel; [4]Medical College Wisconsin, Molecular Genetics, Milwaukee, Wisconsin, USA*

Background: The urea cycle is a conserved mechanism to dispose ammonia and toxic nitric-rich compounds away from the body. Citrullinemia type II (CTLN II), is a urea cycle disorder caused by germline mutations in the *SLC25A13* gene encoding for the protein Citrin. Patients with CTLN II manifest failure to thrive clinically, and high ammonia levels biochemically. Interestingly, children with CTLN II have growth restriction and preferentially avoid carbohydrates in their diet, thus, suggesting a potential connection between Citrin, cell proliferation and glycolysis. While in adults Citrin is mainly expressed in the liver and intestine, we were surprised to found it to be highly expressed in multiple different types of tumors. Citrin is an inner-mitochondrial membrane protein which participates in the malate-aspartate shuttle as an antiporter for glutamate and aspartate, adjusting the NAD/NADH ratio between the cytosol and the mitochondria. We thus hypothesized that Citrin might play an important role in carcinogenesis by regulating the glycolysis/oxidative phosphorylation balance, which consume both NAD and NADH during these processes.

Materials and Methods: Labeled-isotope tracing by GC/MS, NAD/NADH detection by ImageStream, Transmission electron microscopy, immunofluorescent staining, Seahorse™ measurements of basal respiration and matrigel invasion assay.

Results: Using fibroblasts from patients with CTLN II we found that CTLN II patients' cells have decreased glycolysis, along with decreased ATP production. These findings could be attributed to the newly observed autophagic overload. This could also be a possible fascinating explanation for their growth restriction. In attempt to use a more reproducible system than primary fibroblasts, we knocked-down Citrin in cancer cells with *siSLC25A13* and found results similar to those discovered in CTLN II fibroblasts, regarding decreased glycolysis and elevated autophagy, along with decreased basal oxygen consumption rate (OCR), all of which might be explained by decreased NAD/NADH levels in these cells. We suspect that the damage to these energy-consuming processes results in decreased ability of the cells to invade matrigel, which can be salvaged by nicotinamide (NAM) supplementation. We also show that overexpression of Citrin presents a mirror effect to the results Importantly, overexpression of Citrin increases proliferation and invasion. Based on the detrimental conformational changes observed in Citrin protein in CTLNII patients, we next screened for small molecule compounds and found specific Citrin inhibitors which restrict the cancerous phenotypes of proliferation and invasion.

Conclusions: Citrin upregulated expression plays a beneficial role in cancer cells by increasing NAD/NADH availability and is hence a potential specific therapeutic target for cancer.

No conflict of interest

103 (PB-054) Poster

Co-Targeting ER and FGFR in patients with ER+/HER2− FGFR amplified (amp) Metastatic Breast Cancer (MBC)

K. Jhaveri[1], U. Syed[1], S. Wong[2], G. D'andrea[1], D. Lake[1], S. Goldfarb[1], D. Gajria[1], L. Ligresti[1], A. Rogado[1], J. Reis-Filho[3], S. Chandarlapaty[1]. [1]*Memorial Sloan Kettering Cancer Center, Medicine, New York, USA;* [2]*Memorial Slaon Kettering Cancer Center, Medicine, New York, USA;* [3]*Memorial Sloan Kettering Cancer Center, Pathology, New York, USA*

Background: FGFR1 amp (Amp of a region at chromosome 8p12) is present in ~17% of breast cancers (BC) and ~27% of luminal B type BC. Based on TCGA, FGFR2 amp are reported in 2–3% and FGFR3 and 4 amp in 1% of BC. Preclinical studies have demonstrated that FGFR1 amp conferred resistance to endocrine therapies (ET). We hypothesized that combination therapy using selective FGFR inhibitors (Debio 1347) along with standard ET (fulvestrant) may lead to better efficacy in ER+/FGFR-amplified MBC.

Material and Methods: This phase 1b/II study enrolled patients with ER+/HER2 – MBC with tumors with FGFR amp with or without co-occurring 11q amp determined by a CLIA certified laboratory. The phase 1b portion is a standard 3 + 3 design with a starting dose of 80 mg for Debio1347 and standard dosing for fulvestrant. A dose -1 (60 mg) was built in for safety. Primary Objective for Phase 1b: safety and MTD/RP2D. Phase 2 is a Simon 2-stage design with plans to enroll a maximum of 43 pts. Primary objective: assess clinical benefit rate (CBR: CR+PR+SD>24 weeks). Pre/perimenopausal (with concurrent GNRH agonists) and postmenopausal women with measurable or evaluable disease per RECIST 1.1 were eligible. For the phase 2 portion, no prior fulvestrant and no more than 1 line of chemotherapy in the metastatic setting was allowed. All pts underwent a pretreatment biopsy with plans for IHC, mRNA expression and FISH. Peripheral blood was also collected serially for ctDNA and PK analyses.

Results: The phase 1b results are presented. 7 pts (5 with known FGFR1 amp, 1 FGFR2 and 1 FGFR3 amp) were enrolled. 1 patient was replaced due to rapid clinical progression after dosing on C1D1. Median age: 55 years (range 41–69), median ECOG: 0, median lines of prior therapy in the metastatic setting: 4 (range 1–6) including 86% with prior CDK 4/6 inhibitors and 43% with prior fulvestrant. There were no DLTs. The most common AE was hyperphosphatemia – 71% Grade (G)1, 43% G2 and 43% G3 despite prophylaxis with phosphate binding agents starting C1D1. Other common AEs were predominantly G1 and included increased AST/ALT (72%/57%), increased CPK (57%), nausea (43%), alopecia (43%), nail changes (43%), and vomiting (28%). G2 bilirubin increase was seen in 43% and G2 fatigue in 14%. 1 patient (CDK4/6 inhibitor and fulvestrant naïve) with FGFR1 amp (fold change 11.5 by MSK-IMPACT) and extensive liver metastases had RECIST SD and is currently ongoing. PK analyses will confirm drug exposures given the concurrent prophylaxis with phosphate binding agents. PK data and correlative analyses will be presented at the meeting.

Conclusions: The combination of Debio1347 and fulvestrant is safe and the RP2D is 80 mg. Phase 2 will evaluate efficacy in fulvestrant naïve and a less heavily pretreated population.

No conflict of interest

104 (PB-055) Poster

Overcoming resistance to AKT inhibition in Oesophageal Adenocarcinoma

L. Cairns[1], L. Stevenson[1], R. Douglas[1], N. Mccabe[1], E. Sutton[1], R. Kennedy[1], T. Harrison[1], R. Turkington[1]. [1]*Queen's University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom*

Background: The incidence of Oesophageal adenocarcinoma (OAC) in the UK has risen by 6-fold in the last 40 years and is now the highest in Europe. The phosphatidylinositol 3-kinase (PI3K)/Protein kinase B (AKT) signalling pathway is implicated in resistance to chemotherapy in OAC and has been investigated as a potential target to enhance therapy with limited success. We aimed to identify resistance mechanisms to AKT inhibition and develop novel combination therapeutic strategies.

Material and Methods: Parental OAC cell lines were exposed to repeated, increasing concentrations of a novel AKT inhibitor (AKTi) over a period of six months to establish AKTi resistant cell lines (AKTi-R). Sensitivity was assessed by MTT and clonogenic cell survival assays and transcriptional profiling was performed using the ALMAC Diagnostics Xcel Array™. Migration and invasion were measured using the xCELLigence platform and receptor tyrosine kinase (RTK) activation was assessed using a phospho-RTK array. Induction of cell death was evaluated using Annexin V/PI flow cytometry coupled with western blot analysis for PARP cleavage.

Results: AKTi-resistant (AKTi-R) cell lines were confirmed to be 6-fold more resistant compared to their parental counterpart. AKTi-R cell lines displayed a mesenchymal morphology together with an increased migratory and invasive capacity (P = <0.0001) and elevated expression of mesenchymal markers. Functional analysis of gene expression data indicated upregulation of pathways governing epithelial-mesenchymal transitions (EMT). The RTK AXL showed increased activation in the AKTi-R cell lines and, this was validated by western blot and RT-PCR. AXL knockdown reversed the mesenchymal phenotype of the AKTi-resistant cell lines but did not restore sensitivity to AKT inhibition. Further investigation identified the RTK MET as a potential mediator of resistance. MET knockdown restored sensitivity to AKT inhibition and targeting MET in combination with AKTi demonstrated enhanced cell death (P = <0.0001).

Conclusions: Inhibition of AKT in OAC cell lines increases migratory and invasive capacity together with an upregulation of mesenchymal markers and AXL activation. We identified the role of AXL in driving this EMT phenotype and we established that targeting MET restored sensitivity to AKT inhibition and caused enhanced cell death in combination with AKTi. Our work elucidates novel molecular mechanisms of resistance to AKT inhibition and indicates potential, innovative therapeutic combinations in the treatment of OAC.

No conflict of interest

106 (PB-057) Poster

Cyclin dependent kinase inhibition: a novel treatment strategy for glioblastoma

B. Murphy[1], J. Noonan[1], M. Jarzabek[1], F. Lincoln[1], B. Kavanagh[1], P. Arhona[1], L. Young[2], K. Ligon[3], H. Jahns[4], A. Ashkenazi[5], D. Zheleva[6], J. Prehn[1], M. Rehm[7], A. Byrne[1]. [1]*Royal College of Surgeons in Ireland, Physiology, Dublin, Ireland;* [2]*Royal College of Surgeons in Ireland, Surgery, Dublin, Ireland;* [3]*Harvard Medical School, Pathology, Boston, USA;* [4]*University College Dublin, Pathobiology, Dublin, Ireland;* [5]*Genentech, Cancer Immunology, San Francisco, USA;* [6]*Cyclacel, Research and Development, Dundee, United Kingdom;* [7]*University of Stuttgart, Cell Biology and Immunology, Stuttgart, Germany*

Background: Glioblastoma (GBM) is the most common primary brain tumour and no cure presently exists. The average survival rate for patients is only 15 months. The reasons for treatment failure are multi-facetted including, the tumour's extreme resistance to apoptotic death which the treatment strategies of radiation and chemotherapy attempt to induce and the challenge of successfully delivering drugs across the blood brain barrier. Our group has previously described seliciclib, as a first generation cyclin-dependent kinase (CDK) inhibitor, that down-regulates the anti-apoptotic protein, Mcl-1, in GBM via its selective targeting by CDK9. Additionally, we have demonstrated that combining seliciclib the with death receptor ligand, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), re-establishes apoptotic sensitivity in GBM cells grown as monolayers.

Materials and Methods: We have now assessed the effectiveness of first- (seliciclib) and second- (CYC065) generation CDK inhibitors, both alone and in combination with TRAIL receptor ligation, in clinically relevant preclinical models of GBM – patient-derived neurospheres and orthotopic patient-derived xenograft (PDX) model.

Results: Significant levels of apoptosis were observed in patient-derived neurospheres upon treatment with seliciclib and TRAIL, highlighting that 3-D-cultures of patient-derived neurospheres could also be sensitised to the apoptotic-inducing effects of TRAIL upon co-administration of seliciclib. Following the successful establishment of our murine orthotopic PDX model, the co-treatment strategy displayed similar efficacy and critically, substantially extended the survival times of the animals. Furthermore, we also provide evidence that the second-generation CDK inhibitor, CYC065, even when administered alone can induce a significant decrease in the size of patient derived neurospheres and critically is well-tolerated in vivo.

Conclusion: These robust preclinical findings highlight that cyclin-dependent kinase inhibition either alone or in conjunction with death receptor ligand ligation represents an exciting novel approach for anti–GBM cancer therapy.

No conflict of interest

107 (PB-058) Poster

The novel oral Cdc7 inhibitor, SRA141, demonstrates robust efficacy in preclinical cancer models

R. Hansen[1], S. Milutinovic[1], B. Strouse[2], M. Hedrick[1], G. Smith[2], C. Hassig[1]. [1]*Sierra Oncology, Inc., Research, Vancouver, Canada;* [2]*Sierra Oncology, Inc., Preclinical, Vancouver, Canada*

Cell division cycle 7 (Cdc7), a serine-threonine kinase, is a novel therapeutic target with emerging clinical validation in oncology. Cdc7 inhibitors demonstrate evidence of anti-tumor activity in both hematological and solid cancers in preclinical models. Cdc7 plays important roles in regulating DNA replication origin firing during S phase, while also having functions within the DNA Damage Response (DDR) and mitosis. SRA141 is a novel, highly potent, selective and orally-bioavailable inhibitor of Cdc7, with an IC_{50} of 1.4–4.0 nM and slow dissociation kinetics (K_{off} = 0.003 at 5 nM). In cells, SRA141 potently inhibits the phosphorylation of the Cdc7 substrate MCM2 ($EC_{50} \sim 33$ nM), an essential component of the replicative helicase complex involved in DNA replication, while having minimal effects against potential off-target kinases. Coincident with the inhibition of phospho-MCM2, SRA141 inhibits the proliferation of a broad spectrum of tumor cell lines derived from both solid and hematologic cancers. Using four orthogonal assays that measured either DNA content, metabolic activity or enzymatic activity in a subset of solid tumor lines, dose-response studies for SRA141 revealed strong anti-proliferative activity, with IC_{50}s ranging from 0.1–1.0 μM for sensitive cell lines such as Colo-205, SW620, SNU-398, and NCI-H716. Comparison of 24, 48 and 72 h treatment showed improved potency with longer SRA141 exposure. In contrast to the cytotoxic effects of SRA141 (0.1–3.3 μM) on Colo-205 colorectal cancer cells, flow-cytometry studies revealed that human fibroblasts were largely unaffected by drug treatment (apoptotic sub-G1 population >50% in Colo-205 vs <5% fibroblasts), consistent with the anticipated differential cytotoxicity of Cdc7 inhibition on tumor cells compared to non-transformed cells. SRA141 displayed dose-proportional exposure leading to durable partial responses (PR) and numerous complete responses (CR) in a Colo-205 colorectal tumor xenograft, resulting in significant average tumor growth inhibition (TGI = 93%) that correlated with a reduction in tumor pMCM2 levels determined by western blot and immunohistochemistry. SRA141's potent and selective in vitro profile, favorable in vivo pharmacokinetic properties and robust anti-tumor activity supports advancement of the compound into clinical development.

Conflict of interest: Other Substantive Relationships: All authors are employees of Sierra Oncology, Inc.

108 (PB-059) Poster

Modulation of OATP1B1 function by LYN-kinase inhibitors

S. Hu[1], M. Chen[1], A. Gibson[1], A. Sparreboom[1], J. Sprowl[2]. [1]*The Ohio State University, Pharmaceutics and Pharmaceutical Chemistry, Columbus, USA;* [2]*D'Youville College, Pharmaceutical, Social and Administrative Sciences, Buffalo, USA*

Background: Functional modulation of various cellular proteins is regulated by tyrosine phosphorylation, which is a key regulatory mechanism of intra- and intercellular signaling in metazoans. We previously reported the existence of a conserved regulatory mechanism in the drug transporter OCT2, and mass-spectrometric data from global phospho-proteome studies suggest that a substantial fraction of clinically-relevant transporters expressed in humans have conserved tyrosine-phosphorylated sites. We hypothesized that OATP1B1, a hepatic uptake transporter of various cancer drugs, including paclitaxel, may also be regulated by tyrosine-phosphorylation and is vulnerable to modulation by tyrosine kinase inhibitors (TKIs).

Materials and Methods: Putative phospho-tyrosine sites in OATP1B1 were identified utilizing computational prediction tools and site-specific tyrosine-to-phenylalanine (Y-F) mutants of OATP1B1 were constructed by site-directed mutagenesis. Functional studies were performed *in vitro* in HEK293 cells engineered to express OATP1B1, mouse mOatp1b2, or zebrafish zfOatp1d1, using 8-fluorescein-cAMP or estradiol-17β-D-glucuronide as prototypical test substrates, as well as *in vivo* in wild-type and mOatp1b2-deficient [Oatp1b2(-/-)] mice. RNAi kinase-library screens for all known kinase genes (Dharmacon) were performed using reverse transfection of transporter-overexpressing 293T cells.

Results: Mutation at one particular site (Y234F) in OATP1B1, conserved in human OATP1B3, mOatp1b2, and zfOatp1d1, was associated with >90% reduced OATP1B1 function. Based on this observation, we hypothesized that certain TKIs could modulate the activity of OATP1B1. To test this hypothesis, we performed a screen with 32 FDA-approved TKIs and confirmed that several of these agents inhibited OATP1B1 function by at least 75%. Nilotinib was found to influence OATP1B1 function most potently at concentrations that can be achieved clinically (IC_{50}, ~1 μM), and was also able to inhibit OATP1B3, mOatp1b2, and zfOatp1d1. Consistent with a non-competitive inhibitory mechanism, we found that nilotinib is itself a poor substrate of OATP1B1 and OATP1B3, and that the interaction with OATP1B1 is reversible and time-dependent. Nilotinib influenced phosphorylation of known kinase targets in the mouse liver, including ABL, and potently inhibit mOatp1b2 *in vivo*. In line with LYN, identified as a lead hit from the RNAi screen, as the putative kinase responsible for OATP1B1 phosphorylation, we found that the LYN-selective TKI bafetinib (INNO-406) potently inhibited OATP1B1 ($P < 0.05$).

Conclusions: The present studies have uncovered a previously unrecognized role of kinases in the post-translational regulation of OATP1B1. The widespread use of TKIs affecting LYN function in diverse therapeutic areas make OATP1B1 substrates highly vulnerable to phosphorylation-mediated drug-drug interactions.

No conflict of interest

109 (PB-060) Poster

Targeting the inhibitor of apoptosis proteins (IAPs) sensitises oesophageal adenocarcinoma to Akt inhibition

L. Stevenson[1], L. Cairns[1], R. Douglas[1], N. Mccabe[2], G. Gavory[3], T. Harrison[3], R.D. Kennedy[2], R.C. Turkington[1]. [1]*Queen's University Belfast, Centre for Cancer Research and Cell Biology, Belfast, N. Ireland, United Kingdom;* [2]*Almac Diagnostics, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom;* [3]*Almac Discovery, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom*

Background: Oesophageal adenocarcinoma (OAC) is the predominant histological type of oesophageal cancer in North America and Europe and incidence rates have progressively increased 6-fold since 1975. Whole genome sequencing has identified PI3K/AKT as the most frequently mutated oncogenic pathway in OAC with amplifications in upstream receptor tyrosine kinases (RTKs) present in the majority of OAC tumours. Akt is therefore an attractive therapeutic target in OAC. Inhibitor of apoptosis proteins (IAP) are overexpressed in a variety of human cancers and small molecule IAP antagonists that mimic the second mitochondria-derived activator of caspase (Smac) are promising cancer therapeutics. This preclinical study uses a novel allosteric Akt1/2 inhibitor, ALM301, to target Akt in OAC cell lines in combination with chemotherapy or IAP antagonist, BV6. We hypothesised that targeting IAPs could enhance the efficacy of Akt inhibition in OAC.

Materials and Methods: The effect of ALM301 in combination with 5-fluoruracil (5FU), cisplatin (CDDP) or BV6 was examined in a comprehensive panel of 9 OAC cell lines. OE33 and FLO-1 cell lines were continuously treated with increasing concentrations of ALM301 or CDDP over ~3 months to generate ALM301- and CDDP-resistant sub-lines respectively. Cell viability was measured using MTT, Cell Titre GLo® and clonogenic assays. Cell death was determined by propidium iodide/Annexin V flow cytometry and detection of cleaved PARP by Western blot. Cell lines were transcriptionally profiled using the Xcel™ array (Almac Diagnostics) and expression of IAPs was examined by Western blot.

Results: Akt inhibition synergistically sensitised the OAC cell lines to chemotherapy (combination index < 1.0) but this sensitisation was context-dependent. Examination of IAPs following chemotherapy treatment revealed down-regulation of cIAP1/2 and Xiap in the settings which synergised with ALM301 and high intrinsic Xiap expression correlated with resistance to ALM301. In addition, IAP gene and protein expression was up-regulated in the ALM301-resistant cell lines. BV6 was found to deplete cIAP1/2 and Xiap in the OE33, OE19 and FLO1 cell lines and sensitised to ALM301. Further examination in the broader OAC cell line panel showed BV6 $\sim IC_{30(72h)}$ doses sensitised to ALM301. BV6 also sensitised to ALM301 in the ALM301- and CDDP-resistant sublines.

Conclusions: Sensitivity to Akt inhibition is correlated with IAP expression in sensitive and resistant *in vitr*o models. Our preclinical findings support a potential clinical role for Akt inhibition in combination with the IAP antagonist BV6 in the treatment of OAC.

Conflict of interest: Other Substantive Relationships: Nuala McCabe, Richard Kennedy, Gerald Gavory and Timothy Harrison are employees of the ALMAC group.

110 (PB-061) Poster

Development of limited proteolysis, a novel drug target deconvolution strategy

N. Beaton[1], R. Bruderer[1], K. Beeler[2], I. Piazza[3], L. Reiter[1]. [1]*Biognosys AG, Research and Development, Schlieren, Switzerland;* [2]*Biognosys AG, Scientific Business Development, Schlieren, Switzerland;* [3]*ETH Zurich, Institut für Biochemie, Zurich, Switzerland*

Background: High attrition rates in target-centric drug development approaches, as well as a limited number of targets, have shifted the focus of drug development back towards phenotypic screening. In parallel, novel proteomics-based target deconvolution approaches to drug target identification have gained popularity. Limited proteolysis (LiP) is a new target deconvolution technique that exploits protein conformational changes driven by drug binding. A major advantage of LiP is its unique focus on novel "conformotypic" peptides that are generated by a limited, unspecific digestion and subsequently identified by proteomic analysis. Here we demonstrate the reproducibility and performance of LiP using the mTOR inhibitor, rapamycin, as well as the protein phosphatase inhibitor Calyculin A, in human cell lysate.

Materials and Methods: Mechanically sheared HeLa cell lysate was incubated with compound at multiple concentrations. Next, a limited digest was performed using the unspecific protease, proteinase K. Finally, the limited digests were processed to peptides with trypsin for mass spectrometry analysis. A project-specific spectral library was generated using data-dependent acquisition (DDA) mass spectrometry and for quantitative analysis data-independent acquisition (DIA) data was recorded and analyzed using Spectronaut Pulsar X and the project-specific spectral library.

Results: Herein, we demonstrate in HeLa lysate that our modified LiP approach reproducibly identified several conformotypic FKBP1A peptides, the target of the potent immunosuppressant rapamycin, with high confidence (p-value <0.01), a finding not yet shown in a mammalian context. Notably, these same peptides displayed a strong dose-response correlation with increasing rapamycin concentrations (IC50 = 144 nM). A similar dose-response relationship was observed, and several conformotypic peptides could be shown, for the protein targets of the phosphatase inhibitor Calyculin A, with IC50 values of 57 and 23 nM for PP1A and PP2A respectively. Importantly, known concentration differences in target affinity were confirmed via extrapolation of IC50 values. Additionally, a previously unknown target of Calyculin A (PP1B) was identified among the same phosphatase family with a higher IC50 than previously known targets at approximately 74 nM.

Conclusions: Collectively, our data demonstrates that LiP can be used to effectively identify protein targets, both known and novel, and characterize their binding properties (e.g. IC50). These capabilities make LiP a powerful target deconvolution strategy with the potential to become an essential part of the drug development pipeline.

Conflict of interest: Ownership: Nigel Beaton, Roland Bruderer, Kristina Beeler, and Lukas Reiter own stock options in Biognosys.

112 (PB-063) Poster

Relationship between c-kit mRNA expression and prognosis in postoperative patients with rectal carcinoma

Y. Lin[2], Y. Huang[3], M. Ying[2]. [1]*Fujian Medical University Cancer Hospital, Department of Abdominal Surgery, Fuzhou, China;* [2]*Fujian Medical University Cancer Hospital, Department of Radiation Oncology, Fuzhou, China*

Background: There was no consensus on the the relationship between c-kit and the prognosis of rectal carcinoma patients. Our study aimed to investigate the correlation between c-kit mRNA expression and prognosis in patients with rectal carcinoma.

Methods: The expression of c-kit mRNA in rectal carcinoma tissues (n = 66) was detected by multiplex branched-DNA liquid chip method. The patients were classified into the high expression group and the low one, according to the outcome of its expression. Chi-square test was utilized to analyze the relationship between expression and clinic pathological features. Kaplan-Meier estimates and multivariate analysis (Cox) were employed to assess the correlation between the expression of c-kit and prognosis.

Results: The high expression rate of c-kit was 27%(18/66) in tumor tissues. No significant correlation was found between the c-kit expression and gender, age, preoperative carcinoembryonic antigen, preoperative hemoglobin, distance to verge, lymph node metastasis, tumor thrombus, T stage, TNM stage, tumor differentiation ($P > 0.05$). Kaplan-Meier indicated that 1-,3-,5-year survival rate of the high c-kit expression group were respectively 100.0%, 77.8%, 77.8%, and those of the low c-kit expression group were respectively 93.8%, 56.3%, 45.8%. The difference were statistically significant($c^2 = 5.056$, $P = 0.025$). Multivariate analysis (Cox) showed that the expression of c-kit[Hazard Ratio (*HR*) = 0.354, *95% CI*:0.133–0.943, $P = 0.038$] and TMN stage[Hazard Ratio(*HR*) = 6.781, *95% CI*:1.100–41.799, $P = 0.039$] were independent prognostic factor of post-operative rectal cancer patients.

Conclusions: Low expression of c-kit was associated with poor prognosis of rectal carcinoma. It is worth further studying whether it could become a potential target or not.

No conflict of interest

113 (PB-064) Poster

Cell-penetrating Alphabodies targeting the Wnt/β-catenin pathway

S. Loverix[1], S. Pandolfi[2], S. Deroo[3], J. Desmet[1], T. Girardi[2], L. Wurth[3], K. Vandenbroucke[1], I. Lasters[1], Y. Mcgrath[1]. [1]*Complix NV, Research Facilities, Zwijnaarde, Belgium;* [2]*Complix NV, Headquarters, Diepenbeek, Belgium;* [3]*Complix NV, Complix Luxembourg, Esch-sur-Alzette, Luxembourg*

Background: BCL9 is a constitutive member of the WNT pathway, where it serves as a docking port for nuclear β-catenin. Aberrant WNT activation is frequently observed in multiple cancers, and leads to increased proliferation, stemness and metastatic potential of tumor cells. WNT pathway inhibition at the level of the enhanceosome by blockade of BCL9 – β-catenin interaction represents an attractive therapeutic approach to tackle WNT-dependent cancers. Complix has developed Cell Penetrating Alphabodies (CPAB's), a novel and unique class of therapeutic proteins engineered to efficiently enter cells and inhibit intracellular protein-protein interactions, and applied its CPAB platform to identify and functionally characterize binders of BCL9.

Material and Methods: Alphabodies targeting the HD2 domain of BCL9 and its paralog BCL9L were identified via selection of an Alphabody library displayed on phage. Ranking of the best candidates was performed via dual reporter luciferase assays. The effects of both intracellularly expressed Alphabodies as well as extracellularly administered CPAB's on transcriptional activation, cell growth, stemness and migration was assessed by qRT-PCR, cell proliferation and clonogenic assays, cancer stem cell (CSC) self-renewal and wound healing assays.

Results: A biopanning campaign on BCL9 and BCL9L HD2 peptides identified Alphabodies binding with low-nM Kd to each of the two peptides (phage-coated ELISA). Intracellular expression of Alphabodies as well as CPAB treatment inhibited WNT pathway activity in a luciferase reporter assay in HEK293T, HeLa (Wnt3a-ligand responsive), DLD-1 (APC mutant) and HCT116 (β-catenin mutant) cells. Design-based modifications further enhanced the ability of the CPAB to inhibit WNT transcriptional activity, as measured in the luciferase reporter assay in HeLa, MDA-MB-231 cells and Hep3B cells, as well as the transcription of the WNT target *AXIN2* in HeLa cells. The anti-BCL9/9L CPAB inhibited the colony forming ability of DLD-1 cells at 1 mM as well as their ability to form secondary spheres in a dose-dependent manner. Wound healing assays also showed reduction of migration of DLD-1 cells upon anti-BCL9/9L CPAB treatment. Structural modeling of the best performing Alphabody in complex with BCL9 allowed the design of an affinity maturation library for a subsequent biopanning campaign. The characterization of new anti-BCL9/9L AB binders with pM affinity (phage-coated ELISA) is in progress.

Conclusions: Complix has developed an anti-BCL9/9L CPAB able to specifically inhibit the BCL9 – β-catenin interaction in the WNT enhanceosome, with low-nM Kd. Treatment of cancer cells with anti-BCL9/9L CPAB leads to inhibition of WNT transcriptional activity and gene expression, and to suppression of CSC self-renewal, proliferation and migration in WNT-pathway activated cancer cells.

No conflict of interest

114 (PB-065) Poster

Anti-tumor activity of tarloxotinib, a hypoxia-activated EGFR/HER2 TKI, in HER2 driven cell lines

A. Estrada-Bernal[1], A.T. Le[1], A.E. Doak[1], R.C. Doebele[1]. [1]*School of Medicine, University of Colorado Anschutz Medical Campus, Department of Medicine, Div of Medical Oncology, Aurora, USA*

Background: Lung adenocarcinoma is characterized by distinct subsets that can be classified by oncogene status. *ERBB2* (HER2) gene amplification is present in ~3% and *ERBB2*activating mutations in ~3% of the patients. Although HER2-directed therapies are available for breast and gastric cancer, the use of HER2-directed monoclonal antibodies and tyrosine kinase inhibitors (TKIs) have been disappointing in lung cancer. Tarloxotinib (TRLX) is a prodrug that releases TRLX-TKI, an irreversible EGFR/HER2 TKI, under hypoxic conditions. We have previously shown that tarloxotinib can overcome the intrinsic resistance of *EGFR*exon 20 insertion mutations to existing TKIs in vitro and in vivo.

Methods: Using MTS, we evaluated the proliferation of three HER2-driven cell lines (Calu-3 and H2071 with *ERBB2*amplification; H1781 with *ERBB2*p. G776>VC insertion mutation) treated with TRLX-TKI or TRLX or 1st, 2nd or 3rd generation EGFR/HER2 TKIs. We analyzed the on-target and signaling effects elicited by TRLX-TKI via immunoblots of pHER2, pHER3, pERK, and pAKT. Using a nude mice xenograft model, we compared the effect of the TRLX prodrug with other TKIs on tumor growth.

Results: Our results demonstrate that HER2-driven lung cancer cell lines show the highest sensitivity to TRLX-TKI whereas the TRLX prodrug is >50-fold less potent under normoxic conditions, consistent with the required mechanism of hypoxia for activation. Afatinib, which has previously been shown to have poor activity in HER2 mutation positive NSCLC, was the next most potent drug in vitro, followed by osimertinib and gefitinib. In immunoblot analyses, TRLX-TKI inhibits HER2 phosphorylation between 10 and 100 nM whereas afatinib inhibits pHER2 at 100 nM, consistent with the cell proliferation data. Notably, we also observed a concurrent reduction in pHER3 phosphorylation with TKI treatment. Analysis of downstream signaling pathways demonstrated that pERK was not inhibited by any of the TKIs, whereas AKT signaling was inhibited at similar doses to that of upstream HER2. Xenograft data will be presented at the meeting.

Conclusions: TRLX-TKI is a potent HER2 inhibitor in vitro that can inhibit HER2 (and HER3) phosphorylation at low nanomolar doses and with greater potency than currently approved TKIs for NSCLC. This activity was observed in cell lines harboring both amplified or mutant ERBB2. HER2-driven cells depend on the AKT pathway for survival whereas MAPK inhibition was not necessary to inhibit cell proliferation. Tarloxotinib represents a potential new therapeutic approach for NSCLC patients harboring *ERBB2*gene alterations.

Conflict of interest: Ownership: RCD: Rain Therapeutics. Advisory Board: RCD: Ignyta, AstraZeneca, Bayer, Takeda. Other Substantive Relationships: RCD: Licensed Biological Materials, Ignyta Licensed patents: Abbot Molecular, Rain Therapeutics.

115 (PB-066) Poster

In-vitro characterization of the mechanism of action of abemaciclib in human bone marrow progenitors

M.P. Ganado[1], R. Torres-Guzman[1], G.E. Perez[1], C. Baquero[1], S. Barriga[1], B. San Antonio[1], J. Du[1], L. Prieto[1], A. De Dios[1], M.J. Lallena[1]. [1]*Lilly, S.A., Quantitative Biology, Madrid, Spain*

Background: The U.S. Food and Drug Administration (FDA) recently approved palbociclib, ribociclib and abemaciclib, orally active and highly selective reversible CDK4 and CDK6 inhibitors, for HR+ metastatic breast cancer in combination with specific endocrine therapies.

Abemaciclib is structurally different from palbociclib and ribociclib, demonstrating greater selectivity for CDK4 compared with CDK6. Hematologic side effects such as neutropenia seem to be less common with abemaciclib, while fatigue and gastrointestinal-related toxicity are the more frequent adverse effects. The mechanism of action of abemaciclib has been thoroughly studied in ER+ breast cancer models where promotes tumor cell cycle arrest upon inhibition of Rb phosphorylation. Furthermore, altered metabolism and an increase in senescence and apoptosis were observed after prolonged and continuous exposure to the drug.

The majority of human breast cancers over-expresses cyclinD1 where CDK4-associated kinase activity is required to maintain breast tumorigenesis. The role of CDK6 in breast cancer tumorigenesis is not so well stablished although its role in neutrophils innate response has been described. Additionally, cyclinD3, natural partner of CDK6 in neutrophil precursors is essential for its maturation in bone marrow as described in KO mouse models, which presented acute neutropenia. The role of the associated kinase activity is still undescribed.

Materials and Methods: We used flow cytometry to study the effect of CDK4/6 inhibitors on the maturation of CD34+ human progenitors and mature neutrophils in-vitro. Washout studies with GCSF were carried out as potential adjuvant for neutrophil counting recovery.

Results: 1. Different CDK4/6 inhibitors share a common mechanism of action to induce neutropenia, which is distinct from that of chemotherapy agents; it affects directly the maturation of human precursors and not mature cells being rapidly reversible. This reflects a cytostatic effect on neutrophil precursors from bone marrow supporting reversibility in-vivo. **2.** Addition of GCSF is not essential for the full recovery of the CD34+ maturation after treatment with CDK4/6 inhibitors as it was required after chemotherapy agent. **3.** There is a robust correlation between the degree of inhibition of CDK6 kinase activity and prevention of CD34+ maturation in-vitro.

Conclusion: Results described herein contribute to understand abemaciclib safety profile and confirm a clear difference from chemotherapy-induced neutropenia providing clarification on the role of CDK6 inhibition on maturation of myeloid line.

No conflict of interest

117 (PB-068) Poster

Molecular dissection of CDK4/CyclinD1 regulation: Prevention of pathway hyperactivation by continuous CDK4/6 inhibition

S. Gharbi[1], J. Gutierrez[2], A. Espada[2], S.M. Gutierrez[1], R. Torres-Guzmán[1], M.F. Debets[2], L. Prieto[1], J. Du[2], A. De Dios[2], M.J. Lallena[1]. [1]*Eli Lilly and Company, Lilly Research Laboratories, Alcobendas, Spain;* [2]*Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, USA*

Background: CDK4/6 are essential regulators of cell proliferation, permitting the stepwise progression through the cell cycle. Alteration in CDK4/6 pathway occurs frequently in numerous types of cancer and drugs targeting CDK4/6 activity are showing clinical activity. *In vitro* reports suggest however, that CDK4/6 inhibitors (CDK4/6i) can stabilize an active complex and this could induce pathway hyperactivation upon compound removal. In some clinical trials with CDK4/6 inhibitors, a rebound in proliferation activity has been observed in patients during the treatment rest interval.

Goals: Our goal was to explore the mechanism of action of abemaciclib, recently approved by the FDA for HR-positive advanced breast cancer both as monotherapy and in combination with fulvestrant or letrozole. Abemaciclib is an ATP-competitive inhibitor; although similar to other CDK4/6i, its enzyme kinetics suggests a mixed mode of action behavior. We sought to investigate how CDK4/6i regulate CDK4/CyclinD complexes at the molecular level and in clinically relevant cellular models of breast cancer.

Methods: We developed an MS-based workflow including affinity purification-MS (AP-MS) and used hydrogen to deuterium exchange (HDX) to understand the dynamic behaviour of CDK4 during inhibition with abemaciclib and other CDK4/6i inhibitory agents. AP-MS was applied to quantify cellular CDK4 phosphorylation (p-Thr172) and CDK4/CyclinD1 complex composition and HDX to characterize inhibitor-target interactions.

Results: CDK4-CyclinD1 complex is controlled by a coordinated interplay between innate negative regulators such as INK and CIP/KIP family of proteins and mitogenic signals, key events that control CDK4 activity. We show that compounds have affinity for both the active (phosphorylated) and the inactive (non-phosphorylated) form of CDK4. In addition, we confirm that they stabilize the activated complex in a breast cancer model sensitive to CDK4/6i; CDK4 inhibition stabilized the phosphorylated form of CDK4 and reduced levels of p21-bound to CyclinD1/CDK4 complex. This correlated with increased Rb phosphorylation and cell proliferation following compound removal.

Conclusions: Together, our data indicate that stabilization of a pre-activated complex lacking the native negative regulator p21 could lead to rapid pathway activation when compound levels decrease. This highlights the need for constant inhibition of the complex for efficient blockade of the pathway. Importantly, to overcome this rebound effect, continuous dosing schedule may be critical to reach best clinical results.

No conflict of interest

118 (PB-069) Poster

EGFR-mediated alterations to trastuzumab-mediated antibody-dependent cell-mediated cytotoxicity (T-ADCC) in TKI-resistant HER2+ breast cancer cell lines

N. Gaynor[1], A. Canonici[1], M. Mcdermott[1], J. Crown[2], D. Collins[1]. [1]*Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland;* [2]*St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland*

Background: Trastuzumab is a monoclonal antibody therapy used in the treatment of HER2+ breast cancer. Trastuzumab inhibits HER2-related

intracellular signalling pathways and is capable of engaging the immune system through ADCC. Lapatinib is a reversible, small molecule tyrosine kinase inhibitor (TKI) targeting HER-family members EGFR and HER2. Afatinib is an irreversible small molecule pan-HER family TKI. Previous work has shown decreased T-ADCC and increased EGFR protein levels in a lapatinib-resistant HER2+ breast cancer cell line (SKBR3-L). This study examines the impact of EGFR activation on T-ADCC in parental and lapatinib- and afatinib-resistant cell line models (HCC1954-L, SKBR3-A) of HER2+ breast cancer.

Methods: The HER2+ breast cancer cell lines SKBR3 and HCC1954 were exposed to afatinib (150 nM) and lapatinib (1 μM) for 6 months to generate the TKI-resistant SKBR3-A and HCC1954-L cell lines. Proliferation assays were used to determine resistance to TKI treatment. Expression of EGFR and pEGFR y1068 in parental and resistant cell lines was determined by Western blot and analysed by densitometry. For immune cell-mediated cytotoxicity assays, PBMCs were isolated from healthy volunteer blood using a Ficoll-Paque-based method. The target parental and resistant cell lines were treated with and without EGF (10 ng/ml) for 24 hours. The PBMCs and the target cancer cells were incubated at a ratio of 5:1 (PBMC:TC) for 12 hours with cytotoxicity assessed using a flow cytometry-based method. Direct cytotoxicity and T-ADCC were determined against each cell line. Comparative statistical analysis was performed using Student's T test.

Results: Protein levels of EGFR were increased in SKBR3-A ($p = 0.02$) and decreased in HCC1954-L ($p = 0.02$) compared to parental cell lines. pEGFR (y1068) levels in the SKBR3-A cell line were reduced relative to total protein levels *versus* the parental cell line ($p = 0.01$). pEGFR (y1068) levels were unchanged between HCC1954-L and the parental cell line. TKI-resistance resulted in increased direct cytotoxicity and T-ADCC compared to the parental cell lines in both SKBR3-A and HCC1954-L. EGF pre-treatment of target cells increased T-ADCC by 15.1 ±/- 0.4% in the SKBR3 cell line but reduced direct T-ADCC by 12.4 ±/- iso 1.2% in the SKBR3-A cell line. T-ADCC levels were not effected in EGF pre-treated HCC1954 or HCC1954-Lap cell lines.

Conclusions: A TKI resistant phenotype in the cell line models examined resulted in increased susceptibility to T-ADCC. Activation of the EGFR pathway alters susceptibility of SKBR3 parental and afatinib resistant cancer cells to T-ADCC. Further work is warranted to examine the mechanisms and potential mediators of this effect in HER2+ breast cancer models.

Conflict of interest: Corporate-sponsored Research: Dr. Alexandra Canonici has received research funding from Boehringer Ingelheim. Dr. Denis Collins has received research funding from Roche. Prof. John Crown has received research funding from Roche, GSK and Boehringer Ingelheim. Other Substantive Relationships: Prof. John Crown has had a consulting or advisory role with Boehringer Ingelheim.

119 (PB-070) Poster

Chromosomal Aberrations in Chronic Myeloid Leukemia: Response to conventional tyrosine kinase inhibitors and risk of blastic transformation

S. Maqsood[1], N. Siddiqui[1], F. Ali[1]. [1]*Shaukat Khanum Memorial Cancer Hospital and Research Center, Medical Oncology, Lahore, Pakistan*

Background: Chronic Myeloid Leukemia (CML) is a common hematological malignancy with tyrosine kinase inhibitors (TKI) forming the main stay of treatment.

Methods: This is a retrospective chart review of all patients who were diagnosed with CML in chronic phase (CP) with additional chromosomal abnormalities (ACAs) over a period of 5 years from 2010 to 2015 at our institution.

Results: Response to initial TKI.

A total of 283 patients were diagnosed with CML from January 2010 to January 2015. Thirty one patients were found to have ACAs at the time of diagnosis in addition to BCR-ABL fusion gene.

23 patients (74.2%) were males whereas 8 (25.8%) were females. 13 (41.9%) were in the age group of 31–50 years. 30 patients were started on Imatinib and one on Nilotinib. 16 patients (51.6%) achieved complete hematological response within 3 months. However a complete molecular and cytogenetic response was never achieved in 17 (54.8%) and 18 (58.1%) patients respectively.

Cytogenetics Analysis and ACAs.

Conventional cytogenetic analysis revealed that 11 (35.5%) of patients had variant Philadelphia chromosome followed by 7 patients (22.6%) with trisomy 8.5 patients (16.1%) had multiple chromosomal abnormalities including trisomy 8, deletion 1 and isochrome 17q.

Risk of blastic transformation.

Eight patients (25.8%) transformed to acute myeloid leukemia(AML) whereas 3 (9.7%) patients had a transformation to acute lymphoblastic leukemia(ALL).

Therapy and response in transformed patients.

Out of the 11 patients who transformed to AML and ALL, 6 patients underwent induction chemotherapy while 5 were not considered fit for it on account of poor performance score and lack of resources. Out of these 6 patients, none responded to induction chemo.

Conclusion: In summary, this purpose of this project was to study the clinical presentation of patients with CML harboring additional chromosomal abnormalities at diagnosis, cytogenetic analysis, risk of blastic transformation and response to treatment in transformed patients. Despite an early hematological response, more than half of the patients failed to achieve complete molecular and cytogenetic response which clearly shows suboptimal response to the tyrosine kinase inhibitors in this particular set of CML patients. There was transformation to acute leukemia in 35.5% of patients. We were able to give induction chemotherapy to only 6 out of 11 patients and none of them responded to it. This study showed that presence of ACA at the time of diagnosis is a high risk feature for patients with CML and confers poor prognosis when treated with conventional TKI. Further studies are required in our population regarding alternative therapy for such patient population.

No conflict of interest

120 (PB-071) Poster

DCC-2618, a broad-spectrum inhibitor of KIT and PDGFRA mutants, synergizes with inhibitors of the MAPK pathway

A. Gupta[1], C. Leary[1], A. Garcia-Valverde[2], J. Arribas[2], C. Serrano[2], D. Flynn[1], B. Smith[1]. [1]*Deciphera Pharmaceuticals Inc., Biology, Waltham, MA, USA;* [2]*Vall d'Hebron University, Preclinical Research Program, Vall d'Hebron Institute of Oncology, Barcelona, Spain*

Background: Treatment of gastrointestinal stromal tumors (GIST) with the KIT-inhibitor imatinib leads to objective responses or stable disease, but complete responses are rare. Residual signaling through the MAPK pathway via incomplete suppression of KIT or activation of other pathways that feed into MAPK signaling are possible mechanisms allowing GIST cells to escape death. The stasis of GIST tumors in response to treatment with KIT inhibitors can lead to rebound tumor growth in the absence of drug or allow for drug-resistance mutations to arise. Inhibitors of the MAPK signaling pathway, such as MEK inhibitors, have been shown to synergize with imatinib to inhibit GIST cell growth in vitro and in vivo. DCC-2618 is a kinase switch control inhibitor that potently inhibits the spectrum of primary and drug-resistant mutations in exons 9, 11, 13, 14, 17 and 18 in KIT and exons 12, 14 and 18 PDGFRA that occur in GIST and other diseases. We hypothesized that broadly inhibiting clinically-relevant primary and drug-resistant KIT mutations with DCC-2618 would synergize with MEK inhibitors in vitro and in vivo in both imatinib-sensitive and imatinib-resistant cell lines and tumors.

Methods: DCC-2618 and control compounds, alone or in combination with several MEK inhibitors, were tested using a variety of cell-based assays, including apoptosis and clonogenic assays. Levels of phosphorylated KIT and other proteins were determined by Western blot or ELISA. Proliferation was measured using the fluorescent dye resazurin. Xenograft models were performed at MI Bioresearch (Ann Arbor, MI).

Results: KIT inhibitors block proliferation of the imatinib-sensitive cell line, GIST T1, however single-agent treatment does not commit most cells to apoptosis, and quiescent cells grow back when drug is removed. The combination of DCC-2618 with a MEK inhibitor leads to substantially higher apoptosis and cell death. Importantly, DCC-2618 also synergizes with MEK inhibitors in drug-resistant GIST and mastocytosis cell lines, leading to a greater blockade of signaling pathways and increases in apoptosis and cell death.

In vivo, treatment with DCC-2618 in combination with a MEK inhibitor led to complete regression of tumor growth during the dosing period, and long-term reduction in tumor growth after the dosing period in a GIST xenograft model. DCC-2618 also showed additive efficacy with a MEK inhibitor in mastocytosis models.

Conclusions: Combinations of DCC-2618 and inhibitors of the MAPK pathway lead to greater cell death in GIST and mastocytosis cell lines, and may have utility in the treatment of patients with GIST or mastocytosis. DCC-2618 is currently in a Phase 3 clinical trial in advanced GIST patients who have been treated with prior therapies (ClinicalTrials.gov Identifier: NCT03353753).

Conflict of interest: Ownership: A.G., C.L, D.F. and B.S. are full-time employees of Deciphera Pharmaceuticals and have stock/options in the company. Advisory Board: C.S. has served on advisory boards for Deciphera Pharmaceuticals. Corporate-sponsored Research: C.S. has received research funding from Deciphera Pharmaceuticals. Other Substantive Relationships: C.S. is an investigator on a clinical trial sponsored by Deciphera Pharmaceuticals.

121 (PB-072) Poster
RX-5902, a Beta-catenin Modulator, enhances immunotherapy through positive alterations in the tumor immunologic environment in preclinical models of triple negative breast cancer

J. Tentler[1]. [1]*University of Colorado School of Medicine, Medical Oncology, Aurora, CO, USA*

Background: RX-5902 (Supinoxin) is a novel, oral, anti-cancer compound that targets phosphorylated p68 (DDX5) to attenuate nuclear shuttling of b-catenin. As elevated levels of b-catenin signaling have recently been implicated in resistance to immunotherapies, we sought to determine if RX-5902 could enhance or reverse these responses. In the current study, we utilized a novel humanized mouse model bearing MDA-MB231 triple-negative breast cancer (TNBC) xenografts to determine if RX-5902 could enhance the anti-tumor activity of the PD-1 inhibitor nivolumab. Additionally, end-of-study immunologic analyses were performed to assess the potential mechanisms by which RX-5902 augments the therapeutic potential of immunotherapies in models of TNBC.

Methods: Humanized Sirpα mice from the BALB/cRag2-/-IL2Rγc-/- (BRG) strain were used. Newborn pups were humanized through transplantation of 1×10^5 CD34+ cells purified from umbilical cord blood. Mice were evaluated for chimerism at 8 and 12 weeks. At 16 weeks, 3×10^6 MDA-MB231 cells were implanted on the right and left flanks mice. When average tumor size reached a volume of ~150–300 mm^3, mice were randomized into treatment groups according to % chimerism. At sacrifice, blood, bone marrow, lymph nodes, spleen and tumors were harvested for flow cytometry analysis of human immune cells.

Results: We observed significant tumor growth inhibition when RX-5902 was combined with nivolumab compared to either agent alone with an inhibition index of approximately 50 percent ($p < 0.01$). Immunologic analyses indicated that mice treated with RX-5902 demonstrated a significant increase in the number of activated T cells in tumor infiltrating lymphocytes (TILs) and a significant decrease in immunosuppressive myeloid-derived precursor cells (MDSCs) compared to vehicle ($p < 0.05$). In the RX-5902/nivolumab combination group, there was a significant increase in the percentage of CD4+ T cells in TILs and increased, systemic granzyme B production ($p < 0.01$).

Conclusions: RX-5902 enhanced the efficacy of nivolumab in a humanized, preclinical model of TNBC. Several changes in immunologic profiles were noted in mice treated with RX-5902 and the combination, including an increase in activated TILs and a decrease in MDSCs in the tumors. These finding indicate that RX-5902 may have important clinical immunomodulatory as well as anti-tumoral activity in the treatment of TNBC with a checkpoint inhibitor. A single-agent phase 2a clinical study in metastatic TNBC is ongoing.

No conflict of interest

122 (PB-073) Poster
Targeting the AKT/mTOR/STAT3 pathways through a ROS-dependent Ubiquitin proteasome degradation in breast cancer by the natural polyphenol compound, carnosol

R. Iratni[1], A.S. Halima[1], E.H. Hussain[1], A. Khawlah[1], B. Nehla[1], B. Mujeeb Zafar[1], A.D. Yusra[1]. [1]*UAE University, Biology, Al Ain, U.A.E.*

Background: We have previously showed that carnosol significantly inhibited the viability and colony growth of triple negative breast cancer cells and induced ROS-dependent Beclin-1-independent autophagy and subsequent apoptotic cell death. Here were analyzed the molecular mechanism through which carnosol exerts its anti-cancer activity.

Material and Methods: Human breast cancer cell lines, MDA-MB-231 were used for this study. Carnosol at concentration of 25, 50 and 100 mM were used for the treatment. Total and phosphorylated STAT3, AKT and mTOR proteins were detected by Western Blot analyses.

Results: Mechanistically, we found that carnosol inactivated the AKT/mTOR pathway by promoting the proteasome-dependent degradation of both proteins. Strikingly, we also found that carnosol target Stat3 to degradation. Proteasome inhibition restored these proteins to level comparable to control cells. The proteasomal degradation of mTOR, which occurred as early as 30 minutes post-carnosol treatment was concomitant with an overall increase in the level of ubiquitinated proteins and translated stimulation of proteolysis by the proteasome. Interestingly, we found that treatment of the breast cancer cells with N-acetylcysteine, a ROS inhibitor, not only restored AKT/mTOR/Stat3 proteins to level comparable to control cells, but also dramatically reduced carnosol-induced cell death and blocked the activation of autophagy and apoptosis.

Conclusion: Our findings demonstrate that carnosol exerts its anti-breast cancer activity through stimulation ubiquitin proteasome system which consequently triggers both autophagy and apoptosis, making it a potential and valuable source of novel therapeutic cancer drug.

No conflict of interest

123 (PB-074) Poster
Targeting CD205-positive solid tumors with a novel antibody drug conjugate (ADC): OBT076/MEN1309 target expression and activity guides Ph1 trial design

A. Kaplan[1], N. Attanasio[1], A. Bisht[2], L. Deban[3], S.L. Lou[2], J. Berry[4], R. Dusek[1], R. Boyd[5], M. Binaschi[6], A. Pellacani[7], E. Zhukovsky[8], C. Rohlff[9], A. Fandi[10]. [1]*Oxford BioTherapeutics Ltd, Target Validation and Translational Science, San Jose, USA;* [2]*Oxford BioTherapeutics Ltd, Protein Science, San Jose, USA;* [3]*Oxford BioTherapeutics Ltd, Immuno-Oncology, Oxford, United Kingdom;* [4]*Oxford BioTherapeutics Ltd, Target Discovery, Oxford, United Kingdom;* [5]*Oxford BioTherapeutics Ltd, UK Site Head and Scientific Director, Oxford, United Kingdom;* [6]*Menarini Ricerche, Pharmacology Department, Pomezia, Italy;* [7]*Menarini Ricerche, Research & Development, Pomezia, Italy;* [8]*Oxford BioTherapeutics Ltd, Research, Oxford, United Kingdom;* [9]*Oxford BioTherapeutics Ltd, Chief Executive Officer, Oxford, United Kingdom;* [10]*Oxford BioTherapeutics Ltd, Chief Medical Officer, Oxford, United Kingdom*

Background: OBT076/MEN1309 is an antibody drug conjugate consisting of a fully humanized anti-CD205 monoclonal antibody coupled via a cleavable N-succinimidyl-4-(2-pyridyldithio) butanoate linker to DM4, a potent maytansinoid-derived microtubule inhibitor. The antigen is highly expressed in a large range of solid tumors and rapidly internalizes on ADC binding. The increased and highly prevalent expression levels in solid tumor types, compared to normal tissues, provide an acceptable toxicity profile and wide therapeutic window. Preclinical studies indicate promising efficacy and pharmacokinetic (PK) and toxicology studies in non-human primates have shown the ADC to be well-tolerated.

Methods: We interrogated commercial tissue microarrays by immunohistochemistry (IHC) to evaluate target expression level and prevalence in gastric ($n = 87$), bladder ($n = 80$), colorectal ($n = 112$), lung ($n = 72$), pancreatic ($n = 120$), breast ($n = 121$), and ovarian ($n = 125$) cancers. Cases were annotated for stage and included both primary and metastatic tumors. Preclinical efficacy of OBT076/MEN1309 was evaluated by *in vitro* killing assays and *in vivo* xenograft and PDX models.

Results: IHC expression of 2+ or greater was observed in 72% of gastric cancer cases, 63% of bladder, 38% of colorectal, 25% of pancreatic, 21% of non-small cell lung, 6% of breast, and 5% of ovarian cancers. Prevalence in TNBC ($n = 36$) was found to be greater than that observed in breast cancer cases of all subtypes (56% expressing at a level of 2+ or greater). The majority of tumors scoring 2+ or greater showed positivity in at least 50% of the viable tumor. Cancer cell lines with target antigen expression levels equivalent to 2+ intensity by IHC were highly sensitive to specific cell killing by the ADC *in vitro* and in xenograft models. OBT076/MEN1309 was efficacious in TNBC, bladder and pancreatic cancer PDX models.

Conclusions: The above data support the initiation of a US Phase 1 clinical trial. Enrollment eligibility will include a requirement of 2+ expression in at least 50% of the tumor sample as determined by IHC. The dose escalation phase of the study will explore escalating IV doses to determine the maximum tolerated dose (MTD) of OBT076/MEN1309. This will be followed by the expansion phase of the study to further evaluate the safety and preliminary efficacy of OBT076/MEN1309 in patients with solid tumors.

No conflict of interest

124 (PB-075) Poster
Trilaciclib, a CDK4/6 inhibitor, does not impair the efficacy of chemotherapy in CDK4/6-dependent tumor models

J. Sorrentino[1], J. Bisi[2], D. Thompson[2], A. Lai[1], J. Strum[2], P. Roberts[1]. [1]*G1 Therapeutics, Translational Medicine, Research Triangle Park, USA;* [2]*G1 Therapeutics, Preclinical Research and Development, Research Triangle Park, USA*

Background: Trilaciclib (trila) is an IV CDK4/6 inhibitor in development to preserve hematopoietic stem and progenitor cell (HSPC) and immune system function during chemo (myelopreservation). In a randomized, placebo-controlled, double-blind Phase 2 trial (NCT02499770) in patients with small cell lung cancer (SCLC) receiving 1st-line chemo, trila demonstrated robust myelopreservation benefits across multiple hematopoietic lineages without increasing toxicity. The initial clinical development of trila has focused on CDK4/6-independent tumors such as SCLC and triple-negative breast cancer. To evaluate the potential use of trila in patients with

CDK4/6-dependent cancers, we studied the effects of trila on chemo efficacy in animals bearing CDK4/6-dependent tumors.

Material and Methods: A panel of patient-derived (PDX) and cell-based breast cancer xenograft models of varying sensitivities to trila (i.e., CDK4/6-dependence) were treated with different chemos±trila for up to six weeks, and tumor volumes evaluated to determine the impact of trila on chemo efficacy. The cell cycle kinetics of tumor cells and bone marrow cells, including hematopoietic stem cells (HSCs) were also evaluated before and during trila treatment. In the model most sensitive to CDK4/6 inhibition (MCF7), experiments varying the scheduling of trila around chemo administration are ongoing to further expand the results described below.

Results: Single doses of trila administered prior to chemo did not antagonize tumor efficacy in any CDK4/6-dependent model and in some models, trila enhanced efficacy. Cell cycle analyses revealed a significantly higher frequency of proliferating tumor cells compared to HSCs, with ~40% of tumor cells in the SG2M phases vs ~8% of HSCs.

Conclusions: Data reported in this abstract using CDK4/6-dependent tumor models demonstrate that intermittent dosing of trila with chemo does not antagonize tumor efficacy and can enhance efficacy in some models. A potential mechanism for these findings includes the differential cell cycle kinetics of tumor cells vs HSCs with a larger proportion of proliferating tumor cells present at the time of chemo exposure, thereby rendering the tumor more sensitive to the chemo cytotoxic effects. Taken together with previous studies in immune competent models that demonstrate trila increases anti-tumor activity through myelopreservation or direct immune activation, these data support clinical testing of trila + chemo in patients with CDK4/6-dependent tumors.

Conflict of interest: Ownership: γWe are all employees of G1 Therapeutics, Inc.

125 (PB-076) Poster

Expedited development of AVB-S6 through the use of a proprietary biomarker in healthy volunteers to guide dosing in oncology studies

G. Mcintyre[1], L. Bonifacio[1], D. Prohaska[1], J. Shang[2], Y. Yokota[2], A. Moss[3], M. Dodds[3], R. Tabibiazar[1], A. Giaccia[1]. *[1]Aravive Biologics, Inc, Research and Developmnet, Houston, USA; [2]SNBL USA, Analytical Biology, Everett, USA; [3]Certara, Translational and Clinical Pharmacology, Princeton, USA*

Background: Aravive is developing AVB-S6, an AXL "decoy receptor" that binds AXL's activating ligand Growth Arrest Specific 6 (GAS6) with higher affinity than endogenous AXL, effectively sequestering GAS6 and abrogating AXL signaling. AVB-S6 has been shown to reduce invasion/migration of highly metastatic cells *in vitro* and inhibit metastatic disease in aggressive preclinical models of human pancreatic, renal, breast, and ovarian cancers. Due to the observed preclinical relationship between serum GAS6 (sGAS6) depletion and anti-metastatic activity, Aravive has developed a proprietary pharmacodynamic [PD] biomarker assay to assesss GAS6 levels throughout development. Use of this PD assay in combination with establishing the human safety and PK/PD profile in healthy volunteers streamlined the clinical program, guiding dose selection for oncology studies.

Materials and Methods: A Phase 1 single-blind, placebo-controlled safety, PK and PD study in 40 healthy volunteers was conducted with single ascending doses (1, 2.5, 5 and 10 mg/kg) and 4 weekly doses (5 mg/kg) of AVB-S6 (ClinicalTrials.gov Identifier: NCT03401528). The effect of GAS6 on the clearance of AVB-S6 was incorporated into a target-mediated drug disposition model, providing parallel linear and nonlinear clearance of AVB-S6. Simulations of human GAS6 suppression were performed for the dose levels of 1, 2.5, 5, and 10 mg/kg using nonhuman primate data. Considering potentially higher sGAS6 levels in cancer patients and dosing regimens of combination chemotherapies, different AVB-S6 dosing regimens were modeled to predict target coverage with doses to be used in the oncology studies.

Results: Single and 4 weekly doses of AVB-S6 were well-tolerated and there were no serious adverse events or dose–related adverse events. All doses tested were pharmacologically active as they suppressed sGAS6 for at least one week. PK/PD-modeling confirmed selection of dosing regimens for cancer studies that would suppress (>90% reduction) sGAS6 and be compatible with chemotherapeutic dosing regimens. Our planned ovarian cancer study is expected to include a Phase 1b open label portion to evaluate safety of AVB-S6 combined with standard of care chemotherapy in patients with platinum-resistant recurrent disease and confirm the dose of AVB-S6 is pharmacologically active prior to proceeding to the Phase 2 blinded portion.

Conclusions: Use of a proprietary PD assay expedited the AVB-S6 development program by guiding dose selection for cancer patients using healthy volunteer data. This minimizes the number of cancer patients administered potentially pharmacologically inactive doses and identifies different dosing regimens to complement those of combination chemotherapy. The ability to test pharmacologically active doses in Phase 1b offers the potential to assess efficacy much earlier in development.

Conflict of interest: Ownership: Laura Bonifacio, Ray Tabibiazar, Amato Giaccia, Gail McIntyre and David Prohaska are employees of Aravive Biologics, Inc and have stock options.

126 (PB-077) Poster

Somatic mutations in vulvar squamous cell carcinoma and its premalignant lesions

A. Kowalik[1], S. Zięba[1], N. Rusetska[2], K. Zalewski[2,3,4], A. Piaścik[5], E. Bakuła-Zalewska[5], J. Kopczyński[6], M. Kowalewska[2,7]. *[1]Holycross Cancer Centre, Molecular Diagnostics, Kielce, Poland; [2]Maria Sklodowska-Curie Institute, Oncology Center, Department of Molecular and Translational Oncology, Warsaw, Poland; [3]Warsaw Medical University, Chair and Department of Obstetrics, Gynecology and Oncology, 2nd Faculty of Medicine, Warsaw, Poland; [4]Holycross Cancer Centre, Gynecologic Oncology, Kielce, Poland; [5]Maria Sklodowska-Curie Institute, Oncology Center, Department of Pthology, Warsaw, Poland; [6]Holycross Cancer Centre, Surgical Pathology, Kielce, Poland; [7]Warsaw Medical University, Immunology, Biochemistry and Nutrition, Warsaw, Poland*

Background: Vulvar cancer is a rare malignancy of gynaecological tract and thus the molecular mechanisms of its carcinogenesis remain obscure. It originates from the progression of HPV associated high-grade squamous intraepithelial lesions (HSIL, former usual type neoplasia – uVIN) and differentiated-type vulvar intraepithelial neoplasia (dVIN). As a consequence, currently targeted therapies for vulvar cancer are lacking. The aim of the study was to analyse and compare of the profiles of somatic mutations in precancerous vulvar conditions (HSIL and dVIN) in vulvar squamous cell carcinoma (VSCC) using next generation sequencing technology.

Material and Methods: 50 HSIL, 16 dVIN and 81 VSCC tumour samples, were screened for hotspot mutations in 50 genes covered by the Ion AmpliSeq Cancer Hotspot Panel v2 Kit (Thermo Fisher Scientific).

Results: Among the 50 analysed genes, *TP53* (44%, 26%, 44%), *CDKN2A* (28%, 9%, 22%), *PIK3CA* (0%, 13%, 9%), *FBXW7* (5%, 7%, 6%), *FGFR3* (5%, 11%, 1%) were identified as the most often mutated in dVIN, HSIL and VSCC, respectively. The remaining genes – *BRAF, ERBB4, KIT, HNF1A, KRAS*, *HRAS*, *FGFR3*, *STK11*, *AKT1*, *SMAD4*, *FLT3*, *JAK3*, *GNAQ* and *PTEN* – were mutated at much lower frequencies.

Conclusions: Unexpectedly, premalignant vulvar lesions (dVIN and HSIL) were found to harbour mutational profile very similar to that of VSCC. The most common genetic alterations identified in VSCC tumours, mutations of *TP53* and *CDKN2A*, are already present in premalignant lesions. Therefore, these mutations may be considered as an early events during VSCC carcinogenesis.

Acknowledgement: This work was supported by the Polish National Science Centre grant no. 2013/10/E/NZ5/00663.

No conflict of interest

127 (PB-078) Poster

Targeting co-regulators of the androgen receptor as a novel therapeutic approach for prostate and breast cancer

M. Prencipe[1], A. Fabre[2,3,4], E. Corey[5], W. Gallagher[1], W. Watson[4], J. Crown[6]. *[1]UCD Conway Institute, UCD School of Biomolecular and Biomedical Science, Dublin 4, Ireland; [2]St Vincent's University Hospital, Department of Pathology, Dublin, Ireland; [3]UCD Conway Institute of Biomolecular and Biomedical Research, Research Pathology Core, Dublin, Ireland; [4]Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, D4, Dublin, Ireland, UCD School of Medicine, Dublin, Ireland; [5]University of Washington, Department of Urology, Seattle, USA; [6]St Vincent's University Hospital, Department of Medical Oncology, Dublin, Ireland*

Background: Current treatments for prostate cancer mainly target the Androgen Receptor (AR); however, despite initial response, these treatments commonly fail. AR expression has also been associated with poor prognosis in a subset of advanced breast cancers; therefore targeting AR in this setting is attracting increasing interest. Among the multiple mechanisms of resistance, aberrations in AR co-factors and co-regulators represent a promising therapeutic approach since co-factors and co-regulators are not

susceptible to AR resistance mechanisms. We previously identified Serum Response Factor (SRF) as an important transcription factor in an in vitro model of castrate-resistant prostate cancer (CRPC). We showed SRF association with CRPC and survival using TMAs of CRPC tissues. A cross-talk between AR and SRF in vitro and in clinical samples was also demonstrated. Using the SRF inhibitor CCG1423, we showed that combination of CCG1423 and Enzalutamide, a new-generation AR-targeting agent, is significantly more effective than monotherapies.

Material and Methods: A panel of breast cancer (BC) cell lines with different expression levels of SRF and AR were selected to investigate the effect of SRF inhibition on cell viability (MTT assay) and migration (scratch assay). CCG1423 was used to inhibit SRF. Immunohistochemistry (IHC) was used to assess SRF expression in 3 BC TMAs: TMA1 (n = 144) and TMA2 (n = 512) with different subtypes of BC and TMA3 with 138 triple negative BC patients. A prostate patient-derived-xenograft (PDX) mouse model was used to further study the cross-talk between SRF and AR in vivo.

Results: MTT assays showed response to CCG1423 in the two cell lines positive for SRF (MDA-MB-231 and HS578t) with IC50 values of 20 uM, while MDA-MB-453 (SRF negative) did not respond (IC50 > 80 uM). In addition, a synergistic effect on cell viability was demonstrated when CCG1423 was used in combination with MDV3100. Moreover, scratch assays to assess cell migration showed a slower gap-closure post- CCG1423 treatment compared to controls. The analysis of the association of SRF staining with clinical-pathological features in the three BC TMAs described above is currently ongoing.

Transgelin (TAGLN), an actin-binding protein and tumour suppressor, is a transcriptional target of SRF and an AR inhibitor, representing a possible mechanistic link. In vitro data showed a significant increase in TAGLN mRNA following SRF down-regulation and a significant decrease post SRF up-regulation, indicating that SRF acts as a repressor of TAGLN. Preliminary data from a prostate PDX study showed significantly higher AR retention into the cytoplasm and higher TAGLN expression following CCG1423 treatment.

Conclusion: Our data support the rationale for using SRF as an alternative molecular target to AR, alone or in combination with AR-antagonists.

No conflict of interest

128 (PB-079) Poster

Real-world practice for non-small cell lung cancer with targetable genetic alterations in an Irish setting

W. Mullally[1], W. Kee[2], C. Dennehy[1], J. Greene[1], D. Kelly[1], R.M. Bambury[1], B.R. Bird[3]. [1]*Cork University Hospital, Department of Medical Oncology, Cork, Ireland;* [2]*University College Cork, Medicine, Cork, Ireland;* [3]*Bon Secours Hospital, Department of Medical Oncology, Cork, Ireland*

Background: Non-small cell lung cancer (NSCLC) has evolved to include treatments for targetable genetic alterations. These include multiple therapies for *EGFR* and *ALK* mutations and evolving drug development for *BRAF*, *MET*, *ROS1* and *RET* alterations.

Material and Methods: The aim of this multi-centre retrospective study was to determine the (i) incidence of targetable mutations and (ii) treatment outcomes. Medical records were interrogated in two Irish oncology centres from September 2009 though to May 2018.

Results: A total of 370 NSCLC patients were identified which included 298 adenocarcinoma, 29 squamous and 43 patients classified as other histology. The median age at diagnosis was 67 and 63% of patients had locally advanced or metastatic NSCLC. The ECOG performance status was 0 to 1 in 80% of patients. Subsequent molecular testing identified 16.3% *EGFR*, 3.0% *ALK*, 3.5% *BRAF*, 41.6% *KRAS* and 2.3% *ROS1* gene mutations. The most common *EGFR* mutations include Exon 19 deletion (42%), Exon 21 L858R (25%), Exon 20 insertion (21%), followed by the *EGFR T790M* (14%) resitance mutation. There were 11 patients identified with two concomitant and two patients with three concomitnant *EGFR* mutations.

The progression free survival (PFS) for patients treated with first line *EGFR TKI* (n = 28) with one mutation was 6.9 months with a overall survival (OS) of 14.9 months and an objective response rate (ORR) of 46%. The PFS of patients with two concomitant *EGFR* mutations (n = 7) was 9.6 months, OS of 28.7 months and an ORR of 71%. There was four patients with *ALK* mutations who received crizotinib with a median PFS of 14.8 months, OS of 17.3 months and ORR of 75%.

Conclusions: The incidence of NCSLC gene mutations is comparble with other Causcasian populations. Interestingly higher *KRAS* mutations were identifed in this population and concomitant resistance and sensitising in the *EGFR* gene can confer sensitivity to EGFR-TKI's.

No conflict of interest

129 (PB-080) Poster

L-Glutamine's role in the anticancer activity of 4-demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) in non-small cell lung cancer (NSCLC) involving the CNS

L. Morgan[1], R. Weiner[2], T. Mahmood[3], M. Ware[4], A. Rodgers[5], E. Benes[5]. [1]*DEKK-TEC, Inc, Research, New Orleans, USA;* [2]*Tulane University Health Sciences Center, Medicine, New Orleans, USA;* [3]*Detroit Clinical Research Center, Oncology, Owasso, USA;* [4]*Ochsner Medical Center, Neurosurgery, New Orleans, USA;* [5]*DEKK-TEC, Inc., Research, New Orleans, USA*

Background: 4-Demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) is a poly-chlorinated pyridine cholesteryl carbonate that is demonstrating excellent anti-cancer activities in adult subjects with NSCLC involving the CNS [AACR #CT 129, 2017]. The drug associates with RBCs and is transported into the CNS *via* breaks in the blood-brain-barrier (BBB) [AACR #1185, 2013]. DM-CHOC-PEN has a MOA *via bis*-alkylation of DNA @ N^7-guanine and N^4-cytosine [AACR #1185, 2013]. We report here a mechanism for the transport of DM-CHOC-PEN into NSCLC cells growing in the CNS *via* L-glutamine's (GLM) transport system.

Methods and Materials: Human NSCLC and small cell lung cancer (SLSC) cell lines, H-460 & H-2087 and SHP-77 & H-1417, resp., were obtained from the American Tissue Culture Collection and grown in multi-well culture plates (seeded with 2–3000 cells/well) using RPMI-1640 complete medium (10% FBS, 10 mg/mL streptomycin, 10,000 U/mL penicillin and 25 μg/mL Amp. B) ±/- GLM (300 mg/500 mL) at 37°C in a 5% CO_2 moist atmosphere. Multiple concentrations of DM-CHOC-PEN [0.3–5 mcg/mL] were impregnated in ChemoChads® and added to each well with cancer cells growing in the post-exponential phase (>18 h in culture); after 8-hours of drug exposure the medium was replaced with fresh complete medium ±/- GLM and 48 h. later assayed for cytotoxicity with a BD fluorescent-activated cell sorter. Controls were vehicle treated ChemoChads®. Acivicin, an inhibitor of gamma-glutamyl transferase (GGT) was included in the studies to document DM-CHOC-PEN's requirement for GLM to penetrate NSCLC cells and produce maximum anticancer activ mobity.

Results: Both of the NSCLC cell lines growing in GLM-supplemented RPMI medium had excellent cytotoxic responses to DM-CHOC-PEN (IC_{50} 0.35–0.75 mcg/mL). In contrast, both cell lines in GLM-free RPMI medium demonstrated poor cellular growth and DM-CHOC-PEN lacked cytotoxic activities (IC_{50} >5 mcg/mL). Both the poor growth and the lack of cytotoxicity could be reversed by simply replacing the GLM-free RPMI medium with RPMI plus GLM medium. The SCLC cell lines grew well with or without GLM, and were poorly sensitive to DM-CHOC-PEN (IC_{50} >5 mcg/mnvmL), irrespective of the presence or absence of GLM. Acivicin (3 nM) when added to NSCLC cells in cultures containing GLM, inhibited the activity of DM-CHOC-PEN (IC_{50} >5 mcg/mL), however, in GLM-free medium no changes in NSCLC cell growth were noted.

Conclusion: DM-CHOC-PEN requires GLM for transport into NSCLC cells. Complete support data will be presented for a 3-stage mechanism for the drug's entry into cancer cells involving entering the CNS *via* reversible binding with RBCs in the peripheral circulation and transport through cancer associated breaks in the BBB, then penetrating cancer cells in association with L-glutamine. **Supported by NCI/SBIR grants – R43/44CA132257 and R43CA203351**.

No conflict of interest

130 (PB-081) Poster

Co-targeting BRAF and Src family kinases in BRAF-mutant melanoma can provide superior control to block the emergence of BRAF inhibitor-resistant melanoma

C. Feddersen[1], A. Varzavand[2], J. Schillo[1], M. Milhem[3], Y. Zakharia[3], M. Henry[4], A. Dupuy[1], C. Stipp[2]. [1]*University of Iowa, Anatomy and Cell Biology, Iowa City, USA;* [2]*University of Iowa, Biology, Iowa City, USA;* [3]*University of Iowa, Hematology/Oncology, Iowa City, USA;* [4]*University of Iowa, Molecular Physiology and Biophysics, Iowa City, USA*

Background: BRAF/MEK inhibition remains an important treatment option for patients with $BRAF^{V600E}$ mutant melanoma who show disease progression on immunotherapy; however, the majority of patients treated with BRAF inhibitor (BRAFi) therapy develop BRAFi-resistant disease within a year of initiating treatment. Genomic analysis of BRAFi-resistant melanomas has identified somatic mutations associated with resistance, but has yet to identify novel drug combinations that can be used to prevent or reverse resistance.

Materials and Methods: To address this critical gap, we have recently completed a forward genetic screen using the Sleeping Beauty transposon mutagenesis approach to identify novel drivers of BRAFi resistance in A375

melanoma cells. Key results were independently confirmed in multiple melanoma cell lines.

Results: Validating our approach, our screen identified N-terminal truncation of BRAF – a known mechanism of vemurafenib resistance. In addition, *we identified DBL family guanine exchange factors (GEFs) as novel drivers of BRAFi resistance that we have functionally validated in multiple BRAF^{V600E} mutant melanoma cell lines*. Analysis of The Cancer Genome Atlas database suggests that this novel, GEF-driven resistance mechanism may be present in as many as 25% of all melanoma cases. Our novel GEF drivers upregulate active Rac when expressed in melanoma cells, and Rac RNAi or pharmacological inhibition of PAK kinases, the major Rac effector kinases, both block BRAFi resistance. Crucially, many DBL family GEFs are well known to be regulated by Src family kinases (SFKs), and our preliminary data indicate that GEF-driven BRAFi resistance can be blocked by combination treatment with vemurafenib and saracatinib, a selective SFK inhibitor. Expansion of resistant cells in the presence of vemurafenib or vemurafenib with cobimetinib can be reversed by switching to vemurafenib plus saracatinib. Consistently, we find that adding saracatinib converts BRAFi-mediated cytostasis into cell killing and blocks spontaneous BRAFi resistance in responsive melanoma cell lines.

Conclusions: Co-targeting of Src family kinases may provide a potent strategy to block the emergence of BRAF inhibitor-resistant disease in a significant subset of BRAF-mutant melanomas in which our novel GEF-driven resistance mechanism operates.

No conflict of interest

131 (PB-082) Poster

Intervenolin, a novel anti-tumor drug, suppresses cancer cell growth through modulation of tumor microenvironment

M. Kawada[1], J. Yoshida[1], M. Amemiya[1], H. Abe[2], T. Watanabe[2], D. Tastuda[1], M. Shibasaki[2]. [1]*Institute of Microbial Chemistry, Laboratory of Oncology, Tokyo, Japan;* [2]*Institute of Microbial Chemistry, Laboratory of Synthetic Organic Chemistry, Tokyo, Japan*

Tumor-stromal cell interactions are attractive targets for cancer chemotherapy and we have been focusing on small molecules modulating the interactions. Intervenolin (ITV), a novel natural compound, inhibits the growth of cancer cells in the presence of stromal cells (fibroblasts) more strongly than that in the absence of stromal cells. ITV exerts efficient anti-tumor activity in animal models without adverse effects. In this study we examined the mechanism of action of ITV. Since conditioned medium (CM) of stromal cells pretreated with ITV showed strong growth inhibitory effect against cancer cells, we then analyzed what factors could be dysregulated by ITV. As a result, we found that ITV increased the secretion of organic acids such as lactic acid and malic acid from stromal cells and changed the medium to be acidic. In that acidic condition we found that ITV suppressed the activity of p70 S6 kinase, a key enzyme in protein synthesis. Furthermore, concerning the secretion of lactic acid by ITV, we found that ITV inhibited mitochondrial complex I. These results suggest that ITV inhibits cancer growth though the modulation of microenvironment. Now we are studying whether the same phenomena occur in vivo.

No conflict of interest

132 (PB-083) Poster

MERTK-driven oncogenecity in bladder cancer

A. Roy[1], D.M. De Silva[1], M.M. Lee[1], R. Costello[1], A.B. Apollo[1], D.P. Bottaro[1]. [1]*National Cancer Institute, Urologic Oncology Branch, Bethesda, USA*

Bladder cancer (BCa) patients refractory to platinum chemotherapy and immunotherapy with checkpoint inhibitors, are in need of other effective systemic therapies. Identification of novel oncogenic pathways and newer therapeutic targets/strategies in bladder cancer is warranted. Receptor tyrosine kinases (RTKs) are broadly involved in cellular signaling pathways and when deregulated in cancer serve as oncogenic drivers. TAM (Tyro3-Axl-Mer) family of transmembrane RTKs mediate activation of oncogenic signaling pathways and play important roles in acquired resistance to targeted therapies and conventional cytotoxic drugs. Furthermore, evidence that clinical efficacy of cabozantinib was not solely due to inhibition of hepatocyte growth factor receptor (Met), we did a systematic assessment of other cabozantinib targets in BCa. Among other RTKs frequently over-expressed/deregulated in BCa, we focused on TAM family members. Binding of ligand Gas6 to TAM receptors stimulates autophosphorylation, activating PI3K and ERK kinase pathways that can regulate tumor cell survival, proliferation, migration, invasion and angiogenesis, promoting tumor progression and metastasis. The potential oncogenic roles that TAM RTKs may play in BCa are undefined.

Bioinformatic analysis platforms, real time PCR, immunoblotting, electro-chemiluminiscent immunoassays and statistical analysis are used to study the hypothesis that suppression of either Axl or Mer or both would reduce tumorigenic potential of BCa cells. It is known that Mer and Axl inhibitor, UNC2025, has good oral bioavailability, solubility, and other DMPK properties from *in vivo* studies in other cancers. If UNC2025 is a promising candidate for treatment in BCa, would be tested *invitro* by soft agar, migration and invasion assays. Efficacy studies of UNC2025 will be performed if *in vitro* studies suggest an oncogenic role of AXL or MERTK in BCa cell lines.

Bioinformatic analysis of BCa patient data from The Cancer Genome Atlas project ($n = 412$) revealed a strong association between increased expression or gene amplification of TAM family members and poor overall and progression free survival (OS and PFS, respectively). *AXL* and *MERTK* expression vary widely among BCa cell lines. In specific BCa cells with robust TAM RTK expression, siRNA-mediated suppression of *AXL* or *MERTK* reduced expression of anti-apoptotic *BCL-2*. Additionally, accumulation of the pre-apoptotic proteins BAX and BAD is induced on *AXL* or *MERTK* suppression, indicating a pro-survival role of the latter in BCa.

Further analysis of TAM RTKs will clarify their oncogenic capacities and establish their importance as potential therapeutic targets in BCa. Results would also help understand if use of small molecule inhibitor of Axl and Mer TK, UNC2025, would be instrumental in reducing the tumorigenic potential of BCa cells, both *in vitro* and *in vivo*.

No conflict of interest

133 (PB-084) Poster

BAT8001, a potent anti-HER2 antibody-drug conjugate with a novel stable linker for the treatment of HER2-positive gastric cancer

J.C. Yu[1], W. Tang[1], X. Deng[1], Z. Ou[1], J. Gan[1], Q. Dong[1], B. Tan[1], L. Lu[1], B. Chen[1], C. Bao[1], B. Thomas[1], S. Li[1]. [1]*Bio-Thera Solutions, Ltd., Research and Development, Guangzhou, China*

Background: HER2 is overexpressed at meaningful levels in a number of tumors, including breast, gastric, salivary duct carcinomas, non-small cell lung, ovarian and colon cancer. Gastric cancer is the third leading cause of cancer death in the world and represents a significant unmet medical need. Trastuzumab emtansine (T-DM1), an anti-HER2 ADC, has shown efficacy in HER2-positive breast cancer patients and was approved by the FDA and EMA for advanced HER2-positive breast cancer. However T-DM1 causes grade 3 and 4 thrombocytopenia in up to 14.5% of patients as its major toxicity. The thrombocytopenia is likely caused by one of T-DM1's catabolites and payload, DM1, indicating T-DM1's linker can be cleaved. Here we adopted a novel noncleavable linker and created an anti-HER2 ADC, BAT8001, which is expected be efficacious in HER2-positive cancers and have a better side effect profile relative to T-DM1 due to the stability of BAT8001's noncleavable linker.

Material and Methods: The payload of BAT8001, a maytansine derivative, is a fermentation product from a bacteria strain Actinosynnema pretiosum. The maytansine derivative is then connected to the linker through chemical reactions, followed by conjugation with the antibody. Annexin V-staining apoptosis assay, 72-hr cell proliferation assay, mouse tumor models, pharmacokinetic and toxicology studies were performed using standard methods.

Results: BAT8001 is internalized in HER2-positive cells. It inhibits proliferation of HER2-positive tumor cells with IC50s of $\sim$0.1 nM, similar to the potency of T-DM1. BAT8001 also induces apoptosis in HER2-positive cells. In both cell-line and patient-derived mouse xenograft (PDX) models, BAT8001 demonstrates strong inhibition activity on tumor growth. For example, in a PDX mouse model of gastric cancer (GA0060), BAT8001 demonstrates a dose response curve with complete responses in all animals tested at the 15 mg/kg dose level. Pharmacokinetics studies in monkey reveals BAT8001 has similar C_{max}, AUC, and $t_{1/2}$ as T-DM1. The major catabolite of BAT8001 is the Cys-linker-payload containing product. No free payload is observed. This compares favorably with T-DM1 where free DM1, T-DM1's payload, is one of the major catabolites. In a multiple dose toxicity study, BAT8001 had a NOAEL of 15 mg/kg versus 10 mg/kg for T-DM1.

Conclusions: BAT8001 exhibits similar potency to T-DM1 on inhibiting HER2-positive cell proliferation and tumor growth, yet demonstrates better multiple dose toxicity than T-DM1. The improved toxicity profile of BAT8001 suggests that the novel noncleavable linker utilized in BAT8001 is more stable than the linker utilized in T-DM1. BAT8001 is very efficacious in PDX and cell-line xenograft models of gastric cancer. The preclinical profile of BAT8001 warrants further development for the treatment of gastric cancer and other HER2-positive cancers.

Conflict of interest: Ownership: All authors are employees of Bio-Thera Solutions, Ltd and are eligible for incentive stock options. Board of Directors:

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134 (PB-085) Poster

Chemotherapy-induced metastasis: mechanisms and translational opportunities

G. Karagiannis[1], L. Rivera Sanchez[1], Y. Wang[1], V. Sharma[1], J. Burt[1], D. Entenberg[1], M. Oktay[1], J. Condeelis[1]. *[1]Albert Einstein College of Medicine, Anatomy and Structural Biology, Bronx, USA*

Background: We have previously discovered that chemotherapy induces prometastatic changes in the breast cancer microenvironment by promoting the assembly and function of cancer cell intravasation sites called tumor microenvironment of metastasis (TMEM), and by increasing the proportion of the highly-invasive Mena^{INV-HI} tumor cells that utilize TMEM sites for hematogenous dissemination. Consequently, chemotherapy-treated animals have increased circulating-tumor cell and micrometastasis counts. Since the formation of metastases depends on both the presence of functional doorways for dissemination (TMEM) and cancer cells capable of using these doorways (Mena^{INV-HI} tumor cells), we investigated the cellular/molecular context required for chemotherapy-driven TMEM function and Mena^{INV} expression.

Materials and Methods: We used spontaneous models of mouse breast carcinoma (MMTV-PyMT) and patient-derived xenografts treated with chemotherapy and co-treated with Tie2 inhibitor (rebastinib), Cxcl12 inhibitors (AMD3100) or macrophage lineage depletion agents (clodronate). Prometastatic endpoints were measured by intravital imaging, multichannel immunofluorescence and standard metastatic dissemination assays.

Results: Since TMEM function depends on Tie2^{+} macrophages and Mena^{INV} expression on macrophage contact, we focused on inhibiting Tie2 receptors, as well as macrophage influx and/or function to suppress chemotherapy-induced metastasis. We demonstrated that chemotherapy-mediated induction of Mena^{INV-HI} tumor cells depends on the presence of macrophages via multiple methods of macrophage suppression. In particular, the depletion of either the entire macrophage lineage using clodronate liposomes, or the use of specific inhibitors of the Cxcl12/Cxcr4 chemotactic pathway, both resulted in a significant suppression of the prometastatic Mena^{INV-HI} cancer cell subpopulation in all mammary tumors examined. Moreover, inhibition of Tie2 by rebastinib blocked TMEM function and decreased the number of circulating tumor cells and metastatic foci, despite the unaffected macrophage infiltration and the chemotherapy-mediated induction of Mena^{INV-HI} tumor cells. Distance analysis algorithms further demonstrated that direct tumor cell-macrophage touching is required for chemotherapy-mediated Mena^{INV} induction. Accordingly, the acute inhibition of Notch pathway, using gamma-secretase inhibitors along with chemotherapy eliminated the MENA^{INV-HI} prometastatic phenotype.

Conclusions: Our data indicate that both the Mena^{INV-HI} disseminating cancer cell population and the TMEM doorways are necessary but not individually sufficient for metastasis. As such, suppression of either of the two, Mena^{INV-HI} population or TMEM function, can suppress chemotherapy-induced metastasis, thus providing targets to improve clinical care and eliminate all the non-beneficial effects of chemotherapy.

No conflict of interest

135 (PB-086) Poster

Cell cycle intervention beyond palbociclib; preclinical discovery of the CDK2/4/6 inhibitor PF-06873600

S. Dann[1], K. Freeman-Cook[2], N. Miller[1], R. Hoffman[2], J. Chionis[1], K. Eisele[1], Z. Qin[1], C. Liu[1], B. Murray[1], M. Xu[1], S. Ninkovic[2], S. Sutton[2], D. Behenna[2], J. Solowiej[1], P. Wei[1], S. Weinrich[1], N. Asako[2], N. Huser[1], C. Zhang[1], R. Visswanathan[2], B. Boras[2], T. Vanarsdale[1], M. White[1]. *[1]Pfizer, Oncology R&D, La Jolla, USA; [2]Pfizer, Medicinal Sciences, La Jolla, USA*

Background: Phosphorylation of the retinoblastoma protein (RB1) by cyclin-dependent kinases (CDK) 4 and 6 is a critical checkpoint for G_1/S cell cycle progression and commitment to cellular proliferation. Palbociclib (IBRANCE®), a highly selective inhibitor of CDK4/6, significantly improves progression free survival, and is approved for prescription use, in hormone receptor positive (HR+) HER2 negative breast cancer when combined with an anti-hormonal agent. In anticipation of intrinsic and acquired therapy-refractory disease we investigated CDK4/6 inhibitor resistance mechanisms in preclinical models of breast cancer. We sought to expand pharmacological control of the cell cycle by selectively targeting the G_1 kinases CDK2, -4 and -6 with a single small molecule inhibitor.

Material and Methods: A comprehensive drug discovery campaign consisting of high-throughput biochemical screening, hit-to-lead structure based drug design and synthesis, biochemical counter-screening, cellular pharmacodynamic/functional response screening, and *in vivo* pharmacodynamic, tumor growth inhibition and safety studies was completed to generate a candidate molecule for use in human clinical studies.

Results: CDK1 and CDK9 anti-targets share several physical structure characteristics with the G_1 kinases making chemical design iterations particularly challenging. Relying heavily on advanced structure based drug design techniques, PF-06873600, a pyridopyrimidine with potent biochemical activity on CDK2/4/6 and >40 fold selectivity over anti-targets, was characterized in models of CDK2 and CDK4/6 driven tumor growth for pharmacodynamic and efficacy response.

Conclusions: Palbociclib resistance models derived from either expression of CDK2-activating cyclin E or deletion of RB1 are sensitive to genetic interruption of CDK2 signaling. Pharmacokinetic, safety and translational discovery data for PF-06873600, generated as rationale for a phase I clinical investigation (ClinicalTrials.gov Identifier: NCT03519178), will be discussed in detail.

*All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and use committee.

Conflict of interest: Other Substantive Relationships: All authors are employed by and/or own shares of Pfizer.

136 (PB-087) Poster

Inhibiting multifunctional ERK-protein complexes for cancer therapy

T. Kaoud[1], W.H. Johnson[1], N.D. Ebelt[2], A. Piserchio[3], S.V. Ravenstein[1], D. Zamora-Olivares[4], R. Edupuganti[4], R. Sammons[5], M. Cano[1], M. Warthaka[1], C.D. Tavares[6], J. Park[7], P. Ren[5], R. Ghose[3], K.Y. Tsai[8], E.V. Anslyn[4], K.N. Dalby[1]. *[1]The University of Texas at Austin, College of Pharmacy, Chemical Biology & Medicinal Chemistry, Austin, USA; [2]City of Hope National Medical Center, Department of Experimental Therapeutics, Duarte, USA; [3]The City College of New York, Department of Chemistry, New York, USA; [4]The University of Texas at Austin, Department of Chemistry, Austin, USA; [5]The University of Texas at Austin, Department of Biomedical Engineering, Austin, USA; [6]Dana-Farber Cancer Institute, Harvard Medical School, Department of Cancer Biology, Boston, USA; [7]University of Texas MD Anderson Cancer Center, Department of Breast Medical Oncology, Houston, USA; [8]H. Lee Moffitt Cancer Center and Research Institute, Departments of Anatomic Pathology & Tumor Biology, Tampa, USA*

Background: Mutations in pathways that enhance the activity of ERK1 and ERK2 are frequently present in human cancers, reflecting their important roles in tumor initiation, and progression. This notion is reinforced by observations in BRAF V600E mutant melanoma where the majority of the mechanisms of resistance to FDA-approved combination therapies targeting BRAF and MEK involve reactivation of ERK1 and ERK2. Recently, the direct targeting of the ERK enzymes using ATP-competitive inhibitors has emerged as an attractive strategy to overcome acquired resistance to current combination therapies. The ERK enzymes employ unique mechanisms of molecular recognition to engage protein components of the MAPK pathway.

Material and Methods: Chemical, Biochemical, cell biology and in vivo studies have been employed to characterize the mechanism of action of the first-in-class covalent inhibitor of ERK docking interactions.

Results: Here we report the potent targeting of an ERK-protein docking interaction by a small molecule thiotriazole, which abrogates ERK signaling in vivo. The thiotriazole binds covalently to a highly conserved cysteine residue within the D-recruitment site of ERK1/2 with more than a 100-fold discrimination over other MAPKs (e.g. JNK1/2, p38MAPKs and ERK5). Treatment of various BRAF-inhibitor naive or inhibitor-resistant melanoma cell lines expressing BRAF V600E with the thiotriazole for 2 hours induces dose-dependent inhibition of ERK activation and downstream signaling. Inhibition is maintained for up to 5 hours following thiotriazole washout and induces apoptosis and growth inhibition. Treatment of mice carrying a BRAFV600E A375 melanoma xenograft with the thiotriazole blocked tumor growth. Transient expression of a mutant form of ERK2, which is resistant to the thiotriazole, promotes survival of A375 and HEK 293 cells treated with thiotriazole.

Conclusions: This study lays the foundation for developing a new modality for the treatment of solid tumors driven by excessive ERK signaling.

No conflict of interest

137 (PB-088) Poster

EVT601, an allosteric modulator of FGFR with an unprecedented irreversible mechanism of action: the next generation of anti-FGFR therapy?

C. Alcouffe[1], G. Gueguen-Dorbes[2], P. Fons[2], F. Dol-Gleizes[2], D. Sibrac[3], F. Comitani[4], F.L. Gervasio[4], M. Whittaker[5], C. Herbert[3]. [1]*Evotec France SAS, Medicinal Chemistry, Toulouse, France;* [2]*Evotec France SAS, Translational Biology, Toulouse, France;* [3]*Evotec France SAS, In Vitro Biology, Toulouse, France;* [4]*University College London, Chemistry and Structural and Molecular Biology, London, United Kingdom;* [5]*Evotec, Oncology, Abingdon, United Kingdom*

Cell-surface tyrosine kinase receptors (R-TKs) represent a prime target family for drug discovery. Historically, drug identification programs have been dominated by efforts to develop inhibitors that compete for binding with endogenous ligands at orthosteric sites. Despite their advantage over orthosteric antagonists, including greater safety and/or selectivity, allosteric modulators are more difficult to identify.

Here, we report the identification of the next generation of extracellularly-acting allosteric modulator of FGFRs (Fibroblast Growth Factor Receptor) leading to an unprecedented irreversible mechanism of action. Using molecular modeling studies, biochemical and cellular assays, we demonstrated that EVT601 interacts with the D3 domain of FGFRs, then inducing a conformational change of the ectodomain allowing the release of a protease-dependent cleavage site in the juxta-membrane domain of the receptor. Consequently, proteases belonging to the MMP-ADAMs family cleaves FGFRs inducing the release of its ectodomain. This biological process is called shedding. By rerouting a naturally occurring biological process with a small molecule allosteric compound, EVT601 leads to the full blockage of FGFR-dependent signaling pathways. We also demonstrated that EVT601 is active whatever the mutation status of the receptor, inhibiting extracellular and intracellular, FGF-dependent or FGF-independent as well as gatekeeper mutations of FGFRs; addressing most of the FGFRs dysregulations observed in cancer.

Oral administration of EVT601 inhibits angiogenesis, tumor growth and the development of inflammatory diseases like psoriasis and arthritis. Therefore, most of the currently anti-FGFR therapies in clinical trials are pan-FGFR TK inhibitors. Because such molecules interfere with endocrine FGFs capabilities (like FGFR1 in kidney), they provoke adverse effects like soft tissue and vasculature mineralization. By its mechanism of action, EVT601 doesn't not disrupt endocrine FGFs physiology, then exhibiting a safer profile than FGFR-TK inhibitors.

FGFR allosteric antagonism offers a new generation of anti-FGF therapies with an unique mode of action. These results open the way for safer anti-FGFR therapies in the treatment of cancers and FGFR-dependent diseases.

No conflict of interest

138 (PB-089) Poster

Differential dependency of BRAF-mutant melanoma on ERK2 Versus ERK1

M. Crowe[1], T. Zavorotinskaya[1], C. Voliva[1], D. Stuart[1], A. Freeman[1]. [1]*Novartis Institutes for Biomedical Research, Oncology, Cambridge, USA*

Background: Approximately half of advanced human melanomas express BRAF-V600E and are highly dependent on this oncogene for constitutive signaling through the MAPK pathway. Recently, we described a large-scale shRNA screen to determine the dependency of 398 human cancer cell lines on the expression of 7,837 genes. Interestingly, most BRAF-V600E-mutant melanoma lines were dependent on expression of ERK2 but not ERK1, suggesting a differential dependency on these closely related kinases, despite sharing 84% amino acid homology, including 100% identity in kinase domains and both substrate binding domains.

Material and Methods: ERK1 and ERK2 were knocked down by shRNA in a panel of human melanoma lines, after which proliferative ability was assessed. Lentiviral shRNA-resistant cDNAs for ERK1 or ERK2 were then expressed in these lines to assess their ability to rescue ERK2 knockdown. Cells were also treated with an ERK1/2 inhibitor, and the ability of inhibitor-resistant mutants of ERK1 and ERK2 to rescue viability was assessed.

Results: Knockdown of ERK2 led to proliferation arrest in a panel of human melanoma cells, whereas ERK1 knockdown had only a modest effect. Re-expression of either shRNA-resistant ERK1 or ERK2 was able to rescue proliferation, suggesting that ERK1 is able to functionally compensate for ERK2 loss in these lines. Expression of inhibitor-resistant mutants of either ERK1 or ERK2 rescued viability in the presence of an ERK inhibitor. Analysis of expression levels of ERK1 and ERK2 suggests that ERK2 is expressed at higher levels than ERK1 in these lines, and this may account for the differences in sensitivity to loss of these kinases. MAPK signaling, as assessed by Western blot, revealed that ERK2 knockdown led to greater suppression of ERK targets than ERK1 knockdown. Finally, gene expression changes upon treatment with MAPK pathway inhibitors more closely resembles those seen upon knockdown of ERK2, whereas ERK1 knock-down and non-targeting shRNA led to few changes or less complete suppression of MAPK signaling.

Conclusions: We found that melanoma cell lines are dependent on continued expression of ERK2 but not ERK1. This dependency may be due to differences in expression of these kinases, as ERK1 was able to functionally substitute for ERK2 and inhibitor-resistant mutants of both ERK1 and ERK2 could rescue viability of these cells in the presence of an ERK1/2 inhibitor. However, this dependency could potentially be exploited through development of ERK2-selective inhibitors, possibly sparing on-target toxicity of MAPK inhibition in normal tissues in patients. In addition to the therapeutic implications, a deeper understanding of the underlying biology could provide insights into the mechanism of transformation by BRAF-V600E.

No conflict of interest

139 (PB-090) Poster

Development of inhibitors of the activated form of KRAS G12C

R. Pollock[1], M. Stewart[1], N. Perl[2], S.J. Lee[3], L. Xue[3], M. Zhou[3], J. Simon[1], K. Luly[1], S. Grigoriu[3], A. Yuzhakov[3], A. Silver[3], J. Lowe[2], A. Mann[2], G. Verdine[2], A. Rigby[1], M. Mulvihill[2], E. May[3], A. Kohlmann[2], S. Townson[3], M. Jin[2]. [1]*Warp Drive Bio, Inc., Biology, Cambridge, USA;* [2]*Warp Drive Bio, Inc., Chemistry, Cambridge, USA;* [3]*Warp Drive Bio, Inc., Physical Biochemistry, Cambridge, USA*

RAS oncogenes are mutated in ~ 1/3 of all human cancers, however, direct pharmacological inhibition of RAS has proved challenging. RAS mutations impair the ability of the protein to hydrolyze GTP to GDP. As a result, mutant RAS proteins exist predominantly in the activated GTP-bound state leading to constitutive aberrant downstream signaling via interaction with effectors such as RAF. Most RAS mutations occur at glycine 12 of the KRAS isoform. One such mutation in which glycine is mutated to cysteine (G12C) is particularly common in non-small cell lung cancer where it is found in ~15% of lung adenocarcinomas.

We deployed a novel SMART^{TM} (Small Molecule Assisted Receptor Targeting) platform to advance covalent compounds that selectively inhibit G12C mutant KRAS. These compounds bind non-covalently with Cyclophilin A (CypA), an abundant immunophilin present in all human cells. The CypA-SMART ligand binary complex is then able to form an inhibitory ternary complex with the activated (GTP-bound) form of KRAS^{G12C} via covalent targeting of the mutant cysteine. High-resolution crystal structures of KRAS$^{G12C\text{-}GTP}$|Ligand|CypA complexes reveal extensive interactions between CypA, SMART ligand, and the Switch I/Switch II region of KRAS$^{G12C\text{-}GTP}$. Structure-based drug design yielded potent covalent inhibitors of KRAS$^{G12C\text{-}GTP}$ that exhibit >100-fold selectivity for mutant G12C over WT KRAS. The KRAS$^{G12C\text{-}GTP}$|Ligand|CypA complex directly occludes effector binding, and as such the compounds disrupt the KRAS-RAF interaction with nanomolar EC_{50}s (3 hour time point) in biochemical assays. This activity is dependent on CypA, thus underlining the importance of CypA|KRAS^{G12C} protein: protein interactions in driving target engagement.

In cell-based studies, SMART inhibitors cross-linked KRAS^{G12C} and potently inhibited ERK phosphorylation and tumor cell growth in G12C mutant cell lines but had no effect on non-G12C bearing cells. CRISPR knockout of cellular CypA confirmed that these activities were dependent on the presence of endogenous CypA.

To our knowledge, these are the first examples of mutant-selective KRAS inhibitors that target the activated (GTP-bound) state of KRAS^{G12C}. The fact that they do not rely on the presence of a cellular inactive (GDP-bound) mutant KRAS^{G12C} pool to engage their target may offer advantages in developing KRAS^{G12C} selective therapeutics. In support of this, we have demonstrated that the potency of SMART inhibitors in growth inhibition assays is maintained in the presence of growth factor treatments that reduce the cellular KRAS$^{G12C\text{-}GDP}$ pool, in contrast to previously described KRAS$^{G12C\text{-}GDP}$ targeting inhibitors. We are currently optimizing the drug-like properties of these SMART inhibitors and evaluating their activity in in vivo models.

Conflict of interest: Other Substantive Relationships: Employees and/or shareholders in Warp Drive Bio Inc.

140 (PB-091) Poster

Cell panel profiling yields additional drug response biomarkers for kinase inhibitors approved for clinical use

J.C.M. Uitdehaag[1], J.J. Kooijman[1], J.A.D.M. De Roos[1], M.B.W. Prinsen[1], J. Dylus[1], N. Willemsen-Seegers[1], D.M. Jos[1], S.J.C. van Gerwen[1], R.C. Buijsman[1], G.J.R. Zaman[1]. [1]*Netherlands Translational Research Center B.V., Biology, Oss, Netherlands*

Background: Kinase inhibitors are used as precision medicine in specific patient populations that are defined by the presence of predictive drug response biomarkers. The application of these inhibitors could be further extended and refined by the identification of new response biomarkers. One of the best ways to do this, is by *in vitro* profiling in cancer cell line panels.

Materials and Methods: Oncolines is a panel of 102 genetically characterized cell lines from diverse tumour origins, on which proliferation assays are run in parallel. Earlier work has shown that the Oncolines workflow generates highly reproducible data, as necessary for biomarker discovery. Previously, we profiled all kinase inhibitors that were approved before November 2013 in the panel. Here we supplement this study by profiling the seventeen inhibitors that have been approved for oncology indications between November 2013 and June 2018. This includes the ALK inhibitors ceritinib, brigatinib, and alectinib, the CDK4/6 inhibitors palbociclib, abemaciclib, and ribociclib, the BTK inhibitors ibrutinib and acalabrutinib, and nine other novel marketed inhibitors. Assays were based on ATP-lite readout, with a nine-point duplicate dilution series of the compounds. Drug response was quantified by calculation of IC_{50}, GI_{50}, growth rate inhibition and maximum effect. Response was associated with the genomic status of the cell lines as retrieved from the COSMIC and Cancer Cell Line Encyclopedia (CCLE) databases. Mutations in patient hotspot locations and copy number changes in well-characterized cancer genes were used as input for ANOVA tests. Basal gene expression levels of 400 clinically actionable genes were used as input for correlation analysis.

Results: Profiling of CDK4/6 inhibitors confirms previously reported biomarkers, such as loss of CDKN2A and wild-type RB1. Some sensitive cell lines have wild type CDKN2A but exhibit downregulation of CDKN2A on an mRNA level. Our analysis also suggests new biomarkers, such as TP53 mutation and CCNE1 overexpression. ALK-targeting drugs strongly inhibit cell lines with high expression levels of ALK and also of JAK3. The dual ALK/JAK3 inhibitory profile of brigatinib might therefore explain its high cellular selectivity for cell lines with the NPM-ALK translocation, despite its moderate biochemical selectivity.

Conclusions: The profiling of newly approved kinase inhibitors in the Oncolines cell line panel shows that nearly all clinically-used drug response biomarkers can be identified from tumor-agnostic profiling in a hundred cell lines. Clinically useful biomarkers, such as NPM-ALK translocation or mutant BRAF(V600E), are characterized by high potency differences between marker-positive and marker-negative cell lines. The combination of other predictive drug response biomarkers might be necessary to yield a clinically useful sensitivity window.

Conflict of interest: Ownership: Guido Zaman and Rogier Buijsman are founders and shareholders of Netherlands Translational Research Center B.V.

141 (PB-092) Poster

Augmented apoptosis and DNA damage by the combined inhibition of CHK1 and PI3 K/mTOR pathway in high-grade serous ovarian cancer (HGSOC)

T.T. Huang[1], E. Brill[1], C.J. Thomas[2], J.M. Lee[1]. [1]*National Cancer Institute, Women's Malignancies Branch, Center for Cancer Research, Bethesda, USA;* [2]*National Institutes of Health, Division of Preclinical Innovation, National Center for Advancing Translational Sciences, Bethesda, USA*

Background: The majority of HGSOCs have p53 dysfunction, making cells more reliant on the cell cycle checkpoint kinase 1 (CHK1)-mediated G2/M arrest for DNA repair. Thus, CHK1 is a rational therapeutic target for HGSOC. We recently reported an early clinical activity of a CHK1 inhibitor, prexasertib (Prex, LY2606368) in *BRCA* wild-type (*BRCA*wt) recurrent HGSOC patients. We conducted a high-throughput drug screening for potential combinations with Prex in HGSOC cells (1,912 combinations). In this screen, PI3K/AKT/mTOR pathways inhibition showed additive/synergistic cytotoxic effects with Prex. PI3K/AKT/mTOR pathway activation is frequently observed in recurrent HGSOC and is associated with chemoresistance. We therefore hypothesized that the combination of Prex and LY3023414 (LY302), a dual PI3K/mTOR inhibitor, would result in greater cell death compared to single agent alone in HGSOC cells.

Material and Methods: We used a panel of *TP53* mutated HGSOC cell lines [*BRCA*wt (OVCAR5 and OVCAR8), *BRCA2* mutant (PEO1) or *BRCA2* gain-of-function (PEO4)] to validate the screening findings. We examined the cell viability and IC50 of Prex and LY302 by XTT assay. Combination index (CI) was evaluated by CompuSyn software. Caspase 3/7 activity was examined for apoptosis. Immunofluorescence staining of γH2AX was performed for DNA damage. All experiments were performed at least three times. Data were analyzed using Student's *t*-test and shown as mean±SD. $P < 0.05$ was considered statistically significant.

Results: IC50 of Prex and LY302 in all cells ranged from 7 to 53 nM and 276 to 726 nM, respectively. Prex/LY302 combination indicated synergy (CI < 1) in all cells. As shown in table 1, the combination therapy using clinically attainable concentrations of Prex (5 nM) and LY302 (200 nM) attenuated cell viability at least additively compared to single agent alone in all cells. In addition, this combination induced greater caspase 3/7 activation compared to monotherapy alone in all cells. We also observed the combination treatment induced greater DNA damage measured by γH2AX foci formation in OVCAR5, OVCAR8 and PEO1 cells, and a trend in PEO4 cells.

Table 1. The effects of Prex (5nM) and LY302 (200nM) treatments on cytotoxicity, apoptosis and DNA damage

Cell viability(%)	OVCAR5	OVCAR8	PEO1	PEO4
DMSO	100	100	100	100
Prex	60 ± 12	55 ± 14	55 ± 4	82 ± 7
LY302	61 ± 5	57 ± 9	69 ± 5	72 ± 9
Combination	28 ± 7*#	24 ± 5*#	34 ± 7*#	51 ± 3*#
Caspase 3/7 activity (%)				
DMSO	100	100	100	100
Prex	537 ± 41	735 ± 135	130 ± 3	143 ± 9
LY302	146 ± 19	595 ± 66	113 ± 3	122 ± 6
Combination	1,770 ± 20*#	3,142 ± 20*#	170 ± 12*#	168 ± 13*#
Cells with ≥5 **γ**H2AX foci (%)				
DMSO	3 ± 1	5 ± 1	5 ± 1	3 ± 1
Prex	32 ± 3	39 ± 4	44 ± 4	29 ± 2
LY302	19 ± 1	30 ± 3	34 ± 4	7 ± 1
Combination	56 ± 6*#	78 ± 10*#	66 ± 3*#	34 ± 9#

*, # $P < 0.05$; *compared to Prex; #compared to LY302.

Conclusions: Our results suggest that inhibition of CHK1 and PI3K/mTOR pathways together results in greater cytotoxicity in both *BRCA*wt and *BRCA* mutant HGSOC cells by inducing DNA damage and apoptosis.

No conflict of interest

143 (PB-094) Poster

Simultaneous molecular alterations and clinical outcomes in solid tumors with IDH1 or IDH2 mutations

A. Swilling[1], J. de Groot[2], M. Javle[3], M. Penas-Prado[2], R. Shroff[4], R. Luthra[5], K. Banks[6], R. Lanman[6], A. Conley[7], R. Broaddus[8], M. Davies[9], S. Kopetz[3], W.A. Yung[2], J. Heymach[10], S. Fu[1], K. Shaw[11], F. Meric-Bernstam[1], F. Janku[1]. [1]*The University of Texas MD Anderson Cancer Center, Investigational Cancer Therapeutics, Houston, USA;* [2]*The University of Texas MD Anderson Cancer Center, Neuro-Oncology, Houston, USA;* [3]*The University of Texas MD Anderson Cancer Center, Gastrointestinal Medical Oncology, Houston, USA;* [4]*The University of Arizona College of Medicine, Medicine, Tucson, USA;* [5]*The University of Texas MD Anderson Cancer Center, Hematopathology, Houston, USA;* [6]*Guardant Health, Medical Affairs, Redwood City, USA;* [7]*The University of Texas MD Anderson Cancer Center, Sarcoma Medical Oncology, Houston, USA;* [8]*The University of Texas MD Anderson Cancer Center, Pathology, Houston, USA;* [9]*The University of Texas MD Anderson Cancer Center, Melanoma Medical Oncology, Houston, USA;* [10]*The University of Texas MD Anderson Cancer Center, Thoracic Head and Neck Medical Oncology, Houston, USA;* [11]*The University of Texas MD Anderson Cancer Center, Institute of Personalized Cancer Therapy, Houston, USA*

Background: Oncogenic somatic mutations in *IDH1* or *IDH2* genes are prevalent in diverse solid tumors. Therapeutic responses to IDH inhibitors are infrequent plausibly due to presence of simultaneous alterations in compensatory molecular pathways.

Methods: We retrospectively reviewed results from clinical genomic profiling with targeted next-generation sequencing of archival tumor samples (Ion Torrent <50 genes, 300; FoundationOne <343 genes, 29; Oncomine 128 genes, 4) and plasma liquid biopsies (Guardant360 < 73 genes panel, 337 samples) from patients (pts) with solid tumors of all stages. Results from tumor tissue were compared to clinical outcomes (time to treatment failure [TTF] on systemic therapies) and overall survival (OS).

Results: In 333 tumor samples the most represented cancers were gliomas (50%), melanomas (14%), and cholangiocarcinomas (CCA, 11%). In 295 *IDH1*-mutated samples the most frequent simultaneous alterations were in *TP53* (45%), *BRAF* (11%), *KRAS* (9%), and *PIK3CA* (9%). In the most represented *IDH1*-mutated tumor types commonly altered genes were *TP53* (66%) in gliomas, *BRAF* (53% [V600K/R 30%, V600E 19%]) in melanomas, *PIK3CA* (13%), and *CDKN2A* (13%) in CCA. In 38 *IDH2*-mutated tumor samples the most frequent simultaneous alterations were in *TP53* (32%), and *KRAS* (16%). Pts with <1 simultaneous alteration in addition to *IDH* mutations had longer OS than pts with >1 simultaneous alteration ($P = 0.01$). In the tumor specific analysis, longer OS for pts with <1 simultaneous alteration was confirmed in gliomas, but not in other tumor types ($P = 0.03$, $P = 0.29$, respectively). Pts who received IDH inhibitors had longer median TTF compared to their therapies administered at the time of testing ($P < 0.001$).

In 337 plasma samples the most represented cancers were non-small cell lung cancer (NSCLC, 39%), CCA (13%), and breast cancer (8%). In 172 *IDH1*-mutated samples the most frequent simultaneous alterations were *TP53* (40%), *KRAS* (23%), *EGFR* (16%) and *BRAF* (16%). In the most represented *IDH1*-mutated tumors commonly altered genes were *TP53* (48%), *KRAS* (37%) in NSCLC and *TP53* (37%), *KRAS* (24%), *BRAF* (18%) in CCA. In 161 *IDH2*-mutated samples, the most frequent simultaneous alterations were *TP53* (43%), *EGFR* (20%), and *KRAS* (19%). In the most represented *IDH2*-mutated tumors commonly altered genes were *TP53* (43%), *EGFR* (32%), *KRAS* (18%) in NSCLC and *TP53* (18%), *ESR1* (18%), *KRAS* (18%) in breast cancer. There were 4 tumors with *IDH1* and *IDH2* mutations.

Conclusions: *IDH1* and *IDH2* mutations often coexist with simultaneous oncogenic alterations including these in potentially druggable molecular targets and having >1 simultaneous alteration is a negative prognostic factor for survival. Treatment with IDH inhibitors leads to longer TTF than prior therapy.

Conflict of interest: Ownership: Trovagene (F. Janku). Guardant Health (R. Lanman, V. Raymond). Ziopharm Oncology, Gilead (J. de Groot). Advisory Board: Guradant Health, Novartis (F. Janku). DNAtrix (WK A. Yung). Genentech, Celldex, Foundation Medicine, Inc., Novogen, Deciphera, Astrazeneca, Insys Therapeutics, Kadmon, Merck, Eli Lilly (J. de Groot). Eli Lilly, Ignyta (A.COnley). J. Heymach: AstraZeneca, Boehringer Ingelheim, EMD Serono, Genentech, Eli Lilly, Merck, Roche, Spectrum, Guardant Health, Janssen, Novartis, Foundation Medicine. Corporate-sponsored Research: Agios (R. Shroff). Novartis, Agios, Bayer (F. Janku). Sanofi-Aventis, Astrazeneca, EMD-Serono, Eli Lilly, Novartis, Deciphera Pharmaceuticals, Mundipharma (J. de Groot). Ignyta(A.Conley). Other Substantive Relationships: Trovagene (F. Janku). Agios (M. Penas-Prado). Celldex, Deciphera Pharmaceuticals, AbbVie, FivePrime Therapeutics, Inc., GW Pharma, Carthera, Eli Lilly, Boston Biomedical Inc., Kairos Venture Investments, Syneos Health, Monteris, DSMB: VBL Therapeutics, DSMB: Novella, VBI Vaccines, Inc., Ziopharm Oncology (J. de Groot). Novartis (A. Conley)

144 (PB-095) Poster

Autophagy dependence of small molecule angiokinase inhibitors in colorectal cancer (CRC)

A.K. Larsen[1], P. Mésange[1], M. Sabbah[1], L. Louadj[1]. *[1]Centre de Recherche Saint-Antoine CRSA, INSERM U938, Faculté de Médecine, Sorbonne Université, Cancer Biology and Therapeutics, Paris, France*

Background: Autophagy plays an important role in the response of tumor cells to environmental stress. Small molecule angiokinase inhibitors target multiple receptor tyrosine kinases that mediate downstream signaling pathways that can be integrated at the level of mTOR, a central regulator of cell metabolism. We here wish to establish the influence of autophagy on the activity of nintedanib and regorafenib, two angiokinase inhibitors with clinical activity in metastatic CRC.

Material and Methods: The antiproliferative activity of nintedanib and regorafenib was determined by the MTT viability assay and by colony formation. Autophagy induction was determined by immunocytochemistry and Western blot of LC3 and by the Autophagy Blue™ fluorescence assay. CRC cells with attenuated Beclin1 expression were obtained by stable Beclin1 knock-down (sh-Becline1).

Results: Nintedanib and regorafenib showed comparable activity toward a panel consisting of 12 well-characterized CRC cells, with average IC_{50} values between 2 and 2.6 μM. However, the activity profile between the two agents was different. Immunocytochemistry and Western blot of the autophagic marker LC3 as well as the Autophagy Blue™ fluorescence assay showed that nintedanib triggers an autophagic response, which was much less prominent for regorafenib. Interestingly, addition of the autophagy inhibitor 3-methyladenine decreased the cytotoxic activity of nintedanib up to 3-fold, but had no influence on the activity of regorafenib. In agreement, genetic models with attenuated expression of the autophagy regulator Beclin 1 showed up to 3-fold decreased sensitivity to nintedanib, but the same sensitivity to regorafenib.

Conclusions: Autophagy contributes to the cytotoxic activity of nintedanib. Tumor cells with high autophagic flux may be selectively sensitive to nintedanib.

Conflict of interest: Corporate-sponsored Research: This project was financed in part by Boehringer-Ingelheim.

145 (PB-096) Poster

Influence of the epithelial-mesenchymal transition (EMT) on the response to VEGF-targeted agents in first- and second-line treatment of colorectal cancer

A.K. Larsen[1], L. Louadj[1], S. Thouroude[1], B. Chibaudel[2], J.P. Thiery[3], J. Denis[1], A. De Gramont[2], A. Bouygues[1]. *[1]Centre de Recherche Saint-Antoine CRSA, INSERM U938, Faculté de Médecine, Sorbonne Université, Cancer Biology and Therapeutics, Paris, France; [2]Centre de Recherche Saint-Antoine CRSA, INSERM U938, Institut Hospitalier Franco-Britannique, Medical Oncology, Levallois-Perret, France; [3]National University of Singapore, Yong Loo Lin School of Medicine, Singapore, Singapore*

Background: VEGF is a validated target for treatment of metastatic colorectal cancer (mCRC) with bevacizumab and aflibercept being approved for first- and second-line treatment, respectively. Unfortunately, there are no clinically validated biomarkers to predict which patients are likely, or not, to respond to VEGF-targeted agents. Recently, different CRC subtypes have been identified including a mesenchymal subgroup with a high microvascular density and poor prognosis. Clinical findings from other cancer types like ovarian cancer suggest that the tumor microvascular density may be predictive for the response to bevacizumab. We here wish to (1) establish if the mesenchymal phenotype is predictive for the response to VEGF-targeted agents, (2) compare the first-line activity of bevacizumab and aflibercept in CRC models with different levels of EMT, and (3) to determine if aflibercept is active toward CRC xenografts progressing on bevacizumab.

Material and Methods: CRC cell lines were examined for the expression of epithelial (E-cadherin, gamma-catenin, cytokeratin 18) and mesenchymal (vimentin, N-cadherin, fibronectin) markers *in vitro* and in the corresponding tumor xenografts by qRT-PCR and Western blot analysis, while the cellular distribution of E-cadherin, alpha-catenin and beta-catenin was determined by ICC. Five CRC models were selected with a phenotype ranging from pronounced epithelial (HT-29), intermediate (DLD-1, HCT-116) to mesenchymal (HCT-116/5-FU, LS174T) and the tumor growth inhibitory activity of bevacizumab and aflibercept was established. The microvascular density was characterized by quantitative IHC analysis.

Results: The results show that two CRC xenograft models were sensitive to both bevacizumab and aflibercept, two models were sensitive to aflibercept but not to bevacizumab, and one model was resistant to both agents. The mesenchymal phenotype was associated with higher microvascular density, but not with the response to neither bevacizumab nor aflibercept. Cross-over experiments showed second-line activity of aflibercept in two of the three CRC tumors progressing on bevacizumab.

Conclusions: Neither the mesenchymal phenotype nor the initial microvascular density was predictive for the response to VEGF-targeted agents. Aflibercept showed second-line activity in CRC models progressing on bevacizumab.

Conflict of interest: Corporate-sponsored Research: This project was financed in part by Sanofi-Genzyme

146 (PB-097) Poster

Novel c-kit exon 9 mutations in GISTs: is imatinib good for bad?

E. Laurini[1], S. Aulic[1], M. Fermeglia[1], R. Riboni[2], M. Lucioni[2], E. Dellera[2], M. Alessiani[3], V. Perfetti[3], S. Pricl[1]. *[1]University of Trieste, MolBNL@UniTS, DEA, Trieste, Italy; [2]IRCSS Policlinico San Matteo, Department of molecular medicine and anatomic pathology, Pavia, Italy; [3]Ospedale SS. Annunziata, Internal Medicine, Pavia, Italy*

Background: Gastrointestinal stromal tumors are mesenchymal tumors arising in the stomach and small bowel and more rarely in the rectum, esophagus, peritoneum and retroperitoneum. These tumors are characterized by KIT or PDGFRA mutations. KIT mutations are all in frame and lead to constitutive tyrosine kinase domain activation without ligand binding. Mutations concern four exons (9, 11, 13 and 17) but mainly exon 11.

Here we report two new KIT exon 9 mutations, belonging to the D5 domain in the extracellular portion of the receptor – namely the deletion of two residues ΔA502-Y503 and the missense substitution K509N – found in a GIST patient at diagnosis. The patient was treated with the tyrosine kinase inhibitor (TKI) Imatinib, and responded well.

Materials and Methods: Immunoprecipitation and immunoblotting assays were performed to measure the tyrosine kinase activity for wild type and the oncogenic KIT mutants. The relationship between the receptor's half-life and the responsiveness to ligand stimulation was studied by treating KIT-expressing cells with cycloheximide, which prevent protein synthesis, and monitoring the degradation of the KIT receptors. To explore the effect of TKIs on oncogenic KIT activation, IP and IB assays were performed after cellular incubation with imatinib (and sunitinib for comparison). The efficacy of Imatinib against oncogenic KIT mutations were further examined by following the effect of Imatinib on colony formation in soft agar of NIH 3T3 cells harboring oncogenic KIT mutants. To understand the mechanism of ligand independent activation of the oncogenic ΔA502-Y503 and K509I D5 KIT mutants, we performed experiments aimed at comparing the capacity of different variants of KIT D4D5 fragments to form dimers in solution. To the purpose, soluble variants of WT or oncogenic D5D5 mutants were expressed in Sf9 insect cells and purified. Different concentrations of these variants were subjected to size exclusion chromatography and dimerization was monitored using multiangle laser light scattering and refractometer index detectors to obtain their weight average molecular mass (Mw). The corresponding dimerization constants Kd were determined by fitting the Mw.

Results: We found that full length KIT variants harboring these mutations exhibit elevated basal tyrosine kinase activity, which can be further stimulated by SCF binding. The dimerization constant of isolated D4D5 KIT domains of both oncogenic ΔA502-Y503 and K509N are increased by up to 20 fold as compared to WT D5, resulting in elevated tyrosine autophosphorylation of unoccupied KIT.

Conclusions: Both ΔA502-Y503 and K509N are ligand-sensitized oncogenic KIT mutations, and both Imatinib and Sunitinib can suppress – albeit with different potency – SCF-induced mutated KIT tyrosine phosphorylation.

No conflict of interest

147 (PB-098) Poster

Impact of regulatory T cells on cellular cytotoxicity induced by ERY974, a novel T cell–redirecting bispecific antibody targeting glypican-3

Y. Sonobe[1], Y. Miyazaki[1], Y. Sano[1], Y. Kinoshita[1], Y. Azuma[1], T. Tsunenari[1], T. Ishiguro[2], S. Kishishita[3], M. Endo[1], J. Nezu[1]. *[1]Chugai Pharmaceutical Co., Ltd, Research Division, Kanagawa, Japan; [2]Chugai Pharmaceutical Co., Ltd, Translational Clinical Research Division, Tokyo, Japan; [3]Chugai Pharmaceutical Co., Ltd, Project & Lifecycle Management Unit, Tokyo, Japan*

Background: ERY974 is a humanized IgG4 bispecific T cell–redirecting antibody (TRAB) currently in Phase 1 clinical trial (NCT02748837). ERY974 consists of a common light chain and two different heavy chains that respectively recognize glypican-3 (GPC3) and CD3. ERY974 simultaneously binds to GPC3 on the cancer cell surface and to CD3 on the T cell surface, and induces TRAB-dependent cellular cytotoxicity mediated by the effector function of T cells. Because CD3 is universally expressed on T cells, ERY974 theoretically could be capable of also redirecting and activating regulatory T cells (Tregs), which possess immune-suppressive activity. We evaluated the impact of Tregs on TRAB-dependent T cell activation and cytotoxic activity.

Materials and Methods: To evaluate the effect of Tregs on the in vitro cellular cytotoxic activity induced by ERY974, CD4+ CD25+ CD127dim/- Tregs were isolated from human PBMCs by magnetic-activated cell sorting followed by expansion using beads conjugated with antibodies against CD3 and CD28 and with recombinant hIL-2. Then in PC-10, which is a GPC3-expressing tumor cell line, ERY974-induced cellular cytotoxicity and the T cell activation status were evaluated using expanded Tregs or CD4+ T cells derived from the same donor as effector T cells (Teffs). Furthermore, we evaluated the suppressive activity of Tregs against ERY974-activated Teffs in various Teff/Treg ratios.

Results: In the GPC3-positive cancer cell line, CD4+ Teffs showed ERY974-dependent activation, proliferation, and cellular cytotoxic activity, while expanded Tregs did not. In addition, Tregs inhibited ERY974-dependent proliferation and granzyme B production of CD8+ Teffs in a manner dependent on the Teff/Treg ratio. As for the amount of Tregs, ERY974 treatment did not expand the suppressive Tregs.

Conclusions: These preclinical data suggest that ERY974 is unlikely to increase Tregs by CD3 stimulation, but Tregs potentially attenuate the antitumor efficacy of ERY974 against GPC3-expressing tumors. Now, the combinational effect of ERY974 and an anti-CTLA4 antibody is being further investigated in mouse syngeneic tumor models to explore the effect of a Treg-targeting agent on the antitumor activity of ERY974 against GPC3-positive cancer.

No conflict of interest

Tuesday, 13 November 2018

POSTER SESSION

Oncolytic viruses

148 (PB-099) Poster

Clinical and correlative data from a first-in-human (FIH) study of the intratumoral (IT) oncolytic virotherapy Voyager-V1 (VV1) in patients (pts) with refractory solid tumors

M. Patel[1], S. Powell[2], J. Merchan[3], J. Strauss[4], T. Cripe[5], M. Old[6], A. Bexon[7], R. Diaz[7], M. Reckner[8], L. Russell[7], N. Packiriswamy[9], B. Bethany[9], D. Upreti[9], R. Khan[9], L. Suksanpaisan[10], R. Vandergaast[10], K.W. Peng[7], S. Russell[7]. *[1]University of Minnesota, Dept of Medicine, Minneapolis, USA; [2]Sanford Cancer Center, Hematology, Medical Oncology, Sioux Falls, USA; [3]University of Miami, Hematology/Oncology, Miami, USA; [4]Mary Crowley Cancer Research, Clinical Research, Dallas, USA; [5]Nationwide Children's Hospital, Hematology and Oncology, Columbus, USA; [6]Ohio State University Comprehensive Cancer Center, Otolaryngology, Columbus, USA; [7]Vyriad, Clinical Development, Rochester, USA; [8]Bexon Clinical Consulting, Clinical Development, Montclair, USA; [9]Mayo Clinic, Research, Rochester, USA; [10]Imanis Life Sciences, Diagnostics, Rochester, USA*

Background: VV1 is derived from VSV, an RNA virus with low human seroprevalence, engineered to replicate selectively in and kill human cancer cells. VV1 encodes hIFNβ to boost antitumoral immune response, plus the thyroidal sodium iodide symporter NIS gene to allow noninvasive imaging of virus spread. VV1 is synergistic with different anti-PD-(L)1 antibodies in preclinical models. Here we report FIH data from IT monotherapy dose escalation.

Material and Methods: This is a 2-part, 2-arm, open label parallel phase 1 study with a 3 + 3 design. Single VV1 doses from 3×10^6 to 3×10^9 $TCID_{50}$ are injected IT into one target lesion. VV1 is given alone in the 1st escalation and in combination with IV anti-PD-L1 in the 2nd. Objectives include identifying MTD/RP2D alone and in combination, preliminary efficacy, PK by RT-PCR for viral genomes, serum IFNβ levels, and Tc-99m SPECT/CT imaging. Each escalation leads to RP2D expansion in pts with metastatic colorectal cancer (mCRC).

Results: The study is ongoing at dose level 5 (n = 16+). No DLTs have been observed. Most pts were male (69%), white (88%), with ECOG PS 1 (69%) and median 5 lines of prior systemic therapy for head & neck (SCCHN, 5), mCRC (4), pancreas (2), breast (2) or other (3) cancers. AEs (in 81% pts) related to VV1 were mild-moderate, short-lived clinical AEs or transient G2-3 neutro- or lymphopenia at higher doses. Most pts had mild biopsy- or injection-related AEs (pain, bruising, subclinical pneumothorax) and 1 had an SAE related to biopsy (G2 pneumothorax). More pts had related AEs at higher doses, including a G2 cytokine release syndrome. 6 pts at higher doses had positive SPECT/CTs showing viral replication in tumor ±/- concomitant lymphocyte/neutrophil trafficking. 2 pts had IFNß in serum but no positive viral titers or shedding. 1 pancreas cancer pt had tumor cavitation with cystic fluid positive for viral RNA. One SCCHN pt had SD on CT at D43.

Conclusions: A single IT injection of the novel oncolytic virotherapy Voyager-V1 is safe and shows correlative signals at doses up to 3×10^8

Table (abstract 148 PB-099)

VV1-related AE >1 pt n (%)	All (n = 16)	3 × 10^6 DL1 (n = 3)	1 × 10^7 DL2 (n = 3)	3 × 10^7 DL3 (n = 4)	1 × 10^8 DL4 (n = 4)	3 × 10^8 DL5 (n = 2)
Any	13 (81)	2 (67)	1 (33)	4 (100)	4 (100)	2 (100)
Nausea	4 (25)	1 (33)	0	2 (50)	1 (25)	0
Fever	4 (25)	2 (67)	0	0	1 (25)	1 (50)
Hypotension	4 (25)	0	0	1 (25)	1 (25)	2 (100)
Sweating	3 (19)	0	1 (33)	2 (50)	0	0
Rash	3 (30)	1 (33)	0	2 (50)	0	0
Fatigue	3 (19)	2 (67)	0	0	0	1 (50)
Chills	3 (19)	1 (33)	0	1 (25)	1 (25)	0
Hot flashes	2 (13)	0	1 (33)	1 (25)	0	0
Neutropenia	2 (13)	0	0	1 (25)	1 (25)	0
Lymphopenia	2 (13)	0	0	1 (25)	1 (25)	0
Malaise	2 (13)	0	0	0	2 (50)	0

$TCID_{50}$. A combination arm with a checkpoint inhibitor and expansion in patients with MCRC will be initiated later this year.

Conflict of interest: Ownership: SJ Russell and KW Peng are shareholders of Vyriad (Sponsor) and Imanis Life Sciences (Diagnostics Vendor). Advisory Board: NA. Board of Directors: SJ Russell is a member of Vyriad's BOD. Corporate-sponsored Research: This is a Vyriad-sponsored study. Other Substantive Relationships: AS Bexon and M Reckner are paid consultants to Vyriad. AS Bexon is Chief Medical Officer, KW Peng is Chief Technology Officer, SJR is Chief Executive Officer of Vyriad.

Tuesday, 13 November 2018

POSTER SESSION

Vaccination

149 (PB-100) Poster

Novel Apolipoprotein-A1 (ApoA-I) multimers, Cargomer®, as new targeted delivery platform for therapeutic cancer vaccines with tumor neo- and shared-antigens

V. Albinet[1], E. Lacoste[2], R. Baron[3], R. Palmantier[4], M. Frohlich[5], J.L. Dasseux[6]. [1]*Imavita SAS, Research & Development, Toulouse, France;* [2]*Imavita SAS, Chief Scientific Officer///CSO, Toulouse, France;* [3]*RND-Pharma, President, Toulouse, France;* [4]*ImmThera Consulting SAS, President, Toulouse Area, France;* [5]*Former Executive V.P. of Portfolio Strategy at Juno Therapeutics, Research & Development, Seattle, USA;* [6]*Cerenis Therapeutics Holding SA., President and Chief Executive Officer, Balma, France*

Background: Therapeutic vaccination is a highly promising tumor-specific approach in immuno-oncology but has shown insufficient clinical efficacy, so far, in part due to poor cross-presentation efficiency and weak cytolytic CD8 (CTL) response. The development of new technologies to target the delivery of Tumor Antigens (TA) and immunostimulants into antigen-presenting cells (APC) and induce a potent tumor-specific CTL response both alone and in combination with immune checkpoint inhibitors (ICI) remains a major goal.

ApoA-I is the major protein of HDL that plays an essential role in lipid transport and delivery via receptors including the scavenger receptor SR-B1 that is expressed on hepatocytes, macrophages and dendritic cells. Cerenis®'s recombinant human (rh)ApoA-I pre-b discoïdal HDL mimetic, CER-001®, is currently evaluated in phase III clinical trials in patients with genetic HDL deficiencies.

Cargomer® are innovative rhApoA-I multimeric complexes developed and are as fully biocompatible nanoparticles as CER-001®, to enhance the targeted delivery of TA and immunostimulatory molecules to APC.

Methods: TA (TRP2, M27, and M30 peptides) and CpG, a TLR9 agonist, have been chemically coupled to modified phospholipids (PL) and Chol with a functionalized linker. Different Cargomer® preparations of highly purified clinical grade rhApoA-I charged with Chol-CpG and PL-TA were produced and purified. Four days after the SC inoculation of B16F10 tumor cells, mice were immunized SC 3x qw, with Cargomer® alone or in combination with ICI treatment, or with controls. Tumor growth, weight, clinical signs and survival were monitored until euthanasia according to ethical end-points. Tumor late challenge in survivors was performed to assess long term protection.

Results: The mice immunized with Cargomer®/TAs/Chol-CpG showed a significant inhibition of tumor growth, which synergized when combined with the ICI. ICI alone, TAs/CpG-Chol alone (i.e., not formulated in Cargomer®) or rhApoA-I alone did not show efficacy. Survival was increased in all Cargomer®-treated groups and demonstrated synergistic efficacy up to 100% and 62.5% of tumor-free animals at d30 and d70, respectively.

Groups	Median survival (days)
Vehicle	23.5
rhApoA-I	22
TAs+CpG	22
Cargomer® 1:2	26
Cargomer® 1:4	46
Cargomer® 1:2+ICI	>70
Cargomer® 1:4+ICI	56.5
ICI	24.5

Cargomer®/TAs/Chol-CpG elicited a long term immune memory response since survivors had substantially delayed and slowed tumor growth after challenge with new B16F10 cells compared to newly tumor challenged naïve mice.

Conclusions: This work demonstrates that rhApoA-I Cargomer® can be effectively loaded with neo and shared peptide antigens and TLR agonists to generate a robust immune and therapeutic responses.

Conflict of interest: Other Substantive Relationships: Consulting services paid by Cerenis Therapeutics SA.

Wednesday, 14 November 2018

POSTER SESSION

Adoptive Cell Transfer therapy

150 (PB-001) Poster

Biomarkers of Fc-gamma receptors (FcγRs) on mononuclear phagocyte system (MPS) cells in blood of patients with advanced gastric cancer are upregulated as compared to patients with metastatic breast cancer

W. Kirschbrown[1], A. Lucas[2], C. Li[1], S. Girish[1], W. Zamboni[2], A. Garg[1]. [1]*Genentech Research and Early Development, Clinical Pharmacology, South San Francisco, USA;* [2]*UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Division of Pharmacotherapy and Experimental Therapeutics, Chapel Hill, USA*

Background: Nanoparticles (NPs), monoclonal antibodies (mAbs), and antibody-drug conjugates (ADCs) are cleared via the mononuclear phagocyte system (MPS). Variability in MPS function has been shown to predict variability in the pharmacokinetics (PK) and pharmacodynamics (PD) of NPs. The MPS serves as a non-target-mediated mechanism of antibody

clearance via their Fc-gamma-receptors (FcγRs). Due to differences in types and affinity of FcγRs, variation in surface expression can lead to significant differences in the ability of MPS cells to clear mAbs and ADCs, which affects the PK and PD of these agents. Studies have reported lower mAb and ADC trough concentrations/faster clearance in patients with advanced gastric cancer (aGC) compared to metastatic breast cancer (mBC) and other solid tumors, which were not explained fully by patient covariates (e.g. age, gender, anti-drug antibodies). Thus, the difference in biomarkers of MPS cells in blood from patients with aGC and mBC were evaluated.

Material and Methods: Biomarker studies were performed in adult female patients diagnosed with mBC (n = 15) or aGC (n = 15). Patients were excluded if they received NP or biological therapy within the past month or were treatment naïve at the time of sample collection. FcγR expression (CD64, CD32, and CD16) and function (phagocytosis and reactive oxygen species [ROS] generation) for MPS cells (monocytes) in whole blood were evaluated by flow cytometry.

Results: There was high inter-patient variability (2- to 5-fold) in expression of FcγRs. There was higher CD64 expression in patients with aGC compared to mBC (8,437 ± 2,512 v. 6,762 ± 1,344 antibodies bound per cell, respectively; p = 0.037). Additionally, CD16 expression was higher in patients with aGC compared to mBC (10,954 ± 4,385 vs. 7,519 ± 3,302 ABC, respectively; p = 0.029). CD32 expression was similar between the two populations. Similar variability and mean phagocytic function were seen in the two patient populations. The innate ROS production (i.e. unstimulated) within MPS cells was higher in patients with mBC compared to aGC (4,043 ± 1,331 vs. 3,170 ± 617, respectively; p = 0.035), as well as after stimulation. In addition, there was an association between monocyte count and MPS function after stimulation in patients with aGC but not mBC.

Conclusions: Increased expression of CD64 and CD16 FcγRs on MPS cells was observed in patients with aGC compared to mBC. This is significant as CD64 is the only FcγR to bind monomeric IgG_1, the isotype commonly used in therapeutic mAbs. FcγR expression was inversely related to ROS production in MPS cells indicating there may be a compensatory relationship between MPS FcγRs and function. In summary, variability in FcγR expression could be explored as a potential covariate for the faster and clinically relevant clearance difference of mAbs and ADCs observed in patients with aGC.

Conflict of interest: Ownership: (1) Glolytics, LLC has licensed technology on biomarkers of MPS function. WCZ is CSO and holds equity in Glolytics, LLC. Corporate-sponsored Research: Funding for technology and study: (1) Genentech & (2) Eshelman Institute for Innovation. Other Substantive Relationships: (1) WCZ and ATL filed ROI for provisional patent on MPS FcγR technology.

151 (PB-002) Poster

IL13Rα2 chimeric antigen receptor T cells combined with checkpoint blockade to treat glioblastoma in human and canine models

Y. Yin[1], Z. Binder[1], R. Thokala[1], D. O'Rourke[1]. [1]*University of Pennsylvania, Neurosurgery, Philadelphia, USA*

Background: Glioblastoma (GBM) is an inherently invasive tumor with a median overall survival of approximately 15 months, following surgery, radiotherapy, and chemotherapy. T cells can be redirected to kill cancer cells using chimeric antigen receptors (CAR), a promising method to treat solid tumors. IL13Rα2 is expressed in many solid tumors but not normal tissues, providing a tumor-specific target for CAR T cells.

Methods: Human and canine tumor cell lines were screened for IL13Rα2 expression by flow cytometry and RT-PCR. Different IL13Rα2 single-chain variable fragments (scFv) were tested in CARs against human and canine recombinant IL13Rα2 protein to identify a cross-reactive clone. Humanized IL13Rα2 CARs expressed in human and canine T cells showed antigen-specific stimulation by cytokine secretion and target cell lysis. NSG mice bearing subcutaneous and orthotopic xenografts received a single treatment of 0.8–5 million CAR T cells with or without anti PD-1, CTLA-4 or TIM3 mAb checkpoint blockade.

Results: IL13Rα2 was detected on human GBM (D270, U251 and U87), canine osteosarcomas (BW-, CS-, MC- and SK-KOSA) and canine lung cancer cell lines (Cacal3, Cacal5). Two different humanized IL13Rα2 CARs were generated that recognized human IL13Rα2 but not IL13Rα1. Of these, one also recognized canine IL13Rα2. IL13Rα2 CAR T cells demonstrated tumor growth inhibition in human and canine GBM tumors grown both subcutaneously and orthotopically. Use of fewer CAR T cells initially controlled tumor growth, followed by tumor outgrowth correlating with expression of T cell exhaustion markers. Combined treatment of CAR T cells with checkpoint blockade mAb, delivered either systemically or *in situ*, had synergistic effects. Anergic T-cell function was selectively restored by specific checkpoint blockades and improved tumor growth inhibition.

Conclusions: IL13Rα2 is highly expressed on human and canine tumors but not normal tissue. IL13Rα2 specific CAR T cells are successfully activated in response to human and canine tumors and inhibit GBM growth in a xenograft mouse model, although this effect was transient at lower treatment doses. Addition of specific checkpoint blockade mAbs to CAR therapy was beneficial for the treatment of solid tumors. We plan to utilize this treatment in a canine spontaneous cancer preclinical model and translate into clinical trials for patients with GBM.

Conflict of interest: Corporate-sponsored Research: Dr. Donald O'Rourke receives research support from Novartis.

Wednesday, 14 November 2018

POSTER SESSION

Clinical Methodology

152 (PB-003) Poster

Detection of exfoliative cytology and VELscope fluorescence in oral cancer and oral leukoplakia

Z. Sun[1], Y. Liu[1], X. Guan[1]. [1]*Beijing Stomatological Hospital, Capital Medical University, Dept. Oral Mucosa, Beijing, China*

Background: To access the feasibility of exfoliative cytologyand VELscope fluorescence in the early detection of oral squamous cell carcinoma (OSCC) and prediction oral cancer risk of oral leukoplakia (OLK).

Methods: We collected oral mucosa of patients with oral leukoplakia (OLK, n = 57), OSCC (n = 70), and healthy subjects (n = 28). Exfoliative cells were collected and analyzed with traditional DNA quantitative analysis and oral cancer risk index (OCRI) analysis. Meanwhile, VELscope fluorescence was used in other patients. All the results were compared with histopathology to determine the sensitivity and specificity of these two methods.

Results: The sensitivity of DNA quantitative analysis, OCRI and VELscope was 92.68%, 100%,and 93.10% respectively. The specificity was 100% in all groups. Among 28 leukoplakia patients with an OCRI less than 0.5, none of them developed cancer during follow-up.

Conclusions: Exfoliative cytology-based method for quantitative prediction of cancer risk (OCRI) and VELscope fluorescence detection (localization of the lesion) could be used in early detection of OSCC and assessment of cancer risk of OLK patients during clinical follow-up.

No conflict of interest

153 (PB-004) Poster

Circulating tumor cell-free DNA correlates with the total tumor volume and survival in patients with advanced cancers

S. Ahmed[1], N. Elshafeey[2], H.J. Huang[3], K. Madwani[3], S. Fu[3], A.M. Tsimberidou[3], S.A. Piha-Paul[3], A. Naing[3], D. Hong[3], D.D. Karp[3], G. Cabrilo[3], S. Kopetz[4], V. Subbiah[3], B.K. Kee[4], C. Eng[4], V.K. Morris[4], F. Meric-Bernstam[3], R. Colen[1], F. Janku[3]. [1]*University of Texas, MD Anderson Cancer Center, Cancer Systems Imaging, Houston, USA;* [2]*University of Texas, MD Anderson Cancer Center, Diagnostic Radiology, Houston, USA;* [3]*University of Texas, MD Anderson Cancer Center, Investigational Cancer Therapeutics, Houston, USA;* [4]*University of Texas, MD Anderson Cancer Center, GI Medical Oncology, Houston, USA*

Backgrounds: The association between the amount of tumor circulating cell-free DNA (cfDNA) and tumor burden is not well understood partially due to inadequate imaging evaluation tools such as RECIST. We hypothesize that, unlike RECIST, volumetric image analysis can accurately estimate total tumor burden and can correlate with the amount of tumor circulating cfDNA with the ultimate translational goal to use cfDNA assessment as a surrogate marker for response to cancer therapy.

Materials and Methods: We performed a retrospective volumetric assessment of pretreatment clinical imaging (CT and/or MRI) to estimate the Total Tumor Volume (TTV) using the 3D slicer 4.3.1 and MATLAB in patients with advanced cancers with *KRAS*$^{G12/G13}$ mutations. *KRAS*$^{G12/G13}$ mutations were detected in 16 ng of unamplified plasma cfDNA collected at the time of imaging and quantified as % variant allele frequency (VAF) using the multiplex droplet digital PCR screening kit. Correlations between VAF and tumor volumes were performed and results compared to overall survival (OS).

Results: Total of 76 patients (colorectal cancer [CRC]: 51; other cancers: 25) were evaluated. TTVs positively correlated with the $KRAS^{G12/G13}$ VAF in cfDNA ($r = 0.46$, $p = 0.005$). When analyzing individual metastatic sites, Hepatic Tumor Volumes (HTVs) positively correlated with the $KRAS^{G12/G13}$ VAF in cfDNA ($r = 0.46$, $p < 0.001$), but not with Lung Tumor Volumes (LTVs; $r = 0.02$, $p = 0.89$) or Brain Tumor Volumes (BTVs; $r = -0.15$, $p = 0.20$). Patients with higher than median $KRAS^{G12/G13}$ VAF in cfDNA had a shorter median OS compared to patients with lower VAF (5.7 vs 7.3 months, P = 0.03). Patients with higher than median TTVs showed a trend towards shorter OS compared to patients with lower TTVs (5.2 vs 6.6 months, P = 0.07). In the largest disease specific subgroup of 51 patients with CRC, TTVs ($r = 0.38$, $p = 0.006$) and HTVs ($r = 0.56$, $p < 0.001$) also positively correlated with the $KRAS^{G12/G13}$ VAF in cfDNA while LTVs ($r = -0.03$, $p = 0.81$) or BTVs ($r = 0.20$, $p = 0.90$) did not. Patients with CRC and higher than median $KRAS^{G12/G13}$ VAF in cfDNA also had a shorter median OS compared to CRC patients with lower VAF (6.0 vs 9.8 months, P = 0.04) while CRC patients with higher than median TTVs had similar OS to patients with lower TTVs (5.4 vs 7.5 months, P = 0.26).

Conclusions: The amount of mutated $KRAS^{G12/G13}$ in plasma cfDNA correlates with TTVs on imaging and higher % VAF of $KRAS^{G12/G13}$ in cfDNA, but not TTVs were associated with shorter OS.

No conflict of interest

154 (PB-005) Poster

Pharmacokinetics of trabectedin and olaparib given in combination during a phase 1b trial (NCT 02398058) in patients with advanced and non-resectable bone and soft tissue sarcomas

C. Matteo[1], M. Zucchetti[1], L. D'Ambrosio[2], Y. Pignochino[2], G. Dovrtělová[1], F. Tolomeo[2], A. Merlini[2], P. Picci[3], E. Palmerini[3], M. D'incalci[1], G. Grignani[2]. *[1]IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Oncology, Milan, Italy; [2]IRCCS Candiolo Cancer Institute-FPO, Division of Medical Oncology, Candiolo, Italy; [3]Orthopedic Rizzoli Institute, Laboratory of Experimental Oncology, Bologna, Italy*

Background: We conducted a pharmacokinetic (PK) study in patients with advanced bone and soft tissue sarcoma participating to the phase 1b trial of trabectedin (T) and olaparib (OLA) combination. T is an ideal partner of the PARP1 inhibitor OLA, thanks to the induction of single- and double-strand DNA damages. Since both drugs are characterized by extensive liver metabolism and biliary excretion and no data are available on the possible PK interaction between the two molecules, we determined the PK profiles of both drugs given alone (1st cycle) and when given in combination (2nd cycle) to sarcoma patients.

Methods: The PK of T given as 24 h infusion and OLA, given P.O. bi-day (bid), were analyzed in 50 sarcoma patients (age: 19–80 yrs). Twenty-two enrolled in a classical 3 + 3 design phase 1 study, treated with 6 dose-levels of T (range: 0.675–1.3 mg/m^2) and OLA (100–300 mg), and 28 patients during the following expansion phase treated with 1.1 mg/m^2 of T and 150 mg bid of OLA. Highly sensitive HPLC-MS/MS methods were used to determine drugs concentration in plasma samples collected before and at several times after treatments. The PK parameters were obtained by the software NCPKA_V3.

Results: In patients participating to the expansion phase, T achieved C_{max} of 1.4 ± 1.1 and 1.2 ± 0.5 ng/mL in 1st and 2nd cycle, respectively. Then drug plasma concentration declined rapidly within 1 h and was followed by a long elimination phase with half-life (HL) of about 6 days. There was high inter-patient variability of drug exposure, but similar mean PK parameters. Mean ±SD clearance (Cl), volume of distribution and HL at 1st and 2nd cycle were respectively 25.3 ± 10.6 and 22.2 ± 11.2 L/h/m^2, 3732 ± 1731 and 3777 ± 1680 L/m^2, 113 ± 49 and 163 ± 144 h. Superimposable values of the parameters were obtained in patients participating to the dose-escalation phase.

In the 1st and 2nd cycle of the expansion phase OLA achieved Cmax respectively of 5.6 ± 2.4 and 5.2 ± 2.9 µg/ml within 3 h, then drug concentration decayed with an apparent HL of 4–6 h. OLA showed a trend of reduced absorption in presence of concomitant T administration, in both groups of patients of escalation and expansion cohorts. The difference seen in the plasma concentration-time profile (AUC), even if unlikely pharmacologically relevant being the reduced absorption <15%, reaches statistical significance $p \leq 0.04$ considering all dose levels (Wilcoxon test).

Conclusions: In this study despite the high inter-patient PK variability of T and OLA administered alone or in combination to sarcoma patients, the Cl values of T resulted superimposable in the two cycles and were in the range of those previously reported in literature, showing no influence of OLA on PK of T. As regards the PK of OLA there was less intra- than inter- patient variability with only a slight reduction of the plasma AUC of OLA when the drug was combined with T.

No conflict of interest

155 (PB-006) Poster

Genome-wide copy number alteration (CNA) of circulating cell-free DNA (cfDNA) as a prognostic biomarker in esophageal squamous cell carcinoma (ESCC)

H.S. Im[1], K. Jihoon[2], C. Eun-Hae[3], J. Ja-Hyun[3], L. Junnam[3], P. Sook Ryun[4]. *[1]Asan Medical Center, University of Ulsan College of Medicine, Department of Internal Medicine, Seoul, South Korea, [2]Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Division of Hematology/Oncology, Seoul, South Korea; [3]Green Cross Genome, Genome Research Center, Yongin, South Korea; [4]Asan Medical Center, University of Ulsan College of Medicine, Department of Oncology, Seoul, South Korea*

Background: Although neoadjuvant chemoradiation (CRT) followed by surgery or definitive CRT are the standard treatment for locally advanced resectable or unresectable ESCC, the prognosis in those patients remains unsatisfactory. In addition, there are no reliable clinical assessment tools for predicting prognosis or monitoring treatment response for CRT in ESCC. We aimed to identify a biomarker for predicting outcomes using cfDNA in ESCC patients receiving CRT.

Material and Methods: This prospective biomarker study analyzed plasma cfDNA from patients with neoadjuvant CRT followed by surgery (n = 24) and patients with definitive CRT for locally advanced ESCC (n = 21) at Asan Medical Center in South Korea from Feb. 2017 to Feb. 2018. Plasma cfDNA was collected from patients before and after CRT. Low depth whole-genome sequencing of cfDNA was used to identify CNA and I-score was developed to express genomic instability. I-score was defined as the sum of absolute Z-scores of sequenced reads on each chromosome.

Results: The pathologic complete response (pCR) was achieved in 7 patients (29%) in neoadjuvant CRT followed by surgery group, and the clinical complete response (cCR) was achieved in 5 patients (24%) in definitive CRT group. The baseline cfDNA concentrations were no significant association with the clinical T stage (cT1-2 vs. cT3-4; 0.378 vs. 0.419 ng/µL; p = 0.856), pathologic response (pCR vs. non-pCR; 0.338 vs. 0.395; p = 0.799), ypT stage (ypT0-2 vs. ypT3-4; 0.343 vs. 0.443 ng/µL; p = 0.421) and ypTNM stage (ypTNM 0-1 vs. ypTNM 2-4; 0.314 vs. 0.423 ng/µL; p = 0.283). When analyzing cfDNA concentration before and after CRT, cfDNA concentration was significantly increased after CRT (0.383 vs. 0.669; p = 0.007). However, the I-score tended to increase at higher clinical T stage (cT1 vs. cT2-4; 2.487 vs. 648.7; p = 0.071), was also significantly higher at higher ypT stage (ypT0-2 vs. ypT3-4; 43.12 vs. 1,007; p = 0.016) and ypTNM stage (ypTNM 1 vs. ypTNM 2-4; 1.259 vs. 615.8; p = 0.049).

Conclusions: The cfDNA concentration is not a good biomarker for prognostication and monitoring the course of treatment in esophageal cancer patients treated with CRT. But the I-score, an indicator of genomic instability in ctDNA, was a significant predictor of poor prognosis.

No conflict of interest

157 (PB-008) Poster

Exploratory multi-dimensional assessments of tumor response in desmoid tumors: alternatives to RECIST

G. O'Sullivan Coyne[1], S.M. Steinberg[2], I.B. Turkbey[3], G. Lee[4], A. Lih[1], L. Machado-Pichardo[5], L.R. Folio[5], S. Kummar[6], A. Chen[1]. *[1]National Institutes of Health, Division of Cancer Treatment and Diagnosis, Bethesda, USA; [2]National Institutes of Health, Biostatistics and Data Management Section, Shady Grove, USA; [3]National Institutes of Health, Molecular Imaging Program, Bethesda, USA; [4]University of Maryland, School of Medicine, Baltimore, USA; [5]National Institutes of Health, Radiology and Imaging Sciences, Bethesda, USA; [6]Stanford University, Department of Medicine-Oncology, Palo Aloto, USA*

Background: Desmoid tumors are rare, locally invasive, soft-tissue lesions that can cause significant morbidity, and frequently recur following surgery or radiation. The γ-secretase inhibitor nirogacestat demonstrated promising activity in a recent phase I trial in which 5/7 evaluable patients (pts) with desmoid tumors experienced partial tumor responses to the agent. In the present phase II trial in pts with recurrent, refractory desmoid tumors (NCT01981551), we reported prolonged disease stabilization and multiple partial responses as assessed by RECIST criteria (Kummar JCO 2017). However, given the irregular and infiltrative morphology of these tumors, we explored alternative imaging-based assessments to evaluate response in pts following treatment with nirogacestat.

Materials and Methods: A total of 17 pts received 150 mg nirogacestat orally twice a day in 3-week cycles. Objective treatment response was defined by RECIST 1.1 at cycle 1 and after every 6 cycles using CT per the primary study objective; optional MRI assessment was concurrently

performed. For volumetric measurements, single and multifocal desmoid tumors were segmented on MRI and CT images using a PACS lesion management tool (Carestream Health, NY). Subsequently, tumor response was evaluated by MRI volume size (MV, cm^3), CT volume size (CTV, cm^3), World Health Organization criteria (WHO), and CT density (Viable Tumor Volume [VTV], histogram/Hounsfield units). A decrease of ≥30% from baseline volume in cm^3 by either MV or CTV estimated a decrease in at least one volumetric dimension.

Results: Multi-modality results for the first 5 response assessments timepoints are reported here.16/17 pts were evaluable for response; 10 pts remained on study ≥3 yrs. Partial responses (PR) were confirmed in 5 pts (29%) by RECIST, no pts progressed on study. By MV assessment, 9/11 pts who consented to MRI imaging had a reduction in MV at first assessment (range –10 to –63%). Furthermore, 8/11 pts experienced continued volume decrease 30–98% from baseline; no volume increases occurred. By CTV assessment, 5/11 pts had 30–98% volume decrease from baseline. At paired timepoints, both MV and CTV revealed > %change compared to baseline than RECIST (**$p < 0.01$ at assessment 3, others not significant). Using WHO, 4/5 RECIST PRs were confirmed; however, 4/16 pts met criteria for PD. For 13/16 pts, VTV values were associated with non-necrotic density on CT (increased %density, range 2.16%–81.08%).

Conclusions: Pending confirmation in larger trials, volumetric assessment by MV or CTV were found to be more sensitive than RECIST for the assessment of the changing shape and size of desmoid tumors in response to treatment, and MV provides an earlier signal of response. WHO was not representative of clinical benefit.

No conflict of interest

158 (PB-009) Poster

Cell-free DNA testing allows for rapid patient screening, identification, and enrollment on molecularly-targeted clinical trials

A. Franovic[1], J. Christensen[2], V. Tassell[2], K. Banks[1], M. Schillebeeckx[3], M. Gebhardt[3], D. Simon[3], R. Lanman[1]. [1]*Guardant Health Inc., Medical Affairs, Redwood City, USA;* [2]*Mirati Therapeutics Inc., Clinical Science, San Diego, USA;* [3]*Guardant Health Inc., BioPharma Business Development, Redwood City, USA*

Background: The ability to identify and enroll patients on clinical trials in a timely manner is critical for providing patients access to potentially life-saving therapies and the drug development process at large. Requirements for tissue biopsies for molecularly-guided clinical trials impose barriers to patient enrollment related to long test turnaround times, insufficient or inaccessible tumor material, test failure, and biopsy refusal. In a recent study, 30% of patients had clinical deterioration due to this delay or had insufficient tissue for analysis, both rendering them trial ineligible. In contrast, blood-based cell-free DNA (cfDNA) next-generation sequencing (NGS) analysis offers a rapid and non-invasive alternative for genomic testing and may accelerate patient screening and selection.

Materials and Methods: We reviewed data from patients whose blood was screened for clinical trial enrollment, including the Phase 2 trial evaluating glesatinib in lung cancer patients with activating *MET* gene alterations. Patient samples were tested using the Guardant360® 73-gene cfDNA NGS assay (G360) with a turnaround time of 7 days from date of receipt. Data pertaining to the number of clinical trial matches, physicians informed, patients enrolled, and factors contributing to lack of enrollment was collected.

Results: For the Phase 2 glesatinib trial, 615 patients were centrally tested by G360; 33 (5.4%) were eligible based on *MET* positivity and 9 (1.5%) were enrolled. A total of 1559 patients were pre-screened: 53% by tissue and 47% by cfDNA. Patients were accrued over a period of 19 months. Among the 68 patients on study, 20 (29%) were enrolled based on cfDNA test results (9/9/2 central G360/local G360/other assay) and 48 (71%) on tissue results. Across 9 targeted therapy trials contracted for referrals based on standard-of-care G360 cfDNA test results, 1007 molecularly-matched patients were identified from May 2017–2018 and 58% of ordering physicians were successfully contacted. Overall, 40% of physicians were fully informed on trial details with 21% progressing to trial site or sponsor connections; common reasons cited for lack of enrollment subsequent to a match included deteriorating patient health/death, prior treatment, and provider refusal.

Conclusions: Rapid identification of patients with specific molecular profiles, particularly rare alterations, is the first and arguably greatest challenge in biomarker-driven clinical trial enrollment. Patient access to trials is reduced when their performance status declines or their condition requires immediate treatment with chemotherapy. In the glesatinib trial, cfDNA testing provided a means of quickly identifying eligible patients and supplemented tissue testing, accounting for almost 30% of patients enrolled, allowing for accelerated trial enrollment without patient risk of biopsy-driven complications.

Conflict of interest: Other Substantive Relationships: A.F., K.B., M.S., M.G., D.S., and R.L. are employees and shareholders at Guardant Health. J.C. and V.T. are employees and shareholders at Mirati Therapeutics.

159 (PB-010) Poster

The PROCLIPI Study; A prototype registry for rare cancers with global collaboration for establishment of a prognostic index in mycosis fungoides and Sezary syndrome

J. Scarisbrick[1], A. Cozzio[2], R. Willemze[3], Y. Kim[4], Cutaneous Lymphoma International Consortium. [1]*EORTC, Cutaneous Lymphoma Task Force, Brussels, Belgium;* [2]*EORTC, Cutaneous Lymphoma Taskforce, Brussels, Belgium;* [3]*Leiden University Medical Centre, Dermatology, Leiden, Netherlands;* [4]*Stanford Medical Centre, Dermatology, Stanford, USA*

Background: The PROCLIPI Study is a multi-national PROspective Cutaneous Lymphoma International Prognostic Index study to develop a prognostic index in mycosis fungoides (MF) and Sezary syndrome (SS).

Material and Methods: PROCLIPI was launched in 2015 collecting pre-defined clinical, haematological, radiological, immunohistochemical, genotypic, quality of life and treatment data using a secure web based database system. Additionally, central clinical and pathological review is performed by experts to confirm diagnosis and stage.

Results: 956 patients are registered from 46 international sites from 5 continents. 680 early stage (IA-IIA) patients; 1.75 males: 1 female and 276 advanced stage (IIB-IV); 1.65 males: 1 female. The median age at diagnosis of advanced disease was 65 yrs compared to 57 yrs in early stage ($p < 0.0001$). The median time of MF-like lesions prior to diagnosis was 36 months in both early and advanced disease confirming diagnostic delay. Raised serum lactate dehydrogenase (LDH) and large cell transformation (LCT) in skin are among the candidate prognostic factors. LCT is found in 20/680 (3%) early stage patients and in 69/276 (25%) advanced patients $p < 0.01$. 9% of early stage and 30% advanced patients have raised serum LDH at diagnosis ($p < 0.01$). Treatment data including responses and quality of life are collected and will be measured against survival and tumour phenotype (clinical, histological and genotypic) to determine the most efficacious therapies in improving outcomes.

Conclusions: PROCLIPI has confirmed a diagnostic delay in all stages of MF/SS. There is a need to develop improved diagnostic techniques to quicken the diagnosis. Identifying patients with a poorer prognosis should allow optimal treatments, better patient experience and improve survival. PROCLIPI is a prototype registry for rare cancers and this study design may benefit other cancer groups.

No conflict of interest

160 (PB-011) Poster

Complete pathologic response is a strong predictor of event free survival and distant recurrence free survival, regardless of tumor subtype or investigational agent, in women with early breast cancer at high risk for recurrence in the I-SPY 2 TRIAL

A. De Michele[1], D. Yee[2], C. Yau[3], W.F. Symmans[4], C. Isaacs[5], K. Albain[6], Y.Y. Chen[7], S. Wei[8], B. Datnow[9], M. Klein[10], C. Godellas[11], B. Chen[12], K. Adamson[13], J. Perlmutter[14], N. Hylton[15], L.J. van 't Veer[3], S. Asare[16], S. Berry[17], L. Esserman[3], D. Berry[17], I-SPY 2 TRIAL Consortium. [1]*University of Pennsylvania, Hematology Oncology, Phliadelphia, USA;* [2]*University of Minnesota, Medicine, Minneapolis, USA;* [3]*University of California, UCSF Hellen Diller Family Comprehensive Cancer Center U.S, San Francisco, USA;* [4]*University of Texas, M.D. Anderson Cancer Center, Pathology, Houston, USA;* [5]*Georgetown University, Medicine and Oncology, Washington D.C., USA;* [6]*Loyola University, Hematology and Oncology, Maywood, USA;* [7]*University of California, Pathology, San Francisco, USA;* [8]*University of Alabama Birmingham, Pathology, Birmingham, USA;* [9]*University of California San Diego, Pathology, La Jolla, USA;* [10]*University of Minnesota, Pathology, Minneapolis, USA;* [11]*Loyola University, Surgical Oncology, Maywood, USA;* [12]*Mayo Rochester, Pathology, Rochester, USA;* [13]*Swedish Cancer Institute, Pathology, Seattle, USA;* [14]*Gemini Group, Advocate, Ann Arbor, USA;* [15]*University of California, San Francisco, Imaging, San Francisco, USA;* [16]*Quantum Leap Healthcare Collaborative, I-SPY TRIALs, San Francisco, USA;* [17]*Berry Consultants, LLC, Statistics, Houston, USA*

Background: While pathological complete response (pCR) is associated with an improved event-free (EFS) and distant recurrence-free survival (DRFS) with standard, approved treatment in the neoadjuvant therapy of

breast cancer, it is unknown whether this would hold for investigational agents. We tested this hypothesis in nine investigational arms and controls in the ongoing, adaptive, phase 2 platform trial, I-SPY2.

Methods: Women with breast tumors >2.5 cm were adaptively randomized to better performing agents compared with control therapy within molecular subtypes, using HER2, hormone-receptor and the 70-gene assay. Hormone receptor +/HER2–/70-gene low-risk tumors were excluded. EFS and DRFS were evaluated by pCR status within subtypes. Hazard ratios (HR) were based on Cox proportional hazards regression. Associations between pCR and EFS were analyzed by therapeutic arm using Bayesian and hierarchical modeling with adjustments for molecular subtype.

Results: The analysis was restricted to patients who had at least 2 years of follow-up, with 741 patients (of over 1200 randomized) eligible for analysis; median follow-up was 2.7 years. Three-year EFS and DRFS for patients achieving pCRs were 94% and 95%, respectively. For pCR versus non-pCR, mean HR = 0.20 for both endpoints, regardless of modelling method, with the 95% probability interval ranges from 0.1–0.35. HRs were similarly low within subtypes and specific treatment arms. Even when assigned treatments were not superior to control, achieving a pCR on any treatment arm resulted in significantly lower HR compared to non-pCR.

Conclusion: Achieving pCR after neoadjuvant chemotherapy for women at high risk of early recurrence predicts an excellent outcome, regardless of subtype, even with the addition of investigational drugs, with 94% of patients remaining disease-free at 3 years. pCR is an early endpoint that can serve to support individualized de-escalation or escalation strategies for drug development trials designed to optimize outcomes. Results by treatment arm will be shown.

No conflict of interest

Wednesday, 14 November 2018

POSTER SESSION

Combinatorial Chemistry

161 (PB-012) Poster

Results of targeted therapy with Votrient in patients with malignant tumors of soft tissues with metastases in the lungs

V. Protsenko[1], V. Chorney[1]. [1]*Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine, Department of Tumors of the Musculoskeletal System, Kyiv, Ukraine*

Background: Soft tissue sarcomas are characterized by low prevalence (1,0–2,5% of all malignant tumors in adult population), frequent recurrence, multicentric growth, early onset of distant hematogenous metastases, and unfavorable prognosis. The five-year survival rate reaches 87% for highly differentiated sarcomas and 62% for low-differentiated ones. In this regard, treatment for soft tissue sarcomas, as a rule, is complex with the use of all methods of treatment: surgical, polychemotherapy and radiotherapy. With the advent of metastases in patients with soft tissue sarcomas, the drug of choice is Votrient.

Materials and Methods: The results of treatment of 23 patients aged from 19 to 76 years with malignant tumors of soft tissues of limbs and trunk are analyzed. Leiomyosarcoma occurred in 8 (34,8%) patiens, 6 (26,1%) patients had rhabdomyosarcoma, 4 (17,4%) – fibrosarcoma, 3 (13%) – malignant fibrosis histiocytoma, 2 (8,7%) – arrhythmic sarcoma. By localization the distribution was the following: lower limb – 15 (65,2%), upper limb – 4 (17,4%), torso – 4 (17,4%). The treatment of patients was complex: preoperative polychemotherapy and radiotherapy to a total focal dose of 40 Gray, surgical treatment, postoperative polychemotherapy and radiotherapy to a total focal dose of 20 Gray. In the case of metastases in the lungs, patients received targeted therapy with the use of Votrient with a daily dose of 400 mg.

Results: The medianoverall survival of patients with pulmonary metastases was 17 months treatment withVotrient. In 9 (66,7%) patients, stabilization of the disease was noted during the 12-week period of drug administration, in 4 (14,8%), partial reduction of metastatic centers in the lungs was observed, in 5 (18,5%), the single disappearance of metastatic foci in the lungs was revealed, in 5 (18,5%) complete disappearance of metastatic foci in the lungs was marked.

Conclusions: The use of Votrient, an inhibitor of tyrosine kinase, which affects the angiogenesis and proliferation of tumor cells, leads to improved survival in patients with metastases with soft tissue sarcoma in the lungs.

No conflict of interest

Wednesday, 14 November 2018

POSTER SESSION

Cytotoxics

162 (PB-013) Poster

Targeting transporters to ameliorate chemotherapy-induced peripheral neuropathy

A. Leblanc[1], J.A. Sprowl[2], A.A. Gibson[1], A. Sparreboom[1], S. Hu[1]. [1]*The Ohio State University, College of Pharmacy, Columbus, USA;* [2]*D`Youville College, Department of Pharmaceutical, Social and Administrative Sciences, Buffalo, USA*

Background: Many tubulin poisons can induce a chronic, dose-dependent sensory peripheral neuropathy that is characterized by tingling, numbness, increased sensitivity to cold and touch, and burning pain of the distal extremities. The incidence of this side effects is particularly high in patients with breast cancer receiving paclitaxel-based chemotherapy. We previously identified transport by OATP1B-type carriers (OATP1B1 in humans and Oatp1b2 in mice) as a contributor to acute and chronic forms of peripheral neuropathy associated with paclitaxel (Taxol) (Leblanc et al, *JCI* 2018). We hypothesized that these carriers also contribute to peripheral neuropathy associated with related compounds.

Materials and Methods: *In vivo* toxicity studies were performed in wild-type mice and Oatp1b2-deficient [Oatp1b2(-/-)] littermates receiving paclitaxel, formulated in ethanol-Kolliphor EL (Taxol) or as an albumin-bound nanoparticle (Abraxane), or vincristine. Standard tests were used to evaluate chemotherapy-induced peripheral neuropathy at timed intervals before, during, and after treatment, and included assessments of mechanical-induced allodynia (Von Frey test), thermal hyperalgesia (Hargreaves test), and changes in digital maximal action potential amplitudes. Plasma pharmacokinetic studies and comparative tissue distribution profiles were evaluated using validated assays based on LC/MS/MS.

Results: Treatment of wild-type mice with Taxol (10 mg/kg) significantly affected mechanical allodynia and thermal hyperalgesia, and no changes were observed in Oatp1b2(-/-) mice ($P < 0.05$). In contrast, these phenotypes were not observed after acute exposure to Abraxane, even after dose-doubling (20 mg/kg). This is consistent with the finding that concentrations of paclitaxel in plasma were consistently higher by 5- to 7-fold for the Kolliphor-containing formulation compared with Abraxane, and with clinical evidence that the paclitaxel dose-corrected rate of neuropathy is lower with Abraxane compared to Taxol. Pre-treatment with the Oatp1b2 inhibitors nilotinib, pazopanib, or rifampin protected mice against acute and chronic peripheral neuropathy without affecting levels of paclitaxel in plasma. The administration of a single dose of vincristine (0.1 mg/kg, i.v.), a confirmed substrate of Oatp1b2 in overexpressed cells, was also associated with significant changes in mechanical allodynia in wild-type mice but not in Oatp1b2(-/-) mice ($P < 0.05$).

Conclusions: We identified OATP1B-type transport as an essential, previously unrecognized contributor to the peripheral neuropathy associated with several tubulin poisons, including paclitaxel and vincristine. Considering the low expression of these carriers in intrinsically-sensitive tumors, our study also suggests that this debilitating side effect can be prevented by pre-treatment with inhibitors of this transport mechanism.

No conflict of interest

163 (PB-014) Poster

Quantum dots conjugates with unsymmetrical bisacridines enhance cytotoxicity of these antitumor compounds in lung cancer cells and have protecting effects on normal cells

J. Pilch[1], M. Pawłowska[1], P. Bujak[2], A.M. Nowicka[3], E. Augustin[1]. [1]*Gdańsk University of Technology, Pharmaceutical Technology and Biochemistry, Gdańsk, Poland;* [2]*Warsaw University of Technology, Chemistry, Warsaw, Poland;* [3]*Warsaw University, Chemistry, Warsaw, Poland*

Background: In recent years, with the rapid development of nanotechnology and its extensive applications in the medicine, nanocarriers for anticancer drug delivery have gained a great importance. Spherical semiconductor nanocrystals, frequently called quantum dots (QDs) are very attractive nanomaterials for bioimaging applications and they possess properties as potential candidates for drug carrier. Unsymmetrical bisacridines (UAs), synthesized in our laboratory, EP 15461518.1, 2017, are the promising

antitumor agents with high cytotoxic activity against many experimental cellular and tumor models (colon, lung, pancreatic, breast, prostate). Here, we investigated whether QDs conjugated with unsymmetrical bisacridines affect cytotoxicity of these compounds in human lung carcinoma cells as well as normal lung fibroblasts.

Materials and Methods: Two unsymmetrical bisacridine derivatives: C-2028 and C-2045 and two human cell lines: non-small cell lung carcinoma H460 and fetal lung fibroblast MRC-5 were selected for these studies. UAs were non-covalently attached to the Ag-In-Zn-S/MUA nanocrystals. The amount of UAs compounds immobilized at QD nanocrystal surface was determined at the potential *ca.* –0.6 V (cyclic voltammetry). The cytotoxicity against H460 and MRC-5 cells was assessed by the MTT assay following 72 h of incubation with $QD_{red/green}$-C-2028 and $QD_{red/green}$-C-2045 conjugates as well as $QD_{red/green}$, C-2028 and C-2045 alone.

Results: Both compounds exhibited high cytotoxicity against lung cancer H460 cells (IC_{80}: 0.035 μM for C-2028 and 0.273 μM for C-2045), being less active against normal lung fibroblast MRC-5 (IC_{80}: 0.47 μM for C-2028, 0.45 μM for C-2045). C-2028 and C-2045 conjugated with QD_{red} and QD_{green} decreased IC_{80} values of both compounds (QD_{red}: 1.5 and 1.9 fold, QD_{green}: 1.2 and 1.3 fold, respectively). Interestingly, UAs conjugated with both red and green QD were much less cytotoxic against normal MRC-5 cells. IC_{80} value for QD_{red}-C-2028 increased 22 fold, QD_{red}-C-2045 28 fold, QD_{green}-C-2028 2.5 fold and QD_{green}-C-2045 11 fold. Moreover, QDs alone did not influence cancer and normal cells proliferation. Comparing the obtained values from voltammetric measurements with the maximum amount of UAs compounds immobilized at QD surface one can conclude that the efficiency of the nanoconjugates synthesis was higher for QD_{red} nanocrystals.

Conclusions: Our results indicate that conjugation of unsymmetrical bisacridines with QDs improves drugs cytotoxicity in lung cancer cells and protects normal lung fibroblast from drugs action. These effects were more pronounced in the case of QD_{red}, which were more effectively loaded by bisacridines.

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No conflict of interest

164 (PB-015) Poster

BT5528, an EphA2-targeting Bicycle Toxin Conjugate (BTC): profound efficacy without bleeding and coagulation abnormalities in animal models

G. Bennett[1], A. Brown[1], G. Mudd[1], P. Park[2], N. Keen[2]. [1]*Bicycle Therapeutics Limited, Therapeutics, Cambridge, United Kingdom;* [2]*Bicycle Therapeutics Limited, Therapeutics, Lexington, USA*

Background: Ephrin receptor A2 (EphA2) is a member of the Ephrin receptor family of cell-cell junction proteins[MK1] and is highly overexpressed in several solid tumours and associated with poor prognosis. Despite the value of the target, clinical development of an antibody drug conjugate targeting EphA2 (MEDI-547) was stopped after severe adverse events, including bleeding and hepatotoxicity were seen (Annunziata *et al.,* 2013).

Method and materials: Bicycle binders for EphA2 were identified using a proprietary phage display peptide technology consisting of highly diverse phage libraries of Bicycles, conjugated to cleavable linkers & toxins to form Bicycle Toxin Conjugates (BTCs). The small size of BTCs offers a significant advantage over other targeted cytotoxic approaches such as antibody-drug conjugates due to rapid extravasation, renal clearance and improved tumour penetration.

The efficacy of BTCs was evaluated using xenograft models (cell- and patient derived) in balb/c nude mice, with treatments administered intravenously once weekly.

Toxicology studies were performed in Han Wistar rats and Cynomolgus monkeys, with treatments administered intravenously once weekly. Standard evaluations of weight, condition, haematology, clinical chemistry, coagulation were undertaken as well as macro- and micro-scopic pathology and measurement of D-Dimer.

Results: We selected the candidate BTC BT5528 from a panel of >75 BTCs, based on *in vivo* efficacy, tolerability and drug-like properties. BT5528 is effective in EphA2-expressing xenograft models, with complete tumour regression seen from 1 mg/kg weekly.

Expression of EphA2 is high across a range of cancers of high unmet medical need and correlation with efficacy in several tumours including lung, breast, oesophageal, ovarian, prostate, gastric and sarcoma models were shown. No significant efficacy is seen in tumours without EphA2 expression. Efficacy was maintained even when treating very large (>1000 mm^3) tumours and heterogeneous PDX models.

While ADC approaches to EphA2 have been hampered by profound bleeding & coagulation toxicity in the clinic and in preclinical species, BT5528 shows no sign of bleeding in exploratory toxicology studies in rat and NHP. No changes were seen in coagulation parameters or liver enzymes after treatment with BT5528, unlike previously reported data for MEDI-547 [MK2].

Conclusions: The EphA2 targeting BTC BT5528 shows potent anti-tumour activity in a range of solid tumour xenograft models without the limiting toxicity observed with previous Antibody Drug Conjugate approaches. IND-enabling studies for BT5528 are currently underway.

Conflict of interest: Other Substantive Relationships: All authors are employees of Bicycle Therapeutics Ltd.

165 (PB-016) Poster

Opposite effects of garcinol on tumor energy metabolism in oral squamous cell carcinoma cells

G. Zhang[1], Y. Su[1], X. Zhang[1]. [1]*Beijing Stomatological Hospital, Capital Medical University, Beijing Institute of Dental Research, Beijing, China*

Background: Cancer cells employ remarkable alterations in cellular metabolism, and this unique cancer cell feature presents itself a promising chemotherapeutic target. Garcinol is a natural polyisoprenylated benzophenone, which was extracted from the dried fruit rind of *Garcinia indica*. Our previous studies revealed its chemopreventive effect and anti-inflammatory properties on hamster cheek pouch carcinogenesis model, but its exact mechanisms were still need to be identified. In this study we investigated the roles of garcinol in oral squamous cell carcinoma (OSCC) cells and its action on cancer cell energy metabolisms.

Material and Methods: OSCC cell lines SCC-15 and CAL-27 were used in this study. Cell proliferation was detected by MTT assay. Cell apoptosis and cell cycle was determined by flow cytometry. Cell migration and invasion was evaluated using the scratch assay and transwell invasion assay. The measurement of oxygen consumption rate and extracellular acidification rate was evaluated with the Seahorse XFe96 Extracellular Flux Analyzer. The glucose content, lactate production, pyruvate kinase activity was measured by assays kits.

Results: Our cell studies showed that garcinol represses OSCC cell proliferation, cell cycle, migration and invasion, and colony formation. Of note, garcinol directly targeted cancer cell energy producing pathway mitochondrial respiration by significantly inhibiting ATP production, maximal respiration, spare respiration capacity and basal respiration in a dose-dependent manner in oral cancer cells. But simultaneously, garcinol treatment reflexively boosted glycolysis presented by increased glycolysis and glycolytic capacity. The promotion of garcinol on glycolytic pathway is also confirmed presented by elevated lactic acid content and the activity of glycolytic enzyme pyruvate kinase. Furthermore, the expression of glucose transporter1 & 4, and several important genes related to the glycolysis pathway, including HIF-1α, AKT and PTEN, was also up regulated after garcinol treatment.

Conclusions: Taken together, our results indicate that garcinol could significantly inhibit mitochondrial oxidative phosphorylation, and simultaneously enhance glycolysis in OSCC cells.

No conflict of interest

166 (PB-017) Poster

EB1-dependent long survival of glioblastoma cancer stem-like cell tumor-bearing mice after daily oral treatment with the novel Tumor Checkpoint Controller BAL101553

R. Berges[1], A. Tchoghandjian[1], A. Serge[2], D. Figarella-Branger[3], F. Bachmann[4], H. Lane[4], D. Braguer[3]. [1]*Aix-Marseille Univ, Cnrs-inp, Marseille Cedex 05, France;* [2]*Aix-Marseille Univ, CNRS, Inserm, Institut Paoli-Calmettes, CRCM, Marseille, France;* [3]*Aix-Marseille Univ, CNRS-INP, Marseille, France;* [4]*Basilea Pharmaceutica International Ltd, aa, Basel, Switzerland*

Background: Glioblastoma (GBM) are the most aggressive brain tumors in adults, and treatment options are very limited. Cancer stem-like cells, which contribute to GBM invasiveness, represent promising target. We have previously shown that overexpression of microtubule + End-binding 1-protein (EB1) correlates with GBM progression and poor survival and that EB1 is a predictive biomarker of response to BAL101553. BAL101553 is the prodrug of BAL27862, a small molecule that binds microtubules and promotes cell death through activation of the spindle assembly checkpoint. BAL27862 inhibited proliferation and migration *in vitro* of glioblastoma stem-like cells (GBM6) in an EB1-dependant manner. Three *i.v.* administrations of BAL101553 over the course of one week were sufficient to provoke an EB1-dependent survival benefit in tumor-bearing mice. Moreover, BAL27862 inhibited stem-cell properties and triggered astrocytic differentiation of GBM

stem-like cells in an EB1-dependent manner. Here, we evaluate a long-term oral schedule of treatment on this orthotopic mouse model.

Material and Methods: GBM6-GFP downregulating EB1 (GBM6 GFP shEB1) or not (GBM6 GFP sh0), were orthotopically grafted in mice. Thirsty-five days later, BAL101553 (30 mg/kg) or vehicle was orally administered 5 days a week for 100 days. Then survival duration was quantified. At the end of treatment, tumor volumes and invasiveness were analyzed by GFP fluorescence and 3D tumor reconstruction, and immunostaining of stem cell biomarkers were performed. Phenotype of stem cell differentiation during BAL27862 treatment was investigated *in vitro*.

Results: Survival was increased by 326 days and 155 days in GBM6 GFP sh0 and GBM6 GFP shEB1 respectively, as compared with respective controls. Patterns of invasion and quantification of tumor cells in brain demonstrated that the anti-proliferative and anti-invasive effects of BAL101553 were more prominent in mice bearing control tumors than in EB1-downregulated tumors. Moreover, BAL101553 decreased the proportion of stem-like cells (A2B5+) in the tumor in an EB1-expression level-dependent manner, as long as the treatment was administered. Furthermore, BAL27862/BAL101553 had a strong effect on tumor vessels: it inhibited GBM6 endothelial cell differentiation *in vitro* and *in vivo* and it inhibited GBM6 migration on preformed endothelial capillary-like tubes in an EB1-dependent manner.

Conclusion: Long-term oral treatment with BAL101553 provokes a large EB1-dependant survival benefit. Moreover, the drug counteracts tumor angiogenesis by acting on cancer stem like cells. These findings support the potential of BAL101553 for oral treatment of GBM, with EB1 expression as a potential predictive biomarker of response, and provide new insights for the therapeutic targeting of GBM stem-like cells.

Conflict of interest: Ownership: Heidi Lane and Felix Bachmann are shareholders of Basilea Pharmaceutica Ltd. Corporate-sponsored Research: Financial support for Research by Basilea Pharmaceutica Ltd. Other Substantive Relationships: Heidi Lane and Felix Bachmann are full-time employee of Basilea.

168 (PB-019) Poster

CP-506, a next-generation hypoxia-activated prodrug, as a promising novel anti-cancer therapeutic

S. Deschoemaeker[1], S. Thiolloy[1], J. Gilissen[1], A. Stampella[1], L. Dubois[2], A. Yaromina[2], J. Theys[2], A. Ashoorzadeh[3], C. Guise[4], M. Abbattista[4], M. Bull[4], J. Smaill[4], A. Patterson[4], P. Lambin[2], A. Heyerick[1]. [1]*Convert Pharmaceuticals, Liège, Belgium;* [2]*Maastricht University Medical Centre, Dept. of Radiotherapy, The M-Lab Group, GROW – School for Oncology and Developmental Biology, Maastricht, Netherlands;* [3]*University of Auckland, Translational Therapeutics Team, Auckland Cancer Society Research Centre, School of Medical Sciences, Auckland, New Zealand;* [4]*University of Auckland, Translational Therapeutics Team, Auckland Cancer Society Research Centre, School of Medical Sciences – Maurice Wilkins Centre for Molecular Biodiscovery, School of Biological Sciences, Auckland, New Zealand*

Background: Hypoxia, defined as a low level of oxygen occurs in many solid tumors, leads to a more aggressive tumor phenotype and is associated with poor prognosis. Hypoxia-activated prodrugs (HAPs) are anti-neoplastic agents that can solely be activated in hypoxia allowing their targeted delivery to the hypoxic tumor niches. Convert Pharmaceuticals is developing CP-506, a next-generation HAP with improved pharmacokinetic properties and a high bystander effect allowing the active cytotoxic metabolite (CP-506M) to also kill neighboring tumor cells.

Materials and Methods: *In vitro* potency assay: Cells are seeded under anoxic (ANX) (<0.01% oxygen) or normoxic (NRX) conditions. After 2 hours, cells are incubated with a dose range of CP-506 for 4 h under ANX or NRX conditions. Hereafter, the cells are washed and maintained under NRX conditions for 96 h when viability is evaluated using resazurin staining.

In vivo studies: Immunocompromised mice were injected subcutaneously (NCI-H1650, NCI-H69) or orthotopically (MDA-MB-436) with human tumor cells. Treatment was initiated after randomization, once the tumor volume (TV) reached 250 mm^3. TV and body weight were measured and the time to reach 4 times the TV at treatment start was used as surrogate endpoint for survival. To assess tumor hypoxia and DNA damage, tumors from vehicle and CP-506 treated groups were collected 24 h after the 2nd treatment. One hour before sacrifice, mice were injected ip with 60 mg/kg of pimonidazole. Tumors were processed for anti-pimonidazole (hypoxia) or anti-pH2AX (DNA damage) immunostaining and analysis.

Results: CP-506 significantly inhibits the viability of a tumor cell line panel under anoxic conditions in an *in vitro* potency assay. *In vivo* studies in different tumor xenograft models confirms that 5 consecutive administrations of CP-506 at 600 mg/kg results in a significant inhibition of tumor growth and in an increase in survival without significant body weight loss. Histological analysis showed that CP-506-treated tumors presented a significant increase in DNA damage compared to vehicle-treated tumors and as expected CP-506 treatment induced DNA damage in the hypoxic regions of the tumor. Additional dose regimen studies showed that a repeated dose dense treatment regimen can control tumor growth over time without additional body weight loss.

Conclusions: Our pre-clinical data show that CP-506 is a potent highly selective HAP with the highest *in vivo* efficacy when dosed with a repeated dose dense treatment schedule. Building on these results, we are currently identifying the key parameters determining the sensitivity to CP-506 (hypoxia, reductases and sensitivity to the alkylating activity of CP-506) that will be part of an integrative biomarker which aims at selecting potentially sensitive patients to CP-506 and pave the path to a successful clinical development.

Conflict of interest: Other Substantive Relationships: Sofie Deschoemaeker, Sophie Thiolloy, Arne Heyerick, Julie Gilissen and Alessandra Stampella are employees of Convert Pharmaceuticals. Philippe Lambin is shareholder and CSA at Convert Pharmaceuticals. A/Prof Jeff Smaill is a paid scientific consultant of Convert Pharmaceuticals. He is a co-principal investigator of a commercial research contract from Convert Pharmaceuticals related to preclinical research on CP-506. He is also a co-inventor on pending patent applications related to CP-506 and as such stands to benefit financially from the development of CP-506. A/Prof Adam Patterson is a paid scientific consultant of Convert Pharmaceuticals. He is a co-principal investigator of a commercial research contract from Convert Pharmaceuticals related to preclinical research on CP-506. He is also a co-inventor on pending patent applications related to CP-506 and as such stands to benefit financially from the development of CP-506.

169 (PB-020) Poster

Augmentation of NAD+ by NQO1 activation attenuates cisplatin-mediated hearing impairment

Y. Sei-hoon[1], K.B. Kwon[2], H.S. So[2]. [1]*School of Medicine Wonkwang University, Internal Medicine, Iksan-city, Jeonbuk, South Korea;* [2]*School of Medicine Wonkwang University, Center for Metabolic Function Regulation, Iksan-city, Jeonbuk, South Korea*

Introduction: Cisplatin is an extensively used chemotherapeutic agent, and one of its most adverse effects is ototoxicity. However, the precise mechanism underlying cisplatin-associated ototoxicity is still unclear. The cofactor nicotinamide adenine dinucleotide (NAD^+) has emerged as a key regulator of cellular energy metabolism and homeostasis. It remains unclear whether modulation of NAD^+ levels has an impact on cisplatin-induced hearing impairment.

Material and Methods: To investigate whether augmentation of NAD^+ by NQO1 activation using b-Lapachone (b-Lap) attenuates cisplatin-mediated hearing impairment, male C57BL/6 mice and NQO1 knockout mice on a C57BL/6 background were used. We measured the enzymatic activity of SIRT1, PARP1, ROS production, NAD+/NADH ratio, mRNA levels of miR-34a and pro-inflammatory cytokines. Immunohistochemistry and western blot analysis were also performed.

Results and Discussion: We have demonstrated for the first time that both the protein expression level and the activity of SIRT1 were suppressed by the reduction of intracellular NAD^+ levels in cisplatin-treated cochlear tissue. We also found that the decrease in SIRT1 protein expression and its activity after cisplatin exposure were mediated by the increase in transcriptional activity of p53 for miR-34a expression and PARP-1 activation causing NAD^+-depletion, respectively.

Conclusion: Considering that b-Lap itself did not attenuate the tumoricidal effect of cisplatin, these results suggest that the direct modulation of the cellular NAD^+ level by pharmacological agents could be a promising therapeutic strategy for enhancing the efficacy of cisplatin chemotherapy without its adverse effects.

No conflict of interest

170 (PB-021) Poster

TAS1553, a novel class of RNR inhibitor, demonstrates antitumor activity in preclinical models

H. Ueno[1], T. Hoshino[1], S. Tsukioka[1], W. Yano[1], T. Suzuki[1], A. Hashimoto[1], S. Miyahara[1], S. Hara[1], Y. Ogino[1], K. Ichikawa[1], K. Matsuo[1], K. Miyadera[1], T. Utsugi[1], Y. Iwasawa[1]. [1]*Taiho Pharmaceutical Co. Ltd., Discovery and Preclinical Research Division, Tsukuba, Japan*

Background: Ribonucleotide reductase (RNR) is a key metabolic enzyme in nucleic acid metabolism which has long been targeted by antimetabolites. RNR regulates a rate-limiting step for supply of dNTPs via converting

ribonucleotides to deoxyribonucleotides. Because dNTPs are the building blocks of DNA and essential for cancer cell growth and survival, RNR is considered to be a promising therapeutic target for cancer treatment. TAS1553 is a novel class of RNR inhibitor which has a potent inhibitory activity against RNR via abrogating the protein-protein interaction between RNR subunits. *In vitro* study suggests that TAS1553 shows wide-ranging antitumor activity by causing DNA replication stress and apoptosis. Here we describe the antitumor activity of TAS1553 in *in vivo* models.

Material and Methods: TAS1553 was designed and synthesized at Taiho Pharmaceutical Co., Ltd. The antitumor efficacy of TAS1553 was evaluated in athymic nude mice or nude rats bearing MV-4-11 (human acute myeloid leukemia) and HCC38 (human breast cancer). The amounts of nucleotide pools (dATP and ATP) in tumors were measured by LC-MS/MS analysis in order to evaluate RNR inhibitory effect of TAS1553. The protein and phospho-protein expression level in tumors were determined by Western blot analysis to evaluate the induction of DNA replication stress and apoptosis.

Results: The antitumor effect and RNR inhibitory effect of TAS1553 were evaluated in MV-4-11 rat xenograft model. Once daily dosing of TAS1553 not only inhibited tumor growth at 150 mg/kg but also caused tumor shrinkage (and tumor disappearance in 3 of 5 rats) at 300 mg/kg without severe effect on body weight gain. Importantly, single oral administration of TAS1553 at 150 mg/kg caused a marked decrease of the intratumoral dATP pool (T/C < 20%) while the administration did not affect the intratumoral ATP pool. Furthermore, the administration of TAS1553 induced an increase of pChk1 level followed by the increase of cleaved PARP and cleaved caspase-3 in tumors, suggesting that TAS1553 exerts antitumor effect by inhibiting RNR, resulting in DNA replication stress and apoptosis. In HCC38 mouse xenograft model, the daily treatment of TAS1553 at 150 mg/kg was also efficacious and showed a superior tumor growth inhibition compared to weekly administration of paclitaxel at 20 mg/kg.

Conclusions: These findings demonstrated that TAS1553, a novel class of RNR inhibitor, was orally available and had potent antitumor activity in preclinical models for both hematological and solid tumors. Therefore, TAS1553 could be a promising therapeutic option for cancer patients.

Conflict of interest: Corporate-sponsored Research: We are employees of Taiho Pharmaceutical Co., Ltd.

171 (PB-022) Poster

TAS1553, a novel protein-protein interaction inhibitor against RNR, causes the inhibition of tumor cell proliferation via the induction of dATP pool reduction and DNA replication stress

T. Hoshino[1], H. Ueno[1], W. Yano[1], S. Tsukioka[1], Y. Otsu[1], T. Yonekura[1], S. Ito[1], M. Terasaka[1], S. Miyahara[1], S. Hara[1], Y. Ogino[1], K. Ichikawa[1], K. Matsuo[1], K. Miyadera[1], T. Utsugi[1], Y. Iwasawa[1]. [1]*Taiho Pharmaceutical Co. Ltd., Discovery and Preclinical Research Division, Tsukuba, Japan*

Background: Ribonucleotide reductase (RNR) is a key metabolic enzyme that catalyzes a rate-limiting step for dNTP biosynthesis. It is composed of two non-identical subunits, R1 and R2, and the interaction between these subunits is necessary to its enzymatic activity. Based on the role in supplying dNTP for DNA synthesis, RNR is considered as a promising target for cancer therapy. The conventional RNR inhibitors have the shortcomings of low potency and selectivity. Here, we report a novel, selective and highly potent RNR inhibitor TAS1553, which disrupts the protein-protein interaction between RNR subunits.

Material and Methods: Inhibitory effect on enzymatic activity of human RNR was assessed by measuring the formation of dCDP from the substrate CDP. Inhibitory effect on the interaction between human R1 and R2 subunits was evaluated with AlphaLISA assay.

Antiproliferative activity was assessed by CellTiter-Glo® 2.0 assay. Intracellular inhibitory effects on R1-R2 interaction and RNR activity were evaluated by using Fluoppi system and measuring the amount of intracellular dATP pool, respectively. Induction of DNA replication stress and apoptosis was investigated by western blotting and immunofluorescence staining.

Results: TAS1553 inhibited RNR activity in a concentration-dependent manner and the IC_{50} value was less than 100 nmol/L. The inhibitory effect of TAS1553 on RNR activity was the strongest among the evaluated conventional RNR inhibitors including hydroxyurea and 3-AP. TAS1553 also inhibited R1-R2 interaction with an IC_{50} < 100 nmol/L. In addition, TAS1553 showed excellent target selectivity compared to 68 off-target enzymes in the LeadProfilingScreen (Eurofins Panlabs Discovery Services Taiwan, Ltd.).

Cytotoxicity profiling of TAS1553 revealed wide-ranging antiproliferative activity against various human hematological and solid cancer cell lines (GI_{50} = 228–4150 nmol/L). In pharmacodynamic analysis using HCC38 breast cancer cells, TAS1553 treatment inhibited R1-R2 interaction and significantly reduced intracellular dATP pool (T/C < 20%) but not ATP pool within 0.5 hours. Furthermore, TAS1553 caused DNA replication stress as indicated by induction of pChk1, pRPA2 and γH2AX. When treated with TAS1553, formation of pRPA2 foci, which implies replication fork stalling, was observed in the nuclei. Following DNA replication stress, TAS1553 induced apoptosis as indicated by cleavage of PARP and caspase-3.

Conclusions: TAS1553 is a novel and highly potent RNR inhibitor with a unique inhibitory mechanism (R1-R2 interaction inhibition). It is suggested that TAS1553 exerts wide-ranging antiproliferative activity by causing marked reduction of intracellular dATP pool and DNA replication stress, and finally leading to apoptosis. TAS1553 may provide a promising therapeutic option for various cancers.

Conflict of interest: Corporate-sponsored Research: We are employees of Taiho Pharmaceutical Co. Ltd.

172 (PB-023) Poster

The expression of topoisomerase IIα(topo IIα)protein in young breast cancer patients (<35 years) and its relationship with prognosis

Y. Lin[1], H. Yunxia[2], Y. Mingang[1]. [1]*Fujian Medical University Cancer Hospital, Department of Abdominal Surgery, Fuzhou, China;* [2]*Fujian Medical University Cancer Hospital, Department of Radiation Oncology, Fuzhou, China*

Background: There was no consensus on the the relationship between topoisomerase IIα (Topo IIα) and the prognosis of young breast cancer patients. Our study aimed to assessed the long-term prognostic and predictive value of Topo IIα expression in young patients (<35 years) with breast cancer.

Methods: A total of 131 young breast cancer patients from 2001 to 2006 were retrospectively analyzed at our institute. We finally enrolled 50 patients for Topo IIα expression detection by immunohistochemistry technology. The association of Topo IIα expression and clinicopathological features were studied by Chi-square test. The 10-year disease free survival (DFS) and overall survival (OS) of these 50 patients were calculated by Kaplan-Meier analysis. The Cox regression model was employed for multivariate analysis.

Results: There were 42 patients (84%) with Topo IIα positive in the patients we analyzed. There was no correlation between Topo IIα protein expression and age, tumor size, lymph node metastasis, TMN stage, molecular typing, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2(HER-2) ($P > 0.05$). The expression of Topo IIα was only positively correlated to Ki67 ($r = 0.533$, $P = 0.002$) as displayed by Spearman's correlation test. The 1-, 3-, 5-, 10-year DFS in Topo IIα positive *vs.* negative group were 95.2% *vs.* 87.5%, 76.2% *vs.* 37.5%, 66.7% *vs.* 12.5% and 64.3% *vs.* 12.5%. The difference between two groups was statistically significant ($c^2 = 5.056$, $P = 0.025$). There were 16(32%) death in our follow-up, the median OS (mOS) of Topo IIα positive and negative group were 144 months and 105 months ($c^2 = 1.652$, $P = 0.199$). In the multivariate Cox regression model, Topo IIα expression [*RR* = 0.341,*95% CI*:0.126–0.919, $P = 0.033$] was an independent prognostic factor for DFS in young patients with breast cancer. Also,TNM staging [*RR* = 5.920, *95% CI*:1.775–19.738, $P = 0.004$] and lymph node metastasis [*RR* = 5.411, *95% CI*:1.116–26.222, $P = 0.036$] were both independent predictors for OS.

Conclusions: Topo IIα was more likely to have high expression in young breast cancer patients, and positively correlated to ki67 expression. Patients with high expression of Topo IIα presented a favorable DFS.

No conflict of interest

173 (PB-024) Poster

Indole-Biaryl Pyrrolobenzodiazepines (I-BiPs): A potent and well-tolerated class of DNA mono-alkylating payload for antibody-drug conjugates (ADCs)

J. Thomas[1], A. Yurkovetskiy[1], M. Yin[1], N. Bodyak[2], J. Tang[1], M. Protopopova[2], E. Kelleher[1], B. Jones[1], L. Yang[1], D. Custar[3], K. Catcott[3], D. Demady[4], S. Collins[2], L. Xu[5], C. Bu[5], L. Qin[2], E. Ter-Ovanesyan[5], M. Damelin[2], D. Toader[3], T. Lowinger[1]. [1]*Mersana Therapeutics, Discovery Chemistry, Cambridge, USA;* [2]*Mersana Therapeutics, Biology, Cambridge, USA;* [3]*Mersana Therapeutics, Bioconjugation, Cambridge, USA;* [4]*Mersana Therapeutics, Toxicology, Cambridge, USA;* [5]*Mersana Therapeutics, Analytical Chemistry, Cambridge, USA*

Background: Although it has been known for years that pyrrolobenzodiazepine (PBD) monomers and polyheterocycle DNA binders can be synergistically combined to form potent DNA mono-alkylators, such PBD hybrids have not yet been used as ADC payloads. We describe here a new class of PBD mono-alkylator hybrid (referred to as I-BiPs) and demonstrate their use as ADC payloads.

Materials and Methods: A series of biaryl heterocyclic DNA binders were linked to a PBD monomer and the cytotoxic activity of the resulting molecules were evaluated in a panel of solid tumor cell lines. The most potent molecules were incorporated into linked payloads. The payloads were conjugated to monoclonal antibodies and the resulting ADCs[RI1] that displayed desirable physicochemical properties and in vitro activity were evaluated in *in vivo* xenograft models. Payload and ADC processing was assessed in tumor cells in culture as well as in the presence of lysosomal extract and in plasma. Bystander capability of the cytotoxic payload released from the ADC was also assessed in co-culture experiments using antigen-positive and antigen-negative cell lines. The lead ADCs were evaluated in *in vivo* xenograft models, PK, and rat tolerability head-to-head against a known DNA mono-alkylator class (IGN mono-alkylators).

Results: SAR studies led to the identification of a key indole unit in the DNA binder portion that significantly improved potency while providing a site for antibody conjugation. The lead I-BiP series exhibited low picomolar activity in a broad panel of solid tumor cell lines, including cell lines resistant to anti-tubulin agents. These *in vitro* cytotoxicities correlated with *in vivo* activity and the corresponding I-BiP ADCs were also highly active *in vivo* in auristatin-resistant xenograft models. Co-culture experiments with I-BiP ADCs showed that the extent of bystander killing could be modulated *via* simple structural variations on the indole unit. Unlike typical PBD dimers and IGN mono-alkylators, I-BiP ADCs are more hydrophilic and therefore are not limited to DAR 2; DAR 4-5 I-BiP ADCs with high monomeric content were readily achieved without resorting to site-specific conjugation. Pronounced anti-tumor activity was observed for I-BiPs ADCs at single doses of 1 or 3 mg/kg in a variety of solid tumor xenograft models. In toxicology studies in rats, I-BiP ADCs were well tolerated after multiple doses.

Conclusions: Given their potent antitumor efficacy in a variety of solid tumor models, favorable therapeutic index and hydrophilicity relative to PBD dimers and IGNs, I-BiPs are a promising new class of DNA damaging payload for ADCs.

[RI1]Defined in the titled.

No conflict of interest

174 (PB-025) Poster

Testing the COXEN method of predicting drug response with a prospective trial in dogs with osteosarcoma

D. Gustafson[1], K. Collins[2], J. Fowles[1], E. Ehrhart[1], K. Weishaar[1], D. Duval[1], D. Thamm[1]. [1]*Colorado State University, Flint Animal Cancer Center, Fort Collins, USA;* [2]*Colorado State University, School of Biomedical Engineering, Fort Collins, USA*

Background: COXEN is a method of predicting drug response of tumors based on gene expression and drug sensitivity of cell lines (*Proc Natl Acad Sci USA* 104:13086). Testing of predictive algorithms in clinical populations are difficult due to a number of factors including cost and the time associated with patient follow up. Pet dogs with cancer are treated for their disease by specialty trained veterinarians and here we report the use of this population to test the ability of the COXEN algorithm to enhance drug response in canine osteosarcoma (cOSA).

Materials and Methods: Gene expression modeling (GEM) to predict the chemosensitivity of canine cancer cell lines and tumor drug response in cOSA using COXEN has been previously reported (*BMC Bioinformatics* 17:93). Following up on these findings, we designed a prospective clinical trial in dogs with cOSA that involved use of the previously derived COXEN algorithm to predict response to doxorubicin (DOX) and carboplatin (CARBO). Dogs with pathologically confirmed cOSA underwent amputation of the affected limb and tumor samples were collected from independent trephine biopsies. Biopsies underwent pathology review for viable tumor content followed by mRNA isolation and processing for gene expression analysis using the Affymetrix® canine 2.0 array. The previously described COXEN algorithm was then implemented to predict tumor sensitivity to DOX and CARBO. Following surgery and adjuvant chemotherapy with DOX, CARBO or the combination (DOX/CARBO), dogs are evaluated every two months for disease progression by chest x-ray to detect lung metastasis.

Results: Sixty-two canine patients have been evaluated for the trial with 56 of a planned 60 confirmed cOSA patients currently enrolled. Dogs are stratified based on COXEN scores of paired samples to obtain a treatment regimen of DOX, CARBO or DOX/CARBO depending on the predicted probability of drug response. Dogs whose tumors are predicted to not respond to either DOX or CARBO are assigned to receive DOX/CARBO. Twenty-seven dogs have been assigned to receive monotherapy (6 DOX and 21 CARBO) and twenty-nine to the DOX/CARBO combination (7 sensitive and 22 resistant). 30% of the dogs designated as drug sensitive have a disease free interval (DFI) greater than one year whereas 19% of the dogs predicted as resistant have a DFI>1 year. 16/34 dogs predicted as drug sensitive are disease free currently while 8/22 dogs predicted as drug resistant are currently disease free.

Conclusions: This is the first example of a GEM algorithm being used for drug selection in a canine cancer patient population and establishes their use for testing molecular-based therapeutic approaches in the adjuvant setting. Enrolled dogs will continue to be monitored for disease progression and the completion of this study allow for the prospective testing of the COXEN-based approach.

No conflict of interest

175 (PB-026) Poster

Elevated serum substance P levels as a predictive marker for chemotherapy induced nausea and vomiting: prospective cohort study

H.S. Park[1], H.S. Won[2], H.J. An[1], B.Y. Shim[1]. [1]*St. Vincent Hospital, College of Medicine, The Catholic University of Korea, Division of Medical Oncology, Department of Internal Medicine, Suwon, South Korea;* [2]*Uijeongbu St. Mary`s Hospital, College of Medicine, The Catholic University of Korea, Division of Medical Oncology, Department of Internal Medicine, Uijeongbu, South Korea*

Background: Chemotherapy induced nausea and vomiting (CINV) is unbearable side effects. Moderate emetic risk chemotherapy induces CINV in a wide range of frequency (30 to 80%), but prevention strategy is the same to all these patients. Therefore, selection of high emetic risk patients and more active anti-emetics strategies are mandatory to improve tolerability of chemotherapy. The aim of this study is to find predictive clinico-biological marker for CINV.

Material and Methods: In this prospective cohort study, patients who received moderate to high emetic risk chemotherapy were enrolled. All patients were received granisetron patch at the day before start of 1st cycle chemotherapy. As predictive biomarkers, leptin, substance-P and ghrelin were measured at baseline, day 3 and day 14 after 1st cycle chemotherapy. Nausea and vomiting were measured each day during 4 days of 1st cycle chemotherapy, respectively. Logistic regression was used to evaluate the association between clinico-biological marker and CINV. Continuous variables were dichotomized according to the best cut-off value by receiver operating characteristic (ROC) curve analysis.

Results: Eighty eight patients were enrolled and median age was 61. Male was predominant (70.5%), and colorectal cancer (56.8%) and gastric cancer (35.2%) are common cancer type. Most of patients (93.2%) received moderate emetic risk chemotherapy. During 4 days receiving chemotherapy, 50 patients (58.0%) had nausea and 20 patients (22.7%) experienced vomiting. Baseline median level of leptin, substance-P and ghrelin was 1.33 ng/mL (range 0.03–29.03), 302.2 pg/mL (range 54.7–1228.4) and 201.0 ng/mL (12.3–1700.0), respectively. There are no significant differences among biomarkers at different time point, but ghrelin level of day 14 was increased compared to baseline (P = 0.052). Patients with nausea had higher level of substance-P than patients without nausea at baseline and day 3. In univairate analysis, higher level of baseline substance P (≥222.6 ng/mL) is the only significant predictive marker for chemotherapy induced nausea [odds ratio (OR): 2.8, 95% confidence interval (CI): 1.1–7.1, P = 0.027]. Regarding chemotherapy induced vomiting, patients with high level of substance-P had more change of vomiting, but that was not statistically significant [OR: 2.4, 95% CI: 0.7–8.1, P = 0.146]. Regimen of chemotherapy and cancer type [Gastric cancer compared to colorectal cancer, OR: 20.0, 95% CI: 2.2–182.4, P = 0.029] is independent predictive marker for chemotherapy induced vomiting.

Conclusions: Baseline serum substance-P which is target of aprepitant is independent predictive marker for chemotherapy induced nausea. Active anti-emetics strategy should be considered to patients with high level of substance-P and further prospective study is needed to validate the predictive value of substance-P.

Conflict of interest: Corporate-sponsored Research: This study was supported by grant from LG Chem, Ltd (to B.Y. Shim).

176 (PB-027) Poster

Influence of tether variations on the biological activity of tesirine analogues

A. Tiberghien[1], N. Patel[1], B. Vijayakrishnan[1], L. Adams[1], S. Corbett[1], N. Arora[1], J. Hartley[1], P. Howard[1]. [1]*Medimmune, Spirogen, London, United Kingdom*

Background: Pyrrolobenzodiazepine (PBD) dimers are DNA crosslinking agents, and exert potent cytotoxic activity across a wide spectrum of cancer cell lines. For these reasons, they were identified as ideal candidates for targeted delivery by antibody drug conjugates. Amongst many PBD drug-

linkers conjugated to anti-tumour antibodies, SG3249 (or tesirine), has been employed in multiple clinical trials. In this study, we sought to verify whether the established SAR observed with stand-alone PBD dimers, were reflected *in vivo* through ADC delivery. A further goal was to exploit the versatility of the tether unit to introduce a range of molecular motifs that could modulate the activity of the PBD dimers.

Material and Methods: N10 protected PBD building blocks produced during the synthesis of the drug SG3199 (**2a**) and the drug-linker SG3249 (**2b**) were joined with a propyl, benzyl, and pyridyl core. The resulting free drugs **1a**, **2a**, **3a**, and **4a** were investigated *in vitro* in cancer cell-line NCI-N87, whereas the drug-linkers **1b**, **2b**, **3b**, and **4b** were conjugated through a cleavable linker to engineered trastuzumab and evaluated both *in vitro* and in murine xenografts models.

Results: Analogues of SG3199 with a five-carbon tether (either aliphatic or aromatic) were 7 to 20 times more potent that their three-carbon tether counterpart *in vitro* (**1a**, **2a**, **3a**, **4a**: 150 pM, 20 pM, 7 pM, 16 pM). The pattern of activity observed *in vitro* was mirrored *in vivo* with the benzyl tether analogue **3b** showing prolonged tumour stasis for up to 80 days (single injection, 0.3 mg/kg) as compared with 30 days for **2b** and **4b**. At 1 mg/kg, full tumour regressions were observed for **2b**, **3b**, whereas prolonged tumour stasis was observed for **1b**.

Conclusions: This works is consistent with previous studies demonstrating the ability of the 5 carbon tethers to optimally follow the curvature of the DNA. Further interactions between the aromatic tethers and the DNA groove may explain the observed enhancement in activity. The exact level of activity *in vivo* could not be predicted solely by the activity of the free drug observed *in vitro*. Other factors such as lipophilicity and permeability may be taken into account to refine the model. In conclusion, we have shown that the tether linking two PBD monomers is tolerant of chemical modification and may accept a variety of molecular motifs suitable for enhancing activity. Drug-linker **3b** was identified as a more potent analogue of SG3249.

Conflict of interest: Ownership: Authors and co-authors are Medimmune/AstraZeneca employees (exception SC) with ownership interest.

177 (PB-028) Poster

DAR 1 ADCs: Advances and opportunities

B. Vijayakrishnan[1], R. Beard[1], L. Masterson[2], A. Tiberghien[2], N. Dimasi[3], P. Howard[4]. *[1]Spirogen-MedImmune, Conjugation, London, United Kingdom; [2]Spirogen-MedImmune, Chemistry, London, United Kingdom; [3]MedImmune, Research and Development, Technology, Gaithersburg, USA; [4]Spirogen-MedImmune, Chief Scientific Officer, London, United Kingdom*

Background: Antibody-drug conjugates (ADCs) are an exciting new class of anticancer therapeutics. ADCs combine the potency of a cytotoxic agent with the selectivity of an antibody to enable targeted killing of tumour cells. PBD dimers are emerging as a particularly attractive class of payload for the growing ADC field due to the combination of their potency and different mode of action to antimitotic warheads such as auristatin and maytansines. Tesirine (SG3249) is currently being used in multiple clinical trials by MedImmune, external licencing companies and others.

Results and Discussion: Expanding the therapeutic window is a key driver for successful ADCs and frequently narrow therapeutic indices (TI) have been reported for candidates under evaluation in the clinic. This is mainly due to off-target toxicity, which is linked to deconjugation, competition with unconjugated antibody, aggregation and faster clearance due to hydrophobicity. In our quest to improve the TI, we designed a PBD payload to achieve a DAR 1 homogeneous ADC. The unique flexibility of the PBD platform technology allowed us to add two functional maleimides to the potent PBD warhead SG3199, which resulted in the creation of a symmetrical PBD payload SG3710. This double maleimide is capable of bridging site-specific cysteines in an engineered antibody. Conjugation conditions were optimised to produce homogenous DAR 1 ADCs using anti-Her2, anti-EphA2 and non-targeting antibodies.

The resulting DAR 1 ADCs were tested in an in vitro cytotoxic assay along with DAR 2 ADCs using tesirine payload. It is worth noting that both SG3710 and tesirine releases the same potent PBD warhead, SG3199. In Her2 overexpressing gastric cancer cell line NCI-N87 both ADCs showed similar EC50 values (0.2–0.5 ng/mL). In a EphA2 overexpressing prostate cancer (PC3) cell line both ADCs exhibited comparable EC50 values (2.2–5.0 ng/mL). The DAR1 and DAR2 anti Her2 ADCs were evaluated in an NCI N87 human xenograft model. At dose of 0.3 mg/kg the DAR1 ADC was as active as the DAR2 despite delivering only half the amount of SG3199 warhead.

Conclusion: In summary, we have developed a unique, site specific DAR 1 ADC by exploiting the flexible PBD platform and a cysteine engineered antibody. DAR 1 ADC shows similar potency in in vitro cell killing assays and in in vivo mouse xenograft models. This work shows the potential of DAR 1 ADCs and we are currently exploring alternate payloads with varying potency.

Conflict of interest: Ownership: The authors declare the following competing financia interest(s): All authors are employees of MedImmune a division of Astrazeneca and own AstraZeneca stocks.

178 (PB-029) Poster

Preliminary pharmacokinetic assessment of BT1718: A phase I/IIa trial of BT1718 (a first in class Bicycle Toxin Conjugate) in patients with advanced solid tumours

U. Banerji[1], N. Cook[2], T.R.J. Evans[3], I. Moreno[1], R. Patricia[3], C. Kelly[2], N. Sabaratnam[4], R. Passi[4], L. Godfrey[5], L. Sawretse[5], S. Katugampola[5], G. Halbert[6], P. Jeffrey[7], M. Rigby[7], G. Bennett[7], M. Koehler[7], G. Langford[7], M. Pittman[5], S.N. Symeonides[8]. *[1]The Institute of Cancer Research, London, and the Royal Marsden NHS Foundation Trust., Drug Development Unit, Sutton, United Kingdom; [2]University of Manchester & Christie NHS Foundation Trust, Christie School of Oncology, Manchester, United Kingdom; [3]University of Glasgow & Beatson West of Scotland Cancer Centre, Cancer Research Centre, Glasgow, United Kingdom; [4]The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust., Drug Development Unit, Sutton, United Kingdom; [5]Cancer Research UK Centre for Drug Developmen, Drug Development, London, United Kingdom; [6]Cancer Research UK Formulation Unit, University of Strathclyde, Cancer Research UK Formulation Unit, Glasgow, United Kingdom; [7]Bicycle Therapeutics, Development, Cambridge, United Kingdom; [8]University of Edinburgh, Edinburgh UK & Cancer Research UK Centre for Drug Development, London, UK, Cancer Research UK Edinburgh Centre, Edinburgh, United Kingdom*

Background: Bicycles® are novel therapeutics: bicyclic peptides constrained via a chemical scaffold, which confers structural stability leading to high affinity and selectivity. These Bicycles have been used to generate Bicycle Toxin Conjugates (BTC), which bind to cell-surface targets on solid tumour cells and deliver a cell-killing toxin. BTC's low molecular weight results in rapid and extensive delivery of payloads into preclinical solid tumour models, associated with a short duration of systemic exposure and liver-sparing rapid renal elimination.

The BTC, BT1718, is a MT1-MMP binding peptide linked to the maytansinoid toxin DM1 via a cleavable disulphide linker that demonstrated significant anti-tumour activity in a range of preclinical experiments. Following intravenous administration to preclinical species, BT1718 exhibited a short terminal half-life (ca. 20 min in Balb c mice) and a volume of distribution similar to extracellular fluid volume (ca. 0.2 L/kg). Here we present the preliminary initial pharmacokinetic (PK) data from the ongoing phase I/IIa clinical study.

Materials and Methods: This is a first in human, multicentre, dose escalation study in patients with advanced solid tumours. The study aims to establish the recommended Phase II dose (RP2D) for once and bi-weekly dosing of BT1718, along with pharmacokinetics and exploratory pharmacodynamics. Expansions will further explore preliminary efficacy, tolerability and pharmacodynamics of BT1718 in approximately 60–70 patients with MT1-MMP expressing NSCLC, TNBC or other tumours (as assessed by IHC) (Clinical trial NCT03486730).

Results: Dose escalation in Phase I is ongoing and no DLTs have been reported to date (up to cohort 3). The preliminary PK data in patients are in line with the preclinical PK data and confirm dose dependent increases in exposure of BT1718 accompanied with a short terminal half-life.

Conclusions: BT1718 is a first in class BTC and is well tolerated at doses up to 2.4 mg/m^2. The preliminary PK data confirm the expected PK profile based on preclinical data. Updated clinical PK data from the ongoing study will be discussed along with further preclinical tumor and plasma data illustrating mechanism of action of this new treatment modality.

Conflict of interest: Other Substantive Relationships: BT1718 is being developed by Cancer Research UK (CRUK) and Bicycle Therapeutics. A number of the authors are employed by CRUK or Bicycle Therapeutics.

179 (PB-030) Poster

Combinations containing the anti-CD205 antibody drug conjugate MEN1309/OBT076 show strong pre-clinical activity in diffuse large B cell lymphomas

C. Tarantelli[1], E. Gaudio[1], F. Spriano[1], F. Guidetti[2], A. Fiascarelli[3], G. Merlino[3], A.A. Mensah[1], D. Rossi[2], E. Zucca[4], A. Stathis[5], M. Binaschi[3], C. Rohlff[6], R. Boyd[6], F. Bertoni[1]. *[1]Università della Svizzera italiana, Institute of Oncology Research, Lymphoma Genomics, Bellinzona, Switzerland;*

[2]*Università della Svizzera italiana, Institute of Oncology Research, Experimental Hematology, Bellinzona, Switzerland;* [3]*Menarini Ricerche S.p. A., Experimental and Translational Oncology, Pomezia, Italy;* [4]*Oncology Institute of Southern Switzerland, Lymphoma Unit, Bellinzona, Switzerland;* [5]*Oncology Institute of Southern Switzerland, Clinical Research Unit, Bellinzona, Switzerland;* [6]*Oxford BioTherapeutics Ltd., Global Headquarters, Oxford, United Kingdom*

Background: Still too many individuals affected by lymphoma succumb due to their disease: novel therapeutic approaches are needed. MEN1309/ OBT076 is a novel antibody drug conjugate (ADC) consisting of an anti-CD205 monoclonal antibody conjugated to the DM4 maytansine derivative (Bigioni et al, AACR 2017; Bressan et al. AACR 2017). We have previously reported strong *in vitro* and in *vivo* anti-tumor activity with the ADC in diffuse large B cell lymphoma (DLBCL) as a single agent (Gaudio et al., AACR 2017), which is now in phase I for patients with CD205-positive metastatic solid tumors and relapsed/refractory non-Hodgkin lymphomas (NCTNCT03403725). Here, we assessed its combinatorial activity in DLBCL models.

Methods: Four human lymphoma cell lines derived from DLBCLwere exposed to MEN1309 in combination with targeted agents. Cell proliferation was measured with MTT. Synergy was assessed with Chou-Talalay combination index. Xenografts (15×10^6 cells/mouse, 200 μL of PBS) were established sc into the left flanks of female NOD-SCID mice; treatments started with established tumors.

Results: MEN1309 was combined with other targeted agents in tree DLBCL cell lines (HBL1, OCI-LY-10, TMD8). MEN1309 plus the anti-CD20 monoclonal antibody rituximab was synergistic in 3/3. The combination was then evaluated *in vivo* in a DLBCLmodel (OCI-LY-10). Mice were divided in four groups and treated with MEN1309 (2.5 mg/kg IV, D1 and D12), rituximab (3 mg/kg IV on D1; 5 mg/kg IV on D12), MEN1309 plus rituximab (same schedule as single agents), or with vehicle only. The combination induced tumor eradication with significant differences at D17 ($P < 0.05$) versus both single agent arms.

Benefit in all three cell lines was also observed with MEN1309 *in vitro* combined with the BCL2 inhibitor venetoclax (ABT-199) and with the BTK inhibitor ibrutinib. The combinations with the PI3K-delta inhibitor idelalisib and the immunomodulant lenalidomide presented additivity in only one cell line. No benefit was seen combining MEN1309 with the proteasome inhibitor bortezomib.

Conclusions: Very promising preclinical anti-lymphoma activity was observed with MEN1309 in combination with venetoclax, ibrutinib and especially rituximab, providing the bases for further clinical development of this novel ADC in a combinatorial setting.

CT and EG: equally contributed.

Conflict of interest: Board of Directors: Oxford BioTherapeutics Ltd.: Christian Rohlff. Other Substantive Relationships: Menarini employee: Alessio Fiascarelli, Giuseppe Merlino, Monica Binaschi. Oxford BioTherapeutics Ltd. employee: Christian Rohlff, Robert Boyd. Institutional Research funds from Menarini: Francesco Bertoni.

Wednesday, 14 November 2018

POSTER SESSION

Drug Screening

180 (PB-031) Poster

Exploration of small-molecule inhibitors targeting glucose-independent cancer metabolism

M. Kawatani[1], M. Hayashida[2], H. Aono[1], M. Muroi[1], Y. Futamura[1], H. Osada[1]. [1]*RIKEN Center for Sustainable Resource Science, Chemical Biology Research Group, Wako, Japan;* [2]*Saitama University, Graduate School of Science and Engineering, Saitama, Japan*

Background: Cancer cells have metabolic variability and flexibility to sustain their growth and survival. Targeting the metabolic differences between cancer and normal cells holds promise as a novel anticancer strategy. The purpose of this study is to investigate cancer-specific metabolic pathways and to find small-molecule inhibitors targeting those pathways.

Material and Methods: For cell-based screening, human osteosarcoma MG-63 cells were treated with compounds in the presence or absence of glucose for 72 h. Cell viability was measured by WST-8 assay. We used the chemical library of RIKEN NPDepo. Proteomic analysis was performed by using two-dimensional differential in gel electrophoresis (2D-DIGE). Bioenergetic analysis was carried out by using a Seahorse flux analyzer.

Results: We examined the nutrient dependency of various cancer cells and found that MG-63 cells, a line derived from an osteosarcoma, can grow even without glucose. Bioenergetic analysis showed that the glycolytic pathway in MG-63 cells functions normally in response to glucose. These results suggested that, although MG-63 cells have the ability to metabolize glucose, they also have alternative mechanisms to enable their growth and survival under glucose deprivation. Proteomic analysis of glucose-starved MG-63 cells showed that part of the proteomic changes including metabolism-related proteins were different from those of glucose-starved HeLa cells, which grow in a glucose-dependent manner. Using this unique metabolic property, we performed cell-based screening for compounds that selectively inhibit glucose-independent growth of MG-63 cells. We screened more than 20,000 compounds from our NPDepo chemical library and obtained several hit compounds. RCOP8154, a hit compound of this screening, were almost no toxic in the presence of glucose and exhibited potent cytotoxicity in the absence of glucose. We found that RCOP8154 is a compound with autofluorescence and localized to mitochondria. In addition, RCOP8154 decreased intercellular ATP level without inhibiting any of mitochondrial electron transport chain complexes (I-V) directly.

Conclusions: We found that human osteosarcoma MG-63 cells can grow even without glucose. By cell-based screening, we found that RCOP8154 specifically inhibits glucose-independent growth of MG-63 cells.

No conflict of interest

181 (PB-032) Poster

An Agaricus blazei-based mushroom extract protects against intestinal tumorigenesis in the A/J Min/+ mouse

G. Hetland[1], D.M. Eide[2], J.M. Tangen[3], M.H. Haugen[4], M.R. Mirlashari[1], J.E. Paulsen[5]. [1]*Oslo University Hospital, Immunology and Transfusion Medicine, Oslo, Norway;* [2]*Norwegian Institute of Public Health, Chemicals and Radiation, Oslo, Norway;* [3]*Oslo University Hospital, Acute Medicine & National CBRNE Centre, Oslo, Norway;* [4]*Oslo University Hospital, Tumor Biology, Oslo, Norway;* [5]*Norwegian University of Life Science, Food Safety and Infection Biology, Oslo, Norway*

Background: The A/J Min/+ mouse model for human familial adenomatous polyposis (FAP) develops spontaneously multiple adenocarcinomas in colon and small intestine. Agaricus blazei Murill (AbM) mushroom is related to champignon and used in traditional medicine against cancer. It contains immunomodulating β-glucans and has antitumor effects in murine cancer models. Andosan™ is an AbM-based extract.

Materials and Methods: A/J Min/+ and A/J wild-type mice were given tap water with 10% Andosan™ for 22 weeks, exsanguinated, their intestines preserved and serum frozen. Intestines were microscoped blindly and stained for tumor-associated protease, legumain. Serum cytokines were measured by Luminex multiplex analysis.

Results: Andosan™ treated A/J Min/+ mice had significantly fewer intestinal adenocarcinomas and 60% reduced tumor load compared to control. Legumain expression was also reduced in intestines from Andosan™ treated animals. Moreover, Andosan™ had significant cytotoxic/apoptotic effect on human cancer colon cell line, Caco-2. There were significantly increased Th1 type and pro-inflammatory cytokines in Andosan™ treated mice.

Conclusions: The results from this mouse model for colorectal cancer shows significant protection of orally administered Andosan™ Agaricus blazei-based mushroom extract against intestinal cancer development. This is supported by finding of less legumain in intestines of Andosan™ treated mice and increased systemic Th1 cytokine response. The mechanism is probably both immunomodulatory and growth inhibition of tumor cells by induction of apoptosis.

Conflict of interest: Ownership: Geir Hetland is co-founder and shareholder of ImmunoPharma Co, Oslo, Norway. The other authors have no interests to declare. Advisory Board: Geir Hetland is on Advisory Board of ImmunoPharma Co. Board of Directors: No. Corporate-sponsored Research: ImmunoPharma sponsored AndosanTM mushroom extract used in the study. Other Substantive Relationships: No.

182 (PB-033) Poster

A translational platform using primary human immune cells in vitro, syngeneic and humanized models in vivo to support and advance immune-oncology drug discovery

M. O'rourke[1], O. Aziz[1]. [1]*Charles River Labs, Biology, Essex, United Kingdom*

Charles River Laboratories (CRL) are establishing a powerful translational immuno-oncology platform with the capability of progressing biologics or small molecule modulators of immune response from in vitro to in vivo assays using human and mouse variants of current check-point inhibitors and small molecules.

The platform is supported by an internal blood donor panel which ensures highly reproducible data and high quality immune cells which are prepared immediately once sampled.

Our *in vitro* platform includes primary human immune cell assays which profile T cell activation, T cell mediated cancer cell kill, expansion of T cell populations, mixed lymphocyte reactions (MLR), T cell invasion, and antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

The platform is currently being expanded to determine the effect of activated immune cell populations on tumour cell spheroid cultures. We are in the process of developing a range of GFP expressing cell lines which will be used to support co-culture experiments. The platform has been validated with standard of care chemotherapeutics, including anti-CTLA4, anti-PD1 and a selection of small molecule inhibitors of targets known to modulate immune responses including IDO inhibitors.

Ex-vivo analysis of activated mouse splenocytes response to check-point inhibitors measured as cytokine release and modulation of immune cell populations, as measured by flow cytometry supports the translation of important compounds from the bench to pre-clinical models.

Syngeneic mouse tumour models have frequently been used to profile immune responses in tumours, CRL have optimized and profiled existing check-point inhibitors to support immuno-oncology drug discovery using mouse and rat antibody variants of anti-CTLA4 and anti-PD1.

To confirm the translational development of our platform CRL have developed and optimized humanized mouse models using sub-cutaneous implanted patient derived xenografts (PDX) with human engraftment via CD34+ haematopoeitic stem cells in NOG mice which were treated with anti-CTLA4 and anti-PD1. Infiltration of human immune cells and PDL-1 expression was detected by flow cytometry (FC) and immunohistochemistry (IHC) in hematopoietic organs and tumor tissue, supporting the initial in vitro response in primary immune cells.

We present a screening platform which will support translation of compounds from in vitro primary immune cell assays, to modulation of mouse immune cell population in spleen and tumours, resulting in efficacy and tumour immune cell activation in humanized mouse models.

No conflict of interest

183 (PB-034) Poster

In silico screen for predictive ERBB mutations

M. Hakanen[1], D. Chakroborty[1], K. Kurppa[1], K. Elenius[1]. [1]*University of Turku, Biomedicine, Turku, Finland*

Background: Mutations or copy number variations in *ERBB* genes have been identified in several cancer types and aberrant ERBB signaling has been associated with the progression of *e.g.* breast, colorectal and lung cancer. Several ERBB-targeting drugs have been approved for clinical use. These selectively target either EGFR (ERBB1) or ERBB2 (HER2), or inhibit all kinase-competent ERBBs (EGFR, ERBB2 and ERBB4) simultaneously. While targeted therapies can be effective for a subgroup of patients, identification of individuals who benefit from treatments has remained a challenge. The predictive clinical significance for the vast majority of the thousands of mutations in the cancer tissues also remains unknown. Here, we describe an approach to identify predictive biomarkers for ERBB-targeted therapeutics that takes advantage of publicly available *in silico* cell line databases.

Materials and Methods: Three databases collectively including data from 272 different cancer cell lines were used: Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC), and Cancer Therapeutics Response Portal (CTRP). An R script was written to collect data about cell lines with mutations in any of the four *ERBB* genes, the *ERBB* mutations and copy number variations in these cell lines, and drug response data for 10 ERBB-targeting drugs. Area under curve (AUC) value was used as an indicator of the drug response. Responsiveness of cells expressing clinically actionable "standard" predictive EGFR mutations (EGFR L858R or exon 19 deletions) to EGFR-selective tyrosine kinase inhibitors was used as a threshold to identify other *ERBB* mutant cell lines responsive to any of the 10 ERBB inhibitors. Candidate predictive mutations were validated by expressing individual mutants in Ba/F3 cells. The sensitivity of the Ba/F3 cells expressing ERBB mutants to ERBB inhibitor drugs was assessed using MTT assays.

Results: The analysis identified 41 cell lines harboring putatively predictive *ERBB* mutations. Included were 9 out of 17 (53%) *ERBB* mutations previously classified as "oncogenic" by cBioPortal, initially validating the screen. Nine candidate predictive mutations were selected for further functional validation in the Ba/F3 cells. Functional analyses are ongoing and will be followed by biochemical and structural analyses to understand the mechanisms by which the mutations lead to ERBB receptor activation and drug sensitivity.

Conclusions: The method can be used to identify activating and putatively predictive mutations in *ERBB* receptors, and possibly in other oncogenes.

No conflict of interest

184 (PB-035) Poster

Chemical genomic analysis on autophagic regulation mechanism in human lung cancer A549 cells

T. Kataura[1], M. Imoto[1]. [1]*Keio University, Biosciences and Informatics, Yokohama, Japan*

Background: Autophagy, an intercellular bulk degradation system is a key regulator of cellular homeostasis. Recently, autophagy has been reported to involve with various diseases such as cancer. Especially, it is indicated that cancer cells utilize autophagy to acquire drug resistance or adapt to undernutrition. Therefore, elucidation of the mechanism of autophagy regulation in cancer cells is becoming important. Additionally, diverse small molecules have been reported to induce autophagy. However, the predominant mechanism of autophagy induction by each small molecule is still unclear. Thus, we conducted comprehensive analysis on autophagy modulating pathways based on chemical genomic approach.

Material and Methods: GFP-LC3-RFP, a novel fluorescent autophagic probe was recently reported. This probe is divided into GFP-LC3 and RFP by ATG4 endogenous protease. GFP-LC3 is degraded in lysosomes along with autophagy progression while RFP continues to stay in cytosol. Therefore, induction or inhibition of autophagy can be estimated by GFP/RFP fluorescence intensity ratio. We produced A549 cells stably expressing this probe using retrovirus vector transduction and FACS cell sorting. Automated quantification of autophagic activity was performed by using OPERA phenix high content imaging system. Clustering and heatmap analyses were conducted with R programming software.

Results: We first screened autophagy inducers from 400 small molecules. As a result, 30 compounds including several anti-cancer drugs were identified as autophagy inducers as judged from the decreased signal ratio of GFP/RFP. Next, we examined the effect of 300 different number of signal transduction inhibitors on autolysosome formation induced by autophagy-inducing compounds to produce fingerprints. Then, all fingerprints were normalized, and comprehensive analysis was conducted using hierarchical clustering to classify the regulation pattern of autophagy. The results of clustering, each autophagy-inducing compound was grouped depending on its mode of action, indicated that this chemical genomic analysis was validated. From the perspective of the signal transduction inhibitors, we confirmed MAPK and JAK-STAT signaling pathways broadly regulate autophagy as reported. Interestingly, we found that inhibitors of specific pathways such as CDKs selectively inhibited autophagy flux activated by the autophagy inducers including anti-cancer drugs, which categorized in same cluster. It is indicated that these specific pathways regulated the autophagy against cancer chemotherapeutic agents.

Conclusions: We proposed that several signaling pathways selectively regulated autophagy induced by anti-cancer drugs based on chemical genomic approach. Inhibition of these pathways might improve the efficacy of anti-cancer drugs by suppressing autophagy.

No conflict of interest

185 (PB-036) Poster

Genetic background affects the drug efficacy in 3D organoid model

W.W. Chen[1], J.M. Chang[1]. [1]*Development Center for Biotechnology, Department of Pharmacology, Institute for Drug Evaluation Platform, New Taipei city, Taiwan*

Background: For decades, 2D cell culture is developed for drug screening and mechanism study in cancer research. It provides lots of information in

preclinical drug development but few drugs can pass through the clinical trial because the 3D environment of tumor cells. Tumor cells grow in 3D environment which is more resistant to anti-cancer drug than growing in 2D cell culture. Here we developed a 3D organoid model for drug screening, which the size of tumor is larger than other traditional methods using U-bottom or cell spheroid formation.

Materials and Methods: The human large adenocarcinoma cell lines HCC-827 and NCI-H727 were cultured on the 96-well nanoculture plate, which the 3D organoid was formed at 3 days and subject to the treatment of a panel of chemo-drugs, target drugs and immune-modulator drug. For improvement of 3D organoid model, adhesion molecules were added in the 3D organoid culture. A panel of anti-cancer drugs at a range between 0.01 and 10 uM was tested in 3D organoid model for 24 hours including taxol, CPT-11, cisplatin, doxorubicin, gemcitabine, afatinib, gefetinib and erlotinib. The viability of cancer cells was measured by caspase3/7 activity by using cell imaging analysis.

Results: The size of 3D organoid was increased by adding adhesion molecule. In a panel screening, afatinib appeared had a good anti-tumor activity on HCC-827 lung adenocarcinoma cells with carrying the PI3K mutation in E545K and EGFR mutation in T790M, nor other TKI inhibitors and chemodrug. HCC-827 cells with were sensitive to afatinib about 100-fold than NCI-H727 cells with KRAS mutation in G12S and G12V.

Conclusions: In conclusion, afatinib was responsible for wild type of kras but not in kras G12D mutation cells in a panel of drug screening with different genetic background. By using wild type/mutated paired cancer cell lines in 3D organoid screening, the companion diagnostic for predicting the drug efficacy by genome test for mutation can be discovered for the selection of the responder patients.

No conflict of interest

186 (PB-037) Poster

Golgi morphology and drug resistance in cancer cells

J.J. Garcia Gomez[1], M. Esteras[1], J. Rodrigues Simoes da Costa[2], J. Kriston-Vizi[2], R. Ketteler[2], J. Hartley[1], D. Hochhauser[1]. [1]*UCL Cancer Institute, Oncology, London, United Kingdom;* [2]*MRC Laboratory for Molecular Cell Biology, Cell Signalling and Autophagy, London, United Kingdom*

Background: Recent studies suggest a link between the DNA damage response and regulation of the Golgi apparatus. In cancer cells, DNA damage triggers DNA-dependent protein kinase (DNA-PK) phosphorylation and activation of GOLPH3 (Golgi-associated protein) resulting in fragmentation of the Golgi apparatus. Golgi fragmentation increases cell survival after DNA damage making this pathway a potentially useful therapeutic target. Our aim is to identify drugs which block Golgi fragmentation after DNA damage to improve efficacy of DNA-targeting anticancer therapies.

Material and Methods: We examined effect of conventional DNA-damaging anticancer drugs on Golgi fragmentation in colorectal cancer cell lines, and analysed how GOLPH3 pathway modulates cancer cell growth using DNA-PK inhibitors and knockdown of GOLPH3. Additionally, we performed cell-based phenotypic screening using a robotic platform and a high-content confocal microscopy system to test a collection of 1280 small molecules (Prestwick Drug Library) to identify new modulators of Golgi morphology. In our primary screening, Golgi fragmentation was triggered by SN38, the active metabolite of irinotecan. Combining SN38 with the Prestwick Drug Library allowed identification of compounds affecting Golgi fragmentation.

Results: In colorectal cancer cells, DNA damage following treatment with cisplatin, SN38, and ionizing radiation triggered GOLPH3 pathway-dependent Golgi fragmentation. We found that GOLPH3 knockdown abrogates Golgi fragmentation after DNA damage, blocks signalling through PI3K/Akt pathway, and increases sensitivity to SN38. We identified 16 drugs that impeded Golgi fragmentation after DNA-damaging chemotherapy treatment, and 6 additional drugs that increased chemotherapy-triggered Golgi fragmentation. Within the candidates we found catecholamines antagonists, inhibitors of prostaglandin synthesis, and modulators of steroid hormone signalling.

Conclusions: Our results suggest that chemical inhibition of Golgi fragmentation after DNA damage has important therapeutic potential. Our goal is to improve the outcome of DNA-damaging anticancer treatments by combining them with drugs that inhibit Golgi fragmentation to sensitise cells to DNA damage.

No conflict of interest

187 (PB-038) Poster

A pharmacological screening to improve the anti-lymphoma activity of BET inhibitors

G. Golino[1], C. Tarantelli[1], F. Spriano[1], L. Scalise[1], A. Arribas[1], L. Cascione[1], E. Zucca[2], A. Stathis[2], F. Bertoni[1], E. Gaudio[1]. [1]*Università della Svizzera italiana, Institute of Oncology Research, Lymphoma & Genomics, Bellinzona, Switzerland;* [2]*Ente Ospedaliero Cantonale, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland*

Introduction: Despite a widespread preclinical anti-proliferative activity of the bromo- and extraterminal domain (BET) inhibitors in lymphomas, the clinical activity in early trials has been limited. Here, we aimed to identify drugs that improve BET inhibition activity performing a pharmacological screening with the BET inhibitor birabresib (OTX015/MK-8628) in combination with a library of 348 compounds in two lymphoma cell lines.

Material and Methods: Two cell lines derived from germinal center B cell (GCB) diffuse large B cell lymphoma (DLBCL) (OCI-LY-19 and WSU-DLCL2) were exposed to birabresib (single dose, 100 nM) in combination with two different doses (20 and 1,000 nM) of 348 compounds. Compounds giving a 1.5-fold decreased proliferation with the combination than with the individual compounds were further evaluated in additional cell lines (the GCB-DLBCL SU-DHL-8 and FARAGE; the mantle cell lymphoma REC1 and the chronic lymphocytic leukemia MEC1) exposed (72 h) to increasing doses of birabresib alone and in combination with increasing doses of other compounds. Combinations were validated using another BET inhibitor (CPI-0610). Synergy was assessed with Chou-Talalay combination index (CI): strong synergism (<0.3), synergism (<0.9), additive (0.9–1.1), antagonism/no benefit (>1.1).

Results: The combinations of birabresib with a series of compounds achieved improved anti-tumor activity than single agents. Besides HDAC and mTOR inhibitors, in accordance with what previously reported by us and others, the ABL/SRC inhibitor dasatinib, the AKT1/2/3 inhibitor MK-2206, the JAK2 inhibitor TG101209 and the LRRK2 inhibitor LRRK2-IN appeared as active combination partners. The screening results were validated in additional four cell lines. The combination of LRKK2-IN with birabresib and with CPI-0610 was synergistic in 6/6 and 5/6 cell lines (no synergism in WSU-DLCL2), respectively. Dasatinib in combination with birabresib or with CPI-0610 was synergistic/strong synergistic in 5/6 cell lines (no synergism in REC1). MK-2206 in combination with OTX015 or with CPI-0610 was synergistic/strong synergistic in 5/6 cell lines. The JAK2 inhibitor TG101209 in combination with OTX015 and with CPI-0610 was synergistic only in 4/6 and 3/6 cell lines respectively.

Conclusion: A chemical screening has identified novel BET inhibitors – containing combinations with anti-tumor activity in lymphoma cell lines, to be further studied.

Work supported by a San Salvatore Foundation grant.

No conflict of interest

188 (PB-039) Poster

Enzyme adsorption by drug aggregates: probing the molecular basis of inhibitor promiscuity using molecular dynamics simulations

M. Ghattas[1], N. Atatreh[1], S. Alrawashdeh[1], R. Bryce[2]. [1]*Al Ain University of Science and Technology, Pharmacy, Al Ain, U.A.E.;* [2]*University of Manchester, Division of Pharmacy and Optometry, School of Health Sciences, Manchester, United Kingdom*

Background: Promiscuous inhibitors are one of the PAINS (pan-assay interference compounds) that make scientists' life difficult in the early drug discovery process. They cause false positives in inhibition assays, forming colloid aggregates in solution which have the ability to adsorb enzyme molecules. The molecular basis of this promiscuous inhibition is difficult to study due to the transient stability of aggregates.

Methods: Molecular dynamics (MD) simulations were employed to study the interaction between a drug aggregate and enzyme in aqueous solution. A large aggregate of drug was first obtained from a separate MD simulation and then solvated along with the enzyme molecule. These systems were then simulated multiple times from different initial configurations.

Results: In accord with experiment, MD simulations indicate direct interactions between the inhibitor aggregate and the enzyme molecule. The protein showed no major changes in its overall tertiary structure. Interestingly, however, there were changes in its active site conformation after being adsorbed to the drug aggregate, that made it unsuitable for substrate binding.

Conclusions: This study provides initial insights into the molecular mechanism of aggregation-based false inhibition and a basis for further investigations.

No conflict of interest

190 (PB-041) Poster

The NCI Program for natural product discovery: a new resource for cancer drug discovery

B. O'keefe[1], C. Thornburg[2], J. Britt[2], J. Evans[3], R. Akee[2], J. Whitt[2], S. Trinh[2], M. Harris[2], J. Thompson[2], T. Ewing[2], S. Shipley[2], P. Grothaus[1], D. Newman[1], J. Schneider[4], T. Grkovic[2]. *[1]National Cancer Institute, Natural Products Branch, Developmental Therapeutics Program, Frederick, MD, USA; [2]Leidos Biomedical Research, Inc., Natural Products Support Group, Frederick, USA; [3]Data Management Services, Inc., Natural Products Support Group, Frederick, USA; [4]National Cancer Institute, Chemical Biology Laboratory, Center for Cancer Research, Frederick, USA*

The US National Cancer Institute's Natural Product Repository is one of the world's largest, most diverse collections of natural products containing over 230,000 unique extracts derived from plant, marine and microbial organisms that have been collected from biodiverse regions throughout the world. Importantly, this national resource is available to the research community for the screening of extracts and the isolation of bioactive natural products. However, despite the success of natural products in drug cancer discovery, compatibility issues that make crude natural product extracts challenging have reduced enthusiasm for the high-throughput screening (HTS) of crude natural product extract libraries in targeted assay systems. To address these limitations and make the NCI's Natural Products Repository more amenable to HTS, we have initiated the prefractionation of extracts using an automated, high-throughput robotics platform capable of generating a library of 1,000,000 partially purified extracts. The talk will discuss this and other mechanisms to increase the utility of the NCI Natural Products Repository in cancer-related drug discovery.

No conflict of interest

191 (PB-042) Poster

3D growth system of MCF-7 is valuable model for anticancer drugs screening

T. Nikolaienko[1], L. Garmanchuk[1], V. Nikulina[1]. *[1]Taras Shevchenko National University of Kyiv, Educational and Scientific Centre "Institute of Biology and Medicine", Kiev, Ukraine*

Introduction: Gene expression profiles in spheroid cultivated cells are more similar to natural tumors, than profiles of the same cells in monolayer culture. Tumor spheroids are heterogeneous cellular aggregates that, when greater than 500 μm diameter, are frequently characterized by hypoxic regions and necrotic centers. Architecture of three-dimensionally (3D) propagated cells is very similar to avascular tumor areas. The gradient of diffusion in cell aggregates leads to reduced proliferation rates and increased drug resistance. The purpose of the work was to conduct a comparative study between 3D and monolayer growth systems of MCF-7 cells, and prove the value of spheroid model.

Materials and Methods: As experimental model was used adhesion line of breast adenocarcinoma MCF-7. Cells in 2D and 3D culture were incubated during 5 days under conditions of starvation. The number of live cells was evaluated using MTT-colorimetric assay. Apoptotic index was assessed by flow cytometry.

Results: MCF-7 cells growth parameters differ significantly in 2D and 3D growth systems. Cells in 2D system are more sensitive to serum starvation then 3D cultures. Cell viability increases dramatically in 3D system. The level of apoptotic and necrotic cells for 2D growth in serum starvation conditions (39.2 ± 7.3% and 33.5 ± 2.8% respectively) were twice increased in comparison with conditions of complete culture medium (19.0 ± 1.3% and 11.4 ± 1.7% respectively), whereas incomplete medium have no detectible effects on 3D cultured cells. However, the 3D cells percentage in G_0/G_1 phase of the cell cycle was increased in 1.6 times in serum free conditions, whereas it was not changed in complete medium that can indicate similarity to natural tumors.

Conclusions: Therefore, the 3D growth system has been proposed as an adequate and valuable model to study tumor growth and response to therapeutic substances.

No conflict of interest

192 (PB-043) Poster

Quantification of target occupancy in cells and tumor tissue using FCCS

F. Becker[1], K. Hansen[1], M. Croshiere[2], L. Yosef[2], S. Hannus[1]. *[1]Intana Bioscience GmbH, Research, Planegg, Germany; [2]Karyopharm Therpeutics, Research & Translational Development, Newton, MA, USA*

Quantification of target occupancy in cells and tissues is an important parameter to correlate the biochemical signature of a drug with in vivo efficacy. Occupancy data can confirm the target hypothesis and support the identification of the best regimen for drug dosing.

Fluorescence Cross Correlation Spectroscopy (FCCS) has been used successfully in the past to monitor and analyze drug- target interactions. The technology is sensitive, fast and specific and allows the detection of drug target interactions in small sample volumes. Here we adopted FCCS to quantify the occupancy of cells and tumors treated with lead molecules.

The application uses a fluorescently labeled tracer to sense and quantify the amount of unoccupied binding pocket of the target. Additionally, target specificity is confirmed using an antibody with a second fluorescent label. Target occupancy studies can be carried out in different relevant cell lines and xenograft tumors retrieved from treated animals. As such the approach can be applied to study target occupancy in vitro and in vivo.

Focus of the presentation will be the selection and characterization of necessary tools to perform occupancy studies using this technology.

The use of this method to support preclinical and clinical studies will be discussed.

Conflict of interest: Ownership: Founder and Shareholder of Intana Bioscience GmbH. Corporate-sponsored Research: Study was sponsored by Karyopharm.

194 (PB-045) Poster

Synergy between IAP inhibitors and a cytotoxic antibody-based chimeric protein

D. Antignani[1], D.J. Urban[2], J.S. Roth[2], M.D. Hall[2], D.J. Fitzgerald[1]. *[1]NCI, Laboratory of Molecular Biology, Bethesda, USA; [2]NCATS, Division of Preclinical Innovation, Rockville, USA*

Background: Screening of drug libraries on tumor cells is a potent tool to discover new therapeutic opportunities. Immunotoxins are cytotoxic anti-body-toxin gene fusion proteins engineered to target cancer cells via antibody binding to surface antigens. Immunotoxins derived from Pseudomonas exotoxin inhibit protein synthesis via the ADP-ribosylation of the eukaryotic elongation factor (eEF2). Clear clinical benefit has been achieved in patients treated with an immunotoxin targeting CD22 expressed on B-cell malignancies. However, success has not been universal and, when treating solid tumors, immunotoxins have performed less well in producing complete responses. Therefore we conducted a screening of 2000 compounds in combination with an immunotoxin targeting the human transferrin receptor on two different epithelial tumor cell lines. Our goals included identifying agents that enhanced the cytotoxic activity of the immunotoxin and also to discover differences in cellular responses to the drug-immunotoxin combinations to identify biomarkers for susceptibility to immunotoxin-mediated killing. Among the most active hits in the screen, we found that the inhibitors of the IAPs proteins, Birinapant and SM164, strongly synergized with the immunotoxin in MDA-MB-468 cells (triple negative breast cancer) but not in A431cells (epidermoid carcinoma). We sought to understand the different responses in each cell line.

Materials and Methods: Viability was determined using the CellTiter-Glo Luminescent Cell Viability Assay kit (Promega, Madison WI). For immuno-blot analysis, the following primary antibodies were used: caspase 8, caspase 3 and cleaved caspase 3 (Cell Signaling), cIAP1 (Enzo), actin (BD Biosciences). TNFa in the cell medium was detectd using the hTNFa-DuoSet ELISA (R&D System). TNFa transcript level was monitored in real time on a StepOnePlus Real Time PCR system.

Results: We confirmed the results of the drug screening. Combinations of the immunotoxin and Birinapant or SM164 greatly increased the cytotoxic activity on MD-MBA-468 cells, but exhibited only modest effects on A431 cells. We found that the level of TNFa and cIAP1 proteins was higher in MDA-MB-468 than A431. We noted that in 24 hours the immunotoxin drastically reduced the level of cIAP1 and the protein is completed degraded when Biripanant is added in both cell lines. In MDA-MB-468, because of the presence of TNFa, this leads to the activation not only of caspase 3 but also caspase 8 while in A431 we were able to detect only caspase 3 cleavage.

Conclusion: We suggest that TNFa and cIAP1 are critical elements of the synergy between the immunotoxin and IAP inhibitors. The rapid degradation

of cIAP1 induces apoptosis but the presence of TNFa amplifies the cytotoxic effect because both the extrinsic and intrinsic pathway of apoptosis were activated.

No conflict of interest

195 (PB-046) Poster

NPL40330: A novel oxidative phosphorylation inhibitor identified by bioenergetic and proteomic profiling

Y. Futamura[1], M. Muroi[1], H. Aono[1], M. Kawatani[1], H. Osada[1]. [1]*RIKEN, Chemical Biology Research Group, Wako, Saitama, Japan*

Backgrounds: Glycolysis and oxidative phosphorylation (OXPHOS) are two key metabolic processes. Due to Warburg's discovery, glycolysis has so far gained much attention in anticancer therapeutics. However, the recent recognition of metabolic reprograming as a major cancer hallmark led to an increased interest in OXPHOS as an attractive target against cancer stem cells and certain cancer cells as well. Here we constructed a screening system to discover new OXPHOS inhibitors based on bioenergetic profiles: oxygen consumption rate (OCR) and extracellular acidification rate (ECAR); and characteristic proteomic changes. This system was used in investigating small molecules that target OXPHOS.

Material and Methods: Bioenergetic profiles were analyzed by using the XFe96 Analyzer. Proteome analysis of sample-treated cell lysates were performed by a two-dimensional differential in gel electrophoresis. We then profiled the expression patterns of eight glycolytic enzymes, specifically 24 post-translational modification subtypes. To detail the mode of action of the hit compounds, we have also developed a semi-intact assay using digitonin-permeabilized cells and the following substrates and ADP to interrogate specific respiratory activity for each complex: malate and pyruvate for complex I; succinate for II; duroquinol for III; TMPD/ascorbate for IV.

Results: Using the Xfe96 Analyzer, we characterized the contribution of glycolysis and OXPHOS to cellular energy metabolism in several cancer cell lines, and chose HeLa cells for screening, which averagely uses both metabolic process. In the course of screening 559 compounds from the chemical library of NPDepo, we found that NPL40330 remarkably decreased OCR value. Moreover, the proteome profiling revealed that NPL40330 upregulated the expressions of glycolytic enzymes, suggesting that it is an OXPHOS inhibitor. Next, we examined which complex does NPL40330 inhibit through the semi-intact assay. Results showed that NPL40330 selectively inhibited malate/pyruvate-driven respiration; while no inhibition was observed in the respirations activated by other substrates. These perturbation profiles suggest that NPL40330 is classified under complex I inhibitors. Finally, the enzymatic assay *in vitro* confirmed that NPL40330 inhibited the oxidation of NADH catalyzed by complex I.

Conclusions: To find new OXPHOS inhibitors, we constructed a screening system based on bioenergetics and proteomic profiling and found that NPL40330 inhibits mitochondrial respiration. Furthermore, the semi-intact assay for OXPHOS established that the target of NPL40330 is mitochondrial complex I.

No conflict of interest

196 (PB-047) Poster

Expression of monoamine oxidase a is correlated with three-dimensional tumorsphere-formation by human breast tumor cells lines

W. Gwynne[1], J. Wu[1], A. Girgis-gabardo[1], A. Dvorkin[1], R. Hallett[1], J.A. Hassell[1]. [1]*McMaster University, Biochemistry and Biomedical Sciences, Hamilton, Canada*

Background: Breast tumors comprise a phenotypically heterogeneous tumor cell population, wherein only a minor fraction of cells, termed breast tumor initiating cells (BTIC), can initiate and sustain tumorigenesis. These observations have therapeutic implications. Traditional cytotoxic anticancer therapies principally eradicate the non-tumorigenic tumor cells, allowing BTIC to remain dormant before ultimately driving disease relapse. Culturing breast tumor cell lines as clonal, three-dimensional tumorspheres (TMS) using a chemically-defined, serum-free medium maintains a high fraction of BTIC *in vitro*. We previously used TMS-formation as a surrogate assay for BTIC in a high-throughput phenotypic screen and found that antagonists of serotonin biosynthesis and function inhibit BTIC activity. Herein, we report a role for monoamine oxidase A (MAO-A), which plays a role in 5-HT degradation, in BTIC activity.

Methods: The sensitivity of breast tumor cells to pharmacological inhibition of MAO-A was determined using the TMS-formation assay and Prestoblue™ cell viability assays. RNA and protein were isolated from breast tumor cells propagated in serum-containing medium or as TMS in serum-free medium. MAO-A transcript expression levels were detected by Nanostring™ nCounter analysis and at the protein level by Western blotting. To determine whether MAOA transcripts are required for breast tumor cell proliferation *in vitro*, we mined the results of a recent genome-wide shRNA dropout screen in 68 human breast tumor cell lines.

Results: MAO-A was detected in several breast tumor cell lines grown in serum-containing or serum-free conditions. In serum-containing medium MAO-A expression in the luminal MCF7 and BT474 breast tumor cell lines was low but the relative expression levels of MAO-A increased at both the protein and transcript level during their propagation in serum-free medium. By contrast, the basal HCC1954 cell line displayed higher MAO-A expression levels when grown in serum-containing media and these levels did not change when the cells were propagated in serum-free media. Interestingly, HCC1954 cells were more sensitive to pharmacological inhibition of MAO-A activity when grown in serum-containing media, but the sensitivity of all 3 cell lines sensitivity was the same when they were propagated in serum-free medium.

Conclusions: MAO-A is expressed at varying levels in several human breast tumor cell lines, and in some contexts, MAO-A activity appears to increase during their propagation in serum-free media as TMS. Breast tumor cell lines are sensitive to MAO-A inhibition by a small molecule antagonist and increased expression levels are correlated with increased sensitivity. Existing reports on MAO-A expression and breast cancer progression are conflicting, which may reflect disparity in experimental context or the cell line used in the experiments.

No conflict of interest

197 (PB-048) Poster

Broad kinome profiling of drug candidates: single point – vs. IC50 – profiling

D. Müller[1], F. Totzke[1], T. Weber[1], M. Pathe[1], C. Schächtele[1], M.H.G. Kubbutat[1]. [1]*ProQinase GmbH, Biochemical Drug Development, Freiburg, Germany*

Protein kinases constitute one of the largest families of evolutionary related proteins. Approximately 500 human genes code for kinase proteins, corresponding to about 2% of the human genome. Over the past decades, deregulation of numerous protein kinases has been shown to contribute to many human diseases. Today, more than 30 protein kinase inhibitors have been approved for the treatment of cancer emphasizing the significance of kinases as drug targets.

However, most of these kinase inhibitors are ATP-competitive compounds. Due to the structural similarity of kinases especially within the ATP-binding site, many of these kinase inhibitors show limited selectivity. Still, sufficient selectivity within the human kinases is of critical importance e.g. to reduce the risk of adverse side effects during treatment.

Therefore, measuring and improving selectivity of a compound within the kinome already in early drug discovery as well as in the later optimization phase is of pivotal importance in the development of therapeutically relevant kinase inhibitors.

Broad profiling of kinase inhibitors in biochemical activity assays of several hundred kinases is nowadays well established. Typically, kinase profiling is done at one concentration of a test compound and measurement of the relative inhibition of the kinase activity compared to a high and low control.

However, due to the limited dynamic range of this approach, and the challenge to select the most appropriate compound concentration, the resulting data are often of limited information with respect to the differences in the potency of compounds against On-target- and Off-target kinases.

We set up an IC50 kinase profiling approach that consists of measuring the effect of a compound on the activity of 320 human protein kinases at six different compound concentrations in logarithmic dilution steps. We will present data showing the effect of compound concentration on the selectivity score in a traditional one concentration profiling setting compared to IC_{50} profiling. IC50 profiles of kinase inhibitors in different stages of development with a focus on CDKs will be presented. The data demonstrates that an IC50 based profiling allows an improved determination of selectivity of a compound compared to single concentration profiling, and provides significantly improved guidance in the development and optimization of drug candidates.

Conflict of interest: Other Substantive Relationships: Employees of ProQinase GmbH.

198 (PB-049) Poster

A high-throughput pharmaceutical screening identifies compounds with specific toxicity against SMARCA4-deficient tumors

A. Wrobel[1], A. Mazan[1], M. Mikula[2], M. Kulecka[3], M. Dabrowska[2], K. Pyziak[1], A. Sroka-Porada[1], M. Ogorek[1], A. Dreas[1], M. Mikulski[1], G. Satala[1], K. Wiatrowska[1], N. Lewandowska[1], M. Milik[1], J. Ostrowski[3], K. Brzozka[1], T. Rzymski[1]. *[1]Selvita S.A, R&D, Krakow, Poland; [2]Maria Sklodowska-Curie Memorial Cancer Center, Department of Oncological Genetics, Warsaw, Poland; [3]Medical Center for Postgraduate Education, Department of Gastroenterology, Warsaw, Poland*

Background: SMARCA4 gene which encodes ATP-dependent chromatin remodeler BRG1 is mutated in virtually all cases of small cell carcinoma of the ovary hypercalcemic type (SCCOHT) and inactivating mutations have been reported in 15% to 35% non-small cell lung cancers (NSCLCs) as well as in 5% to 10% of lung adenocarcinomas. High occurrence of inactivating mutations prompted several screening projects focused on synthetic lethality interactions with other proteins. This led to the identification of SMARCA2 (BRM) as an essential gene in SMARCA4 mutated cancers. Several studies have shown that the ATPase domain of SMARCA2 is the therapeutic target for SMARCA4-deficient cancer. We have used phenotypic screening of a library of small molecule compounds with biological activity in order to identify compounds active in synthetic lethality manner in the context of SMARCA4-inactivating mutations.

Material and Methods: We have generated cell lines carrying knockout (KO) of SMARCA4 locus using CRISPR/Cas9 technology. Applicability of the cellular model for the synthetic lethality screening was validated in a series of SMARCA2 gene knockdown experiments. Miniaturization and automation of seeding and growth conditions enabled rapid screening of small molecule probes and tool compounds.

Results: SMARCA2 knockdown leads to rapid and irreversible loss of viability in SMARCA4 KO cells. Gene expression changes induced by the knockdown of SMARCA2 in this model recapitulated many changes previously observed in cells with inactivating mutations of SMARCA4. In the next step, we used SMARCA4 KO cell line to screen the LOPAC library (The Library of Pharmacologically Active Compounds) of drug-like compounds by measuring viability as a surrogate readout of antitumor activity. Cell line without SMARCA4 inactivation (WT) was used as a control. Compounds active in SMARCA4 KO cells were validated by measuring a dose response curves and compared with the activity on SMARCA4 WT cells. We identified several compounds showing differential cytotoxicity effect in SMARCA4 mutant cell lines.

Conclusions: Phenotypic screening is a feasible alternative to identification of potent leads for diseases driven by virtually undraggable targets. Identified compounds could be promising candidates for further development as drugs targeting tumors with loss-of-function mutations in SMARCA4.

Conflict of interest: Board of Directors: Krzysztof Brzozka.

199 (PB-050) Poster

Curcumin treatment alone or in combination of imatinib may circumvent the side effects associated with imatinib therapy in chronic myeloid leukaemia

S.A. Guru[1], M.A. Bhat[1], M.P. Sumi[1], P. Yadav[1], N. Gupta[1], A. Saxena[1]. *[1]Maulana Azad Medical College, University of Delhi, Biochemstry, New Delhi, India*

Background: Despite the outstanding survival benefits of imatinib in CML patients, a significant number of patients encounter some major side effects of the imatinib treatment viz, neutropenia and thrombocytopenia bear priority. The molecular mechanisms of these imatinib side effects are associated with its ability to inhibit other tyrosine kinases such as PDGFRα. Here, in this study, we tried to explore the role of PDGFRα and its downstream signalling components in the development of imatinib induced thrombocytopenia in imatinib treated CML patients.

Methodology: We studied PDGFRα mRNA expression and its activation (Tyr^{754}; p-PDGFRα) in K562 cells and CML patients. The effect of deregulated mRNA expression and its dephosphorylation on the expression of PDGFRα's downstream signalling molecules like PI3K, AKT1 and AKT2 was also studied and compared in thrombocytopenic and nonthrombocytopenic CML patients.

Results: The effect of imatinib, curcumin and imatinib+curcumin exposure on mRNA expression of *PDGFRA, PI3K, AKT1* and *AKT2* genes was analyzed in vitro in K562 cells. A significant down regulation of expression of these genes in all the three groups of treatment was observed compared to untreated group, except for *AKT2* gene expression in case of curcumin treatment. Analysis of effects of imatinib, curcumin and imatinib+curcumin exposure in K562 cell line on PDGFRα protein (Tyr754 – p-PDGFRα) phosphorylation/activation showed that these treatments led to significantly reduced levels of PDGFRα protein (Tyr754 – p-PDGFRα) phosphorylation/activation compared to untreated group. However, we observed a higher concentration of curcumin inhibiting PDGFRα than that of imatinib.

Conclusion: Thus it is suggested that curcumin or its derivatives could be used for CML treatment in combination with imatinib to avoid the development of side effects associated with imatinib therapy.

No conflict of interest

200 (PB-051) Poster

A Novel TACC3 inhibitor as an anti-cancer agent in breast cancer

Ö. Akbulut[1], D. Lengerli[2], B. Çalışkan[2], E. Banoğlu[2], O. Şahin[1], Banoğlu Group. *[1]Department of Molecular Biology and Genetics, Faculty of Science, Bilkent University, Ankara, Turkey; [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey*

Background: Transforming Acidic Coiled-Coil Containing Protein 3 (TACC3) is an essential mitotic gene which is concentrated at centrosomes where it ensures chromosomal segregation and microtubule stability. Being amplified or mutated in a broad spectrum of cancers, TACC3 has a potential to be a therapeutic target. There is a wide variety of *in vitro* and *in vivo* studies together with patient data about the oncogenic profile of TACC3, but there is currently no inhibitor of TACC3 used in clinics. KHS101, a small molecule inhibitor against TACC3, has been shown to reduce GBM xenografts tumor growth; similarly, SPL-B, another TACC3 inhibitor, was found to suppress tumor growth in ovarian cancer xenografts. However, none of these TACC3 inhibitors is being tested in clinics due to low systemic stability or high IC_{50} (50% inhibitory concentration) values. Hereby, by combining rational drug design approaches with screening, we aimed to identify a novel TACC3 inhibitor which is more effective in *in vitro* and *in vivo* systems and can be used as a mitotic blocker in breast cancer (BC).

Material and Methods: A classical design approach was combined with screening to develop new TACC3 inhibitors where some chemical fragments of already available TACC3 inhibitors changed with their isosteric equivalents with the aim of improved potency as well as drug-like properties. Then, these compounds were tested in *in vitro* settings where different subtypes of BC cells were screened for the effects on viability. Moreover, these candidate agents were compared with the other TACC3 inhibitors in terms of the general cellular processes that TACC3 involved. Finally, the most promising agents were tested in *in vivo* settings to test their anti-tumorigenic effects.

Results: High level of TACC3 is found to be associated with worse survival in BC patients. Due to the urgent need for a more potent TACC3 inhibitor, we developed and screened dozens of compounds. *In vitro* assays with BC cells belonging to different subtypes showed the lowest IC_{50} value for BRP-OZG-264 (our novel TACC3 inhibitor) and the superior effects on the cellular processes such as mitotic arrest, DNA damage and apoptosis when compared to the other available TACC3 inhibitors. Importantly, we showed that oral administration of this novel inhibitor significantly suppressed the tumor growth in breast cancer xenografts better than the already reported compounds. Currently, we are testing the pharmacokinetics and toxicity of this inhibitor as well as detailed molecular characterization of TACC3 inhibition-mediated anti-tumor effects.

Conclusions: Overall, our preclinical studies suggest that this new compound (BRP-OZG-264) presumably acting as a TACC3 inhibitor, has a potential to be developed as a novel mitotic blocker for the treatment of breast cancer.

No conflict of interest

201 (PB-052) Poster

Study of the effect of non-steroidal antiinflamatory drugs in combination with elfornithine over non-small cell lung cancer cells viability and migration

R. Lopez-Muñoz[1], R. Garcia-Jaramillo[1], F. Lopez-Contreras[1], M. Muñoz[1], A. Martin[1], J. Perez[1], M. Peña-Espinoza[1]. *[1]Universidad Austral de Chile, Facultad de Ciencias Veterinarias, Instituto de Farmacologia y Morfofisiología, Valdivia, Chile*

Background: Non-small cell lung cancer (NSCLC) is the most lethal and prevalent form of lung cancer. It is widely documented that in NSCLC the levels of polyamines (putrescine, spermidine and spermine) are increased and play a pivotal role tumor proliferation. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to have antitumor activity *in vitro* in different types of cancer, which has been related to the induction of the expression of an enzyme called Spermidine/Spermine Acetyltransferase (SSAT). SSAT is a key player in the catabolism of polyamines, allowing the exit of acetyl-polyamines from the intracellular milieu, through the SLC32

transporter. On the other hand, eflornithine is a classic inhibitor of ornithine decarboxylase (ODC), pacemaker enzyme in the synthesis of polyamines. In this work, we evaluated the effect of eflornithine and its combination with three NSAIDs: celecoxib (a selective cyclooxygenase-2 inhibitor) sulindac (a non-selective cyclooxygenase inhibitor) and indomethacin (a selective cyclooxygenase-1 inhibitor) on H1299 cells (a human cell line. Derived from lymph node metastasis of NSCLC).

Material and Methods: We evaluated the cell viability using the tetrazolium reduction technique, and the level of interaction between combined drugs (additivity, synergism or antagonism) was analyzed using Compusyn software. Also, cell migration was evaluated by scratch assay and transwell migration.

Results: We compared the effect of the combinations at a 90% of NSCLC cell mortality (Fa = 0.9). At this level, celecoxib showed the best combinatory activity with eflornithine. In addition, these drugs were combined with four antitumor drugs used against NSCLC: gemcitabine, taxol, pemetrexed and carboplatin. Of these combinations, sulindac and celecoxib showed the best combination profile with classic antitumors, whereas indomethacin showed antagonism in almost all combinations with antitumor drugs. Polygonograms were constructed to evaluate the triple interaction between eflornithine, NSAID and each antitumor, of these drug triads, the best combinatory profile was given by celecoxib/gemcitabine/eflornithine. When migration ability of cells was evaluated, we find that all NSAIDs were able to significantly reduce cell migration, and the best combination profile was shown by sulindac/eflornithine.

Conclusions: These results indicate that the sulindac/eflornithine and celecoxib/eflornithine combinations could be a good strategy for future studies in animal models of NSCLC. This work was funded by the National Comitee of Science and Technology of Chile (CONICYT), through the Fondecyt grant #1160807.

No conflict of interest

202 (PB-053) Poster

Nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) as potential chemotherapy drugs against non-small cell lung cancer (NSCLC) cells

R. Lopez-Muñoz[1], A. Martin[1], F. Lopez-Contreras[1], M. Muñoz[1], J. Perez[1], R. Garcia-Jaramillo[1], M. Peña-Espinoza[1]. [1]*Universidad Austral de Chile, Facultad de Ciencias Veterinarias, Instituto de Farmacologia y Morfofisiología, Valdivia, Chile*

Background: Non-small cell lung cancer (NSCLC) is the most lethal and prevalent type of lung cancer. Because current therapy increases survival only about 15% at 5 years since start of treatment, it is necessary to carry out research for new chemotherapeutic approaches. Nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO•-NSAIDs) have shown greater antitumor efficacy over traditional NSAIDs in ovarian and colon cancer cells. However, the effect of NO•-NSAIDs has not been evaluated in NSCLC cells. The aim of this study is to compare the effect of three NO•-NSAIDs: NCX-4040 and NCX-4016 (derived from acetylsalicylic acid) and NCX-2121 (derived from indomethacin) on NSCLC cancer cells.

Material and Methods: We studied the viability (by a tetrazolium salt reduction) and proliferation (by uptake of 5-bromo-2′-deoxyuridine) of NSCLC cells, after exposition of drugs by 96 or 24 hours, respectively. Also, we studied the migratory ability of cells by transwell migration. The nitric oxide (NO•) and oxygen-derived reactive species (ROS) were measured by fluorescence using the 4,5-Diaminofluorescein diacetate (DAF-2A) and 2′,7′-Dichlorodihydrofluorescein diacetate (DCFDA) probes, respectively. Finally, we evaluated the combinatory profile of these drug with erlotinib using the Combenefit software.

Results: When viability and proliferation was assessed, NO•-NSAIDs were 10–200-fold more potent than their non-NO•-releasing counterparts. Also, aspirin-derived NO•-NSAIDs were able to reduce the cell migration. Interestingly, NCX-4040, the most potent compound was the one that produced more NO• and ROS. Thus, we explored the effect of the NO•-NSAIDs in prescence of Carboxy-PTIO (a NO• scavenger), which partially reverted the effect of NCX-4040, showing that NO• release is part of the mechanism of action of this drug. Finally, we studied the combination of the three compounds with chemotherapeutics such as erlotinib; where NCX-4040 was showed the best combinatory profile with this drug.

Conclusions: These results suggest that NCX-4040 is an interesting drug to study over *in vivo* models of NSCLC. This work was funded by the National Comitee of Science and Technology of Chile (CONICYT), through the Fondecyt grant #1160807.

No conflict of interest

Wednesday, 14 November 2018

POSTER SESSION

Drug Synthesis

203 (PB-054) Poster

Synthesis and evaluation of imidazo[1,2-a]pyridine analogues of the phosphatidylinositol 3-kinase inhibitor ZSTK474

G. Rewcastle[1], S. Gamage[1], J. Spicer[1], J. Flanagan[1], W. Denny[1], P. Shepherd[1], W. Lee[2]. [1]*University of Auckland, Auckland Cancer Society Research Centre and Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand;* [2]*University of Auckland, Department of Molecular Medicine and Pathology, Auckland, New Zealand*

Background: The phosphatidylinositol 3-kinase (PI3K) pathway is frequently activated in cancers, although several small molecule inhibitors of the pathway have now been identified. Our earlier work showed that replacement of one of the morpholine groups of the PI3K inhibitor 2-(difluoromethyl)-1-(4,6-di-4-morpholinyl-1,3,5-triazin-2-yl)-1*H*-benzimidazole (ZSTK474) with sulfonamide-containing substituents, coupled with the addition of a methoxy group at the 4-position of the benzimidazole group, produced an active class of potent PI3Kα and dual PI3Kα/mTOR inhibitors. This work culminated in the clinical trial of our lead candidate PWT33597 in 2011. Here we describe our investigation of analogues of this class of inhibitors where the benzimidazole portion of the molecule is replaced by an imidazo[1,2-*a*]pyridine group.

Material and Methods: Compounds were prepared using a heteroaryl Heck reaction procedure that involved the palladium-catalysed coupling of 2-(difluoromethyl)imidazo[1,2-*a*]pyridines with chloro, iodo or trifluoromethanesulfonyloxy (trifloxy) substituted 1,3,5-triazines or pyrimidines. Compounds prepared included imidazo[1,2-*a*]pyridine analogues of the known inhibitors ZSTK474, PWT33597, SN32976, MIPS-9922 and AS2541019. The compounds were tested for their inhibitory activity against the p110α, p110β, and p110δ isoforms of PI3K using Homogeneous Time Resolved Fluorescence (HTRF) assays.

Results: The imidazo[1,2-*a*]pyridine compounds followed their benzimidazole analogues in terms of selectivity for the PI3K isoforms, but in general showed less potency in the HTRF assays.

Conclusions: Using a scaffold-hopping approach we have investigated imidazo[1,2-*a*]pyridine analogues of the ZSTK474 class of PI3K inhibitors. The new compounds maintain the isoform selectivity of their benzimidazole analogues, but in general show less potency.

No conflict of interest

Wednesday, 14 November 2018

POSTER SESSION

Immunecheckpoints

204 (PB-055) Poster

IL-1β inhibition by canakinumab may be effective against diverse molecular subtypes of lung cancer

J. Baum[1], C. Wong[2], M. Beste[3], B. Dharan[4], Y.A. Wang[5], X. Wang[6], A. Silvestro[7], R. Leary[8], M.F. Prescott[9], L. Krajkovich[10], M. Dugan[11], W. Winckler[8], P.M. Ridker[12], A.M. Martin[13], E.C. Svensson[14], S. Wong[15]. [1]*Novartis Institutes for BioMedical Research, Precision Medicine, Lung Cancer, Cambridge, Massachusetts, USA;* [2]*Novartis Institutes for BioMedical Research, Precision Medicine, Immunology, Cambridge, Massachusetts, USA;* [3]*Novartis Institutes for BioMedical Research, Translational Medicine, Biomarker Development, Cambridge, Massachusetts, USA;* [4]*Novartis Pharmaceuticals Corporation, Biostatistics, Oncology, East Hanover, USA;* [5]*Novartis Institutes for BioMedical Research, Biostatistics, Cambridge, Massachusetts, USA;* [6]*GCE Solutions, Biostatistics, Bloomington, USA;* [7]*Novartis Institutes for BioMedical Research, Precision Medicine, Oncology, Cambridge, Massachusetts, USA;* [8]*Novartis Institutes for BioMedical Research, Next Generation Diagnostics, Cambridge, Massachusetts, USA;* [9]*Novartis Pharmaceuticals Corporation, Cardio-Metabolic Development, East Hanover, USA;* [10]*Novartis Pharmaceuticals Corporation, Biomarker and Imaging Operations, East*

Hanover, USA; [11]Novartis Pharmaceuticals Corporation, Global Drug Development, East Hanover, USA; [12]Brigham and Women's Hospital, Harvard Medical School, Center for Cardiovascular Disease Prevention, Boston, USA; [13]Novartis Pharmaceuticals Corporation, Precision Medicine, Oncology, East Hanover, USA; [14]Novartis Institutes for BioMedical Research, Translation Medicine, Cardiovascular, Cambridge, Massachusetts, USA; [15]Novartis Institutes for BioMedical Research, Precision Medicine, Diagnostics, Cambridge, Massachusetts, USA

Background: Chronic inflammation plays key roles in lung cancer development and progression. In the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), inhibition of the interleukin-1β (IL-1β) inflammatory pathway by canakinumab, an anti-IL-1β antibody, significantly reduced the lung cancer incidence and mortality. To gain further insights, a molecular characterization of the lung cancer patients from CANTOS was undertaken.

Methods: Serum levels of C-reactive protein (CRP), IL-6, IL-18, IL-1RA, TNF-α, leptin, adiponectin and plasma levels of fibrinogen and plasminogen activator inhibitor-1 from patients diagnosed with lung cancer in the CANTOS trial (n = 116) were determined. Circulating tumor DNA (ctDNA) in plasma samples collected at the baseline visit and/or time closest to lung cancer diagnosis from patients diagnosed with lung cancer (n = 71) were assayed using the Guardant360 platform.

Results: At both baseline and 3 months, patients in the highest quartile of CRP or IL-6, both downstream of IL-1β signaling, showed a trend toward shorter time to lung cancer diagnosis compared to patients from the lowest quartile. Other inflammation markers at baseline did not correlate with time to lung cancer diagnosis. Circulating tumor DNA was detected in 83% of patients, with 73% (48 of 66) of patients having detectable ctDNA at time closest to diagnosis and 66% (44 of 67) having detectable ctDNA at baseline. Patients with ctDNA at baseline had a median time to lung cancer diagnosis of 628 days (n = 44), compared to 942 days (n = 23) in patients without baseline ctDNA. A typical driver mutation profile was observed in ctDNA from lung cancer patients in CANTOS. At baseline, p53 mutations were found in 47% of patients with detectable ctDNA, while mutations in NF1 and EGFR were found in 14% and 12% of patients, respectively. Importantly, there was no evidence of treatment-specific enrichment in any mutation following treatment with canakinumab.

Conclusion: Taken together, these results provide further evidence for the importance of IL-1β-mediated inflammation in lung cancer. In addition, since 66% of patients diagnosed with cancer had ctDNA present at entry into the CANTOS trial, these results suggest canakinumab's effect may be mediated by delaying the progression of diverse molecular subtypes of lung cancer.

Conflict of interest: Ownership: Stock Ownership: Jason Baum, Connie Wong, Michael Beste, Bharani Dharan, Ying A Wang, Rebecca Leary, Margaret F Prescott, Lynne Krajkovich, Margaret Dugan, Wendy Winckler, Anne-Marie Martin, Eric C Svensson and Stephane Wong. Advisory Board: Paul M Ridker (Honoraria – Novartis). Corporate-sponsored Research: Paul M Ridker (Novartis). Other Substantive Relationships: Employee at Novartis: Jason Baum, Connie Wong, Michael Beste, Bharani Dharan, Ying A Wang, Angela Silvestro, Rebecca Leary, Margaret F Prescott, Lynne Krajkovich, Wendy Winckler, Anne-Marie Martin, Eric C Svensson and Stephane Wong. Employee at GCE solutions: Xiaoshan Wang.

205 (PB-056) Poster

Insights on the regulation of immune checkpoint genes – potential implications for cancer immunotherapy

R. Leibowitz-Amit[1,2], A. Layani[2], P. Dobosz[2], K. Slabodnik Kaner[1,2], Y. Sidi[1,2], D. Avni[2]. *[1]Tel-Aviv University, Medicine, Tel-Aviv, Israel; [2]Sheba Medical Center, Cancer Research Center, Tel-Hashomer, Israel*

Background: The interface between T lymphocytes and cancer/antigen presenting cells (the "immunological synapse") comprises of both co-inhibitory and co-stimulatory transmembrane proteins ("checkpoint proteins") that modulate the signal transmitted to T lymphocytes, leading to either activation or exhaustion. Monoclonal antibodies against checkpoint proteins have anti-neoplastic activity in many malignancies, but not all cancers and not all patients within a given cancer respond. Our aim is to study the factors regulating the expression of checkpoint genes at the immunological synapse at both the transcriptional and post-transcriptional level, and their relationship with tumor immunogenicity and response to immunotherapy.

Materials and Methods: We analyzed all 33 cancers of the "tumor cancer genome atlas (TCGA)" for the expression of 22 checkpoint mRNAs known from the literature to be expressed at the cancer side of the synapse, focusing on co-expression of checkpoint mRNAs and micro-RNAs in melanoma, a highly immunogenic cancer, and in bladder cancer, a less immunogenic cancer. Co-expression was calculated with Spearman rho correlations, and expression results were corroborated in-vitro. Survival was assessed by Kaplan-Meier curves. Direct targeting of mRNAs by miRNAs was assessed by luciferase reporter assays. Transcription factor binding motifs were analyzed on the putative promotor sequences of checkpoint mRNAs using tools from MEME suit.

Results: In all cancers analyzed, we found networks of co-expressed checkpoint mRNAs that differed in their components and extent. In melanoma, we found that that two co-stimulatory checkpoint mRNAs are directly regulated by mir-16. High expression of mir-16 and low expression of checkpoint mRNAs was associated with worse survival, suggesting that mir-16 affects the immunogenicity of the synapse. In bladder cancer, there was a negative correction between checkpoint mRNA expression and mir-15b (sharing an identical seed sequence with mir-16). Additionally, in bladder cancer all co-expressed checkpoint mRNAs have, in their putative promotor regions, binding motifs for the transcription factor BACH-2, which is also co-expressed with the checkpoint mRNAs and associated with survival.

Conclusions: Our work suggests that there are networks of checkpoint genes that are co-regulated at both the transcriptional and post-transcriptional levels. We hypothesize that immunogenicity of tumors partly stems from the specific components of these co-expression networks. Moreover, our work may help point to novel checkpoints at the cancer side of the synapse that must be co-targeted with the PD-1L/PD-1 axis in order to further potentiate the activation of the immune response against cancer.

No conflict of interest

207 (PB-058) Poster

A Canadian Cancer Trials Group phase IB study of durvalumab plus tremelimumab given concurrently or sequentially in patients with advanced, incurable solid malignancies (IND.226)

M. Smoragiewicz[1], P. Bradbury[2], P. Ellis[3], J. Laskin[4], C. Kollmannsberger[4], D. Hao[5], R. Juergens[3], G. Goss[6], P. Wheatley-Price[6], S. Hotte[3], K. Gelmon[4], A. Tinker[4], P. Brown-Walker[1], I. Gauthier[1], D. Tu[1], X. Song[7], L. Seymour[1]. *[1]Queen's University, Canadian Cancer Trials Group, Kingston, Canada; [2]Princess Margaret Cancer Centre, Division of Medical Oncology, Toronto, Canada; [3]Juravinski Cancer Centre, Department of Oncology, Division of Medical Oncology, Hamilton, Canada; [4]BCCA Vancouver Cancer Centre, Division of Medical Oncology, Vancouver, Canada; [5]Tom Baker Cancer Centre University of Calgary, Department of Oncology, Section of Medical Oncology, Calgary, Canada; [6]The Ottawa Hospital Cancer Centre, Division of Medical Oncology, Ottawa, Canada; [7]MedImmune, Clinical Pharmacology, Pharmacometrics & DMPK, Gaithersburg, USA*

Background: Immune checkpoint inhibitors are established therapies in many advanced cancers. The primary objective of this study was to evaluate the safety and tolerability of durvalumab (Durva), a PD-L1 inhibitor, plus tremelimumab (Treme), a CTLA-4 inhibitor, given either sequentially (SEQ) or concurrently (CON). Sequential administration of multiple agents increases total chair time adding costs overall and inconvenience for patients. The serum pharmacokinetic (PK) profile and safety of SEQ vs CON of Durva and Treme administration was evaluated in a cohort of patients (pts).

Methods: Pts with advanced solid tumours, regardless of tumour PD-L1 status or number of prior therapies, were enrolled and randomized to either SEQ Treme 75 mg IV over 1 hr followed by Durva 1500 mg IV over 1 hr q4wks, or CON administration over 1 hr. PK samples were drawn on cycle (C) 1 day (D) 1 (0,1, 2, 4 hr)/2/3/8/15/22, and C2/3 D1 (pre-dose & end of infusion).

Results: Fourteen pts (median age = 65 (range 29–74); 8 male, ECOG 0 (1pt)/1(13pts)) were randomized to SEQ (7pts/24 cycles) and CON (7 pts/23 cycles). There were no infusion related reactions. Drug related adverse events (AEs) were mainly £Grade (G) 2 and manageable, and comparable in frequency between SEQ/CON- fatigue (43/57%), rash (43/43%), pruritus (43/29%) and nausea (29/29%). Potentially related ³ grade 3 AEs included 2 in SEQ (G3 diarrhea, G3 ALT/AST (complicated by steroid induced psychosis)) and 3 in CON (G3 rash, G3 pneumonitis, G5 respiratory failure). Of note, the pt with the G5 AE (C1D30) had G2 dyspnea, lymphangitic carcinomatosis and pleural effusions requiring frequent drainage at baseline which gradually worsened on study, but contribution from Durva/Treme could not be excluded as the pt was not fit for further investigation. One pt in each cohort discontinued treatment due to toxicity. The PK profiles of Durva and Treme were similar between CON and SEQ (Table 1) and to historical reference data.

Conclusions: Concurrent administration of Durva and Treme over 1 hr is safe with a comparable PK profile to sequential administration.

Conflict of interest: Ownership: Dr. Song owns stock in AstraZeneca. Advisory Board: Dr. Goss has received honoraria from the following organizations: Astrazeneca, Boehringer Ingelheim, Pfizer, BMS and Celgene. Dr. Juergens has received honorario from the following

Table 1 (abstract 207 PB-058): The serum pharmacokinetic profile of sequential vs concurrent durvalumab and tremelimumab administration

	Tremelimumab 75 mg IV Q4W First dose			Durvalumab 1500 mg IV Q4W First dose		
Dosing Regimen	C_{max} (μg/mL)	C_{trough} (μg/mL)	$AUC_{0\text{-}28d}$ (Day*μg/mL)	C_{max} (μg/mL)	C_{trough} (μg/mL)	$AUC_{0\text{-}28d}$ (Day*μg/mL)
Concurrent administration	23.4 (3.90) (n = 7)	3.55 (0.62) (n = 6)	243 (43.2) (n = 6)	450 (69.4) (n = 7)	72.7 (17.9) (n = 6)	4526 (829) (n = 6)
Sequential administration	21.1 (5.42) (n = 7)	2.96 (1.29) (n = 6)	233 (102) (n = 6)	377 (94.4) (n = 6)	53.6 (22.2) (n = 6)	4049 (1621) (n = 6)

organizations: Bristol-Myers Squibb, Boehringer Ingelheim, AstraZeneca, Roche Canada, Merck Sharkp & Dohme, Lilly, Amgen, EMD Serono, Novartis Canada Pharmaceuticals Inc. She has also acted in a consulting/advisory role for AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Lilly, Merck Sharp & Dohme, Novartis, Pfizer, Amgen, Roche Canada and Takeda. Dr. Tinker has received an honoraria and grant from AstraZeneca. Corporate-sponsored Research: Dr. Seymour and the Canadian Cancer Trials Group received funding from AstraZeneca for this trial. Other Substantive Relationships: Dr. Song is an employee of MedImmune.

208 (PB-059) Poster

Suppression of regulatory T cells (Tregs) in vivo by small molecule targeting of the mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1)

N. Finnberg[1], F. Öberg[1], S. Mangsbo[2], I. Kerzeli[2], E. Fletcher[2], A. Kallin[1], A. Christodoulidou[1], S. Sedig[1], A.K. Sternbeck[3], J. Bylund[3], S. Moses[4], L. Salvador Oden[5], M. Kullman-Magnusson[1], I. Henderson[1], H.G. Beisel[6], K. Ersmark[5], S. Karlström[5], R. Bethell[7], M. Albertella[1]. *[1]Medivir AB, Biology, Huddinge, Sweden; [2]Science for Life Laboratory and Uppsala University, Department of Pharmaceutical Biosciences, Uppsala, Sweden; [3]Medivir AB, Dmpk, Huddinge, Sweden; [4]Medivir AB, Non-clinical Drug Safety, Huddinge, Sweden; [5]Medivir AB, Medicinal Chemistry, Huddinge, Sweden; [6]Medivir AB, Computational Chemistry, Huddinge, Sweden; [7]Medivir AB, Discovery Research, Huddinge, Sweden*

Background: Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a protease that signals downstream of the B and T cell receptors and promotes NF-kappaB activation. MALT1-deficiency in mice has been linked to reduced levels of regulatory T (Treg) cells. Pharmacological targeting of MALT1 may therefore reduce the numbers of Tregs in cancer patients and thereby represent a new immunotherapeutic approach to cancer therapy.

Methods: Compound A was used as a small-molecule tool in order to investigate the effects of MALT1 inhibition. Inhibition of human and mouse MALT1 activity was assessed in biochemical assays, and in an assay of intracellular cleavage of HOIL1 in stimulated Jurkat cells. To assess the impact of MALT1 inhibition on Treg differentiation, CD4+ naïve T cells (CD4 +CD45RA+CD127+CD25-) were sorted from healthy donor PBMCs and stimulated in IL-2 and TGF-beta containing medium with anti-CD3/CD28 for 5 days, rested for 5 days without stimulation, and analysed for FoxP3 and CD25 expression. In vivo, the capacity to inhibit TCR-signalling was assessed by quantification of serum IL-2 following a single IV bolus of the anti-CD3 agonist antibody 145-2C11. The pharmacodynamic effects on FoxP3+ Tregs in the tumour, tumour-draining lymph nodes (TDLN) and distal lymph nodes (LN) were evaluated in the MB49 syngeneic mouse bladder cancer model. Pharmacokinetics were performed using standard methods.

Results: Compound A is a potent and selective inhibitor of MALT1 (human K_i = 9 nM and mouse K_i = 5 nM), and retains potent activity in cell culture, as measured by inhibition of cleavage of the endogenous substrate HOIL1 (IC_{50} = 14 nM), and inhibition of IL-2 (IC_{50} = 12 nM). In vitro, MALT1 inhibition suppressed CD25+/FoxP3+ Treg development without impact on proliferation and viability of CD4+ T cells. Administration of 30 μmol/kg PO of **compound A** to mice was well-tolerated and gave an average 24 hr free plasma exposure twenty-one times greater than the mouse K_i. This dose inhibited serum IL-2 release triggered by 145-2C11 approximately 62% and 80% at 2.5 and 5 hrs post treatment, respectively, confirming effective in vivo inhibition of MALT1. Inhibition of MALT1 in MB49 tumour-bearing mice triggered selective dose-dependent reduction of Treg cells in both LN (fourfold, P < 0.0001) and TDLN (tenfold, P < 0.0001) without apparent toxicity. A trend towards reduced levels of FoxP3+ Tregs in MB49 tumours (4.11 vs 1.62%; P = 0.0727) after treatment was also observed.

Conclusions: Pharmacological inhibition of MALT1 by small molecules enables selective suppression of tumour-associated Tregs and has potential applications for the stimulation of anti-tumour immunity. We are currently progressing a small-molecule chemistry program with the aim to select a final molecule for clinical development.

Conflict of interest: Other Substantive Relationships: NF, FÖ, AK, AC, SS, AKS, JB, SM, LSO, MKM, IH, HGB, KE, SK, RB and MA are employees of Medivir AB, Huddinge Sweden.

209 (PB-060) Poster

Efficacy of pembrolizumab in patients with adrenocortical carcinoma

M. Habra[2], M. Campbell[3], C. Jimenez[2], D. Karp[1], D. Hong[1], V. Subbiah[1], S. Pant[1], J. Rodon Ahnert[1], A. Alshawa[1], J. Painter[1], S. Khan[1], M. Rejon[1], B. Stephen[1], C. Tapia[4], T. Mendoza[5], R. Colen[6], K. Hess[7], F. Meric-Bernstam[1], A. Naing[1]. *[1]The University of Texas MD Anderson Cancer Center, Investigational Cancer Therapeutics, Houston, USA; [2]The University of Texas MD Anderson Cancer Center, Endocrine Neoplasia and Hormonal Disorders, Houston, USA; [3]The University of Texas MD Anderson Cancer Center, Genitourinary Medical Oncology, Houston, USA; [4]The University of Texas MD Anderson Cancer Center, Translational Molecular Pathology, Houston, USA; [5]The University of Texas MD Anderson Cancer Center, Symptom Research, Houston, USA; [6]The University of Texas MD Anderson Cancer Center, Cancer Systems Imaging, Houston, USA; [7]The University of Texas MD Anderson Cancer Center, Biostatistics, Houston, USA*

Background: Adrenocortical carcinoma (ACC) is an orphan endocrine malignancy with poor prognosis and limited response to chemotherapy. Targeting select pathways has not resulted in any meaningful response in ACC and clinical trials using small molecule kinase inhibitors did not result in any treatment breakthroughs. However, in a limited set of ACC tumor samples, programmed death-ligand 1(PD-L1) expression was seen in about 11% of primary ACC tumors after surgical resection. This finding opens new avenues in the treatment of ACC as several studies have demonstrated correlation between PD-L1 expression and treatment outcomes in patients treated with programmed death-1 (PD-1) signaling pathway inhibitors. So far, there is no published clinical trial about using immunotherapy in ACC.

Methods: We are currently enrolling patients in a phase II study of pembrolizumab in patients with rare tumors that includes a pre-specified ACC cohort (http://ClinicalTrials.gov identifier, NCT02721732). Patients received pembrolizumab 200 mg intravenously once every 3 weeks. Response was assessed every 9 weeks using RECIST1.1. The primary objective of the trial was to evaluate efficacy by evaluation of non-progression rate (NPR) at 27 weeks (9 cycles), defined as the percentage of patients who are alive and progression-free at 27 weeks as assessed by RECIST 1.1. The primary end point is progression-free survival at 27 weeks (PFS27wk). Mandatory biopsies are taken at baseline, on cycle 1 day 15–21, and at the time of progression.

Results: At the time of analysis, a total of 15 patients were enrolled and treated in the ACC cohort. The patients remained on the study for a median duration of 18 weeks (range, 5–95 weeks). Among the 14 patients evaluable for response, the NPR at 27 weeks was 29% (4/14 patients). Of the 4 patients who were progression-free at 27 weeks, 2 patients had partial response. The first patient had 37% tumor reduction and is still continuing on therapy at 95 weeks and the second patient had tumor reduction of 41% and duration of response was 42 weeks. The remaining 2 patients had stable disease. Ten patients had evidence of disease progression within 27 weeks of starting the study (median duration 17 weeks; range, 8–27 weeks). Three of the 4 patients with PFS27wk had no evidence of hormonal excess at the time of their initial diagnosis. The safety profile of pembrolizumab was very favorable and only 1 patient had grade 3 or 4 immune-related adverse event (pneumonitis, grade 3).

Conclusion: Single agent pembrolizumab may be an effective option for a subset of ACC patients. As less response is seen in patients with cortisol-producing tumors further study is required to confirm this initial observation. Translational data will be presented at this meeting.

No conflict of interest

210 (PB-061) Poster

Efficacy of pembrolizumab in patients with cutaneous squamous cell carcinoma

R. Ferrarotto[1], B. Glisson[1], G. Blumenschein[1], D. Hong[2], S. Piha-Paul[2], J. Rodon Ahnert[2], S. Pant[2], A. Tsimberidou[2], F. Janku[2], A. Alshawa[2], D. Jain[2], M. Rejon[2], K. Hess[3], R. Colen[4], C. Lu[1], B. Stephen[2], C. Tapia[5], T. Mendoza[6], F. Meric-Bernstam[2], A. Naing[2]. *[1]The University of Texas MD Anderson Cancer Center, Thoracic/Head & Neck Med Onc, Houston, USA; [2]The University of Texas MD Anderson Cancer Center, Investigational Cancer Therapeutics, Houston, TX, USA; [3]The University of Texas MD Anderson Cancer Center, Biostatistics, Houston, USA; [4]The University of Texas MD Anderson Cancer Center, Cancer Systems Imaging, Houston, USA; [5]The University of Texas MD Anderson Cancer Center, Translational Molecular Pathology, Houston, USA; [6]The University of Texas MD Anderson Cancer Center, Symptom Research, Houston, USA*

Background: Cutaneous squamous cell carcinoma (cSCC) is the second most common form of skin cancer in the US. While the majority of patients with localized lesions are cured with surgery, the prognosis is poor in the metastatic setting as the role of chemotherapy in the treatment of metastatic cSCC is limited. The median survival is less than 2 years and up to 70% of patients die as a consequence of the disease in the metastatic setting. Furthermore, the incidence of cSCC has risen dramatically by up to 200% in the past 3 decades in the US because of lifestyle changes leading to increased voluntary exposure to sunlight. However, the high-mutational burden, the presence of tumor-infiltrating lymphocytes, and evidence of direct immunosuppressive effects of UV radiation suggest that patients with cSCC may benefit from treatment with immune checkpoint inhibitors.

Methods: This is a phase II study of pembrolizumab in patients with rare tumors including a pre-specified cohort for patients with cSCC (http://ClinicalTrials.gov identifier, NCT02721732). Patients received pembrolizumab 200 mg intravenously once every three weeks. The response was assessed every nine weeks using RECIST1.1. The primary end point was progression-free survival at 27 weeks (PFS27wk), defined as the percentage of patients who are alive and progression-free at 27 weeks (9 cycles) as assessed by RECIST 1.1. Mandatory biopsies are taken at baseline, on cycle 1 day 15–21, and at the time of progression.

Results: At the time of analysis, a total of 18 patients were enrolled and treated in the cSCC cohort. The patients remained on the study for a median duration of 16 weeks (range, 4–93 weeks). Among the 15 patients evaluable for response, the NPR at 27 weeks was 33% (5/15 patients). Three of the 5 patients had complete resolution of target lesions and are still continuing on treatment. The other 2 patients had 80% and 65% reduction in the size of target lesions from the baseline. The median duration of response was 67 weeks (range, 55–93 weeks). The remaining 10 patients had evidence off progressive disease within 27 weeks of starting the study. The safety profile of pembrolizumab was very favorable and only 1 patient had grade 3 or 4 immune-related adverse event (arthralgia, grade 3).

Conclusion: Single agent pembrolizumab may be an effective therapeutic option for patient with advanced cSCC. Translational data will be presented at this meeting.

No conflict of interest

211 (PB-062) Poster

Immune-related pneumonitis in non-small cell lung cancer (NSCLC) patients treated with anti-PD(L)1: the impact of previous thoracic radiotherapy

A. Botticella[1], T. Ibrahim[2], L. Mezquita[2], L. Hendriks[2], J. Le Pavec[3], R. Ferrara[2], C. Caramella[4], J. Remon[5], S. Champiat[6], J.M. Michot[6], P. Lavaud[2], F. Aboubakar[2], P. Gustin[1], P. David[2], A. Gazzah[6], A. Marabelle[6], E. Deutsch[1], B. Besse[2], C. Le Pechoux[1]. *[1]Institut Gustave Roussy, Department of Radiation Oncology, Villejuif, France; [2]Institut Gustave Roussy, Department of Medical Oncology, Institut d'Oncologie Thoracique IOT, Villejuif, France; [3]Centre Chirurgical Marie Lannelongue, Laboratoire de Chirurgie Expérimentale-UPRES EA-2705, Le Plessis Robinson, France; [4]Institut Gustave Roussy, Department of Radiology, Villejuif, France; [5]Vall d'Hebron University Hospital, Department of Medical Oncology, Barcelona, France; [6]Institut Gustave Roussy, Département D'Innovation Thérapeutique et des Essais Précoces DITEP, Villejuif, France*

Background: Pneumonitis is a life-threatening adverse event of anti-PD(L)1, occurring in 1–5% of patients enrolled in clinical trials. The risk factors for the occurrence of pneumonitis are unknown. Many NSCLC patients treated with anti-PD(L)1 for metastatic disease had previous thoracic radiotherapy (RT), but little is known about the interactions between anti-PD(L)1 and thoracic RT. The main aim of this study is to assess the correlation between the onset of immune-related pneumonitis and previous thoracic RT.

Materials and Methods: Between December 2012 and November 2017, 318 consecutive non-small cell lung cancer (NSCLC) patients received ICI in our Institution and their charts were retrospectively analyzed. Primary endpoint was to determine whether previous radiotherapy had an effect on the occurrence of immune-related pneumonitis. Pneumonitis was assessed by Common Terminology Criteria for Adverse Events version 4.0.

Results: Median age at ICI start was 63 years. 205 patients (64,5%) were males, 103 (32,4%) smokers and 250 (78,6%) with PS $\leq$1; 206 (64,8%) had adenocarcinoma and 76 (23,9%) squamous; 79 (24,8%) were KRAS mutated, 18 (5,5%) EGFR mutated and 5 (1,6%) ALK positive. PDL1 was $\geq$1% by immunohistochemistry in 86 (27%), negative in 37 (11,6%) and unknown in 196 (61,3%) patients. ICI treatment was median 3rd line (range: 1–12), 89,4% monotherapy PD-(L)1 inhibition. Median follow-up was 32.8 months [95%CI: 5–190].

72 patients (22,6%) received a thoracic RT: 62 out of the 72 RT patients (87,5%) were irradiated with a curative intent. 53 patients (73,6% of the RT patients) received thoracic 3D-conformal RT or intensity modulated RT (normo- or mildly hypofractionated), whereas 9 received SBRT.

The occurrence of all grades was higher in previously irradiated patients compared to non-irradiated patients (16,7% versus 2.4%, p = 0.001).

The median interval between the onset of the immune-related pneumonitis and the end of the RT was 22,4 months.

Conclusions: Prior thoracic RT with curative-intent is associated with a higher risk of immune-related pneumonitis in NSCLC patients treated with anti-PD(L)1.

No conflict of interest

213 (PB-064) Poster

Characterization of tumor-infiltrating immune cells and the efficacy of pembrolizumab in preclinical models of primary and bone metastatic triple-negative breast cancer

T. Kähkönen[1], M.I. Suominen[1], J. Mäki-Jouppila[1], J.M. Halleen[1], A. Tanaka[2], M. Seiler[2], J. Bernoulli[1]. *[1]Pharmatest Services, Oncology, Turku, Finland; [2]Taconic Biosciences, Taconic, Rensselaer, USA*

Background: High number of tumor-infiltrating lymphocytes (TILs) is associated with improved survival, and targeting of programmed cell death protein 1 (PD-1) has shown promising results in treatment of triple-negative breast cancer (TNBC). TNBC patients typically have a high incidence of bone metastasis. As immune regulation in bone is different compared to other organs, it is essential to understand immune cell infiltration also to metastatic location. The aim of this study was to assess the efficacy of anti-PD-1 therapy (pembrolizumab, Keytruda®) in the growth of primary and bone metastatic TNBC in preclinical models, and to characterize immune cell infiltration into these tumors to support immuno-oncology drug discovery.

Materials and Methods: MDA-MB-231(SA)-luc human TNBC cells were inoculated orthotopically into the mammary fat pad (primary tumor model) or tibia bone marrow (bone metastasis model) of female huNOG mice (Taconic Biosciences). Treatments with pembrolizumab or IgG4 isotype control (5 mg/kg, i.p., Q5D, n = 8) were started at day 3. Tumor growth was monitored by caliper measurements or X-ray imaging of tumor-induced bone lesions for 24–21 days in the primary tumor and bone metastasis models, respectively. Immunohistochemical stainings were performed at endpoint for human CD4, CD8, CD163, Granzyme B, PD-1 and PD-L1. TILs and tumor-associated macrophages (TAMs) were assessed by 4-scale immunoscoring system and PD-L1 by tumor proportion score (TPS).

Results: Pembrolizumab decreased tumor growth in the primary tumor model but had no effects in the bone metastasis model. About 38% of the mice responded to treatment. PD-1 expression was low in the control-treated orthotopic tumors and absent in the pembrolizumab treated mice due to antibody blocking of the epitope. PD-L1 expression was moderate in both tumor types (TPS 1–49%). Moderate number of CD4+ and CD8+ TILs (scoring 2–3) was observed and granzyme B expression correlated with CD8 positivity. In these tumors, corresponding number of CD4+ and granzyme B+ TILs were observed. However, in the bone microenvironment less CD8+ TILs (scoring 0–1) were observed and PD-1 expression was variable. Generally, intratumoral and peritumoral variation of expression and localization of TILs and TAMs was observed, especially in the bone tumors.

Conclusions: Orthotopic tumors responded to pembrolizumab treatment but bone metastatic growth was not inhibited. Bone marrow has a unique immune cell microenvironment that is different from primary tumor. The lack of efficacy of pembrolizumab could be explained by low number of CD8+ and PD-1 positive cells in tumor growing in bone. These results highlight the importance of validation of immune cell infiltration to metastatic location, and

using preclinical metastasis models to have predictive data before entering clinical trials.

No conflict of interest

214 (PB-065) Poster

Development of AO-176, a next generation humanized anti-CD47 antibody with novel anti-cancer properties and negligible binding to red blood cells

M.N. Bouchlaka[1], R. Puro[1], B. Capoccia[1], M. Donio[1], R. Hiebsch[1], A.J. Carter[1], K.S. Crowley[1], W.C. Wilson[1], P. Chakraborty[1], P.T. Manning[1], R. Karr[1], V. Sung[2], D.S. Pereira[1]. [1]*Arch Oncology, Research, St. Louis, USA;* [2]*Arch Oncology, Translational Research, San Francisco, USA*

To date, inhibitors of immune checkpoints have shown significant advances as cancer treatments and have focused mainly on enhancing adaptive immune responses. CD47, a cell surface glycoprotein, is an innate immune checkpoint receptor broadly expressed on normal tissues and over-expressed on several tumors. Binding of CD47 to signal regulatory protein alpha (SIRPα) on macrophages and dendritic cells triggers a “don’t eat me” signal that inhibits phagocytosis. Several hematologic and solid tumors escape innate immune surveillance by overexpression of surface CD47 preventing engulfment and clearance of tumor cells by phagocytes. Blocking the interaction between SIRPα and CD47 has been shown to promote phagocytosis leading to reduction in tumor burden in numerous xenograft and syngeneic animal models.

We have developed a next generation humanized anti-CD47 antibody, AO-176, that not only blocks the CD47/SIRPα interaction and induces tumor cell phagocytosis, but also exhibits several unique functional properties. The first property is the ability of AO-176 to induce direct tumor cytotoxic cell death in hematologic and solid human tumor cell lines by a cell autonomous mechanism (not ADCC). Secondly, AO-176 exhibits preferential binding to tumor versus normal cells, including red blood cells (RBCs), T cells, endothelial cells, skeletal muscle cells and epithelial cells. AO-176 also does not affect the function of any of these primary cells when assayed ex vivo. The negligible binding of AO-176 to RBCs versus Jurkat tumor cells (∼30–50 fold lower Bmax and ∼16–88 fold lower Ec50) is particularly profound and different from other reported anti-CD47 antibodies. AO-176 also does not induce hemagglutination of RBCs. These properties are expected not only to decrease the antigen sink, but also to minimize on-target clinical adverse effects observed following treatment with other reported RBC-binding anti-CD47 antibodies. When tested in cynomolgus monkeys, AO-176 was well tolerated with no adverse effects. A third novel property of AO-176 is its enhanced binding to tumor cells at acidic pH. AO-176 binds to human tumor cell lines ∼3–49-fold higher at an acidic pH of 6.5 compared to a physiological pH. Because the microenvironment of solid tumors has an acidic pH of 6.4–7.2, this enhanced binding of AO-176 at low pH has the potential added advantage of tumor-specific targeting. Lastly, we show that AO-176 demonstrates dose-dependent anti-tumor activity in tumor xenograft models.

Taken together, the unique properties and activity of our next generation anti-CD47 antibody, AO-176, bodes well as this candidate progresses to clinical development.

Conflict of interest: Other Substantive Relationships: Employees of Arch Oncology.

215 (PB-066) Poster

Prognostic and predictive biomarkers of efficacy for Durvalumab in patients with metastatic or advanced head and neck cancer

A. Baverel[1], I. Bartelink[2], V. Pierre[3], Y. Zheng[2], T. Salehi[4], L. Shi[5], J. Buenconsejo[4], C. Morehouse[6], B. Higgs[6], N. Standifer[2], J. Englert[7], S.C. Chang[8], L. Roskos[3], R. Narwal[3]. [1]*MedImmune, Clinical Pharmacology Department, Cambridge, United Kingdom;* [2]*MedImmune, Clinical Pharmacology Department, San Francisco, USA;* [3]*MedImmune, Clinical Pharmacology Department, Gaithersburg, USA;* [4]*AstraZeneca, Global Medicines Development, Gaithersburg, USA;* [5]*MedImmune, Clinical Statistics, Gaithersburg, USA;* [6]*MedImmune, Translational Medicines, Gaithersburg, USA;* [7]*MedImmune, Clinical Development, Gaithersburg, USA;* [8]*MedImmune, Immuno Oncology Franchise, Gaithersburg, USA*

Background: Durvalumab (Imfinzi®), an anti-PDL1, fully human IgG1 mAb, is currently being investigated for the treatment of metastatic or recurrent head and neck squamous cell carcinoma (HNSCC) and in other indications as monotherapy or in combination with immunomodulating agents. We aimed to characterize the relationship between tumor kinetics and survival, and to evaluate prognostic and predictive biomarkers for efficacy outcomes in HNSCC patients.

Methods: Pooled data from 232 HNSCC patients treated with single-agent durvalumab at 10 mg/kg Q2W IV from 3 clinical studies (CD-ON-MEDI4736-1108 [NCT01693562], CONDOR [NCT02319044], and HAWK [NCT02207530]) were used to develop population tumor kinetic, dropout and survival models based on nonlinear mixed-effects modeling approach, including a multivariate covariate analysis.

Results: Tumor kinetic modeling revealed that tumor shrinkage was associated with 2 predictive biomarkers that were statistically significant and deemed clinically relevant: low tumor burden and high PD-L1 expression in the tumor. Similarly, survival benefit (after accounting for individual tumor kinetic profiles) was associated with ($p < 0.01$) low neutrophil/lymphocyte ratio (NLR), high albumin levels (both known prognostic biomarkers) and high PD-L1 scores on either immune cells or tumor cells.

Conclusions: A tumor kinetic model coupled with dropout and survival models adequately described clinical outcomes in HNSCC patients treated with durvalumab and enabled identification of disease specific risk-factors and biomarkers potentially predictive of immune therapy. The modeling framework can be a useful tool to guide patient selection/enrichment strategies for immune-oncology therapies.

No conflict of interest

216 (PB-067) Poster

Converting PD-L1-induced T-lymphocyte inhibition into CD137-mediated costimulation via PD-L1xCD137 bispecific DART® molecules

A. Berezhnoy[1], L. Huang[2], K. Shah[2], D. Liu[1], J. Dichiara[2], C.Y.K. Lam[3], L. Liu[1], R. La Motte-Mohs[1], J. Li[4], V. Ciccarone[5], J. Tamura[5], R. Alderson[5], G. Diedrich[2], E. Bonvini[5], S. Johnson[2], P. Moore[1]. [1]*Macrogenics INC, Cell Biology and Immunology, Rockville, USA;* [2]*Macrogenics INC, Protein Engineering, Rockville, USA;* [3]*Macrogenics INC, Protein Engineering, Brisbane, USA;* [4]*Macrogenics INC, Cell Biology and Immunology, Brisbane, USA;* [5]*Macrogenics INC, Research, Rockville, USA*

Background: PD-1/PD-L1 axis blockade is a clinically proven cancer therapeutic strategy, but can be insufficient to fully activate tumor-specific T cells. CD137 co-stimulation synergistically increases the activity of PD-1 blockade in mouse tumor models. Clinical application of such an approach, however, may be limited by toxicity associated with the systemic administration of CD137 agonists. Here we demonstrate that bispecific DART molecules comprising anti-PD-L1 and CD137 mAb specificities provide PD-1 axis blockade concomitantly with PD-L1-dependent CD137 co-stimulation.

Materials and Methods: PD-L1xCD137 bispecific DART molecules were constructed based on PD-L1 blocking mAbs and CD137-engaging mAbs and evaluated for binding to their respective antigens and in reporter assays, as well as in CD3 or SEB-driven T-cell activation and MLR assays. Anti-tumor redirected T-cell activity was evaluated in combination with anti-CD3 based DART molecules. RNAseq was performed to characterize T-cell gene expression.

Results: PD-L1xCD137 DART molecules bind and block PD-L1, reversing PD-1-mediated T-cell inhibition equipotently to the effect of approved PD-L1 benchmark mAbs. They also bind CD137, but, without secondary cross-linking or clustering induced by PD-L1+ cells, fail to induce CD137 signaling. In the presence of PD-L1-expressing cells, however, PD-L1xCD137 DART molecules drive CD137 activation and immune cell co-stimulation. Robust T-cell activation and cytokine secretion was induced by PD-L1xCD137 DART proteins, with significantly greater activity than that observed with the combination of PD-L1 blocking and CD137 agonistic mAbs. Notably, when combined with tumor targeted anti-CD3 based DART molecules, PD-L1xCD137 bispecific molecules enhance activation of effector cells in the presence of tumor cells and increase tumor growth inhibition. Transcriptome studies revealed a gene expression profile uniquely induced by the PD-L1xCD137 bispecific protein but not by the mAb combination.

Conclusions: These data show that PD-L1xCD137 bispecific DART molecules can switch on CD137 co-stimulation in a PD-L1-dependent fashion. While tumor adaptive resistance via PD-L1 induction promotes immune escape, PD-L1xCD137 DART molecules can exploit the checkpoint ligand up-regulation and further amplify checkpoint blockade by contributing a co-stimulatory signal. Further investigations as a potential therapeutic approach to overcome limitations of existing PD-1/PD-L1-targeting strategies is warranted.

Conflict of interest: Other Substantive Relationships: Full-time employee of Macrogenics, INC.

217 (PB-068) Poster

Distinct immunological properties of the two histological subtypes of adenocarcinoma of the ampulla of vater

M.H. Kim[1], M. Jang[2], H. Kim[2], W.J. Lee[3], C.M. Kang[3], H.J. Choi[1]. [1]*Yonsei University College of Medicine, Division of Medical Oncology, Department of Internal Medicine, Seoul, Korea;* [2]*Yonsei University College of Medicine, Department of Pathology, Seoul, Korea;* [3]*Yonsei University College of Medicine, Department of Hepatobiliary and Pancreatic Surgery, Seoul, Korea*

Background: Adenocarcinoma of the ampulla of vater (AOV) is classified into intestinal type (IT) and pancreatobiliary type (PB), but the immunological properties of these subtypes remain to be characterized. Here, we evaluated the clinical implications of PD-L1 expression and $CD8^+$ T lymphocyte density in adenocarcinoma of the AOV tumors, and their potential association with Yes-associated protein (YAP).

Materials and Method: We analyzed 123 adenocarcinoma of the AOV patients who underwent surgical resection, and tumors were classified into IT type and PB type. Tumor or inflammatory cell PD-L1 expression, $CD8^+$ T lymphocyte density in the cancer cell nest (intratumoral) or in adjacent stroma, and YAP localization and intensity were analyzed by immunohistochemical staining.

Results: PB type tumors showed higher tumoral PD-L1 expression than IT type tumors, and tumoral PD-L1 expression was associated with shorter disease-free survival (DFS: hazard ratio [HR], 1.77; $p = 0.045$) and overall survival (OS: HR, 1.99; $p = 0.030$). Intratumoral $CD8^+$ T lymphocyte density was higher in IT type than in PB type and associated with favorable DFS (HR, 0.47; $p = 0.022$). The nuclear staining pattern of YAP in tumor cells, compared to non-nuclear staining patterns, was more frequently associated with PB type and increased tumoral PD-L1 expression. Nuclear YAP staining was a significant prognostic factor for OS (HR, 2.21; $p = 0.022$).

Conclusions: These results show that the two subtypes of adenocarcinoma of the AOV exhibit significant differences in tumoral PD-L1 expression and intratumoral $CD8^+$ T lymphocyte density, which might contribute to their distinct clinical features.

No conflict of interest

218 (PB-069) Poster

A novel immunomodulatory strategy of targeting glyco-immune checkpoints with EAGLE technology

L. Peng[1]. [1]*Palleon Pharmaceuticals, Biotherapeutics Discovery, Waltham, USA*

Cancer therapy has been revolutionized by inhibiting immune-checkpoints to harness the power of the immune system in fighting cancer. However, the majority of patients are resistant to the current immuno-oncology drugs. There is a strong need to identify novel mechanisms of cancer immune evasion and explore novel therapeutic modalities. Glyco-immune checkpoint axis plays a critical role in modulating innate and adaptive immune responses against cancer. However, this pathway is underexplored for therapeutic interventions of cancer, because the complexity and heterogeneity of glycan-ligands on tumor cells pose grand challenges for conventional therapeutic modalities.

Here we described a multi-functional antibody-like novel therapeutic modality, named EAGLE (Enzyme-Antibody Glyco-Ligand Editing), which can overcome the heterogeneity and complexity problems and specifically edit tumor-specific glycans. We evaluated the efficacy of EAGLE molecule and studied its mechanism of actions in a breast cancer EMT6 syngeneic tumor model.

Systematic delivery of the EAGLE molecule decreased the amount of immunosuppressive glycan-ligands on tumor cell surfaces, and increased T-cell infiltration and activation in the syngeneic tumor models. EAGLE treatment led to 50% complete regressions of established tumors as a monotherapy and 100% cures in combination with an anti-PD1 mAb. Furthermore, cured mice from EAGLE treatment completely rejected the rechallenge of tumor cells, suggesting that EAGLE induced anti-tumor immunological memory.

In summary, the novel therapeutic modality, EAGLE, blocked the glyco-immune checkpoint pathway in the tumor microenvironment and potentiated innate and adaptive antitumor immunity, offering a novel immunomodulatory strategy to treat cancer.

No conflict of interest

219 (PB-070) Poster

CANscript is an ex vivo human tumor culture platform capable of accurately capturing response to immune modifying agents

M. Paris[1]. [1]*Mitra Biotech, Director, Translational Applications, Woburn, USA*

Clinically-relevant tumor models are urgently needed to interrogate mechanisms of action, and accurately predict real-world efficacy of anticancer agents. This is particularly true for emerging drugs such as immunotherapies, which suppress tumor growth by releasing the brakes on the immune system. To meet these needs, we have developed a novel ex vivo patient tumor culture model using intact tumor fragments cultured with autologous plasma and PBMC's. Our model is the result of a multi-year development effort focused on the contextual preservation of native-state proliferation, morphology and viability. Most importantly, we have defined and validated a series of phenotypic response assessments which are coupled to a machine learning algorithm to quantitate drug response in a way that allows prediction of clinical efficacy with 90% accuracy. Our assay was trained on more than 2,000 patients and is currently validated across 10 indications (solid and heme malignancies) and >80 approved therapeutic regimens. Furthermore, we leverage the tissues and culture supernatants to explore mechanistic questions that deepen our understanding of the drugs tested. The data presented herein features responses seen in squamous cell carcinoma of the head and neck (SCCHN) challenged with clinically-approved anti-PD1 agents. Our observations are mechanistically consistent with known functions for anti-PD1 and mirror published clinical response rates for Pembrolizumab in SCCHN. Finally, we demonstrate that our platform preserves known mechanisms of resistance that represent opportunities for testing new modalities and rational combinations in the immuno-oncology space.

No conflict of interest

220 (PB-071) Poster

Targeting myeloid-associated immunosuppression with a novel immunoglobulin-like transcript 4 (ILT4)-specific monoclonal antibody

D. Wilson[1], B. Joyce-Shaikh[2], H. Cherwinski[2], Y. Chen[2], E. Oldham[3], J. Grein[1], X. Du[4], E. Hsieh[4], G. Ayanoglu[4], M. Beaumont[4], S. Zhang[4], J. Punnonen[2], M. Rosenzweig[5], R. Kastelein[2], R. Altura[6], D. Cua[2], L. Zuniga[2]. [1]*Merck & Co., Dept. of Profiling and Expression, Palo Alto, CA, USA;* [2]*Merck & Co., Dept. of Immuno-Oncology, Palo Alto, CA, USA;* [3]*Merck & Co., Dept. of Discovery Planning, Palo Alto, CA, USA;* [4]*Merck & Co., Dept. of Bioanalytics, Palo Alto, CA, USA;* [5]*Merck & Co., Dept. of Immuno-Oncology, Boston, MA, USA;* [6]*Merck & Co., Dept. of Clinical Research, Rahway, NJ, USA*

Background: Immunotherapy has dramatically improved efficacy in human malignancies, especially melanoma and lung cancer. However, current approved immunotherapies targeting T lymphocytes are not effective in many cancers, as tumors can employ additional mechanisms to limit host immune responses and escape immunosurveillance. Myeloid-derived suppressor cells are an important factor mediating immunosuppression in the tumor microenvironment and are considered a prime target for therapeutic intervention. ILT4/LILRB2 is an inhibitory member of the leukocyte immunoglobulin-like receptor (LILR) family that is expressed primarily by myeloid cells, including monocytes, granulocytes, and dendritic cells. ILT4 interacts with major histocompatibility (MHC) class I complexes and angiopoietin-like (ANGPTL) ligands, and its signaling is associated with the induction of a tolerogenic phenotype in antigen presenting cells. Although ILT4 has been proposed as a putative target for cancer immunotherapy, the utility of targeting this pathway for anti-tumor efficacy has not been previously demonstrated *in vivo*.

Material and Methods: Here we describe the discovery and characterization of antibody clone 1E1 – a fully human monoclonal antibody of the IgG4 subclass that specifically binds ILT4. Primary human tumor tissue, blood, and serum were extensively profiled for the expression of ILT4 and its ligands. Primary human PBMC assays were utilized to assess the ability of clone 1E1 to enhance myeloid-associated cytokine responses *in vitro*. Due to the absence of a homologous ortholog of the ILT4 gene in other model species, we relied on a humanized mouse model system to evaluate mechanism of action of ILT4 antagonism.

Results: We show that clone 1E1 is specific to ILT4 and does not bind to other LILR-family receptors. Clone 1E1 blocks ILT4 binding to Human Leukocyte Antigen (HLA)-G, other MHC class I molecules (HLA-A, -B, and -F), as well as ANGPTL ligands. Moreover, clone 1E1 reverses ILT4-mediated suppression of signal transduction. Blocking ILT4 *in vitro* dose-dependently enhances proinflammatory cytokine expression of GM-CSF and TNFα in LPS-stimulated human PBMC cultures. Utilizing a novel human immune system SK-MEL-5 tumor mouse model, we demonstrate the

presence of ILT4+ myeloid cells both in the periphery and in the tumor infiltrate. Treatment with clone 1E1 results in approximately 50% reduction in tumor growth, alterations in the both splenic and tumor myeloid subset distributions, as well as changes in myeloid-centric chemokine and cytokine profiles.

Conclusions: These data support a role for ILT4 antagonism as a new strategy for enhancing anti-tumor immune responses by targeting a myeloid-associated immunomodulatory pathway.

Conflict of interest: Corporate-sponsored Research: All authors are current or former employees of Merck & Co (also known as MSD in Europe/EU). This body of work was financially supported by Merck & Co (also known as MSD in Europe/EU).

221 (PB-072) Poster

Identification of novel inhibitors of Arginase-1 for cancer immunotherapy by high-throughput screening

Y. Grobben[1], J.C.M. Uitdehaag[1], N. Willemsen-Seegers[1], W.W.A. Tabak[1], J. van Groningen[2], J. Friesen[2], H. Rutjes[2], J. de Man[1], R.C. Buijsman[1], G.J.R. Zaman[1]. *[1]Netherlands Translational Research Center B.V., Biology, Oss, Netherlands; [2]Pivot Park Screening Centre, Oss, Netherlands*

Background: Arginase-1 (Arg-1) is an important drug target for cancer immunotherapy. Expression of Arg-1 by tumour-infiltrating myeloid-derived suppressor cells induces local L-arginine depletion, which results in reduced T-cell and natural killer cell proliferation. Despite increased insight in the role of Arg-1 in tumour immune suppression, there is still a lack of drug-like Arg-1 inhibitors. To identify novel inhibitors, a new high-throughput screening (HTS) assay was developed (Arginase Gold™) and used to screen a 93,000-compound diversity library.

Materials and Methods: ABH and (3R,4S)-3-Amino-1-((S)-2-aminopropanyoyl)-4-(3-boronopropyl)pyrrolidine-3-carboxylic acid (AABC) were synthesized in-house. Arginase Gold is a homogenous mix-and-measure assay based on a novel and proprietary chemical probe. In contrast to previously reported arginase assays, this assay requires only two addition steps, short incubation times and mild reaction conditions. Moreover, it is a gain-of-signal fluorescence assay and allows for kinetic measurements. Binding of compounds to Arg-1 was further studied by thermal shift assay, surface plasmon resonance and protein crystallogrophy.

Results: The Arginase Gold assay technology was validated by testing a set of published Arg-1 inhibitors (i.e., ABH, nor-NOHA and AABC), resulting in potencies consistent with those in the classical urea detection assay. Nevertheless, we found that inhibitor potencies varied considerably depending on the exact conditions in both assays, demonstrating the importance of Arg-1 stability and a minimal incubation time with L-arginine. Kinetic experiments performed with the screening assay showed that ABH does not stably inhibit Arg-1 over time, which explains why IC_{50} values of ABH vary significantly in scientific literature. Furthermore, we observed that AABC has slow association kinetics at pH 9.5, but not at pH 7.4, which was confirmed with surface plasmon resonance. The pH-dependent binding character of AABC could be related to the boric acid-borate equilibrium.

To demonstrate the use of the assay for HTS, a library of 93,000 compounds was screened. Of the initial 621 hits, 297 were confirmed at a single test concentration. Through cheminformatic “nearest neighbour” analysis, 49 additional compounds were selected, followed by dose-response measurements. Side-by-side determination of the activity of hits in the Arg-1 assay and a control assay without enzyme allowed the efficient de-selection of fluorescent artefacts, resulting in the identification of a few novel, genuine Arg-1 inhibitors.

Conclusions: A novel fluorescence-based arginase screening assay was developed that is suitable for 384-well automated HTS. The assay was validated using known Arg-1 inhibitors and revealed new insights into their binding kinetics. Furthermore, new inhibitors of Arg-1 could be identified using the novel HTS assay.

No conflict of interest

222 (PB-073) Poster

Bispecific CD40/FAP DARPin® molecule for tumor-restricted immune activation

N. Rigamonti[1], A. Schlegel[2], S. Barsin[1], J. Schwestermann[1], J. Krieg[1], S. Mangold[2], M. Paladino[2], S. Bruckmaier[2], V. Calabro[2], V. Levitsky[1], S. Plyte[1], M. Stumpp[1], C. Metz[2]. *[1]Molecular Partners, Biology, Schlieren, Switzerland; [2]Molecular Partners, Lead Generation, Schlieren, Switzerland*

CD40 is a member of the tumor necrosis factor receptor (TNFR) superfamily which can activate both innate and adaptive tumor immunity, making it an attractive target for cancer immunotherapy. Systemic administration of agonistic CD40 antibodies (Ab) has shown signs of activity in cancer patients, but dose-limiting toxicities have impaired the clinical efficacy. New therapeutic approaches to achieve tumor-restricted activation through intratumoral administration of CD40 Ab are now in early clinical development, aiming to reduce systemic toxicity and increase efficacy. However, there are clear limitations, such as tumor accessibility. Here, we report an alternative approach based on systemic administration of a bispecific DARPin® molecule targeting human CD40 and a tumor antigen (TA), enabling CD40 pathway activation exclusively in the presence of TA-expressing cells. Using fibroblast activation protein (FAP) alpha, a glycoprotein abundantly expressed in many solid tumors, as a TA, we generated a bispecific CD40/FAP DARPin® molecule able to functionally activate the human CD40 receptor in (1) a reporter cell assay, (2) primary lymphocytes and (3) primary monocytes. The CD40 activation only took place in the presence of FAP-expressing cells, confirming a mechanism of action strictly dependent on FAP-mediated cross-linking. In order to properly address the *in vivo* activity, a surrogate mouse-specific CD40/FAP DARPin® molecule was also generated and tested in different *in vitro* assays showing a FAP-dependent activation and similar results of the human counterpart. Experiments are ongoing to assess the efficacy and mechanism of action of a tumour-restricted CD40 agonistic DARPin® molecule. In conclusion, we have generated bispecific agonist CD40/FAP DARPin® molecules able to activate the CD40 pathway in cellular assays with a targeting-dependent mechanism of action, supporting the hypothesis that these DARPin® molecules could lead to a tumor-localized immune activation *in vivo*. Data of the ongoing *in vivo* experiments in mouse tumor models to test this hypothesis will be also shown at the meeting.

No conflict of interest

223 (PB-074) Poster

MAPK pathway activity plays a key role in programmed death ligand-1 expression of EGFR wild-type non-small cell lung adenocarcinoma cells

T. Stutvoet[1], A. Kol[2], E. de Vries[1], M. de Bruyn[2], R. Fehrmann[1], A. Terwisscha van Scheltinga[3], S. de Jong[1]. *[1]Cancer Research Center Groningen, University of Groningen, Medical Oncology, Groningen, Netherlands; [2]Cancer Research Center Groningen, University of Groningen, Obstetrics and Gynaecology, Groningen, Netherlands; [3]University of Groningen, University Medical Center Groningen, Clinical Pharmacy and Pharmacology, Groningen, Netherlands*

Background: Immune checkpoint inhibitors targeting the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) interaction have improved survival of patients with epidermal growth factor receptor (EGFR) wild-type non-small cell lung cancer (NSCLC). Still, many patients do not respond to these inhibitors. The aim of the present study is to improve understanding of PD-L1 regulation in EGFR wild-type NSCLC, which may provide a rationale for combination therapy of immune checkpoint inhibitors with other (targeted) agents.

Materials and Methods:

1. Publicly available RNA-seq data of EGFR wild-type lung adenocarcinoma (n = 197) and squamous cell lung carcinoma (n = 172) from The Cancer Genome Atlas (TCGA) was used to investigate the correlation between PD-L1 gene expression and RAS, MEK, and PI3K pathway activity scores, or interferon gamma (IFNγ) signaling related gene expression.
2. Next, the influence of EGF, an activator of the wild-type EGFR signaling pathway, and IFNγ on PD-L1 mRNA (by qPCR), total protein (by Western blotting) and membrane expression (by flow cytometry) was determined in a panel of 5 lung adenocarcinoma cell lines after treatment for up to 72 hours. Small molecule inhibitors and siRNAs of the JAK/STAT, MAPK and PI3K pathway were used to modulate activity of their respective targets. Results were validated using cocultures of tumor cells with peripheral blood mononuclear cells (PBMCs).

Results: Analysis of TCGA RNA-seq data revealed that gene expression of transcriptional targets of IFNγ signaling correlated with PD-L1 gene expression in EGFR wild-type lung adenocarcinoma and squamous cell lung carcinoma, but inferred MAPK activation correlated with PD-L1 gene expression only in lung adenocarcinoma. In our tumor cell line panel, stimulation with EGF or IFNγ increased PD-L1 mRNA, protein, and membrane expression levels. These were further enhanced by combining EGF and IFNγ, raising PD-L1 mRNA levels 66–88 fold and membrane expression levels 3.5–31 fold compared to untreated controls across our cell line panel. Similarly, coculture with PBMCs increased tumor cell PD-L1 membrane expression. Inhibition of the MAPK pathway, using EGFR-inhibitors cetuximab and erlotinib or mitogen-activated protein kinase kinase

1 and 2 (MEK1/2) inhibitor selumetinib, prevented PD-L1 mRNA, protein, and membrane upregulation. Interestingly, MAPK pathway inhibition did not influence IFNγ-induced MHC-I membrane expression of tumor cells.

Conclusions: Inhibition of MAPK pathway signaling disrupts IFNγ- and growth factor-induced PD-L1 expression in EGFR wild-type lung adenocarcinoma, without affecting MHC-I expression. These results support further investigation of MAPK pathway inhibition to improve efficacy of immune checkpoint inhibitors.

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No conflict of interest

224 (PB-075) Poster

Novel, small molecule PD-L1 inhibitors for cancer immunotherapy

D. Sivanandhan[1], S. Garapaty[1], G.P. Seerapu[1], R. Das[1], R. Kar[1], A.K. Singh[1], V. Ashok Kumar[1], C. Venkateshappa[2], R. Putta[2], M. Pendyala[3], T. Jadhav[3], A.E. Wani[3], S. Ghanta[3], S. Birudukota[1], H. Gogoi[1], H. Nadig[1], R. Gangaiah[4], Z. Mohd[4], J. Athisayamani[2], S. Rajagopal[1]. *[1]Jubilant Biosys Ltd, Biology, Bangalore, India; [2]Jubilant Biosys Ltd, Medicinal Chemistry, Bangalore, India; [3]Jubilant Chemsys Ltd, Chemistry, Noida, India; [4]Jubilant Biosys Ltd, ADME/Tox, Bangalore, India*

Background: The PD-1/PD-L1 molecular pathway is one of the primary mechanisms of immune evasion deployed by cancer cells. Activation of PD-1/PD-L1 pathway induces apoptosis of activated T-cells, inhibits their proliferation, facilitates T-cell anergy and exhaustion and enhances the function of regulator T-cells. Therefore, blocking this pathway restores the proliferation and cytotoxicity of CTLs, inhibits the function of Tregs and results in decreased T-cell apoptosis. Few mAbs targeting PD-1/PD-L1 have been approved for a number of malignancies. But, these approved therapies require bolus intravenous injections, are administered in high dose and have a long half-life, which could contribute to the well-documented drug-related adverse effects. Small molecule inhibitors, can therefore, provide increased oral bioavailability, increased bio-efficiency and shorted half life activity for a more controllable treatment, particularly in the case of auto-immune or other adverse effects.

Methods: Rational design approaches were used to design novel PD-1/PD-L1 pathway modulators; potency of these inhibitors was assessed in an *in-vitro* TR-FRET assay. Checkpoint signaling assays as well as *ex-vivo* co-culture assays were used to assess the ability of the compounds to restore T-cell proliferation and function. Syngenic cancer models were used to assess tumor growth inhibition in vivo.

Results: Three novel series of potent PD-L1 inhibitors are being developed for the treatment of cancer. One of the leads, JBI-426 exhibited an IC_{50} of <0.1 μM on PD1:PD-L1 binding and no cytotoxicity against cancer cell or immune cells. JBI-426 showed good *in vitro* ADME properties in terms of aqueous solubility and metabolic stability and excellent oral bioavailability in mouse pharmacokinetics. JBI-426 restored IFN-g level that was depleted by co-culture of cancer cells and immune cells. Oral administration of JBI-426 at 50 mg/kg resulted in a strong tumor growth inhibition, in RENCA and CT26 syngeneic models and was well tolerated. This tumor growth inhibition was associated with an increase in CD4+ as well as CD8+ cytotoxic T lymphocytes in the tumor. Similar effect was observed with leads from other two chemical series as well.

Conclusions: Since the therapeutic benefit of PD1/PD-L1 pathway has already been well established with mAbs, orally bioavailable small molecule inhibitors certainly provide a significant therapeutic benefit in treating cancer and in enhancing the quality of life of these patients.

No conflict of interest

225 (PB-076) Poster

A novel set of 7 homopolymer indels for detection of MSI is associated with tumor mutation burden and total indel load in endometrial and colorectal cancers

H. Zhao[1], B. De Craene[2], X. Sagaert[3], F. Amant[4], G. Maertens[2], D. Lambrechts[1]. *[1]VIB, Center for Cancer Biology, Leuven, Belgium; [2]Biocartis, Create, Mechelen, Belgium; [3]University Hospital Gasthuisberg, Division of Pathology, Leuven, Belgium; [4]University Hospital Gasthuisberg, Division of Gynaecologic Oncology, Leuven, Belgium*

Background: Immune checkpoint blockade was recently approved for the treatment of unresectable or metastatic, microsatellite-instability-high (MSI-H) tumors regardless of site or histology. Observed response rates were ~40%. Currently, there is no FDA-approved test to detect MSI status. MSI-H tumorsshare histopathological characteristics, such as high lymphocytic infiltration and high tumor mutation burden. Specifically, these tumors have a high number of insertion-deletion (indel) mutations, which are known to be highly immunogenic leading to an abundance of neoantigens. High indel rates in MSI-H tumors may therefore predict response to anti-PD1 therapy.

Material and Methods: We previously characterized the mutation landscape of 14 endometrial and colorectal MSI-H tumors by whole-exome sequencing (Zhao et al., Elife 2014), and identified indels in homopolymers that recurrently affect MSI-H tumors (frequency up to 80% per tumor type). We selected 7 of the most robust indels as a set of markers to detect MSI-H and correlated them with mutation burden, as determined by whole-exome sequencing.

Results: An additional 19 MSI-H tumorswere positive for at least 3 out of 7 indels, while MSS tumors were positive for none of them. We further correlated the number of positive indels in all available MSI-H tumors (n = 19 + 14) with mutation load. This revealed a positive correlation for both non-synonymous and indel mutation load (r = 0.68 and r = 0.75 with $p < 1e^{-5}$ and $p < 2e^{-16}$ respectively). Per additional indel marker that was positive, we observed an increase in indel mutation rate of ~120 indels, starting with a median of ~350 indels as of 2 markers positive.

Conclusions: A selection of 7 indels reliably detects MSI-H in endometrial and colorectal cancer, while the number of positive indels serves as a proxy, not only for mutational burden, but also for total indel load, and may thus be used as a test for tumor neoantigen load predictive of response to anti-PD-1 therapy in MSI-H tumors. These 7 markers will be available as a fully automated Idylla™MSI test to detect MSI status and could be used as a companion diagnostic to predict immunotherapy outcome in MSI-H tumors.

No conflict of interest

226 (PB-077) Poster

Generation of human immune checkpoint double knock-in mice (dKI HuGEMM) for preclinical efficacy assessment of combinatorial therapeutic antibodies

L. Zheng[1], D. He[1], R. Sun[2], A. An[1], J. Fei[2], H. Li[1], D. Ouyang[1]. *[1]Crown Bioscience Inc., Transnational Oncology, San Diego, USA; [2]Shanghai Model Organisms Inc., Research & Development, Shanghai, China*

Introduction: Immune checkpoint inhibitors (ICIs), *i.e.* PD1, PDL1 and CTLA4 therapeutic antibodies, have led to long-term survival in many late stage solid tumor patients. Despite their revolutionary clinical impact, the overall response rate is still low. One major roadblock is that compensatory immune inhibitory pathways are turned on to protect tumor cells from being attacked by T cells. Various combinatory ICI treatments have now been investigated in clinical trials to tackle this challenge. We have previously reported the development of immune checkpoint target humanized mice (HuGEMM) through CRISPR-Cas9 knock-in. We have generated PD1 and CTLA4 HuGEMM mice and used them for efficacy assessment of corresponding therapeutic antibodies. We have also generated human PDL1 expressing MC38 cells and PDL1 HuGEMM, and demonstrated robust efficacy of several PDL1 therapeutic antibodies.

Methods: Here we report the establishment of ICI double knock-in (KI) mice, i.e., PD1/PDL1 and PD1/CTLA4 dKI HuGEMM, and use them as tools to test immunotherapies with PD1/PDL1 and PD1/CTLA4 combos.

Results: Similar to clinical benefit of PD1 and CTLA4 combo shown in the melanoma and NSCLC patients, we found combined treatment of Nivolumab and Ipilimumab antibody leads to 103% TGI in the corresponding PD1/CTLA4 dKI-HuGEMM, with complete tumor remission in 80% of treated mice. These mice remained disease free throughout a tumor re-challenge study for over 40 days. We have also developed PD1/PDL1 dKI HuGEMM and tested combined treatment of Atezolizumab and Nivolumab. Interestingly, we observed improved efficacy in the combo treatment group despite the two ICIs are thought to target the same PD1/PDL1 axis. Monotherapies may not fully release the PD1/PDL1 blockade due to complicity of multiple compatible ligands & receptors, including PDL2 and B7-1. We have also tested PD1/OX40 combo treatment with dKI HuGEMM, which is reported separately.

Conclusion: Our dKI HuGEMM models offer robust tests on ICI combinations, as well as combos of ICIs with other anti-tumor therapeutic modalities.

Conflict of interest: Corporate-sponsored Research: Crown Bioscience Inc., Shanghai Model Organisms Inc.

227 (PB-078) Poster

Association of immune checkpoint expression, tumor STING expression and DNA damage repair deficiency in breast cancer

E. Gilmore[1], F. Orafidiya[1], S. Mcquaid[2], L. Mcilreavey[2], G.W. Irwin[3], L. Knight[4], M. Salto-Tellez[2], R. Kennedy[1], N. McCabe[1], E. Parkes[1]. *[1]Queen's University Belfast, Centre for Cancer Research and Cell Biology,*

Belfast, N. Ireland, United Kingdom; [2]Queen's University Belfast, Northern Ireland Molecular Pathology Laboratory, Belfast, N. Ireland, United Kingdom; [3]Belfast City Hospital, Surgery, Belfast, United Kingdom; [4]Almac Group, Almac Diagnostics, Craigavon, United Kingdom

Background: We previously developed a 44-gene expression assay capable of identifying a subgroup of breast cancer with DNA damage repair deficiency due to loss of the Fanconi Anemia/BRCA repair pathway. This assay was retrospectively validated as predictive of improved relapse-free survival following DNA damaging chemotherapy. This 44-gene assay is immune driven, and expression of immune genes is dependent on the cytosolic DNA-sensing cGAS-STING pathway. We previously demonstrated association of the immune checkpoint PD-L1 with the DNA damage repair deficient (DDRD) subgroup in breast cancer. We sought to identify other immune targets associated with the DDRD subgroup and their association with STING expression.

Material and Methods: DNA microarray data from FFPE samples was used to identify immune checkpoints associated with the DDRD subgroup. We used isogenic breast cell line models to identify the effect of endogenous DNA repair deficiency on checkpoint expression. PD-L1 (Roche SP263), PD-1, ICOS, TIM3, LAG3, IDO1 and STING were identified by immunohistochemistry (IHC) on a previously described breast TMA of 164 breast tumor samples, of which 58 were in the DDRD subgroup.

Results: We found upregulation of gene expression of PD-L1, IDO1, TIM3 and LAG3 in the DDRD subgroup. Cell line modelling revealed upregulation of PD-L1 and LAG3 in the *BRCA1* mutant MDA-MB-436-EV cell line compared to its *BRCA*-reconstituted pair. Moreover, upregulation of PD-L1 and LAG3 was shown to be dependent on STING using siRNA knockdown ($p < 0.05$). IHC analysis revealed a significant association of PD-L1 and IDO1 tumor and stromal expression ($p < 0.0001$), and LAG3, ICOS, PD-1 ($p < 0.001$) and TIM3 ($p = 0.0007$) stromal expression, with the DDRD subgroup in breast cancer. STING tumor expression identified by IHC was significantly associated with the DDRD subgroup ($p < 0.0001$), although not stromal expression of STING. STING tumor expression was associated with upregulation of the immune checkpoints PD-L1, IDO1 and LAG3.

Conclusions: Here we show the association of immune checkpoint expression and tumor STING expression with loss of DNA damage repair capability as assessed by the DDRD assay. Moreover, tumoral STING expression was significantly associated with checkpoint upregulation on IHC. We propose the DDRD subgroup as a target for combination immune checkpoint therapy. Tumor STING loss may be important in the efficacy of STING agonists in combination with immune checkpoint targeted therapy.

No conflict of interest

228 (PB-079) Poster

EOS100850, an insurmountable and non-brain penetrant A2A receptor antagonist, inhibits adenosine-mediated T cell suppression, demonstrates anti-tumor activity and exhibits best-in class characteristics

E. Houthuys[1], R. Marillier[1], T. Deregnaucourt[1], B. Margreet[1], P. Basilico[1], R. Pirson[1], J. Marchante[1], S. Prasad[1], A. Hermant[1], F. Nyawouame[1], J. Preillon[1], K. Frederix[1], A.C. Michaux[1], F. Lambolez[1], J. Swiercz[1], G. Driessens[1], C. Martinoli[1], M. Detheux[1], X. Leroy[1], S. Crosignani[1]. *[1]iTeos Therapeutics, Research and Development, Gosselies, Belgium*

High levels of extracellular adenosine in the tumor microenvironment promote tumor immune evasion. We and others have shown that adenosine, predominantly through the A_{2A} receptor (A_{2A}R), suppresses the Th1 cytokine production of T cells and monocytes and cytolytic activity of T and NK cells. We demonstrated that A_{2A}R antagonists initially designed for Parkinson's disease but repurposed for immuno-oncology dramatically lost potency in a high adenosine environment. We therefore developed EOS100850, a novel, non-brain penetrant and highly selective inhibitor of A_{2A}R with sub-nanomolar Ki. Using experimental conditions that mimic tumor environment, we have shown that EOS100850 potently inhibited A_{2A}R signalling in human T lymphocytes independently of adenosine concentrations, and rescued cytokine production, even in the presence of high concentrations of A_{2A}R agonists. iTeos A_{2A}R antagonist potently rescued Th1 cytokine production in human whole blood treated by A_{2A}R agonists, and increased $CD8^+$ T cell cytotoxicity in a co-culture assay of effector $CD8^+$ T cells and target cancer cells.

An *in vivo* pharmacodynamic assay based on phosphorylation of CREB (pCREB) in mouse peripheral $CD8^+$ and $CD4^+$ T cells was developed and qualified as a readout for A_{2A}R activation. EOS100850, 30 minutes after oral gavage at doses ranging from 0.03 to 1 mg/kg, demonstrated 80 to 100% inhibition of pCREB induced by the ex vivo addition of A_{2A}R agonist. Remarkably, 12 hours after gavage at 1 and 3 mg/kg, when the EOS100850 antagonist was no longer detectable in the plasma, more than 50% of inhibition of pCREB was still observed. These results demonstrate that EOS100850 has a PD activity that extends well beyond its PK based on a long residence time.

iTeos's A_{2A}R antagonist, uniquely designed to address the challenge of counteracting elevated adenosine concentrations in tumors, was tested for the first time in a mouse A20 lymphoma model. iTeos's A_{2A}R antagonist in combination with anti-PD-L1 demonstrated significant tumor growth suppression ($p = 0.0008$) compared with anti-PD-L1 alone, with a 6-fold decrease in tumor volume compared to anti-PD-L1 alone (median tumor volume = 105 ± 79 mm^3 vs 696 ± 427 mm^3 on day 23, respectively).

EOS100850 represents a novel, potent, insurmountable and best-in-class A_{2A}R blocker, specifically optimized for immuno-oncology indications, that deserves studies in Human.

Conflict of interest: Other Substantive Relationships: The co-authors are employees of iTeos Therapeutics.

229 (PB-080) Poster

Chemotherapy beyond immune checkpoint inhibitors in patients with metastatic colorectal cancer

P. Martin Romano[1], E.D. Yolla[1], B. Capucine[1], V. Andrea[1], V. Perrine[1], A. Eric[1], B. Rastilav[1], G. Anas[1], C. Stephane[1], A. Jean Pierre[1], P.V. Sophie[1], M. Aurelien[1], S. Jean Charles[1], D. Michel[2], B. Valerie[2], M. David[2], M. Christophe[1], H. Antoine[1]. *[1]Gustave Roussy, DITEP, Drug Development Department, Villejuif, France; [2]Gustave Roussy, Department of Medical Oncology, Villejuif, France*

Background: Immune checkpoint inhibitors (CPI) have changed the landscape of cancer treatment, yet only a small subset of patients (pts) with microsatellite instability–high (MSI-H) metastatic Colorectal Cancer (mCRC) seem to benefit from CPI. Thus, most mCRC patients have microsatellite stable (MSS) tumors and are refractory to CPI. Many clinical trials are evaluating different approaches to overcome primary immunoresistance of mCRC patients. We aimed to assess whether sequential CPI followed by chemotherapy (CT) may be an alternative therapeutic approach in this subset of patients.

Material and Methods: We retrospectively evaluated response upon chemotherapy after immunotherapy failure. All pts with mCRC treated by chemotherapy after failure of anti-PD(L)1 alone or in combination at the early Drug Development Department at Gustave Roussy were included. Primary endpoints were RECIST objective response rate (ORR), progression-free survival (PFS), and overall survival (OS).

Results: Twenty-three mCRC patients were treated with CT between 2014 and 2018. Median number of previous lines including CPI were 4 (range, 2–7). Median age was 58 years old (25–88), 19 pts (83%) were male and 87% had MSS tumors. CT regimens included TAS-102 (12 pts), FOLFIRI (1 pt), FOLFIRI plus targeted therapy (4 pts: 1 pt Bevacizumab; 3 pts Cetuximab), FOLFOX plus targeted therapy (3 pts: 1 pt Panitumumab; 2 pts Bevacizumab), Regorafenib (2 pts), and Carboplatin (1 pt BRCAm). Median interval between CPI and CT was 0.5 months (range, 0.3–3); 21/23 pts were assessable for the analysis. Overall response rate was PR in 4 pts (19%), SD in 9 pts (43%) and PD in 8 pts (38%). Patients with PR had received FOLFOX-bevacizumab (2 pts), FOLFIRI-bevacizumab (1 pt) and TAS-102 (1 pt) and had MSS tumors. Median PFS and OS post-CPI were 2.7 months (95% CI, 1.2–3.5) and 5.5 months (95% CI, NA), respectively.

Conclusion: Chemotherapy after CPI failure is feasible in patients with immunorefractory mCRC. The results of this small retrospective cohort need to be validated in independent retrospective cohorts as well as prospectively. The role of immunotherapy as a modifier of both tumor cells and microenvironment in mCRC deserves further research.

Conflict of interest: Other Substantive Relationships: Pr Soria is full time employee of Medimmune since sept 2017.

230 (PB-081) Poster

Oral immune checkpoint antagonists dually targeting TIGIT and PD-1 pathways for cancer therapy

P. Sasikumar[1], C. Gundala[1], S. Patil[2], S. Naremaddepalli[1], R. Ramachandra[3], N. Gowda[3], S. Tangela[1], S. Adurthi[4], A. Dhudashia[5], S. Dodheri[6], N. Gowda[4], K. Nellore[2], M. Ramachandra[7]. *[1]Aurigene Discovery Technologies Limited, Medicinal Chemistry, Bangalore, India; [2]Aurigene Discovery Technologies Limited, Cell and Molecular Biology, Bangalore, India; [3]Aurigene Discovery Technologies Limited, Pharmacology, Bangalore, India; [4]Aurigene Discovery Technologies Limited, Immunology, Bangalore, India; [5]Aurigene Discovery Technologies Limited, Pharamacokinetics, Bangalore, India; [6]Aurigene Discovery Technologies Limited, Pharmacokinetics, Bangalore, India; [7]Aurigene Discovery Technologies Limited, Preclinical Biology, Bangalore, India*

Advances in harnessing the immune system for cancer treatment have been spectacular in the recent years with the achievement of highly durable clinical responses with antibodies to checkpoint receptors such as CTLA4 and PD1. Apart from PD-1 and CTLA-4, there are several other checkpoint proteins in the tumor microenvironment that play a role in dampening the anti-tumor immune response. In addition to PD1 and CTLA4, upregulation of other inhibitory pathways prevent effective immunity. T cell Ig and ITIM domain (TIGIT) is a recently identified co-inhibitory receptor expressed by activated T cells, Tregs, and NK cells. TIGIT binds two ligands, CD112 (PVRL2, nectin-2) and CD155 (PVR), and these ligands are expressed by T cells, APCs, and tumor cells. TIGIT is upregulated on tumor antigen-specific (TA-specific) $CD8^+$ T cells and $CD8^+$ tumor-infiltrating lymphocytes (TILs) in various cancer types and TIGIT receptor/poliovirus receptor (PVR) ligand interaction signaling inhibits cytotoxicity mediated by NK and $CD8^+$ T cells. Interestingly, TIGIT-expressing $CD8^+$ T cells often co-express the inhibitory receptor PD-1. Therefore simultaneously blocking of both TIGIT and PD-1 pathway could potentially result in better anti-tumor activity.

We sought to discover and develop small molecule immune checkpoint antagonists capable of simultaneously targeting TIGIT and PD-L1 pathways. We reasoned that such therapeutic agents will be amenable for oral dosing, likely show greater response rate due to dual antagonism and allow better management of irAEs due a shorter pharmacokinetic profile. Herein we report the pharmacological evaluation of the first-in-class small molecule antagonists capable of targeting both PD-L1 and TIGIT immune checkpoint pathways. The design hypothesis for generating a dual antagonist is primarily based on approach of truncating high affinity peptides or critical fragments from the interface of TIGIT/PVR interactions to arrive at the shortest pharmacophore. Considering the pockets of sequence similarity of PDL1 and TIGIT proteins a focused library of small molecule compounds, based on shortest pharmacophore, mimicking the interaction of checkpoint proteins was designed and synthesized to achieve compounds exhibiting dual antagonism towards TIGIT and PD-1 pathways.

We have identified novel antagonists demonstrating dual TIGIT and PD-L1 inhibition with potent rescue of PVR-mediated inhibition of IL-2 production from T cells and PD-L1 mediated IFN-γ production. The SAR optimized lead compounds exhibits desirable invitro ADME and DMPK profile including oral bioavailability and better tumor distribution. The lead compounds exhibit significant anti-tumor activity in a syngeneic tumor model and demonstrated profound immune PD in vivo on both T and NK cells. Additional biomarker characterization and efficacy studies in additional tumor models are ongoing.

No conflict of interest

231 (PB-082) Poster

Targeting TNFR2 – A key regulator of the tumor immunosuppressive microenvironment

A. Raue[1], R. Fulton[1], J. Sampson[1], A. Camblin[1], J. Richards[1], Y. Jiao[1], L. Xu[1], C. Wong[1], A. Koshkaryev[1], V. Paragas[1], L. Luus[1], S. Ghassemifar[1], M. Razlog[1], A.S. Dugast[1], F. Depis[1], M. Muda[1], E. Tam[1], D. Drummond[1]. [1]*Merrimack Pharmaceuticals, Inc., Research, Cambridge, USA*

Background: Despite the dramatic anti-tumor responses observed for immune checkpoint inhibition in subsets of patients, there remains an unmet medical need for the larger patient population. Resistance to checkpoint inhibitors include multiple immunosuppressive mechanisms that are present in the tumor microenvironment, many of which are linked to regulatory T cells (Tregs). Here, we have identified TNF receptor 2 (TNFR2) as a key regulator of the immunosuppressive microenvironment and have observed significant anti-tumor responses using a monoclonal antibody.

Materials and Methods: Human and mouse tissues were profiled for TNFR2 expression in the tumor versus periphery. Antibodies against murine TNFR2 were generated by screening an antibody library and by rabbit immunization. Antibodies were characterized for affinity, ability to block TNF and for developability. A selected number of antibodies were expressed as murine IgG2a and evaluated for efficacy in multiple syngeneic mouse tumor models. The mechanism of action of the most potent murine surrogate antibody was investigated further both *in vitro* and *in vivo*.

Results: TNFR2 was highly expressed in both human and murine intratumoral Tregs compared to other T cell subpopulations. Notably, TNFR2 expression in the periphery was low for all T cell populations. The murine surrogate antibody Y9 showed robust anti-tumor activity at a single injection of 300 micrograms in multiple syngeneic mouse tumor models, including anti-PD-1 resistant models. Y9 treatment broadly downregulated several suppressive markers such as PD-1, GARP/LAP, and CTLA-4 on tumor infiltrating T cells. Treg depletion was not consistently observed across responding tumor models, suggesting it may not be the dominant mechanism. The mechanism of action required CD8T cell and NK cell responses and led to long-term memory in re-challenge experiments.

Conclusions: Treatment with our murine surrogate anti-TNFR2 antibody led to robust responses in multiple syngeneic mouse tumor models both alone and in combination with checkpoint inhibition. The effect was specific to the tumor microenvironment, as no effect on Tregs in the periphery was observed. A human anti-TNFR2 antibody was modeled after our murine surrogate and is being developed to potentially become an efficacious and safe treatment option for patients that are refractory to, or relapsing on, currently approved immune checkpoint inhibitors.

Conflict of interest: Corporate-sponsored Research: All co-authors are employees of Merrimack Pharmaceuticals, Inc.

232 (PB-083) Poster

Green tea and decitabine in the treatment of triple negative breast cancer: pre-clinical studies on alterations in tumor wnt signaling and immune recognition properties

A. Yee[1], M. Alamoudi[2], M. Chipman[1], K. Li[3], A. Applebaum[1], D.F. Francesca[1], C. Carland[1], K.E. Paulson[1]. [1]*Tufts University School of Medicine, Developmental. Molecular and Chemical Biology, Boston, USA;* [2]*Tufts University School of Medicine, Graduate Program in Pharmacology and Experimental Therapeutics, Boston, USA;* [3]*Tufts University School of Medicine, Graduate Program in Pharmacology and Drug Development, Boston, USA*

Background: Triple negative breast cancer (TNBC) is an aggressive subtype with extensive metastases. The incidence of fatal brain metastases has dramatically increased with improved clinical management of non-brain metastases. A major barrier is the lack of most treatments to cross the blood brain barrier (BBB). We have identified a pre-clinical combination therapy of epigallocatechin-3-gallate (EGCG; active ingredient of green tea) and decitabine (DAC; DNA demethylation). EGCG/DAC is effective at reducing primary tumors and brain metastases. Both compounds cross the BBB and are in cancer clinical trials or FDA approved for other malignancies. Mechanistically, EGCG/DAC alters the signaling landscape of the treated TNBC xenograft tumor and unexpectedly predicts new susceptibility to immune checkpoint inhibitors.

Results: Increased Wnt signaling is associated with TNBC and brain metastases, often due to epigenetic silencing of Wnt pathway inhibitors. Wnt signaling inhibitors might be increased by EGCG/DAC, because DAC reactivates SFRP1, etc. and EGCG increases the HBP1 transcriptional repressor/Wnt pathway inhibitor (our work). We determined the maximum tolerated dose (MTD) for EGCG/DAC efficacy in a pre-clinical model of human TNBC xenografts in immune compromised mice. The clinically relevant EGCG/DAC MTD reduced primary tumors and brain and non-brain metastases. We demonstrated reduced Wnt signaling in tumors, concomitant with the re-induction of SFRP1, HBP1, etc. We next used RNA seq with bioinformatics analyses to comprehensively delineate the molecular mechanisms. In addition to reduced Wnt signaling, there was significant up-regulation in (1) antigen-presenting pathways (numerous genes; direct MHC staining); (2) cancer antigens (e.g. NY-ESO1, MAGE6); and (3) interferon/JAK-stat pathway (numerous genes; P-stat3). Notably, these were tumor-intrinsic changes. Lastly, treatment of TNBC tumors in a syngeneic mouse model (with an intact immune system). EGCG/DAC resulted in (1) reduced syngeneic tumor size; (2) decreased Wnt signaling; (3) increased antigen presentation machinery; and (4) increased interferon/JAK-Stat signaling. There was increased CD8+ T cell infiltration—a biomarker that predicts efficacy for anti-PD1 and other immune checkpoint inhibitors.

Conclusion: Studies in other cancers revealed that increased Wnt signaling and a disabled interferon pathway are impediments to immune checkpoint inhibitor efficacy. EGCG/DAC treatment altered both Wnt and interferon signaling and predict sensitization to immune checkpoint inhibitors: (1) Heightened tumor interferon signaling causes increased antigen presentation capability; (2) increased immunogenicity upon induction of cancer antigens; (3) decreased Wnt signaling; (4) CD8+T-cell infiltration. These studies may advance the use of immune checkpoint inhibitors to improving breast cancer patient outcome.

No conflict of interest

Wednesday, 14 November 2018

POSTER SESSION

New Therapies with Pleiotropic Activity

233 (PB-084) Poster

Characterization of MP1000, a unique bioactive lipid nanoparticle targeting multiple cancers

J. Duex[1], C. Johnson[1], G. Miknis[1], M. Kandasamy[1], C. Hudson[1], J. Staszak-Jirkovsky[1], N. Goodwin[1]. *[1]Machavert Pharmaceuticals, Biology, Aurora, USA*

Background: Lipids and their associated pathways are increasingly found to be critical to cancer etiology. In particular, intestinal, gastric, colon, hematological and other KRAS-dependent cancers have been shown to be dependent upon lipid metabolism for disease progression. We have pursued the identification of bioactive lipid compounds that simultaneously target multiple metabolic pathways and subsequently disrupt tumor progression. From our investigations we have identified MP1000, a unique mixture of bioactive lipids, that has undergone lead optimization and formulation as stable nanoparticles and is being pursued as a new anti-cancer agent.

Materials and Methods: MP1000 nanoparticles formed by thin film hydration and extrusion methods. It has been extensively characterized including dynamic light scattering (DLS), zeta potential, liquid chromatography mass spectrometry (LC-MS), and electron microscopy analytical methods and shown to be comprised of 90 nm diameter particles that can be stably stored frozen long-term. MP1000 was tested in immunocompromised mice for in vivo efficacy and in a non-GLP acute safety study in rats.

Results: MP1000 has demonstrated monotherapy efficacy in in vitro 3D tumor cell culture assays including colorectal and pancreatic cancer cell lines ($P < 0.05$). MP1000 is being evaluated for monotherapy efficacy in multiple in vivo xenograft models and has shown efficacy when administered orally or injected intravenously in immunocompromised mice xenografted with pancreatic carcinoma models ($P < 0.05$). MP1000 has demonstrated high potential for loading with small molecule therapeutic payloads to form synergistic nanoparticles that have multiple anti-tumor mechanisms of action (MOA). To date MP1000 has successfully been loaded with immune cell activating payloads and cell cycle arrest payloads.

Conclusions: Based on preliminary efficacy and its single-agent and combination efficacy in multiple tumor xenograft models, MP1000 and multiple payload formulations of MP1000 are in preclinical development in preparation for clinical trials.

Conflict of interest: Other Substantive Relationships: All authors are employees of Machavert Pharmaceuticals, LLC.

234 (PB-085) Poster

Imprime PGG, a soluble yeast beta-glucan PAMP, converts the immunosuppressive myeloid tumor microenvironment into an immunoactive one: translation of preclinical findings to melanoma and triple-negative breast cancer (TNBC) patients

M. Uhlik[1], A. Chan[1], B. Harrison[1], A. Jonas[1], K. Gorden[1], X. Qiu[1], N. Ottoson[1], R. Walsh[1], M. Gargano[2], M. Chisamore[3], J. Lowe[4], B. Osterwalder[5], N. Bose[1], J. Graff[1]. *[1]Biothera Pharmaceuticals Inc., Research and Development, Eagan, USA; [2]Biothera Pharmaceuticals Inc., Program Management, Eagan, USA; [3]Merck & Co. Inc., Oncology Early Development, Kenilworth, USA; [4]Biothera Pharmaceuticals Inc., Clinical Development, Eagan, USA; [5]B.O. Consulting GmbH, Clinical Development, Riehen, Switzerland*

Background: Imprime PGG (Imprime), an IV administered soluble yeast β-1,3/1,6 glucan is being clinically developed in combination with tumor-targeting antibodies, anti-angiogenics, and checkpoint inhibitors (CPI). Currently Imprime is in multiple Ph2 clinical studies in combination with the anti-PD-1 CPI, pembrolizumab (Pembro). Imprime acts mechanistically as a PAMP enlisting innate immune functions including cytotoxic effector mechanisms, reversal of immunosuppression and cross-talk with the adaptive immune system. Imprime has been shown to polarize monocyte-derived macrophages to an anti-tumor M1-like orientation in *ex vivo* studies. Here we expand these findings in a pre-clinical tumor model as well as translationally in on-treatment tumor biopsies obtained from melanoma and TNBC patients treated with Imprime and Pembro.

Methods: Imprime was tested in combination with anti-PD1 mAb in a MC38 colon cancer model. Imprime's M1-polarization effects were evaluated by RNA expression and flow cytometric analyses of spleens and tumors from the different treatment groups. T cell activation effects were assessed by anti-CD3/CD28 proliferation assay. Clinically, the activation of monocyte/macrophage population was assessed in samples collected in an ongoing Ph2 clinical trial (NCT02981303) combining Imprime with Pembro in metastatic melanoma patients (prior CPI-failed) and for TNBC patients (prior chemoTx-failed, but CPI naïve). From these patients, we obtained pre- and on-treatment PBMCs and biopsies to assess biological changes within the periphery (flow cytometry) and tumor site (multispectral imaging).

Results: Imprime combined with anti-PD-1 elicited a robust control of tumor growth which was greater than either agent alone. Macrophages evaluated from Imprime-treated tumors downmodulated M2-related genes (Arg-1, CCL17, IDO etc.) and upregulated M1-genes (iNOS, TNF-α, IL-12B etc.). Flow cytometry revealed increased M1 markers (CD86, PD-L1, iNOS, and MHCII) with Imprime treatment compared to vehicle. Furthermore, macrophages from Imprime/PD-1-treated tumors showed significantly less T cell immunosuppressive functionality than those from PD-1 treated tumors. In clinical samples, higher frequency of monocytes and enhanced expression of CD86 and HLA-DR were observed in the periphery with Imprime/Pembro Tx. Compared to pre-Tx samples, tumor biopsies from Imprime/Pembro on-Tx samples show increased myeloid infiltration and polarization to M1 phenotype (CD80+/CD206-), as well as consistently increased T cell infiltration and activation (Ki67+ and/or GranzymeB+).

Conclusions: Collectively, these data show consistency between preclinical and clinical observations that demonstrate that Imprime/Pembro remodels the immunosuppressive myeloid tumor microenvironment and triggers the infiltration and activation of immune cells in preclinical and patient tumors.

Conflict of interest: Ownership: All authors with the exception of Michael Chisamore and Bruno Osterwalder are employees of Biothera and own Biothera company share options and/or stock. Bruno Osterwalder owns Biothera share options. Michael Chisamore is an employee of Merck and owns Merck stock.

235 (PB-086) Poster

Target discovery of natural product inspired phyllanthusmins for treatment of high grade serous ovarian cancer

A. Young[1], S. Kurina[1], A. Huntsman[2], R. Rathnayake[3], M. Korkmaz[3], A.D. Kinghorn[2], L. Aldrich[3], S. Cologna[3], J. Fuchs[2], J. Burdette[1]. *[1]University of Illinois at Chicago, Medicinal Chemistry & Pharmacognosy, Chicago, IL, USA; [2]Ohio State University, Medicinal Chemistry & Pharmacognosy, Columbus, OH, USA; [3]University of Illinois at Chicago, Chemistry, Chicago, IL, USA*

Background: High grade serous ovarian cancer (HGSOC) is a lethal gynecological malignancy with a need for new therapeutic agents. Many of the most widely used chemotherapeutic drugs are either derived from or are semi-synthetic derivatives of natural products. We developed potent synthetic analogs (PHYs) of the phyllanthusmin class inspired by prior natural product isolated from *Phyllanthus poilanei* Beille.

Materials and Methods: HGSOC cell lines, OVCAR3 and OVCAR8, and non-tumorigenic controls, IOSE80 and FT33, were used in this study. Cytotoxicity assays included sulforhodamine B assay, and annexin X/PI staining and Western blotting for confirmation of apoptosis induction. A photo affinity labeling method was used to attach PHY analogs to solid phase support. Targets were isolated using a pulldown technique and mass spectrometry. CRISPR-Cas9 genome editing was used to knockout and confirm putative targets.

Results: The most potent analog, PHY34, has nanomolar potency in HGSOC cell lines *in vitro* and displayed cytotoxic activity through late-stage autophagy inhibition and activation of apoptosis. PHY34 was readily bioavailable through intraperitoneal administration *in vivo* where it significantly reduced HGSOC tumor burden. Targets were identified using photo affinity labeling-aided protein pulldown and mass spectrometry, and confirmed by generating knockout cell lines of targets.

Conclusions: This class of compounds holds promise as a potential, novel chemotherapeutic approach and demonstrates the effectiveness of pleiotropically targeting autophagy and apoptosis as a viable strategy for combating high grade serous ovarian cancer.

No conflict of interest

237 (PB-088) Poster
Development of a synthetic water-soluble curcumin mono-beta-D-glucuronide (CMG), as an innovative anticancer prodrug targeting KRAS-NF-kappaB signaling pathway

H. Kakeya[1], A. Imaizumi[2], M. Kanai[3]. [1]*Kyoto University, Graduate School of Pharmaceutical Sciences, Kyoto, Japan;* [2]*TheraBioPharma Inc., Research and Development Hedquarters, Kanagawa, Japan;* [3]*Kyoto University, Graduate School of Medicine, Kyoto, Japan*

Recent well-designed preclinical studies have demonstrated that KRAS mutations can activate the canonical NF-κB signaling pathway, which is a pivotal role in tumor progression. Therefore, inhibition of this KRAS-NF-κB axis would be a promising target to develop a new molecular targeted anticancer agent. Curcumin, a naturally occurring polyphenol derived from the plant *Curcuma longa*, has been shown to block NF-κB signaling pathway via at least two mechanisms; inhibition of IkB phosphorylation and inhibition of ubiquitin-dependent degradation of IκB. Several preclinical studies have clearly demonstrated that curcumin exhibited anticancer effects in colorectal/pancreatic cancer models through NF-κB inhibition. However, since curcumin is highly lipophilic and orally administrated curcumin has very low bioavailability, its clinical application in cancer patients has been hampered.

To overcome this problem, we focused on developing a synthetic water-soluble curcumin mono-β-D-glucuronide (CMG) to administer this promising agent intravenously. First CMG was chemically synthesized from a starting material vanillin in three steps. Then CMG was injected intravenously into SD rats at 30 mg/kg and measured a concentration of active free-form curcumin. As a result, the level of active free-form curcumin was more than 1,000-higher than those with orally administered curcumin. Finally we found that the intravenous injection of CMG exhibited the in vivo antitumor effects in the mouse xenograft model bearing human colorectal cancer cells HCT116 without loss of body weight.

These results suggested that CMG could be developed as an anticancer agent targeting KRAS-NF-κB axis without the serious side effects. The combination chemotherapy regimens of CMG with conventional anticancer agents are currently undertaken.

Conflict of interest: Other Substantive Relationships: H.K. and M.K. own equity in and scientific consultants for TheraBioPharma Inc.

238 (PB-089) Poster
Novel small molecule based modulators of protein phosphatase 2A (PP2A) for cancer treatment

G. Narla[1]. [1]*Case Western Reserve University, Medicine, Cleveland, USA*

Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase that functions as a tumor suppressor by negatively regulating multiple oncogenic signaling pathways. PP2A is a heterotrimeric enzyme composed of a scaffolding A-subunit, catalytic C-subunit, and one of four classes of regulatory subunits. PP2A is functionally inactivated in several human malignancies through multiple mechanisms, including increased expression of endogenous PP2A inhibitor proteins, somatic mutation, epigenetic silencing, and post-translational modifications of various subunits.

We have used a series of cellular and *in vivo* models to examine the functional significance of some of the most commonly mutated residues in PPP2R1A, the gene encoding the PP2A Aα scaffolding subunit, such as R183 and P179. Mutant R183W Aa bound different PP2A regulatory subunits compared to wild type, forming alternative holoenzymes, suggesting a rededication of functions. The formation of complete holoenzymes was impaired for the P179R mutant due to significant, nearly global loss of interaction with regulatory B-subunits. Additionally, expression of wild type Aα, but not Aα R183W or Aα P179R, suppressed tumorigenesis.

Furthermore, our lab has developed and characterized small molecule activators of PP2A (SMAPs) as both chemical tools to probe PP2A regulation and for the treatment of PP2A dependent diseases. We have used molecular modeling, hydroxyl radical footprinting and cryo-electron microscopy to show that SMAPs protect the regulatory c-terminal tail of the catalytic subunit. This results in the methylation of the terminal leucine (L309) which induces the holoenzyme composition and substrate directed catalysis.

Our studies using isogenic lung carcinoma cell lines suggest that Aα-R258H tumors are responsive to SMAP therapy, while Aα-R183W tumors are non-responsive. This data, in combination with the altered interactome of Aα-subunit mutations, suggests that the anti-tumorigenic activity of SMAPs is mediated through a distinct subset of holoenzymes. This principle forms the basis of our ongoing studies, where we propose to identify PP2A holoenzyme complexes that modulate pathways necessary for SMAP efficacy and the tumor suppressive activity of PP2A. The successful completion of these studies may provide the foundation for patient stratification to SMAP therapies based on patient specific genetic alterations to the Aα-subunit.

Conflict of interest: Ownership: RAPPTA therapeutics – licensed small molecule PP2A activators.

239 (PB-090) Poster
Role of cereblon in in vitro angiogenesis and mediating the anti-angiogenic activity of immunomodulatory drugs

P. Huang[1], S. Beedie[1], E. Harris[1], J. Strope[1], C. Mahony[2], C. Chau[1], N. Vargesson[2], W. Figg[1]. [1]*National Institutes of Health, National Cancer Institute, Bethesda, USA;* [2]*University of Aberdeen, Institute of Medical Sciences, Aberdeen, United Kingdom*

Background: Cereblon (CRBN) is a substrate recruiter element of the E3 cullin 4-RING ubiquitin ligase complex, and a binding target of immunomodulatory drugs (IMiDs). In recent years, CRBN has been studied extensively because it is involved in many biological processes and is responsible for the pleiotropic effects of IMiDs; however, its function in angiogenesis and in mediating the anti-angiogenic effects of IMiDs remains unclear. We investigated the role of CRBN in *in vitro* angiogenesis, and in propagating the anti-angiogenic effects of IMiDs.

Material and Methods: siRNA-mediated CRBN knock down or over-expression were performed in endothelial cells (HUVEC or HMVEC-L) and assessed for *in vitro* cell proliferation, migration, and tube formation. Microvessel outgrowths were determined using an *ex vivo* rat aortic ring model using tissue from CRBN knockout ($CRBN^{-/-}$) vs wildtype ($CRBN^{+/+}$) mice.

Results: Following cereblon knock down in endothelial cells, no changes in cell survival or functional abilities (including migration, proliferation, and tubule formation) were identified. No differences in microvessel outgrowth were observed in $CRBN^{-/-}$ mice aortic ring outgrowths, as compared to those from $CRBN^{+/+}$ mice. Loss of cereblon did not affect thalidomide activity *in vitro*, but restricted lenalidomide's inhibition of tubule formation. We also show that a loss of cereblon corresponded with a decrease in the gene expression of the microRNA regulator, AGO2.

Conclusions: This data shows that although loss of cereblon does not affect *in vitro* angiogenesis, there appears to be a link between cereblon and AGO2 in endothelial cells, and a potential role for cereblon in lenalidomide's anti-angiogenic activity. Overall, our data indicates that additional factors/conditions or a multi-gene coordinated effort is needed for CRBN to mediate the anti-angiogenic effects of IMiDs.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Animal Models

240 (PB-001) Poster
Establishment and application of a panel of PBMC-humanized mouse tumor models in immune-oncology and targeted cancer immunotherapy

L. Zhang[1], S. Qi[1], Y. Jin[1], H. Wu[1], F. Chen[1], L. Zhao[1], X. An[1], W. Tan[2], X. Fu[2], M. Qiao[1], Q. Shi[1], W. Yang[1]. [1]*Crown Biosciences, Inc, Cancer Biology, Taicang, Suzhou, China;* [2]*Taicang Blood Center, Taicang Blood Center, Taicang-Suzhou, China*

Background: To meet the rapidly growing I/O market, the demands for fast, relevant and cost effective mouse tumor model systems are also increasing. We developed a panel of straightforward humanized tumor models, designated as MiXeno platform. Validation and characterization of MiXeno models is essential and will help to apply these models in the I/O field.

Method: CrownBio has established a sizable collection of MiXeno models where human PBMCs were reconstituted in the mouse system for the evaluation in vivo activity of immune checkpoint inhibitors or immune-modulators. These MiXeno models were characterized with tumor response to anti-PD-1 and anti-CTLA4 antibodies, and onset of possible graft versus host disease (GVHD) or graft versus tumor response (GVT) under different settings. To engage both host immune system and tumor antigens, we have developed some specific Mixeno tumor models by inoculating tumor cells over-expressing specific anti-tumor antigens (e.g. EGFR, CD47, Braf or PD-L1) into PBMC-humanized immunocompromised mice. Moreover, to

improve capacity and consistency of MiXeno platform, we are conducting studies to characterize and validate commercialized frozen PBMCs for MiXeno model establishment. In addition, we are comparing T cells reconstitution in immunocompromised B2M mice with NOG or NCG mice by profiling specific human/mouse antigens, such as CD45 and CD3.

Results: Models with over-expression of a variety of tumor antigens (e.g. EGFR, CD47, Braf, PD-L1, etc.) were used to develop specific Mixeno tumor models. Meanwhile, in order to overcome the limitation of PBMC shortage, commercialized PBMC has been purchase and implanted into several xenograft models, and exhibit consistent tumor growth with fresh PBMC, as well as human immune component reconstitution. Up to date, a variety of test articles of different categories, including checkpoint inhibitors, T cell modulators and bispecific T cell engagers (e.g. EpCam-CD3, CD47/CD3, BCMA/CD3) have been evaluated using this platform. Merchandized I/O drugs, such as Pembrolizumab, are being tested in commercialized PBMC implanted immunocompromised mice. Evaluation of immunocompromised B2M application for better reconstitution are also in progress.

Conclusions: MiXeno platform are valid model system for the human immuno-modulatory drugs including bispecific antibodies evaluation and will be optimized by introduction of specific Mixeno tumor models, commercial PBMC and B2M mice. Further studies are needed to expand model collections and to extend their applications in I/O space. Several outstanding questions remain to be further addressed, e.g. PBMC donor recruitment limitation, feasibility of in vitro PBMC donor screening, and selection of suitable animal strain for better reconstitution, etc.

No conflict of interest

241 (PB-002) Poster

Characterization of a large panel of syngeneic tumor models and validation of an in vivo screening platform optimized for efficacy or immune-modulation evaluation for novel I/O drugs

Y. Jin[1], L. Zhang[1], H. Wu[1], B. Mao[2], A. An[3], S. Guo[2], W. Yang[4], Q. Shi[1]. [1]*Crown Bioscience Inc, Cancer Pharmacology, Taicang, Suzhou, China;* [2]*Crown Bioscience Inc, Bioinformatics, Taicang, Suzhou, China;* [3]*Crown Bioscience Inc, Biomarker Technology, Taicang, Suzhou, China;* [4]*Crown Bioscience Inc, Cancer Pharmacology, Taicang, Suzhu, China*

Background: Syngeneic model is a useful tool for evaluating the anti-tumor effect of cancer immunotherapy. Crownbio's MuScreen is the first in vivo screening tool to test novel I/O drug candidates utilizing multi-syngeneic models. To better understand interplay between immune-modulation and anti-tumor phenotype in an in-depth fashion, better model characterization is required to capture dynamic changes of immune cell presence and infiltration within different compartments of tumor-bearing mice upon treatment with checkpoint inhibitors.

Method: Anti-PD-1 antibody was tested in 12–13 murine syngeneic models to evaluate the antitumor efficacy and the profiling of tumor infiltrated lymphocytes, as well as the percentage change of immune cell population in spleen and whole blood were evaluated. Anti-PD-1 antibody at 10 mg/kg was dosed twice weekly via intraperitoneally injection. Both of PBS and isotype were included as control groups. Both efficacy and BM modulation were examined. Samples were collected at 4 different timepoints (baseline, day 7, 14 or 28 post the first treatment). Tumor, blood and spleen samples were analysed using multi-color FACS to identify the percentage change of different immune cell populations after treatment.

Results: The antitumor activity of anti-PD-1 antibody in the treatment of syngeneic models were evaluated. No vehicle effect was observed in neither PBS or hIgG groups. Comprehensive and dynamic immune cell populations were determined at basal level or at different time points post treatment, revealing trend of immune status change in tumor bearing animals. Highly variable data was observed in aPD-1 Ab treated groups. Data analysis with more sophisticated bioinformatics methods is in process. Correlational analysis was attempted to reveal dynamic relationship between immune-profiling changes and anti-tumor effects.

Conclusion: Muscreen represents a powerful screening platform for I/O drug evaluation using a collection of well-characterized syngeneic tumor models. The data set from a large scale validation study using syngeneic models reveals dynamic changes of both immune-profiling and anti-tumor response, which will help to make strategic decisions not only on the preclinical model selection but also on combinatorial drug selection.

No conflict of interest

243 (PB-004) Poster

Development and characterization of a palbociclib-resistant luminal A breast PDX model from a clinically resistant patient

A. Moriarty[1], M. Quinn[1], M. Rundle[1], K. Papadopoulos[2], D. Rasco[2], A. Patnaik[2], M.J. Wick[1]. [1]*START, Preclinical Research, San Antonio, USA;* [2]*START, Clinical Research, San Antonio, USA*

Background: Palbociclib is a CDK 4/6 inhibitor approved in combination with letrozole in hormone receptor-positive breast cancer. Although this combination therapy has been found effective in some patients, resistance often develops. To aid in developing new therapies for palbociclib-resistant breast cancer and better understand resistance mechanisms, we established a PDX model from a patient with luminal A breast cancer at time of progression, following response to palbo/letrozole therapy for six months. This model, designated ST3164B, was developed in athymic nude mice and characterized for receptor expression, genomic alterations and in vivo drug sensitivity.

Methods: ST3164B was established from pleural fluid taken from a 63 year old Asian woman pretreated with various therapies including eribulin, an investigational therapy and palbociclib/letrozole. The resulting model was passaged and challenged with palbociclib at various doses to confirm resistance. Receptor expression was determined immunohistochemically. Genomic analysis including NGS and RNAseq were tested at early and later passages to identify mechanisms of resistance.

Results: The ST3164B model retained high ER/PR staining (2+) expression over tested passages with similar histology compared with an archival clinical sample. DNA/RNA sequencing identified a number of conserved variants; however, none have been currently identified as known mechanisms for palbociclib resistance.

Conclusion: We have established and characterized a palbociclib-resistant breast PDX model designated ST3164B which can be utilized as a valuable tool in better understanding CDK4/6i resistance and in developing new therapies for CDK4/6 inhibitor resistant patients.

No conflict of interest

244 (PB-005) Poster

Assessment of functional signal transduction pathway activity in patient-derived tumor xenografts to predict and evaluate therapy response

W. Verhaegh[1], A. van de Stolpe[1], L. Holtzer[1], B. De Regt[1], J. Wrobel[1], M. Posch[2], N. Caushaj[2], A. Maier[2], T. Metz[2]. [1]*Philips Research, Precision and Decentralized Diagnostics, Eindhoven, Netherlands;* [2]*Charles River, Discovery Research Services Germany, Freiburg, Germany*

Background: Patient-derived xenograft (PDX) models are becoming the cornerstone of preclinical profiling of anti-cancer agents, facilitating efficacy tests of agents targeting cellular signal transduction pathways. Selecting the right PDX models, usually based on molecular characteristics, is key for the success of such preclinical studies. However, molecular alterations found in PDX models do not necessarily imply activation of the respective signal transduction pathways, a prerequisite for sensitivity of a PDX model to a targeted drug. Hence better tests are needed of functional pathway activity in individual PDX and also clinical samples.

Method: We developed computational models to assess functional activity of signal transduction pathways, using Bayesian networks that look at mRNA levels of pathway target genes resulting from activation (Verhaegh et al, Cancer Res 2014:2936–45), for the AR, ER, FOXO-PI3K, Hedgehog, TGFbeta, Wnt, and NFkB pathways. Calibration of network parameters was done on samples with known ground truth pathway activity. Gene and probeset selection was fine-tuned for application on PDX models to avoid undesired effects of the tumor microenvironment when compared to patient samples. After calibration, biological validation was performed on various healthy and diseased cell and tissue types.

Pathway activity was inferred for PDXs from public data sets and from the Charles River PDX collection. Activity scores were compared between PDXs representing different tumor stages, following different treatments and carrying various genetic aberrations, and were correlated to response data of PDXs and corresponding 3D cell cultures.

Results: Pathway activity clearly varied between different PDXs. For example, AR pathway activity differed between androgen-dependent, castration-induced and castration-resistant prostate PDXs. Comparison of WNT pathway activity with alterations of the APC gene in the same PDX model revealed which gene alterations affect pathway activity and which ones do not. Even in the absence of genetic alterations, sensitivity of ex vivo 3D PDX cultures to PI3K inhibitors correlated with FOXO-PI3K pathway activity, and sensitivity to porcupine inhibition correlated with WNT pathway activity. Pathway activity reduction was generally observed in PDXs following

exposure to agents targeting a given pathway, demonstrating the effectiveness of such compounds on a biological level. Taken together, PDX models displaying a high pathway activity score tend to be sensitive to agents targeting this pathway, irrespective of genetic alterations.

Conclusion: Signal transduction pathway activity scores are an excellent tool to select PDX models for preclinical profiling of targeted anti-cancer agents. They complement standard molecular characterization of PDXs and consequently have been incorporated in the Charles River PDX collection database.

No conflict of interest

245 (PB-006) Poster

Immune characterization and diet-variants of a stage-defined, transgenic immunocompetent mouse model of HCC (ASV-B)

A. Tijeras-Raballand[1], C. Hobeika[2], B. Rousseau[3], P. Hainaud[4], P. Bonnin[5], A. Rodrigues[3], F. Ladfil[3], M. Pocard[2], V. Paradis[6], A. de Gramont[1], E. Raymond[7], E. Dupuy[4], C. Eveno[2], S. Faivre[8]. [1]*AFR Oncology, Preclinical and Translational Department, Paris, France;* [2]*Inserm U965-Lariboisière University Hospital, Digestive and Cancer Surgery Department, Paris, France;* [3]*Inserm U955-Mondor University Hospital, Medical Oncology and Clinical Pharmacology Department, Créteil, France;* [4]*Inserm U965-Institut des Vaisseaux et du Sang, Preclinical Department, Paris, France;* [5]*Inserm U965, Lariboisière University Hospital, Clinical Physiology-Functionnal Investigations Department, Paris, France;* [6]*Inserm U1149-Beaujon University Hospital, Pathology Department, Clichy, France;* [7]*Saint-Joseph Paris Hospital, Medical Oncology Department, Paris, France;* [8]*Beaujon University Hospital, Medical Oncology Department, Clichy, France*

Background: Hepatocellular carcinoma (HCC) is a complex multistep malignancy often arising on underlying chronic liver disease and is the main cause of death in patients with non-alcoholic steatohepatitis (NASH). Due to the obesity epidemics, NASH incidence is rising and is predicted to become the leading cause of HCC in the next decades. Therefore, there is an urgent need for robust animal models fully recapitulating the NASH-related HCC carcinogenesis. In this study, we further characterize and develop our transgenic HCC mouse model, focusing on immune landscape and specific diet-induced variants.

Methods: ASV-B is a transgenic mouse model (C57BL/6J) that spontaneously develops, upon SV40 T-Ag oncogene expression in hepatocytes, a reproducible stage-defined HCC: hyperplasia at week(W)8, nodular stage at W12, and diffuse carcinoma at W16-20. RNA was extracted from frozen livers at W20 for immune markers analysis using qRT-PCR (LightCycler, Roche). Immune populations were also assessed using automated immunohistochemistry (IHC) (Bond Max, Leica). To mimic NASH, we exposed mice to 5 different diet variants: classic diet (yellow), a high-fat diet (blue), a diet enriched with saturated fatty acids + 1.25% cholesterol (green), a diet containing 22% of vegetal oil + 0.2% cholesterol (orange), and a 1.25% cholesterol diet containing 21% of milkfat (red).

Results: In ASV-B, we showed an increase of liver volume and angiogenesis using ultrasound and Doppler, compared to control. Moreover, ASV-B livers harbor marked arterialization (increased arterial flow) and capillarization (tortuous and dilated sinusoids, surrounded by activated hepatic stellate cells). We further assessed 40 immune markers on 7 tumor specimens at W20, using qRT-PCR. As frequently observed in human inflammatory HCC, we observed an increase of CD8, Foxp3, INOS, CD11b, PD-1, PD-L1, IL1β, IFN-γ, TNF-α, IL17A and IL17F mRNA expression. In addition, IHC staining showed intratumoral infiltration of CD8+ lymphocytes and F4/80+ macrophages.

To develop NASH, we fed 10 ASV-B and 5 control mice with 5 different diet variants. All mice fed with special diets also received 30% fructose in the drink water. ASV-B mice receiving yellow, blue, and green regimens seemed to have similar liver volumes and weights, whereas red and orange regimens appear to be associated with increased liver volume, liver weight and higher morbidity and mortality. At the conference, we will show the morphologic changes of the livers using HPS staining (necrosis, steatosis, fibrosis, etc.), and the immune landscape in the livers of the diet-variants.

Conclusion: ASV-B transgenic mouse model mimics several characteristics of human HCC developing on healthy liver including inflammatory reaction and immune cell infiltration. In the ASV-B model, we have been able to develop specific-diets variants aiming at mimicking NASH.

No conflict of interest

246 (PB-007) Poster

Patient-derived xenograft (PDX) models from metastatic castration-resistant prostate cancer (mCRPC) patients reflect clinical disease and therapeutic outcomes

D. Ciznadija[1], P. Kipp[2], M. Kohli[3], S. Tati[1], M. Mancini[1], A. Davies[2]. [1]*Champions Oncology, Research, Hackensack, USA;* [2]*Champions Oncology, Medical Affairs, Hackensack, USA;* [3]*Mayo Clinic, Oncology, Rochester, USA*

Background: Prostate cancer (PC) is a leading cause of cancer-related mortality in men and prognosis for mCRPC remains poor despite the availability of novel therapies including androgen receptor (AR) pathway inhibitors. Integration of drug screening and sequencing in PDX models may allow for precision medicine in mCRPC by advancing our understanding of resistance mechanisms (*de novo* and acquired) and identification of clinical response biomarkers. Here we highlight a cohort of PDX models developed from the bone metastases of mCRPC patients and demonstrate alignment of genomic features with specific clinical genetic elements as well as recapitulation of parent tumor histology and patient responses to therapy.

Methods: A total of 9 mCRPC PDX models, along with matched clinical specimens, were evaluated by next-generation sequencing for genomic alterations (mutations, amplifications/deletions, fusions, and gene expression). PDXs were also analyzed by immunohistochemistry to determine expression and localization patterns for the androgen receptor (AR) and prostate-specific membrane antigen (PSMA). Drug screening of models was performed against the AR inhibitor enzalutamide, with tumor regression (TR) and RECIST criteria determined and correlated with patient outcomes.

Results: Nine PDX models developed from 7 mCRPC patients, including matched models from two patients pre- and post-abiraterone progression, were interrogated. Strong and extensive nuclear AR and membrane PSMA staining was observed in each PDX evaluated to date, correlating with clinical tumor grade. The common TMPRSS2-ERG and TMPRSS2-ETV4 fusions were identified in 4/9 models and the expression profile of one model suggested possible neuroendocrine features. In matched patient models, 94% of mutations from the pre-abiraterone model were retained in the model developed post-treatment. Two mCRPC models developed from patients with acquired and *de novo* resistance to enzalutamide also demonstrated a lack of response to the same agent *in vivo*.

Conclusion: Our study demonstrates alignment of genomics between PDX models and clinical metastases, as well as reflecting patient responses to clinical therapy, highlighting the application of these models for translational modeling and *in vivo* clinical trial simulation. The availability of matched PDX models developed from single patients enables insights into the molecular changes governing disease resistance. Comprehensive sequencing (WES and RNA) and standard of care drug testing of these and additional PC models developed from tissue and circulating tumor cells (CTCs) under our two-year NCI award (N44CA170013) is planned to gain further understanding of such mechanisms, as well as permit biomarkers of response to be identified. In this context, application of mCRPC models to support future drug development will remain important and continue to evolve.

Conflict of interest: Ownership: Daniel Ciznadija, Peter Kipp, Swetha Tati, Maria Mancini, Angela Davies.

247 (PB-008) Poster

Establishment and characterization of the HCC78 NSCLC tumor xenograft model

A. Moriarty[1], L. Gamez[1], M. Quinn[1], M. Rundle[1], K. Papadopoulos[2], D. Rasco[2], A. Patnaik[2], M.J. Wick[1]. [1]*START, Preclinical Research, San Antonio, USA;* [2]*START, Clinical Research, San Antonio, USA*

Background: The HCC78 cell line is widely used in vitro for targeted drug development in cancers harboring ROS1-fusions including non-small cell lung cancer (NSCLC). However, lack of an accessible, matched xenograft has limited in vivo evaluation of potential therapies. To this end, we established the HCC78 NSCLC xenograft model in athymic nude mice. The resulting model was characterized and compared with the parent line using immunohistochemistry and genomic analysis. In vivo studies were performed using the xenograft model comparing various therapies targeting ROS1, ALK, and EGFR and relevant chemotherapies.

Materials and Methods: The HCC78 NSCLC xenograft model was established and serially passaged in athymic nude mice. Receptor expression was determined using immunohistochemistry and compared with the parent cell line. For single agent in vivo studies, we evaluated the following targeted therapies: crizotinib, ceritinib, alectinib, brigatinib, erlotinib, afatinib, osimertinib, cetuximab and the chemotherapies docetaxel and cisplatin. Dosing was initiated on Day 0 and continued until control groups

reached designated tumor volume endpoint and drug response reported as %T/C values.

Results: Immunohistochemistry on the cell line and model passages reported intensity values of (2+) for ROS1 receptor expression. The following in vivo %T/C values were reported: Crizotinib = (−11%), Ceritinib = (35%), Alectinib = (100%), Brigatinib = (−51%), Erlotinib = (72%), Afatinib = (30%), Osimertinib = (100%), Cetuximab = (100%), Docetaxel = (8%) and Cisplatin = (60%). Tumor regressions were reported with brigatinib treatment.

Conclusion: We have established a reproducible xenograft model of the HCC78 cell line which can be used for developing new therapies to ROS1-fusion positive cancers.

No conflict of interest

248 (PB-009) Poster

Whole exome sequencing of canine cancer cell lines identifies common oncogenic mutations

D. Duval[1], S. Das[1], R. Idate[1], D. Gustafson[1]. [1]*Colorado State University, Clinical Sciences, Fort Collins, USA*

Background: Cancer cell cultures have served as a backbone in research exploring cancer cell biology and have informed the vast majority of preclinical studies to test cancer therapeutics. Preclinical analysis of human cell lines in conjunction with whole exome sequencing has helped to evaluate oncogene addiction and sensitivity to drugs that target oncogenic pathways. Similarly, we have conducted whole exome sequencing in a panel of canine cancer cell lines to identify somatic variants that contribute to the pathogenesis of canine cancers and to determine the prevalence of common human oncogenic mutations.

Material and Methods: Genomic DNA was isolated from 33 canine cell lines, exonic regions were captured using the Agilent Sure Select XT Canine V 2.0 Exome Capture (43.45 Mbp), and the Illumina HiSeq 2500 platform was used to generate 151 bp paired end reads. Reads were mapped to CanFam 3.1 and variants were called using FreeBayes. Germline variants were removed by screening against 3 canine germline databases and 13 local normal samples. Somatic variants were further screened to eliminate synonymous variants and variants that fell outside the coding region and scored for functional impact using Sift4G. Somatic variants were filtered for known drivers of human cancers from the COSMIC database (v83) and cross referenced for known oncogenic variants listed on cBioportal.

Results: Mean on-target coverage for these 33 samples was 181x. Somatic mutation profiles identified in this screen were on average 62% SNPs which were dominated by C/G < T/A changes. Mutation frequency ranged from 22 to 129 mutations/captured MB. A total of 9,849 variants in 5554 genes were identified across 33 cell lines. Sift scoring categorized 34.8% of the missense mutations as deleterious. Filtering for COSMIC curated cancer genes identified 232 genes with 447 variants in the 33 cell lines ranging from 3 cancer gene variants in CML10C2 canine melanoma to 43 in OSW canine lymphoma cell line. The most frequently mutated cancer gene was TP53 (11 mutations in 10 cell lines). By cross-referencing the identified variants on cBioPortal, we identified 60 candidate oncogenic mutations in 30 genes including activating mutations in BRAF, EIF1AX, ERBB2, KIT, KRAS, MET, NRAS, PIK3CA, PPM1D, PTPN11, RAC1, and inactivating mutations in ARID1A, ASXL1, ATRX, CCND3, CDKN1A, CIC, EP300, FBXW7, KDM6A, KMT2C, KMT2D, MED12, NF1, PTEN, SETD2, SPEN, SRGAP3, TET2 and TP53. Oncogenic drivers were identified in 30 out of 33 cell lines. Selected mutations were confirmed using Sanger sequencing. Microarray and Western blot analysis revealed decreased expression in the 4 cell lines with NF1 frameshifts.

Conclusions: Whole exome screening of canine cancer cell lines has identified driving mutations that can be targeted for the treatment of canine cancer and development of novel therapies.

No conflict of interest

249 (PB-010) Poster

Prevention of tumor ulceration by tumor cell inoculation into the mammary fat pad

C. Obodozie[1], J. Beshay[1], S. Ruf[1], G. Bijelic[1], S. Moor[1], B. Giesen[1], U. Leisegang[1], H. Weber[1]. [1]*ProQinase GmbH, In vivo Pharmacology, Freiburg, Germany*

Currently, cancer research focus on development of novel cancer immunotherapies. In several tumor models tumor ulceration as a corollary of subcutaneous tumor growth occurs very often in an early stage of tumor growth. Ulcerated tumor-bearing mice must be euthanized according to animal ethical rules before the potential highest tumor volume can be reached. Hence, the number of existing/remaining animals is too low to get statistical significant calculations at study end and study duration is too short to study the effects on the immune system, which needs time to react after test substance exposure.

We observed for implanted 4T1 and EMT6 cells, both originated from breast cancer, that tumor ulceration appeared with lower frequency and at a later timepoint when implanted into the mammary fat pad in comparison to subcutaneous implantation. This brought us to the idea to implant also tumor cells other than breast cancer cells into the mammary fat pad (MFP). Both methods (subcutaneous and MFP inoculation) are heterotopic and can be calipered.

We compare 8 syngeneic tumor cell lines from various entities when inoculated into the MFP and the subcutaneous space, respectively, with respect to tumor volume, occurrence of tumor ulceration and potential therapeutic window. In detail, in the CT26wt tumor model, tumor volume was >2-fold increased reaching 1500 mm^3 and tumor ulceration was reduced >50% by MFP compared to subcutaneous inoculation. Likewise, study duration for the LL/2 tumor model was prolonged by 4 days after MFP implantation compared to subcutaneous. Here, a 5-fold increase in tumor volume and a 3-fold decrease in tumor ulceration was observed when cells were inoculated into the MFP. Implantation of MC38-CEA tumor cells into the MFP did not lead to any tumor ulceration, whereas MC38-CEA s.c. implantation resulted in 7 tumor ulcerations out of 12 tumors. The outcome for additional syngeneic cell lines AB12, Hepa1-6, RENCA, B16F10, CloneM3 is listed. Pathohistological examinations verified the absence of ulcerations in MFP-tumors and revealed the "protecting" effect of MFP. Additionally, examples for the impact of immune checkpoint inhibitors like anti-PD-1, anti PD L1 and/or anti-CTLA-4 on MFP implanted tumor growth are presented as well as the analysis on immune cell populations determined by flow cytometry analysis.

In summary, tumor ulceration could be prevented up to 100% by using the mammary fat pad as an alternative injection site. Thus, using MFP implantation extends study duration which is now mainly limited by tumor size. In addition, tumor growth appears better with less heterogeneity. In conclusion, the heterotopic MFP implantation of tumor cells is superior to the traditional subcutaneous implantation resulting in an improved meaning- and powerful tumor model.

Conflict of interest: Other Substantive Relationships: All authors work at ProQinase GmbH.

250 (PB-011) Poster

Establishment of mouse prostate homograft tumor models for efficacy evaluation of combinatory immunotherapies

J. Wang[1], H. Sun[1], A. An[1], L. Zhang[1], H. Li[1], D. Ouyang[1]. [1]*Crown Bioscience Inc., Transnational Oncology, San Diego, USA*

Introduction: Despite the early approval of Sipuleucel-T for metastatic castration-resistant prostate cancer, which often perceived as a milestone achievement in cancer immunotherapy, subsequent progress in prostate cancer immunotherapy development has been limited by disappointing results with tumor vaccines and its resistance to immune checkpoint inhibitors, such as PD1 & PD-L1. It is now generally accepted that we need to tackle prostate cancer by combinatorial approaches of chemo-, targeted- and immuno-therapies. Highly relevant preclinical models are very much needed for proof of principle efficacy evaluation. Genetically engineered mouse models (GEMM) recapitulate some aspects of human prostate cancer in both histo-/molecular pathology. Among those, PTEN loss of function (LOF) in prostate epithelium is one of the central events in human prostate cancer. PTEN haploinsufficiency in mice is sufficient to drive mouse prostatic intraepithelial neoplasia (PIN) formation, while loss of both alleles of PTEN in mouse prostate leads to hyperplasia at 4 weeks, PIN at 6 weeks, and fully invasive adenocarcinoma from 12 weeks of age. PTEN null tumors are also resistant to androgen depletion. In the meantime, although KRAS mutation are not often seen in human prostate cancer, activation of MAPK pathway often happens in advanced tumor. Mutant KRAS or BRAF can robustly promote mouse prostate cancer progression. *Pten null* and *Kras G12D; Pten null* mouse prostate cancer models have been well characterized by a number of labs. However, parental GEMM models are difficult to be used for pharmacological studies due to the spontaneous nature of tumor onset and progression. The compound mutant mice are also costly to breed.

Method: We have generated transplantable mouse prostate cancer homograft models by passaging the primary tumor subcutaneously in the C57BL/6 mice GEMM.

Results: These mouse tumors featuring *Pbsn-Cre;LSL-Kras*$^{G12D/+}$; *Pten*$^{flox/flox}$ or *Pbsn-Cre;Pten*$^{flox/flox}$ retain morphological similarity to moderate to poorly differentiated human prostate cancer. Growth of these mouse prostate tumors are resistant to androgen depletion, but sensitive to treatments with mTOR inhibitor. They are also moderately responsive to immune checkpoint antibodies, i.e. PD1 and CTLA4. We are now testing a

variety of combinatory therapies with chemotherapies, immune modulators and immune checkpoint antibodies. The results will be presented at the meeting.

Conclusions: Mouse prostate tumor homograft derived from GEMM could become an important tool for evaluating anti-prostate cancer pharmacology of different treatment strategies, including the combinatorial immunotherapies.

Conflict of interest: Corporate-sponsored Research: Crown Bioscience Inc.

252 (PB-013) Poster

Novel preclinical model to study mechanistic link between breastfeeding and triple negative breast cancer: Could Elf5 be the culprit?

B. Ramaswamy[1], M. Basree[2], N. Shinde[1], J. Stephens[3], J. Zhang[3], P. Shields[1], G. Sizemore[4], S. Majumder[5]. [1]*The Ohio State University, Internal Medicine, Columbus, USA;* [2]*University of Pikeville, College of Medicine, Pikeville, USA;* [3]*The Ohio State University, Biostatistics, Columbus, USA;* [4]*The Ohio State University, Radiation Oncology, Columbus, USA;* [5]*The Ohio State University, Cancer Biology, Columbus, USA*

Background: Epidemiological studies strongly link higher parity and lack of breastfeeding to higher risk of triple negative breast cancer (TNBC). Although multiple animal studies have demonstrated that abrupt involution (AI, no breastfeeding) results in a highly inflammatory environment, no preclinical model has been developed to show how parity and gradual involution (GI, prolonged breastfeeding) results in distinct changes that reduces the risk of developing TNBC. Using FVB/N mice we designed a novel modeling to study changes occurring in the mammary glands upon AI vs GI following single/multiparity and we present our findings.

Methods: Female FVB/N mice (8 weeks) were mated once and within 24 hours of partum, pups were normalized to six per dam. For the AI cohort, all six pups were removed from the dam on postpartum (PP) day 7, while for the GI cohort pups were removed on PP day 28 and 31, 3 at a time. For multiparity, females were mated again on PP day 31, and pups were removed as described. Each mouse underwent three pregnancies. Mammary glands were harvested on PP day 28, 56 and 120. Whole mounts were used for gross morphological analysis and FFPE section were used for immunohistochemistry. Single cell suspension of mammary glands were analyzed for mammary epithelial subpopulations using Fluorescence activated cell sorting. Gene Set Enrichment Analysis (GSEA) was used to analyze gene expression data (Affymetrix) from mouse epithelial subpopulation and normal breast tissue from healthy women.

Results: The AI mammary gland showed denser stroma, marked increase in collagen deposition, immune cell infiltration, inflammatory markers and higher proliferation index when compared to GI glands. Alveolar hyperplasia and squamous metaplasia was observed in the AI glands four months after partum. AI resulted in a dramatic restructuring of the mammary epithelial subpopulations with a massive expansion of the luminal progenitor (LP) cell compartment, the putative cell of origin of basal like breast cancer. A significant increase in expression of ETS –like transcription factor Elf5, one of the most highly ranked genes in the LP gene signature in both human and mouse, was observed in the precancerous lesions in the AI glands. GSEA of the mouse mammary LP cells revealed enrichment of Notch and Hedgehog pathway. Similar enrichment of these pathways was observed in breast tissue obtained from women who breast fed for <6 months (AI) vs. >6 months (GI).

Conclusion: We have successfully modeled AI and GI of breast in mice that showed striking differences not only in proliferative and inflammatory markers but also in mammary epithelial composition and Elf5 expression that could explain the link between breastfeeding and TNBC. This model can be used to study prevention strategies that could be translated to women who are unable to breastfeed after pregnancy.

No conflict of interest

253 (PB-014) Poster

Measurement of adenosine and metabolites in tumors of freely moving mice utilizing in vivo microdialysis

M. Heins[1], N. Morisot[2], H. Janssens[2], G. Flik[1], J. Roeser[2], A. Rassoulpour[2]. [1]*Charles River Laboratories, Discovery, Groningen, Netherlands;* [2]*Charles River Laboratories, Discovery, South San Francisco, USA*

Background: Monitoring changes in biochemical elements within tumors is crucial to understanding cancer biology and to helping with the development of novel therapies. Yet to date, experimental techniques enabling sensitive and quantitative measurement of the levels of small molecules contributing to tumor development have been limited in preclinical oncology models.

Materials and Methods: We implemented in vivo microdialysis to measure signaling molecules and oncometabolites in the tumor microenvironment of freely moving rodents. To this aim, a small dialysis probe is implanted in syngeneic tumors in mice. Probes are perfused with artificial dialysate fluid and dialysate containing molecules from the tumor microenvironment is collected at regular intervals. Levels of biochemicals in the dialysate are then quantified by liquid chromatography-mass spectrometry (LC-MS).

Results: First, we tested whether implantation of dialysis probe affects in vivo tumor growth. To do so, tumor volume from probe-implanted and non-implanted MC38 allografts in mice was monitored daily for up to 10 days following probe implantation. We found that the growth curve from probe-bearing MC38 tumors was similar to probe-free tumors, revealing that probe implantation did not influence tumor size progression. Next, the levels of metabolites that are known to be involved in tumor development were measured in tumor allografts of freely-moving mice using in vivo microdialysis. Relevant levels of arginine, ornithine and putrescine were quantified by LC-MS. Levels of key molecules of the adenosine signaling and tryptophan/kynurenine pathway metabolites in MC38 tumors were also quantified. To better understand the homogeneity of this particular allograft model, we measured the concentration of the above-mentioned molecules in different regions of a tumor. To this end, two separate probes were implanted in the center and the peripheral area of the same 300 mm^3 MC38 allograft. We found that levels of measured molecules were similar in dialysates collected from both probe locations, suggesting homogenous levels of biochemicals throughout the microenvironment of these non-necrotic tumors.

Conclusions: Our proposed experimental approach allows for a quantitative and sensitive measurement of key biochemical mediators of tumor progression. In vivo microdialysis in murine tumor models may be used to elucidate the mechanisms by which therapies, such as chemotherapy and immune checkpoint inhibitors, modulate the tumor microenvironment. Our efforts in preclinical cancer research has the potential to bring new insights on the mechanisms underlying cancer development and help the discovery of next-generation therapies for cancer.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Chemoprevention

254 (PB-015) Poster

Biochemical targets and biomarkers of liver cancer and therapeutic strategies with leaf extracts of justicia adhatoda L

S. Arora[1]. [1]*Guru Nanak Dev University Amritsar, Botanical and Environmental Sciences, Amritsar, India*

Liver cancer or hepatocellular carcinoma (HCC) is one of the commonest cancers in the world especially in countries like India which have a high incidence of hepatitis B infection. Apart from Hepatitis B, it may be caused by other diseases that lead to cirrhosis of the liver such as hepatitis C infection, and alcohol abuse. Unfortunately, like many other cancers, liver cancer may go undetected until a late stage. To explore the targets and biomarkers of liver cancer, *in vivo* anticancer studies were done in rats using various biochemical markers. The leaves of the plant *Justicia adhatoda* were extracted serially with the solvents of increasing polarity and vasicine was isolated from most active n-butanol fraction by column chromatography and structure elucidation was also done The effect of vasicine was analyzed on Phase I, Phase II, antioxidant enzymes and serum parameters in rats treated with CCl4. However, it has been found that rats treated with lower and higher doses of vasicine along with CCl4 reduced the specific activity of enzymes to the normal level. Moreover, vasicine significantly lowered their specific activity in a dose dependent manner. These findings reveal that administration of vasicine modulates both phase I and phase II enzymes as well as antioxidative enzymes in rats. Phase I enzymes which mediate oxidation, reduction or hydrolysis reaction converts lipophillic chemical compounds into hydrophillic products, so it can be effectively eliminated by the kidneys. One of the most common modifications is hydroxylation catalysed by the cytochrome P-450-dependent mixed-function oxidase system. In the present study, CCl4 administration resulted in significant elevation in the level of both SGOT and SGPTin a dose dependent manner. Administration of vasicine for 12 days along with the CCl4 administration restored the activities

of SGOT and SGPT at higher dose of vasicine (100 mg/kg b.w.). These hepatoprotective results were further supported by histipathological studies in which sections of control group showed the normal hepatic architecture. However, CCl4 treated group showed various histological changes like fatty changes, steatosis and lobular inflammation. These injuries were markedly reduced in the liver sections of vasicine treated rats. Vasicine was found to be a potential hepatoprotective candidate. The future endeavour is to substantiate the work already done by collaborating with the medical practitioner to know the efficacy of the compounds in other relevant models for the drug development.

No conflict of interest

255 (PB-016) Poster

Zerumbone inhibits Epithelial-mesenchymal transition and cancer stem cells properties by inhibiting β-catenin pathway through miR-200c

F. Karimi Dermani[1]. [1]*Hamadan University of Medical Sciences, Molecular Medicine and Genetics, Tehran, Iran*

Background: Colorectal cancer (CRC) is one of the most lethal and rampant human malignancies in the world. Zerumbone, a sesquiterpene isolated from subtropical ginger, has been found to exhibit an antitumor effect in various cancer types. However, its effects on the biological properties of CRC, including epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs), have not been fully elucidated. Here, we investigated the inhibitory action of ZER on the EMT process, CSC markers, and β-catenin signaling in the presence and absence of miR-200c.

Methods: The effect of zerumbone on the viability of HCT-116 and SW-48 cells was examined by MTT assay. The effects of zerumbone on EMT-related genes, CSC markers, cell migration, invasion, sphere-forming, and β-catenin signaling were explored. To evaluate the role of miR-200c in these anti-cancer effects, miR-200c was down-regulated by LNA-anti-miR-200c.

Results: Zerumbone significantly inhibited cell viability, migration, invasion, and sphere-forming potential in HCT-116 and SW-48 cell lines. It significantly suppressed EMT and CSC properties as well as down-regulated β-catenin. Silencing of miR200c reduced the inhibitory effects of zerumbone on EMT and CSCs.

Conclusion: These data indicate that zerumbone may be a promising candidate for reducing the risk of CRC progression by suppressing β-catenin pathways via miR-200c.

Conflict of interest: Ownership: This study was funded by a grant from Hamadan University of Medical Sciences (9603231780).

256 (PB-017) Poster

In vitro anticancer effects of compounds isolated from Ricinodendron heudelotii

O. Yakubu[1], A. Adebayo[1], E. Iweala[1], T. Dokunmu[1], Y.J. Zhang[2]. [1]*Covenant University, Biochemistry Department, Ota, Nigeria;* [2]*Kunming Institute of Botany, State Laboratory of Phytochemistry, Kunming, China*

Background: This study was designed to explore the *in vitro* anticancer effects of the bioactive compounds isolated from *Ricinodendron heudelotii* on selected cancer cell lines.

Materials and Methods: The leaves of the plant were extracted with ethanol and partitioned in sequence with petroleum ether, ethyl acetate and n-butanol. The ethyl acetate fraction was phytochemically studied using thin layer chromatography (TLC) and column chromatography (CC). Structural elucidation of pure compounds obtained from the ethyl acetate fraction was done using mass spectra, ^{1}H NMR and ^{13}C NMR analysis. The isolated compounds were subsequently applied to HL-60, SMMC-7721, A-549, MCF-7 and SW-480 cells to assess their cytotoxic effects.

Results: Ten compounds were isolated and structurally elucidated as 1,6-di-*O*-galloyl glucose, gallic acid, gallic acid ethyl ester, corilagin, quercetin-3-*O*-rhamnoside, myricetin-3-*O*-rhamnoside, 1,3,6-tri-*O*-galloyl glucose, 3,4,6-tri-*O*-galloyl glucose, 1,2,6-tri-*O*-galloyl glucose and 4,6-di-*O*-galloyl glucose. Compound 4 (corilagin) exhibited the most cytotoxic activity with IC_{50} values 33.18 μg/mL against MCF-7 cells which were comparable to cisplastin with an IC_{50} value of 27.43 μg/mL.

Conclusion: The result suggests that corilagin isolated from *R. heudelotii* have the potential to be developed as an effective therapeutic agent against the growth of breast cancer cells.

No conflict of interest

257 (PB-018) Poster

Natural antibiotic Tetrocarcin-A downregulates Junctional Adhesion Molecule-A in conjunction with HER2 and inhibitor of apoptosis proteins and inhibits tumour cell growth

S.H. Vellanki[1], R. Cruz[1], H. Jahns[2], L. Hudson[1], G. Sette[3], A. Eramo[3], A. Hopkins[1]. [1]*RCSI, Department of Surgery, Dublin, Ireland;* [2]*University College Dublin, UCD School of Veterinary Medicine, Dublin, Ireland;* [3]*Istituto Superiore di Sanità, Department of Oncology and Molecular Medicine, Rome, Italy*

Background: Overexpression of the adhesion protein Junctional Adhesion Molecule-A (JAM-A) has been linked to aggressive disease in breast and other cancers, but JAM-targeting drugs remain elusive. The purpose of the study was to identify JAM-A-targeting compounds by means of a natural compound chemical library screen.

Materials and Methods: The effects of Tetrocarcin-A on cell viability, colony formation and semi-*in-vivo* tumor xenograft growth were examined in MCF-7 HER2 and BT474-Trastuzumab-resistant breast cell lines, in addition to breast cancer primary cultures and lung cancer stem cells. The impact of Tetrocarcin-A on the protein expression of signalling effectors controlling cell fate was examined by Western blotting, and compared with that of transient JAM-A gene silencing. Finally, apoptotic pathways were investigated downstream of Tetrocarcin-A treatment.

Results: Screening of a natural compound library identified the antibiotic Tetrocarcin-A as a novel downregulator of JAM-A and the human epidermal growth factor receptor-2 (HER2) protein expression in breast cancer cells. Lysosomal inhibition partially rescued the downregulation of JAM-A and HER2 caused by Tetrocarcin-A, and attenuated its cytotoxic activity. Tetrocarcin-A inhibited c-FOS phosphorylation at Threonine-232, its transcriptional regulation site, and downregulated expression of the inhibitor of apoptosis proteins (IAP). This translated into Tetrocarcin-A-induced caspase-dependent apoptosis. To begin evaluating the potential clinical relevance of our findings, we extended our studies to other models. Encouragingly, Tetrocarcin-A downregulated JAM-A expression and caused cytotoxicity in primary cultures derived directly from breast cancer patients and in lung cancer stem cells. It also inhibited the growth of breast cancer cell xenografts in a semi-*in vivo* model involving invasion across the chicken egg chorioallantoic membrane.

Conclusions: Taken together, our data suggest that Tetrocarcin-A warrants future evaluation as a novel cancer therapeutic by virtue of its ability to downregulate JAM-A expression, induce apoptosis and reduce tumourigenic signalling.

This work was financially supported by Science Foundation Ireland (grant 13/IA/1994 to AMH). Tetrocarcin-A was provided by the NCI/DTP Open Chemical Repository https://dtp.cancer.gov. *(NSC 333856).*

No conflict of interest

258 (PB-019) Poster

An improved formulation of GLG-302, a STAT3 antagonist, with chemopreventive activity in preclinical models of mammary cancer

R. Shoemaker[1], J. Fox[1], H. Gomez[2], J. White[3], F. Moeinpour[4], C. Grubbs[4]. [1]*National Cancer Institute Division of Cancer Prevention, Chemopreventive Agent Development Research Group Division, Bethesda, MD, USA;* [2]*GLG Pharma, Headquarters, Jupiter, FL, USA;* [3]*MRIGlobal, DCP Repository, Kansas City, USA;* [4]*UAB, Chemoprevention, Birmingham, AL, USA*

Background: GLG-302 was initially identified as a potential STAT3 antagonist using in silico screening. Subsequent in vitro and in vivo mechanistic studies verified the mechanism. Because of the potential role of this signaling pathway in mammary carcinogenesis, we evaluated GLG-302 in preclinical models of mammary cancer.

Materials and Methods: We first tested a carboxymethylcellulose suspension of GLG-302 in mice and rats to establish the ability of orally administered compound to affect STAT signaling in normal mammary tissue and to determine tolerated doses for chronic administration. In both species, after two weeks of treatment immunohistochemical studies indicated the pharmacodynamic markers pSTAT3 and Ki67 were reduced in normal ductal epithelium. In both species the compound was well tolerated at doses as high as 500 mg/kg/day.

Results: Preliminary cancer prevention efficacy testing was performed in mice using daily oral administration of a suspension of GLG-302 in carboxymethylcellulose. Cancer prevention efficacy was indicated in the spontaneous MMTV/Her2 mouse model and in rat DMBA-induced tumor model with compound administered in the feed. No body weight loss or other toxicity was observed. We then evaluated alternative approaches to formulation of GLG-302 and demonstrated that a Trizma salt form could be produced with aqueous solubility to 50 mg/ml. While subject to rapid

elimination from plasma, this formulation demonstrated better bioavailability than the suspension. We tested this formulation in the rat and mouse mammary tumor models and verified retention of activity on pharmacodynamic markers. Striking preventive activity was observed in the MMTV/Her2 mouse model.

Conclusion: GLG-302 given orally to mice and rats can modulate STAT3 signaling and proliferation in mammary epithelium. Additional studies, especially toxicology studies, will be needed to assess the potential of this class of compounds for chemopreventive use.

Conflict of interest: Ownership: Hector Gomez and GLG Pharma are owners of GLC-302. A joint patent exists on the Trizma salt formulation of GLG-302 with NCI, MRIGlobal and GLG staff as inventors.

260 (PB-021) Poster

Insulin-like growth factor binding protein-3 (IGFBP-3) promotes the migration and invasion of advanced prostate cancer cells under hypoxia

R. Singh[1], A. Dheeraj[1]. [1]*Jawaharlal Nehru University, School of Life Sciences, Cancer Biology Laboratory, New Delhi, India*

Backgrounds: Worldwide, cancer has become the most common disease and accounts for millions of death every-year. It has become one of the leading cause of deaths in both economically developed and developing countries. Prostate cancer (PCa) is a major epithelial cancer among men with the second highest incidence rate. The high ratio of Insulin-like growth factor-1(IGF-1)/Insulin-like growth factor binding protein-3 (IGFBP-3) correlates with increased risk of many cancers including prostate cancer.

Materials and Methods: In present study, we have evaluated the role of IGFBP-3 and effect of Fisetin, a phytochemical in prostate cancer progression under the hypoxic state. Western blotting, immunocytochemistry, overexpression and knock-down systems were used.

Results: Under the hypoxic conditions (1% Oxygen), prostate cancer PC3 cells showed increased levels of IGFBP-3 when compared to normoxic conditions (21% Oxygen) in time-dependent manner. Hypoxia is a major regulator of prostate cancer progression through modulating the angiogenic, migratory and invasive potential of cancer cells. We down-regulated IGFBP-3 using ShRNA construct against it in PC3 cells. The knock-down of IGFBP3 did not considerably affect the growth pattern of PC3 cells as compared to scrambled control but addition of fisetin (25–100 μM) decreased the cell viability in both normoxic and hypoxic conditions for time period up to 72 h. The down-regulation of IGFBP-3 resulted in the increased expression of E-cadherin, a biomarker of epithelial phenotype. The wound scratch assay showed the promigratory role of IGFBP-3 in cancer cells and treatment with Fisetin inhibited the migration of these cells under the hypoxic condition. The knockdown of IGFBP3 resulted in the decreased invasive potential of cancer cells in comparison to cells in hypoxic state.

Conclusions: The overall results suggested the possible role of IGFBP-3 in promoting the migration and invasion of prostate cancer cells under the hypoxic condition.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Drug Delivery

261 (PB-022) Poster

Tofacitinib enhances delivery of antibody-based therapeutics to tumor cells through reduction of tumor-associated inflammatory cells

D. Fitzgerald[1], N. Simon[1], A. Antignani[1], S. Hewitt[2], M. Gadina[3], C. Alewine[1]. [1]*National Cancer Institute, Laboratory of Molecular Biology, Bethesda, USA;* [2]*National Cancer Institute, Laboratory of Pathology, Bethesda, USA;* [3]*National Institute of Arthritis and Musculoskeletal and Skin Diseases, Office of Science and Technology, Bethesda, USA*

Background: Antibody-based therapeutics play important roles in treating cancer. However, the routes by which tumor-targeted antibodies gain access to malignant cells in the complex environment of an epithelial tumor are not well defined. Compounds that increase the efficiency of delivery to malignant cells are likely to constitute important avenues of advancement. Here, we report on the action of tofacitinib, an FDA-approved JAK3/JAK1 inhibitor.

Materials and Methods: Tumor cells were injected into mice, treated with tofacitinib or vehicle and then treated with an antibody-based therapeutic agent. Tumor growth was measured following one of four treatments: vehicle only, tofacitinib only, antibody agent alone or a combination of antibody and tofacitinib. Tumors from treated mice were analyzed by flow cytometry for the uptake of fluorescently-labeled antibodies into malignant cells. Further, tumors were removed from treated animals and stained by immunohistochemistry with antibodies inflammatory cells. Immune-related transcripts from tofacitinib-treated mice were quantified via Nanostring analysis. Likewise, serum cytokines were measured by ELISA.

Results: Tofacitinib altered the tumor microenvironment by reducing the number of tumor-associated inflammatory cells and allowed increased delivery of antibody-based agents to malignant cells. Alone, tofacitinib exhibited no antitumor activity, but combination treatments with recombinant immunotoxins (IT) or an antibody drug conjugate (ADC) resulted in increased anti-tumor responses compared to either antibody-based drug alone, confirming *in vivo* synergy. Further, fluorescently-labeled antibody-based agents accumulated in a higher percentage of malignant cells following tofacitinib treatment. The action of tofacitinib was microenvironment-dependent as there was no enhancement of killing or antibody uptake in tissue culture. Nanostring profiling of tofacitinib-treated tumors indicated that the levels of several cytokine transcripts involved in immune cell chemotaxis were reduced. Reductions in arginase, IL6, CCL2, CCL4, and CXCL2 were confirmed at the protein level. Histological analysis of tumor tissue revealed that tofacitinib reduced the number of neutrophils, monocytes, and macrophages within the tumor microenvironment thereby allowing greater access of therapeutic antibodies to malignant cells.

Conclusions: Present findings serve as a rationale for designing human trials in cancer patients where short-term treatments with tofacitinib could be administered in combination with antibody-based therapies to increase their uptake into malignant cells.

No conflict of interest

262 (PB-023) Poster

New non-covalent complex with atractyloside based on the Oncoshuttle technology using AFP as carrier – The results of Aimpila drug development and preclinical study

S. Tsurkan[1], J. Tcherkassova[1], H. Treshalina[2], E.Y. Grigor'eva[2], I. Treshchalin[3], E. Pereverzeva[3]. [1]*Pharmaceutical Research Center "PharmAccess", lab, Moscow, Russian Federation;* [2]*Federal State Budgetary Institution, N.N. Blokhin Medical Research Center of Oncology of the Ministry of Health of Russian Federation, Lab, Moscow, Russian Federation;* [3]*Gause Institute of New Antibiotics, Lab, Moscow, Russian Federation*

Background: The Oncoshuttle™ technology uses a non-covalent native (porcine) AFP complex with therapeutic agents showing antitumor activity due to selective targeting of cancer cells via AFP-receptor (AR) mediated endocytosis. We've studied AFP-atractyloside (ATR) complex Aimpila (AMP) during drug development and preclinical study.

Material and Methods: The complex was standardized by the anti-proliferative activity of HepG2 cells. Biodistribution of ^{125}I-AMP and ^{125}I-AFP was studied using a direct radiometry after oral drug administration. AR was measured by chemiluminescence. AMP efficacy was studied using the s.c. human tumor xenografts, murine P388 tumor (oral & iv), rabbit VX2 tumor in acid resistant capsules(ARC).

Results: The AMP is the equimolar complex of ATR and AFP showing low dissociation and immunologically is not different from AFP. It shows intestinal absorption proved by the rats isolated inverted small intestinal sac model. The AMP dosage form (DF) was developed containing 1 mg of AMP (app. 10 nmol) and pharm components in ARC and showed enough stability. Acute toxicity test in mice showed no toxicity. The results of long term toxicity study demonstrate that AMP does not produce any changes in examined parameters. Liver, renal, cardio, pancreatic and gastrointestinal toxicity have been observed only in groups of rats, treated with high dose of the AMP. These abnormalities were reversible withing a month. Local toxicity and special toxicity tests showed no restrictions to Phase 1. The efficacy studies of oral daily doses 0,1–0,4 mg/kg for 5–10 days showed AMP caused a significant tumor growth inhibition *in vivo* depending of AR expression in the tumors: T47D (T/C = 22%) > HepG2 (T/C = 51%) > SW620 (T/C = 70%) (criterion T/C ≤ 42%) corresponding featuring different expression of AR. The effect of AMP for non-human tumor models was observed but didn't match the criterion. A biodistribution study in healthy mice showed different absorption in organs/tissues with lower tropism to the liver and jejunum (AUCtis/ser < 1.0) and higher to the kidneys, lungs and mammary gland (MG) (AUCtis/ser = 1.11, 2.08, and 1.13). Biodistribution differs In xenografts. In the tumor and MG course of accumulation/elimination within first 3 h after administration AMP was absorbed in the tumor significantly

faster than in the MG. The mean rate of AMP elimination from T47D and MG was similar between 6 and 9 h after drug administration, after which it was slower, and by 24 h the ID/g in the tumor was 5 times higher than in the MG (ID/g = 0.87 vs 0.18). The retention period was longer for the tumor as well (MRT = 12.3 h vs 9.5 h).

Conclusions: High efficacy of low doses orally administrated AMP was achieved due to the targeting and receptor mediated endocytosis yielding retention of AMP in T47D. Oral treatment of AMP showed intestinal absorption and sufficient bioavailability.

No conflict of interest

263 (PB-024) Poster

Pre-beta HDL discoidal mimetic, CER-001®, and novel Apolipoprotein A-I (ApoA-I) multimers, Cargomer®, as new targeted delivery vehicles for therapeutic cancer medicines

V. Albinet[1], E. Lacoste[2], R. Baron[3], R. Palmantier[4], J.L. Dasseux[5]. *[1]Imavita SAS, Research & Development, Toulouse, France; [2]Imavita SAS, Chief Executive Officer and CSO, Toulouse, France; [3]RND Pharma, President, Toulouse, France; [4]ImmThera Consulting SAS, President, Toulouse Area, France; [5]Cerenis Therapeutics Holding SA., President and CEO, Balma, France*

Background: Despite the huge potential of nanoparticles, the delivery efficiency of their payload to tumors has plateaued at 0.7% in the last decade. The ability to produce and manufacture recombinant human (rh)ApoA-I to design a new type of nanovehicles, CER-001® and Cargomer®, deploying the HDL natural features of targeted delivery system opens the way for improved means of drug delivery.

ApoA-I is the major protein of HDL that plays an essential role in reverse cholesterol (Chol) transport, in particular via the scavenger receptor SR-B1 that is expressed on hepatocytes and macrophages and is highly over-expressed on cancer cells. Based on HDL capabilities to transport lipophilic molecules as well as micro-RNA and deliver esterified-Chol into the cytosol of SR-B1-expressing cells, CER-001® and Cargomer® have been developed to optimize the delivery of cytotoxic drugs, siRNA or radiotracer to tumors.

CER-001® is a negatively charged pre-b HDL mimetic, which is being evaluated in phase III clinical trial (TANGO) in patients with genetic HDL deficiencies and in phase II (TARGET) as ^{89}Zr-nanocarrier for tumor imaging in patients with esophageal cancer. Cargomer® are innovative rhApoA1 multimeric nanocomplexes developed for targeted drug delivery.

Methods: Paclitaxel, a poorly soluble cytotoxic drug was complexed in CER-001® (CER-Pacli) through a proprietary production process. MDA-MB-231, a human breast cancer cell line, was engrafted orthotopically into NOD-SCID mice. At d25, mice were administered IV with either CER-Pacli, paclitaxel protein-bound (Abraxane®) as positive control, both groups at 10 mg paclitaxel/kg, or vehicle, 1 × qd for 5d, then, 2x qw until euthanasia at d57, 24H after last administration.

Cargomer® have been loaded with the siRNA oncogenic KRAS mutant G12D or STAT-3 DNA oligo antisense both coupled to modified Chol with a functionalized linker. A KRAS G12D mutated human pancreatic cancer cell line, PANC-1, has been treated *in vitro* with Cargomer®-siRNA. KRAS expression was evaluated by WB at 72 h.

Results: In contrast to the mice in the vehicle group, tumor growth was inhibited in the mice treated with CER-Pacli as well as with Abraxane®.

	tum vol (mm^3)	tum vol (mm^3)	paclitaxel : tumor (ng : mg)
groups	D25	D57	D57
vehicle	150	864	1.2
Abraxane®	143	105	23.5
CER-pacli	143	141	31.7

The prepared Cargomer®-siRNA or -DNA elute in a single major pick by SEC. Preliminary data show a reduction of KRAS expression in PANC-1 treated by Cargomer®-siRNA compared to Cargomer®-scrambled siRNA.

Conclusions: CER-001-Pacli is at least as efficacious as Abraxane® nanoparticles to deliver and treat tumor-bearing mice. The Cargomer® is a flexible targeting nanoparticle platform with promising delivery activities.

Conflict of interest: Other Substantive Relationships: Consulting services paid by Cerenis Therapeutics.

264 (PB-025) Poster

Liposomal Gemcitabine, FF-10832, Overcomes Gemcitabine resistance by improving pharmacokinetics

T. Matsumoto[1], T. Ioroi[1], T. Komori[1], Y. Yoshino[1], H. Kitahara[1], Y. Izumi[1], S. Shimoyama[1], K. Takada[1], S. Hagiwara[1], T. Hara[1]. *[1]FUJIFILM Corporation, Pharmaceutical & Healthcare Research Laboratories, Kanagawa, Japan*

Background: Gemcitabine (GEM) based therapies are standard treatment for advanced pancreatic cancer. However, it is thought that rapid clearance of GEM is one of the reasons for poor clinical outcomes or drug resistance. We developed FF-10832 (F832) that achieved long circulation in plasma, high accumulation and payload release in tumors. F832 demonstrated anti-tumor effects in both Capan-1[GEM-sensitive] and BxPC-3[GEM-resistant] human pancreatic cancer xenograft models.

The aims of this study are to demonstrate the mechanism of F832 to overcome GEM-resistance and the potential of combo therapy for maximizing the efficacy.

Materials and Methods: Expression levels of GEM activation related molecules were compared between Capan-1 and BxPC-3 tumors by qRT-PCR.

The active form of GEM [dFdCTP] in F832 or GEM-treated tumors were quantified by LC/MS to analyze the correlation of anti-tumor effects and Cmax, AUC or mean residence time (MRT) which represents the average time of dFdCTP staying in tumors.

For the identification of F832 internalized cells, FACS and IHC with fluorescent dye labeled F832 were used.

The anti-tumor effects of F832 and GEM in combo with immune checkpoint inhibitor (ICI) were examined in a syngeneic GEM-resistant mouse tumor model (EMT6).

All animal studies were approved by the FUJIFILM animal committee.

Results: We found that mRNA levels of ENT1, a major transporter for GEM, was significantly low in the BxPC3 model, compared to the Capan-1 model. The lower expression of ENT1 is thought to result in the reduced Cmax, AUC and MRT of dFdCTP, and GEM resistance in the BxPC3 model.

On the other hand, MRT of dFdCTP in F832 treated tumors in the BxPC3 model was comparable to that in the Capan-1 model, although Cmax and AUC were reduced. These results suggested that the efficacy of F832 in the BxPC3 model can be attributed to longer MRT of dFdCTP than that after GEM administration.

In addition, we clarified the mechanism of F832 uptake and payload release in tumors. FACS and IHC data showed that F4/80 positive tumor-associated macrophages preferentially internalized F832. Macrophages that internalized F832 completely released GEM. These results indicated that macrophages would act as a reservoir of GEM and contribute to longer exposure of tumors to dFdCTP.

Then, we examined the combo effect with ICI in the EMT6 model. F832 + ICI demonstrated statistically significant anti-tumor effects compared to GEM + ICI. Several animals of F832 + ICI showed complete regression of tumors, though the others didn't.

Conclusions: We indicated that F832 could overcome GEM-resistance through longer exposure (MRT) of tumors to dFdCTP, presumably by the EPR effect and the following tumor macrophages related reservoir function. Then, the potential of combo therapy with ICI showed enhanced efficacy in GEM-resistant models. We have started a Phase1 study (NCT03440450).

Conflict of interest: Other Substantive Relationships: All authors are employees of FUJIJFILM Corporation.

265 (PB-026) Poster

Anti-vascular effects of calcium electroporation in murine melanoma

B. Staresinic[1,2], T. Jesenko[1], U. Kamensek[1], S. Krog Frandsen[3], G. Sersa[1,4], J. Gehl[3,5], M. Cemazar[1,6]. *[1]Institute of Oncology Ljubljana, Department of Experimental Oncology, Ljubljana, Slovenia; [2]University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; [3]Zealand University Hospital, Department of Clinical Oncology, Roskilde, Denmark; [4]University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia; [5]Faculty of Health and Medical Sciences, University of Copenhagen, Department of Clinical Medicine, Copenhagen, Denmark; [6]University of Primorska, Faculty of Health Sciences, Izola, Slovenia*

Background: Calcium electroporation (CaEP) is a novel anti-tumor approach, which follows the principles of the electrochemotherapy (ECT) to enhance local cytotoxicity and reduce systemic toxicity of the drug. Here, electroporation is used to load cells with large quantities of calcium and induce cell death. Anti-tumor effectiveness of CaEP has been demonstrated

in vitro, *in vivo*, and in a clinical trial; however, its effects on tumor vasculature have not been studied yet, which was the aim of the study.

Material and Methods: Dense cell suspensions of B16F1 murine melanoma cells or HUVEC human endothelial cells were prepared in medium containing 1–5 mM $CaCl_2$, placed between electrodes (2 mm gap) and subjected to 8 square-wave electric pulses (260 V, 100 μs, 1 Hz). After *in vitro* treatment, cytotoxicity and ATP depletion were determined. Additionally, the anti-vascular effect was evaluated by tube formation and wound healing assay *in vitro* and by intravital microscopy of normal and tumor vasculature in dorsal window chamber (DWC) model in C57Bl/6 mice *in vivo*. B16F1-GFP tumors were induced by intradermal injection of cells (3.5×10^5) into the skin in DWC. The treatment was performed by intratumoral or intradermal injection of either 5 μl of MiliQ water or $CaCl_2$ (50 mM, 168 mM, 250 mM) then electric pulses (4+4 square-wave pulses, 520 V, 100 μs, 1 Hz) with 4 mm gap plate electrodes were applied. After treatment, DWC were imaged for 3 days, then Rhodamine B labeled dextran was injected into the orbital plexus and images of fluorescent vessels were taken. Mice were euthanized on day 3, skin in the region of DWC was excised, paraffin-embedded sections were prepared and stained with H&E.

Results: CaEP was cytotoxic and caused ATP depletion in a dose-dependent manner in both cell lines, corresponding to the results of previous studies. Furthermore, anti-vascular effects were demonstrated by reduced migration of cells in wound healing assay and reduced tube formation of HUVEC cells. *In vivo*, CaEP caused immediate tumor necrosis and affected both normal and tumor blood vessels, where severe damage to smaller vessels and skin was observed; however, larger vessels appeared to be less affected.

Conclusions: We have demonstrated that CaEP is highly cytotoxic to tumor cells and also has an anti-vascular effect on both normal and tumor blood vessels. Our study provides further evidence for the use of CaEP in clinical settings for the treatment of cutaneous tumors.

Conflict of interest: Ownership: Competing financial interests: A patent has been submitted – PCT/DK2012/050496 Therapeutic applications of calcium electroporation to effectively induce tumor necrosis (co-inventors: S.K. Frandsen and J. Gehl).

267 (PB-028) Poster

FF-10850, a novel liposomal topotecan, dramatically improved pharmacokinetics, anti-tumor effects, and the safety profile of topotecan

S. Shimoyama[1], M. Mori[1], K. Okada[1], T. Kimura[1], K. Makita[1], T. Ioroi[1], N. Kasagi[1], S. Matsuda[2], S. Nakayama[1], T. Kobayashi[3], A. Inomata[1], S. Hagiwara[1], K. Takada[1], T. Hara[1]. [1]*FUJIFILM Corporation, Pharmaceutical & Healthcare Research Laboratories, Kaisei-machi, Japan;* [2]*FUJIFILM Corporation, Safety Evaluation Center, Minami-Ashigara, Japan;* [3]*FUJIFILM Pharmaceuticals U.S.A., Inc., Drug Development, Cambridge, USA*

Background: Topotecan has been approved and used as a treatment option for ovarian, cervical, and small cell lung cancers. The major clinical issues of topotecan are rapid clearance from plasma and serious hematotoxicity. These issues significantly limit the clinical use of topotecan. In order to maximize the clinical benefit of topotecan by overcoming these issues, we designed FF-10850, a novel liposomal topotecan, expecting preferential distribution in the tumor tissue through enhanced permeability and retention (EPR) effect.

Material and Methods: FF-10850 was formulated with a proprietary and optimized composition of dihydrosphingomyelin (DHSM), cholesterol, and polyethylene glycol. The anti-tumor effects of FF-10850 were compared to topotecan in the ES-2 ovarian cancer xenograft mouse model. The anti-tumor effects of FF-10850 in combination with the immune checkpoint blockade were also examined in a syngeneic mouse tumor model. Hematotoxicity and systemic toxicity of FF-10850 were evaluated in rats and compared to the toxicity of topotecan. All animal experiments were approved by the FUJIFILM animal experiment committee.

Results: The introduction of DHSM as a lipid component for the liposomal shell of FF-10850 allowed stable encapsulation of topotecan in the interior of the liposomal shell and prolonged the plasma half-life compared to the conventional liposomal formulation with hydrogenated soy phosphatidylcholine.

Weekly administration of FF-10850 at 0.5 mg/kg demonstrated almost equivalent anti-tumor effects to topotecan at the maximum tolerated dose (2 mg/kg, daily for five consecutive days) in the ES-2 mouse xenograft model. Higher concentrations of FF-10850 up to 4 mg/kg were tolerated and induced stronger anti-tumor effects resulting in tumor regression. In addition to the monotherapy treatment, FF-10850 achieved an induction of robust and durable anti-tumor effects in combination with the immune checkpoint blockade even after completion of treatment in a syngeneic mouse model.

Toxicity studies in rats demonstrated that FF-10850 improved the toxicity profile of topotecan, including hematotoxicity, which is a serious clinical issue, whereas the target organs of toxicities identified in FF-10850 treated rats were similar to those identified in topotecan treated rats. Improvement of the safety profile was supported by pharmacokinetics and pharmacodynamic analysis measuring phospho-H2AX, which is a DNA damage marker induced by topotecan.

Conclusions: FF-10850 dramatically improved the anti-tumor effects and toxicity of topotecan by modulating the pharmacokinetic profile. FF-10850 also demonstrated the potential of combination therapy with the immune checkpoint blockade. Based on these results, IND-enabling studies are currently underway to move FF-10850 forward to the Phase 1 clinical trial in 2019.

Conflict of interest: Other Substantive Relationships: All authors are employees of FUJIFILM Corporation.

269 (PB-030) Poster

Dual hydrophobization formulation strategies improve intravenous half-life, safety, and tumor delivery of siRNA nano-polyplexes

M. Jackson[1], S. Bedingfield[1], F. Yu[1], M. Stokan[1], R. Miles[1], E. Hoogenboezem[1], E. Curvino[1], T. Giorgio[1], C. Duvall[1]. [1]*Vanderbilt University, Biomedical Engineering, Nashville, USA*

Background: While RNA-based drugs hold great promise for targeted, personalized medicine, they possess significant delivery challenges due to rapid clearance from circulation and poor tissue biodistribution. Encapsulation of siRNA in nano-sized, electrostatic polymer complexes (si-NPs) can improve pharmacokinetic profiles and delivery to solid tumors in mice, but the impact of formulation parameters (i.e., ratio of carrier: cargo or cargo chemical modification) on *in vivo* pharmacokinetics and safety have been minimally studied. Herein, we sought to optimize polyplex si-NP formulation using a dual carrier-cargo hydrophobization strategy to improve both circulation time and safety of si-NP formulations *in vivo*.

Materials and Methods: Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization was used to synthesize diblock copolymers [PMPC-DB] containing homopolymer poly(2-methacryloyloxyethyl phosphorylcholine) [PMPC] and random copolymer core block composed of poly(dimethyl amino ethyl methacrylate-co-butyl methacrylate) [DB]. Polyplexes (si-NPs) with either normal or palmitic acid conjugated-siRNA (PA-siRNA) were formulated at polymer: siRNA ratios of 10, 15, and 20. Cy5-labeled si-NPs were injected in male CD-1 mice and their circulation times were monitored by intravital microscopy. Toxicologic properties of si-NPs were monitored upon repeat injection in mice by sampling serum liver enzymes, cytokines, complete blood counts, body weight, and tissue histology. Tumor uptake of fluorescent si-NPs was measured by flow cytometry.

Results: Doubling the si-NP polymer: siRNA ratio increased circulation half-life five-fold. However, as amount of polymer was increased, associated liver toxicities increased. For polyplexes formulated at polymer: siRNA ratios of 10 or 15, PA-siRNA increased si-NP half-lives to the equivalent of polyplexes formulated at 20, thereby reducing the amount of injected material while maintaining superior pharmacokinetic profiles. This dual hydrophobization strategy further reduced the toxicity of polyplex formulations, which were well-tolerated in a 6- injection repeat dose study in mice. Optimally formulated PMPC-DB polyplexes also exhibited greater than two-fold increased uptake in orthotopic MDA-MB-231 xenografts compared to the commercial transfection agent, *in vivo* Jet-PEI.

Conclusions: Our work demonstrates that varying polymer:siRNA ratio and employing dual hydrophobization strategies significantly impact siRNA polyplex pharmacokinetics and carrier-associated toxicities in mice. By utilizing hydrophobic moieties on both carrier polymers and cargo siRNA, large doses of siRNA can be delivered to tumors using less total polymer. These data have implications on maximizing the therapeutic index of new molecularly-targeted, siRNA-based cancer therapies.

No conflict of interest

270 (PB-031) Poster

Drug delivery to malignant brain tumor by a peptide specific to Annexin A1

M. Nonaka[1], K. Hideaki[1], M. Fukuda[2]. [1]*Kyoto University, Graduate School of Pharmaceutical Sciences, Kyoto, Japan;* [2]*National Institute of Advanced Science and Technology, Laboratory for Drug Discovery, Tsukuba, Japan*

Background: Annexin A1 (Anxa1) is a ubiquitous protein which is normally expressed in the cytoplasm and nucleus in the cell. In the tumor vasculature, Anxa1 translocated onto the cell surface of endothelial cells, indicating

Anxa1 is the most specific marker of tumor vasculature [Oh *et al., Nature*, 429: 629–35, 2004]. Previously, we reported that a carbohydrate mimetic peptide, designated as IF7, targets to various solid tumors through binding to Anxa1 on the tumor endothelial cells [Hatakeyama *et al. PNAS*108:19587–92, 2011]. IF7 rapidly crossed endothelial cells via transcytosis and was incorporated in the tumor cells. IF7 conjugated with SN38, an anti-cancer drug, efficiently regressed tumor, indicating IF7 becomes a novel drug delivery system.

Materials and Methods: Brain tumor xenograft model of rat glioma C6 cell was established. Fluorescence-tagged IF7 was injected intravenously into the brain tumor-bearing mice. For dual xenograft model, mouse melanoma B16 cells, which stably express luciferase, were injected subcutaneously and into the brain in one mouse. IF7 was conjugated with SN-38, which is an active metabolite of irinotecan, a topoisomerase I inhibitor and then IF7-SN38 was injected intravenously at a low dose (7 nmol/g/mouse) everyday.

Results: In this study, we hypothesized that IF7 crosses blood-brain barrier by transcytosis and delivers anti-cancer drug to tumor stroma. When IF7-labeled with fluorescent dye was injected into the mice of glioma xenograft model, fluorescence signal accumulated in the tumor site but not in the normal brain. We next established the dual tumor xenograft model by implanting C6 glioma cells subcutaneously and in the brain. Of note, IF7-SN38 suppressed growth of both brain and subcutaneous tumors at a low dose.

Conclusions: These results suggest that IF7 can cross blood-brain barrier and efficiently deliver anti-cancer drugs into malignant brain tumor. A new peptide with increased stability *in vivo* is now under developing by applying phage display technology.

No conflict of interest

271 (PB-032) Poster

The conjugation of newly developed unsymmetrical bisacridine antitumor agents with quantum dots nanoparticles to improve the drug delivery

E. Matysiak[2], A. Kowalczyk[2], P. Bujak[3], A. Nowicka[2], E. Augustin[1], Z. Mazerska[1]. [1]*Gdansk University of Technology, Chemical Faculty, Department of Pharmaceutical Technology and Biochemistry, Gdansk, Poland;* [2]*University of Warsaw, Faculty of Chemistry, Warsaw, Poland;* [3]*Warsaw University of Technology, Faculty of Chemistry, Warsaw, Poland*

Background: The search of our group for the potential anticancer drugs among acridine derivatives resulted in the synthesis of new promising unsymmetrical bisacridine derivatives (UAs), EP 15461518.1, 2017, which exhibited high cytotoxic activity against a lot of tumor cell lines and high antitumor efficacy against several types of human cancer xenografts in nude mice. To improve the drug delivery conditions the nanoconjugates of newly developed UAs, compounds C-2028, C-2045, with red (QD_{red}) and green (QD_{green}) quantum dots nanoparticles were synthesized. There is known, that nanoparticles penetrate the cell membrane by endocytosis. Therefore, we aimed to answer the question, which properties are crucial in the improving of drug-conjugate transport into the cell. The stability of the synthesized nanoconjugates were tested at various pH values: 7.4, 5.5 and 4.4, corresponding to the outer cell layer, endosomes and lysosomes.

Materials and Methods: UAs were non-covalently attached to Ag-In-Zn-S-MUA nanocrystals. The amounts of UAs anchored to nanocrystals were determined from voltammetric analyses using the charges of cathodic peak at −0.6 V. The dynamic light scattering (DLS) and zeta potential (ZP) measurements were performed with a He-Ne (4 mV) laser at 632.8 nm, in buffer at 25°C. Quartz crystal microbalance with dissipation (QCM-D) analysis, was carried out in the flow system, rate 100 mL min^{-1}, temp. 21°C. To modify the QCM electrode with QD nanocrystals, the droplet of 150 μL of QD solution, 1 mg mL^{-1} was placed on the Au surface and left to dry. The FTIR spectra were acquired in a transmission mode with the spectral resolution of 4 cm^{-1}.

Results: DLS, ZP and FTIR investigations confirmed the effectiveness of the conjugation procedure between UAs compounds and QD nanocrystals. The increase of QD diameter value at pH 7.4 evidenced that the conjugate was formed. However, at pH 4.0 the low hydrodynamic diameter indicated that the drug was released from the nanoconjugate, except of QD_{green}-C-2045 nanoconjugate. The changes of ZP values in the pH range from 4.0 to 8.4 demonstrated that QD_{green} are stable at whole tested pH range, while the QD_{red} only in the pH range 5.5–8.4. The QCM-D experiments showed that the most probable way of transport of QD-UAs conjugates to the cell is endocytosis. In the acidic pH values (5.5 in endosomes and 4.0 in lysosomes) the decomposition of nanoconjugate took place. The release of C-2028 from QD nanoconjugates was more effective than C-2045 in the case of QD_{red} at pH 5.5 and for QD_{green} at pH 4.0.

Conclusions: Under physiological conditions, pH 7.4, the UAs-QD nanoconjugates are stable. Inside the endosomes, pH 5.5, the nanoconjugate stability significantly decrease according to the drug structure. Under acidic conditions in lysosomes, pH 4.0, 80% of the drug is released from the nanoconjugate.

No conflict of interest

272 (PB-033) Poster

Discovery of the novel, homogeneous payload platform dolasynthen for antibody-drug conjugates

M. Kozytska[1], A. Yurkovetskiy[1], M. Yin[1], N. Bodyak[2], P. Conlon[1], B. Du[1], C. Stevenson[1], D. Gumerov[3], M. Nazzaro[3], D. Lee[3], W. Lee[2], L. Qin[2], K. Catcott[1], D. Demady[4], S. Collins[4], J. Xu[4], J. Duvall[1], M. Damelin[2], D. Toader[1], T. Lowinger[1]. [1]*Mersana Therapeutics, Discovery Chemistry, Cambridge, USA;* [2]*Mersana Therapeutics, Biology, Cambridge, USA;* [3]*Mersana Therapeutics, Analytical, Cambridge, USA;* [4]*Mersana Therapeutics, Pharmacology, Cambridge, USA*

Background: Dolasynthen is a novel, proprietary, homogeneous payload platform enabling the creation of antibody-drug conjugates (ADC) with drug-to-antibody ratios (DAR) ranging from 6 to 22. We previously reported a high DAR payload platform, Dolaflexin, which is a polymer-based, polydisperse scaffold incorporated into the ADCs XMT-1522 and XMT-1536 currently in clinical trials. We disclose here efficacy, safety and PK data for Dolasynthen, a second-generation Auristatin F hydroxypropylamide (AF-HPA) payload platform with a defined, fully homogeneous structure that allows for the creation of ADCs with diverse, defined DAR values.

Materials and Methods: A set of payload platforms was synthesized that use AF-HPA cytotoxic warhead. These payload platforms contain highly biodegradable and biocompatible polypeptide frameworks and can be varied with respect to overall charge and the identity and characteristics of hydrophilic moieties to balance overall physicochemical properties. ADCs were prepared by conjugating the payload platforms to IgG1 antibodies through stochastic bioconjugation, or site-specifically to engineered anti-bodies. The resulting ADCs displayed DARs in the range of 6–22 and their overall properties and activity in xenograft animal models were determined. ADCs prepared with lead payload platforms were evaluated in mouse xenograft efficacy models, non-human primates (NHP) tolerability and TK studies.

Results: SAR data showed a strong correlation between the structure of the payload platform and observed activity *in vitro* and *in vivo* and allowed for further optimization of hydrophilic modifiers and other physicochemical enhancing components. PK evaluation in non-human primates showed a strong correlation of the ADC exposure to the net charge of the payload platform. Lead ADCs showed excellent efficacy in mouse xenograft models and desired tolerability in NHPs at doses and DARs comparable to Dolaflexin conjugates. The lead payload platform was used to generate homogeneous ADCs that showed the expected pM activity *in vitro* and excellent efficacy *in vivo*. The ADCs showed exceptional plasma stability and good exposures in tumor bearing mouse models and NHP tolerability studies.

Conclusions: The novel, fully homogeneous AF-HPA based payload platform Dolasynthen showed potent *in vivo* antitumor activity and excellent tolerability in NHP. The Dolasynthen platform is amenable to the generation of ADCs with enhanced homogeneity including fully homogeneous ADCs. The hydrophilic nature of the structurally defined framework coupled to careful design of the payload led to identification of a platform that shows great promise for future clinical use.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Drug Design

273 (PB-034) Poster

Design and synthesis of benzopyrrolo[2,1-a]phthalazine hybrids as potent anitcancer agents to small cell lung cancer

T.L. Chen[1], Y.W. Lin[1], T.L. Su[1], T.C. Lee[1]. [1]*Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan*

Combination therapy that combine two or more anti-cancer drugs enhance efficacy (via synergistic or an additive mode) compared to the mono-therapy approach. Hybrid molecules conjugate two different pharmacophores and display dual mode of action via interaction with two targets. Thus, hybrid

molecules may have advantage over combination therapy on enhanced efficacy and reduced adverse side-effects. Recently, we have synthesized a series of hybrids, pyrrolo[2,1-*a*]phthalazine, which consist of phthalazine (anti-antiogenesis moiety) and 1,2-bis(hydroxymethyl)pyrrole (DNA cross-linking moiety). We demonstrated that these hybrids possessed dual mode of action and displayed potent therapeutic efficacy against human small cell lung cancer (SCLC) cells in mouse xenograft models. Here, we further used benzolog approach to design and synthesize novel hybrids, namely 1,2-bis (hydroxymethyl)benzo[*g*]pyrrolo[2,1-*a*]-phthalazines with various secondary amine side chains at C6 and their ethylcarbamate derivatives for anticancer evaluation. We found that these new hybrids exhibited significant cytotoxicity against the growth of a panel of human cancer cell lines in vitro. The values of IC_{50} of these compounds were ranged from dozens of picromolar to submicromolar to leukemia and several SCLC cell lines. Mechanistically, the newly synthesized benzolog hybrids remained their DNA crosslinking and anti-angiogenesis activities. Among these compounds, a potent anticancer activity of compound **8** was noteworthily noticed. We observed complete tumor remission of SCLC H526 xenograft in mice treated with compound **8** at the dose of 20 mg/kg (5 days/week for 2 weeks) and only a minor body weight loss. These results revealed that compound **8** is more potent than cisplatin in terms of antitumor efficacy and safety. In summary, our present study further confirms that designing hybrid molecules with dual mode of action is a promising strategy in the new anticancer drug discovery. Moreover, it suggests that hybrid **8** may have high potential for further development as a useful anticancer drug for the treatment SCLC patients.

No conflict of interest

274 (PB-035) Poster

Discovery and characterization of potent and selective small-molecule inhibitors of ecto-nucleotidase CD73 for cancer immunotherapy

K. Lawson[1], J. Kalisiak[1], E. Lindsey[1], E. Newcomb[1], B. Rosen[1], N. Walker[2], L. Jin[1], E. Scaletti[3], N. Strater[3], A. Chen[4], J. Tan[5], M.J. Walters[5], S. Young[4], U. Schindler[5], M. Leleti[1], J.P. Powers[1]. *[1]Arcus Biosciences, 3928 Point Eden Way, Hayward, CA 94545, Chemistry, Hayward, USA; [2]Arcus Biosciences, 3928 Point Eden Way, Hayward, CA 94545, Protein Science, Hayward, USA; [3]University of Leipzig, Center for Biotechnology and Biomedicine Institute of Bioanalytical Chemistry, Leipzig, Germany; [4]Arcus Biosciences, 3928 Point Eden Way, Hayward, CA 94545, Quantitative Biology, Hayward, USA; [5]Arcus Biosciences, 3928 Point Eden Way, Hayward, CA 94545, Chemistry, Biology, USA*

Introduction: Adenosine (ADO) is a potent inhibitor of T cell and NK cell activation and is present in high concentrations in the tumor micro-environment (TME) resulting in an immunosuppressed phenotype. In the TME, generation of ADO relies on the sequential hydrolysis of ATP by two ecto-nucleotidases, CD39 (ATP→AMP) and CD73 (AMP→ADO). Inhibition of CD73 eliminates a major pathway of ADO production and can reverse ADO-mediated immune suppression. Here we present the discovery, drug design, and characterization of a highly potent and selective series of small molecule inhibitors of CD73.

Materials and Methods: The potency of CD73 inhibitors was evaluated by measuring AMP hydrolysis by CHO-CD73 cells using a malachite green assay. Potency was similarly measured using soluble recombinant CD73. In the presence of human serum, CD73 inhibition was measured by quantitation of AMP hydrolysis via luminescence. Selectivity against related ecto-nucleotidases was also assessed. The pharmacokinetic properties of select inhibitors were evaluated in rodents. X-ray co-crystal structures of select inhibitors were obtained by co-crystallizing them with recombinant human CD73.

Results: The medicinal chemistry team at Arcus Biosciences has developed a concise series of potent and selective CD73 inhibitors via interrogation of structure activity relationships (SAR) and structure-based drug design. Key molecular interactions were identified from high resolution X-ray structure data of select inhibitors bound to human CD73. These inhibitors were found to occupy the adenosine pocket and form an array of hydrogen bonds with CD73 residues D506, R354, and N390. Furthermore, a strong hydrophobic π-stacking interaction between two phenylalanine residues (F417 and F500) and coordination of the di-zinc catalytic center was integral to retain high potency. A001202, a representative member of this series, potently inhibits soluble and membrane-bound CD73 (IC_{50} = 0.86 and 2.6 nM, respectively). Similar potency was measured in cell lines (IC_{50} = 0.55 nM, SKOV-3). A001202 exhibits a favorable preclinical profile. A001202 is >10,000-fold selective against related ecto-nucleotidases (NTPDase 2,3,8 and CD39), does not inhibit the major CYP450 isoforms, and its pharmacokinetic properties in rodents are characterized by low clearance and a long half-life.

Conclusions: We have discovered a novel, potent, and selective series of small molecules capable of inhibiting CD73. These efforts have expanded the understanding of the structural underpinnings of small-molecule CD73 inhibition. Members of this series exhibit a favorable pharmacokinetic profile and are apt for further development.

Conflict of interest: Other Substantive Relationships: Employees of Arcus Biosciences.

275 (PB-036) Poster

Progress towards orally bioavailable, potent, and selective small-molecule inhibitors of CD73 for cancer immunotherapy

E. Lindsey[1], K. Lawson[1], R. Thomas-Tran[1], J. Beatty[1], J. Fournier[1], D. Mandal[1], X. Yan[1], S. Drew[1], J. Jeffrey[1], N. Walker[2], N. Sträter[3], S. Moschütz[3], A. Chen[4], J. Tan[5], L. Jin[1], M. Walters[5], S. Young[4], U. Schindler[5], M. Leleti[1], J. Powers[1]. *[1]Arcus Biosciences, Chemistry, Hayward, USA; [2]Arcus Biosciences, Protein Science, Hayward, USA; [3]Leipzig University, Center for Biotechnology and Biomedicine Institute of Bioanalytical Chemistry Faculty of Chemistry and Mineralogy, Leipzig, Germany; [4]Arcus Biosciences, Quantitative Biology, Hayward, USA; [5]Arcus Biosciences, Biology, Hayward, USA*

Introduction: Extracellular adenosine (ADO) is present in high concentrations within the tumor micro-environment (TME), and ADO is a potent inhibitor of T cell and NK cell activation, resulting in suppression of immune function. The generation of ADO is dependent upon the ecto-nucleotidases CD39 (transforms ATP to AMP) and CD73 (transforms AMP to ADO). Inhibition of CD73 represents a promising therapeutic approach for preventing ADO-mediated immunosuppression in the TME. Here we present the discovery of a novel class of small molecules that are capable of inhibiting CD73.

Methods: The potency of CD73 inhibitors was evaluated by measuring AMP hydrolysis by CHO-CD73 cells using a malachite green assay. Potency was also measured using human T cells and soluble recombinant CD73. Selectivity against related ecto-nucleotidases was also assessed. Pharmacokinetic parameters were determined in preclinical species.

Results: A novel class of small molecule CD73 inhibitors has been developed starting from a high throughput screening hit. Several potent small-molecule CD73 inhibitors have been co-crystallized with recombinant CD73. They have been found to bind to the closed form of CD73 and to occupy the adenosine binding pocket, maintaining the same π-π stacking interaction observed with the CD73 inhibitor AMPCP. This class of molecules also demonstrates a key hydrogen bond with CD73 residue ASP506 to provide a drastic improvement in CD73 inhibition. Compounds exhibit potent CD73 inhibition in multiple biochemical and cell based assays providing IC_{50} values of less than 20 nM. Inhibitors have also been screened against various NTPDases as well as the adenosine receptors and were found to be highly selective for CD73. Select molecules do not show significant inhibition of the major CYP450 isoforms. Pharmacokinetic properties in rodents indicate the potential for oral bioavailability with low to moderate clearance.

Conclusions: Efforts have resulted in the discovery of a novel class of small-molecule CD73 inhibitors. These compounds are capable of inhibiting both soluble and membrane-bound CD73 and represent a step forward in the development of an orally bioavailable CD73 inhibitor for use in the treatment of cancer.

Conflict of interest: Other Substantive Relationships: Employees of Arcus Biosciences.

276 (PB-037) Poster

The discovery of potential CDK2-Spy1 inhibitors via structure-based drug design

N. Atatreh[1], M. Al Sorkhy[1], S. Al Rawashdah[1], M. Ghattas[1]. *[1]Al Ain University of Science and Technology, College of Pharmacy, Al Ain, U.A.E.*

Background: Spy1 is required for cell proliferation and recent findings have demonstrated that Spy1 is highly elevated in invasive ductal carcinoma of the breast, brain and many other cancers. The effect of this protein is initiated by binding to CDK2 and p27. Herein, we propose to interfere with this partnership, particularly with the two big proteins spy1-CDK2, using small molecules inhibitors in an attempt to prevent tumor formation and progression which could be a novel and effective approach in regiments of cancer treatments.

Methods: The crystal structure of CDK2-Spy1 binary complex was assessed via the SiteMap software in order to predict the most well-defined cavity in the contact area. Then, this cavity was targeted via a drug-like ligand library that was screened through an intensive docking protocol using GLIDE. A ligand-based virtual screening approach was also conducted based on the structure of the amino acid that fitted that pocket, using the MOE software.

Results: Interestingly, a moderately well-defined pocket was identified on the CDK2 structure where a Spy1 glutamate residue is bound to. This residue was found to form several electrostatic interactions with multiple positively charged residues in the identified cavity. The CDK2 pocket and the bound Spy1 residue were then screened to reveal a number of hits from various structural scaffolds that have all required features for inhibiting Spy1 binding to CDK2.

Conclusions: Potential hits obtained from this computational study will be tested *in-vitro* to prove their usefulness in cancer treatment.

No conflict of interest

277 (PB-038) Poster

Pyrrolo[2,1-a]phthalazine hybrids with anti-angiogenesis and DNA crosslinking induction activities are potent anitcancer agents

T.C. Lee[1], T.L. Chen[1], S.M. Chang[1], H.B. Pidugu[1], Y.W. Lin[1], M.H. Wu[2], H.C. Wu[3], T.L. Su[1]. [1]*Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan;* [2]*Industrial Technology Research Institute, Biomedical Technology and Device Research Laboratories, Hsinchu, Taiwan;* [3]*Academia Sinica, Institute of Cellular and Organismic Biology, Taipei, Taiwan*

Hybrid molecules are composed of two distinct biologically active molecules that act at different targets and thus may display dual mechanism of action. Using hybrid anticancer drug for treatment cancer can mimic the combination therapy, because hybrid molecule may have increased potency or selectivity profiles for biological targets as compare to the corresponding single drugs. While angiogenesis as a hallmark of tumor development and metastasis is a validated target for cancer treatment, the outcomes of anti-angiogenic therapy are still far away from the desired overall benefits. On the other hand, the adverse effects of DNA crosslinking agents also limit their clinical application. The development of novel cytotoxic drugs with increased efficacy but lowered in incidence of adverse events is an unmet need. Here we designed and successfully synthesized pyrrolo[2,1-*a*]phthalazine derivatives comprising two pharmacophores that suppress tumor growth via different mechanisms: anti-angiogenesis and induction of DNA crosslinks. Our data demonstrated that the hybrid pyrrolo[2,1-*a*]phthalazine derivatives are cytotoxic to a batch of cancer cell lines by inducing DNA damage, arresting cell cycle progression at the G2/M phase, and triggering apoptotic death. These hybrid molecules also inhibit angiogenesis by targeting VEGFR-2 on endothelial cells. We also demonstrated that compound **29d**, encapsulated in a liposomal formulation, significantly suppressed the growth of small cell lung cancer cells (H526) in mouse xenograft models. At the dose used, there is no body weight lost in mice, supporting its low toxicity. By immunohistochemical staining, the tumor xenografts in mice treated with compound **29d** showed time-dependently decreased intensity of CD31, a marker of blood vessel, but increased that of g-H2AX, a marker of DNA damage. Our present data suggest that the conjugation of anti-angiogenic and DNA damaging agents are potential hybrid agents for the treatment of cancer.

No conflict of interest

278 (PB-039) Poster

A peptide based on transmembrane domain of S1PR1 as a tumor cell growth inhibitor

N. Bailey Kobayashi[1,2,3], M. Sawada[4,5], Y. Hasegawa[6,7], T. Yoshida[1,2,3]. [1]*Toagosei Co., Ltd., Institute for Advanced Sciences, Tsukuba, Japan;* [2]*Toagosei Co., Ltd., JST/CREST, Institute for Advanced Sciences, Tsukuba, Japan;* [3]*Keio University, Laboratory of Gene Medicine, School of Medicine, Tsukuba, Japan;* [4]*Nagoya University, Research Institute of Environmental Medicine, Nagoya, Japan;* [5]*Nagoya University, JST/CREST, Research Institute of Environmental Medicine, Nagoya, Japan;* [6]*Nagoya University Graduate School of Medicine, Department of Respiratory Medicine, Nagoya, Japan;* [7]*Nagoya University Graduate School of Medicine, JST/CREST, Department of Respiratory Medicine, Nagoya, Japan*

Background: Many anti-tumor agents are used in chemotherapy for cancers. However, they also have serious side effects because they express similar growth inhibitory activity in normal cells. Meanwhile, the development of molecular target drugs inhibiting biomolecules relating to tumor growth, invasion, and metastasis has been proceeding with the goal of selectively attacking cancer cells. It has been revealed that the proliferation of cancer stem cells involved in redevelopment, invasion, and metastasis is promoted by sphingosine-1-phosphate (S1P). We focused on the trans-membrane domains of sphingosine 1 phosphate receptor 1 (S1PR1), and hypothesized that peptides which utilized these domains would exhibit a selective anti-tumor effect in cancer cells. We designed peptides based on the transmembrane domains of S1PR1 and evaluated whether the peptides showed tumor cell growth inhibitory activity against various kinds of human tumor cells.

Material and Methods: We chemically synthesized conjugation peptides of the human S1PR1 transmembrane domain sequences and a cell-penetrating peptide HIV TAT. The peptides were evaluated for growth inhibitory activity against human melanoma, non-small cell lung cancer (NSCLC), renal cancer, and prostate cancer by WST assay.

Results: The peptide based on the S1PR1 transmembrane domain 2 exhibited the strongest anti-tumor activity in the synthesized peptides based on the domain from 1 to 7. The result was that more than 95% of the tumor cells were inhibited in cells treated with a concentration of 25 μM for 48 hours.

Conclusions: We demonstrated a novel strategy for discovering an anti-tumor molecule by focusing on transmembrane domains of proteins involved in the proliferation of cancer stem cells. In particular, the peptide based on the transmembrane domain 2 of S1PR1 exhibits remarkable anti-tumor activity against human melanoma, NSCLC, renal cancer, and prostate cancer, which suggests that the peptide could be a candidate for a new anti-tumor agent.

No conflict of interest

279 (PB-040) Poster

PROTAC small-molecule degraders of MDM2 protein

A. Aguilar[1], J. Yang[2], Y. Li[3], D. McEachern[1], S. Przybranowski[1], L. Liu[1], C.Y. Yang[1], S. Wang[3]. [1]*University of Michigan, The Rogel Cancer Center and Department of Internal Medicine, Ann Arbor, USA;* [2]*University of Michigan, The Rogel Cancer Center and Department of Pharmacology and Internal Medicine, Ann Arbor, USA;* [3]*University of Michigan, The Rogel Cancer Center and Departments of Medicinal Chemistry and Internal Medicine, Ann Arbor, USA*

The tumor suppressor function of p53 is compromised in essentially all human cancers. In about half of human cancers, wild-type p53 status is retained, however, its function is effectively inhibited by the murine double minute 2 (MDM2) protein through a direct protein–protein interaction. Therefore, inhibition of MDM2 is an attractive cancer therapeutic target and this drove the discovery of several highly potent small molecule MDM2 inhibitors that have entered clinical development for cancer treatment. Since MDM2 is a direct transcriptional target of p53, MDM2 inhibitors' activation of p53 leads to upregulation of MDM2 mRNA and accumulation of MDM2 protein limiting their potential clinical efficacy and also may cause unwanted effects due to the oncogenic activity of MDM2 protein.

Here we describe our design, synthesis and evaluation of small-molecule MDM2 degraders based on the proteolysis targeting chimera (PROTAC) concept and our investigation of their therapeutic potential and mechanism of action *in vitro* and *in vivo*. Our MDM2 degrader effectively induces rapid degradation of MDM2 resulting in accumulation of wild-type p53 protein and activates p53 transcriptional activity in leukemia cells without accumulation of MDM2 protein. Consistent with its design to effectively degrade MDM2, our MDM2 degrader potently inhibits cell growth and induces apoptosis at low nano-molar concentrations in ALL and AML cell lines >10–100 times more potent than the best MDM2 inhibitors; strongly activates wild-type p53 in RS4;11 xenograft tumor tissue in mice; and achieves complete tumor regression in RS4;11 xenograft model in mice at well tolerated dose-schedules. Our data suggests that targeting MDM2 degradation is a novel and very exciting therapeutic approach for the treatment of ALL and AML.

Conflict of interest: Other Substantive Relationships: Shaomeng Wang, Angelo Aguilar, Jiuling Yang, and Yangbing Li, are co-inventors for MDM2 degraders disclosed in this study. The University of Michigan has filed a number of patent applications related to these MDM2 degraders, which have been licensed to Oncopia Therapeutics. Shaomeng Wang is a co-founder, stock holder, and consultant for Oncopia Therapeutics. Angelo Aguilar is a consultant for Oncopia Therapeutics.

280 (PB-041) Poster

Potent anti-tumor activity correlated with inhibition of DNA damage response genes with highly selective and orally bioavailable CDK12 covalent inhibitors

R. Poddutoori[1], S. Rajagopalan[2], S. Mukherjee[1], S. Marappan[2], D. Samiulla[2], S. Nayak[2], S. Sivakumar[2], M. Ravindra[1], S. Gore[1], A. Dhudashiya[2], K. Charamanna[2], G. Daginakatte[2], S. Chelur[2], M. Ramachandra[1], S. Samajdar[1]. [1]*Aurigene Discovery Technologies Ltd., Medicinal Chemistry, Bangalore, India;* [2]*Aurigene Discovery Technologies Ltd., Pre-clinical Biology, Bangalore, India*

Cyclin-dependent kinase 12 (CDK12) regulates transcription and plays a critical role in DNA damage response, splicing and pre-mRNA processing. During transcription CDK12 regulate transcription elongation by phosphorylating RNA polymerase II (RNAP II) at Serine 2 in the C-terminal domain (CTD). Overexpression of CDK12 in various tumor types supports the possibility that CDK12 has oncogenic properties similar to other transcription-associated kinases. Based on its critical role in transcription and RNA processing, CDK12 is considered as an attractive therapeutic target for cancer.

Several series of potent and selective CDK12 covalent inhibitors were identified by structure-guided and iterative medicinal chemistry approaches. Compounds were further optimized towards attaining good physicochemical properties, high potency, good selectivity and desirable pharmacokinetic profile to achieve anti-tumor activity.

Optimization of two distinct chemical series resulted in very potent and highly selective CDK12 inhibitors. The covalent mode of action for these biochemically potent compounds has been confirmed by CDK12 target engagement assay in the cellular context. These selective inhibitors showed significant anti-proliferative activity in TNBC and other cancer cell lines including those with ETS fusion, which correlated with the inhibition of pS2 (RNAP II). Anti-proliferative activity also correlated well with down-regulation of a number DNA damage response genes including *BRCA1, RAD51, ATM*and *FANC1*. Consistent with the inhibition of genes involved in DNA damage repair, a highly synergistic anti-proliferative activity was observed when treated in combination with cisplatin. In vivo efficacy in a mouse model of TNBC as a single agent and in combination with DNA-damaging agents are currently underway and the data will be presented.

Conflict of interest: Ownership: All authors are employees of Aurigene Discovery Technologies Limited.

281 (PB-042) Poster

Natural product inhibitors of hypoxia inducible factor-2a for the treatment of renal cell carcinoma

M.M. Lee[1], D.C. Rabe[1], T.C. Mckee[2], G.M. Woldemichael[2], J.R. Vasselli[1], J.B. McMahon[2], D.M. De Silva[1], A. Roy[1], W.M. Linehan[1], D.P. Bottaro[1]. [1]*National Cancer Institute, Urologic Oncology Branch, Bethesda, USA;* [2]*Frederick National Laboratory for Cancer Research, Molecular Targets Laboratory, Frederick, USA*

Almost all sporadic ccRCC tumors harbor a loss of *von Hippel Lindau* (*VHL*) gene function, resulting in constitutive activation of hypoxia inducible factor-alpha subunits (HIF-a) which contributes to tumorigenesis. In particular, HIF-2a acts as an oncogene, and upregulation of HIF-2a in ccRCC is often associated with poor prognosis. HIF-2a turns on transcription of a large number of HIF-regulated genes, many of which contribute to RCC progression, including *VEGF*, *GLUT1*, and *TGF-a*. Two structurally similar HIF-2a selective inhibitors are currently undergoing clinical investigation with promising phase I results reported, but efficacy and potential resistance pathways in some ccRCC preclinical models is yet to be fully understood. A cell-based high throughput screen (HTS) of the National Cancer Institute's Natural Products Repository was performed to identify HIF-2a inhibitors for use as research tools and drug development leads. An engineered ccRCC cell line with a luciferase reporter construct downstream of hypoxia response elements from the human vascular endothelial growth factor (*VEGF*) gene promoter was used to identify natural product samples with inhibitory activity and minimal cellular toxicity. HTS leads were chromatographically separated into component structures yielding ~40 pure compounds with micromolar to submicromolar IC_{50} values, >80% HIF-2a inhibition, and <10% cell toxicity. Cell lines engineered to express either HIF-1a, HIF-2a, or both, were used to study patterns of mRNA expression of HIF-1a, HIF-2a and several HIF target genes to help identify HIF-2a-selective inhibitors and determine mechanism of action. Three lead compounds were found to signicantly reduce *VEGF* gene transcription and protein secretion, and selectively downregulate HIF-2a target genes. Using ICM-Pro protein/small molecule docking software, the three lead compounds were found to bind only to the HIF-2a PAS B domain, and not to the HIF-1a protein, suggesting a mechanism of action similar to the clinical compounds in which the inhibitor prevents HIF-2a:ARNT dimerization in *VHL* deficient cells. Additionally, cultured cell-based assays showed the compounds to be active against ccRCC proliferation, motility, and invasion. Interestingly, treatment of ccRCC cell lines with the three lead compounds results in significant downregulation of *HIF2A* levels, a phenomenom not seen with other HIF-2a inhibitors, and a potential mechanism to circumvent or overcome resistance to this newly targeted oncogenic signaling pathway.

No conflict of interest

282 (PB-043) Poster

A journey of developing a new class of active and potent PI3Kalpha inhibitors

S. Gamage[1], A.C. Giddens[1], K.Y. Tsang[1], J.U. Flanagan[1], J.D. Kendall[1], W.J. Lee[2], B.C. Baguley[1], C.M. Buchanan[2], S.M.F. Jamieson[1], P.R. Shepherd[2], W.A. Denny[1], G.W. Rewcastle[1]. [1]*University of Auckland, Auckland Cancer Society Research Centre, Auckland, New Zealand;* [2]*University of Auckland, Department of Molecular Medicine and Pathology, Auckland, New Zealand*

Background: Phosphoinositide 3-kinase (PI3K) is a lipid kinase, a central component in the PI3K/AKT/mTOR signalling pathway. The targeting of PI3K, and more specifically the PI3Kα isoform, with small molecule inhibitors has been widely explored as developing anti-cancer therapeutics. Several PI3K inhibitors are currently under evaluation in human clinical trials. 2-(2-difluoromethylbenzimidazol-1-yl)-4, 6-dimorpholino-1,3,5-triazine (**ZSTK474**) is a known small molecule pan Class I (PI3K) inhibitor. Using **ZSTK474** as the starting point we endeavoured to design and develop a series of new analogues to inhibit PI3Kα.

Material and Methods: We used established chemistry to form various substituted 2-(difluoromethyl)-1*H*-benzo[*d*]imidazole moieties. Reacting these with 4,4′-(6-chloro-1,3,5-triazine-2,4-diyl)dimorpholine we synthesized a series of **ZSTK474** analogues.

Then we replaced one of the morpholine groups of the **ZSTK474** analogue, 4,4′-(6-(2-(difluoromethyl)-4-methoxy-1*H*-benzo[*d*]imidazol-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**SN 30378**). We synthesized 4-(4-chloro-6-(2-(difluoromethyl)-4-methoxy-1*H*-benzo[*d*]imidazol-1-yl)-1,3,5-triazin-2-yl)morpholine by reacting 2-(difluoromethyl)-4-methoxy-1*H*-benzo[*d*]imidazole with 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine. This was then reacted with various groups to form a new class of PI3K inhibitors.

Results: By doing a SAR study and replacing one of the morpholine rings of **ZSTK474**, with sulphonamide containing substituents obtained a new Class of active and potent PI3Kα inhibitors eg: 4-(4-(2-(difluoromethyl)-4-methoxy-*1H*-benzo[*d*]imidazol-1-yl)-6-(4-(methylsulfonyl)piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**SN 32539**).

Conclusions: The initial SAR study identified 4,4′-(6-(2-(difluoromethyl)-4-methoxy-1*H*-benzo[*d*]imidazol-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**SN 30378**) as the lead compound of the study. By changing one of the morpholine groups to different moieties we identified a new class of active PI3Kα inhibitors. Finally we improved the solubility of these to obtain a potent PI3Kα inhibitor 2-((4-(4-(2-(difluoromethyl)-4-methoxy-1*H*-benzo[*d*]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)sulfonyl)-N,N-dimethylethan-1-amine (**SN 32976**).

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Other

283 (PB-044) Poster

Characterization of KDM6A loss in urothelial carcinoma

H.C. Wise[1], W. Hu[2], P.S. Gao[2], A. Ahmad[2], V.A. McPherson[2], S. Dason[2], E.J. Pietzak[2], G. Lyer[2], H. Al-Ahmadie[2], D.B. Solit[2]. [1]*Louis V. Gerstner Graduate School of Biomedical Sciences, New York, NY, USA;* [2]*Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Background: Almost all bladder cancers harbor mutations in genes involved in the regulation of chromatin state. KDM6A is among the most commonly mutated of these chromatin-modifying genes in urothelial cancers, and KDM6A alterations are by far more common in urothelial cancer than in any other human cancer type. However, little is understood of the role of KDM6A mutation in promoting bladder cancer initiation, progression and its impact on drug response.

Material and Methods: To define the temporal sequence of mutational events in bladder cancers from non-invasive to invasive tumors to metastatic disease, we performed next generation sequencing analysis of paired tumor samples collected at varying clinical states from individual bladder cancer patients. To gain further insight into the clonality of individual alterations, we inferred the cancer cell fraction of mutations in individual tumors identified by our paired sequencing and in bladder tumors analyzed by the TCGA. To explore the functional consequences of KDM6A loss of function, we used

CRISPR/Cas9 to generate an isogenic KDM6A knockout model in the RT4 bladder cancer cell line.

Results: In our paired sample analyses, KDM6A loss was always identified in the primary tumor and early non-invasive recurrences, suggesting KDM6A loss is an early oncogenic event in bladder cancer pathogenesis. KDM6A mutation often co-occurs with mutations in other chromatin modifying genes. In patients with both KDM6A and ARID1A mutation, the ARID1A mutation was exclusive to the metastatic samples in four out of nine patients, suggesting that ARID1A mutations may play an important role in metastatic progression. Further supporting the hypothesis that KDM6A mutations typically precede those in ARID1A in bladder cancer progression, analysis of the TCGA whole-exome data from primary muscle invasive tumors demonstrated that KDM6A loss is typically a clonal alteration present in all cancer cells whereas ARID1A were more likely to be subclonal. Functional analysis of KDM6A loss in the RT4 bladder cancer cell line demonstrated that knockout of KDM6A resulted in accelerated cell growth and increased colony formation, as well as a significantly altered RNA expression profile compared to parental RT4 cells. Gene Set Enrichment Analysis (GSEA) identified significant downregulation of a number of negative feedback regulators of RAS/MAPK signaling, consistent with an increase in RAS/MAPK signaling.

Conclusions: Our data show that KDM6A loss is an early event in bladder cancer development that promotes cell growth and rewiring of the RNA expression signature whereas ARID1A mutations are a later genetic event that may play a role in metastatic progression. In the future, we hope that a deeper understanding of the functional consequences of KDM6A loss will provide viable targets for new therapeutics.

Document not received.

284 (PB-045) Poster

Genetic alterations of driver genes as an independent prognostic factor for disease free survival in patients with resected non-small cell lung cancer

A. Ono[1], M. Isaka[2], H. Kojima[2], K. Nakashima[1], S. Omori[1], K. Wakuda[1], H. Kenmotsu[1], T. Naito[1], H. Murakami[1], K. Omae[3], M. Serizawa[4], K. Urakami[4], Y. Ohde[2], T. Nakajima[5], M. Kusuhara[4], T. Takahashi[1]. [1]*Shizuoka Cancer Center, Division of Thoracic Oncology, Shizuoka, Japan;* [2]*Shizuoka Cancer Center, Division of Thoracic Surgery, Shizuoka, Japan;* [3]*Kyoto University, School of Public Health, Graduate School of Medicine, Kyoto, Japan;* [4]*Shizuoka Cancer Center, Research Institute, Shizuoka, Japan;* [5]*Shizuoka Cancer Center, Division of Diagnostic Pathology, Shizuoka, Japan*

Background: Shizuoka Cancer Center launched the first prospective pan-cancer molecular profiling study in Japan in January 2014, identifying patient-specific molecular signatures via multi-omics analysis, with a view to developing cancer precision medicine. By May 2018, whole-exome sequencing (WES) for over 4,600 pts had been completed across all types of tumors. This study aims to assess the association between molecular signature and clinical information in pts with postoperative relapse-free survival (p-rfs) of non-small cell lung cancer (NSCLC) for identification of novel prognostic factors, focusing on the association with driver mutations (mt).

Materials and Methods: Between February 2014 and September 2015, 242 pts with NSCLC, including 192 pts with adenocarcinoma (Ad) and 50 pts with squamous cell carcinoma (Sq), underwent surgery and were enrolled in this study. Surgically resected tissue was subjected to WES using an ion torrent proton platform (Thermo Fisher Scientific). Mt detected in 138 cancer-related genes listed in Vogelstein *et al.*(Science. 2013 339:1546) were evaluated as driver mt. Multivariate analysis using the multi-state model was used to establish the association between co-variables and p-rfs.

Results: P-rec was observed in 49 (20.2%) and 19 (7.9%) pts with Ad and Sq, respectively. Median follow-up period of all censored cases (range) was 2.5 years (2.0–3.5). Median relapse-free survival times was 2.3 years (0.05–3.5). Patient background [recurrence (rec); non-recurrence (non-rec)]: median age (range) 71 (50–87); 74 (39–87), male 56; 65%, smoker 75; 68%, pathological stage (p-stage) (I/II/III) 44/28/28%; 77/18/5%, histological type (Ad/Sq) 72/28%; 82/18%, adjuvant chemotherapy (yes/no) 44/56%; 28/72%, driver mutation (presence/absence) 96/4%; 79/21%. In univariate analysis, age ($<70/\geqq 70$), smoking history (yes/no), p-stage (I, II/III), histological type (Ad/Sq), and driver mutation (presence/absence) were favorable prognostic factors ($p = 0.017$, $p = 0.048$, $p = 0.0002$, $p = 0.006$, $p = 0.029$ respectively). A multivariate analysis also revealed significant association between driver mutation status and p-rfs ($p = 0.046$, odds ratio [OR]: 2.86, 95% CI 1.02–8.08), when adjusted by histological type ($p = 0.10$), smoking status ($p = 0.09$), gender ($p = 0.51$), age ($p = 0.008$) and p-stage ($p = 0.00003$).

Conclusion: Driver mutation status may be an independent prognostic factor of p-rfs in NSCLC.

No conflict of interest

285 (PB-046) Poster

Molecular profiling of tumour and ctDNA in a gastrointestinal cancer cohort at an academic centre

S. Rack[1], D. Rothwell[1], A. Wallace[2], L. Carter[1,3], G. Brady[4], F. Thistlethwaite[1,3], C. Kelly[3], D. Graham[3], N. Tinsley[3], A. Vickers[3], S. Aruketty[3], R. Kurup[3], M. Ayub[4], C. Dive[4], M. Krebs[1,3], N. Cook[1,3]. [1]*University of Manchester, Faculty of Medical and Human Sciences, Manchester, United Kingdom;* [2]*Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [3]*The Christie NHS Foundation Trust, Experimental Cancer Medicine Team, Manchester, United Kingdom;* [4]*Cancer Research UK, Manchester Institute, Manchester, United Kingdom*

Background: Tumour characterisation is typically performed on archival tumour biopsies however circulating tumour DNA (ctDNA) can provide a minimally invasive liquid biopsy that may better capture tumour heterogeneity. The Tumour chARacterisation to Guide Experimental Targeted Therapy Trial (TARGET) was set up to test the hypothesis that ctDNA molecular profiling can be used to guide selection of experimental medicines and monitor tumour response for patients that receive a matched targeted therapy. We report results of ctDNA and tumour molecular profiling from a gastrointestinal subset of patients recruited to TARGET to date.

Methods: Eligible patients (pts) referred for phase 1 trials were consented for blood sampling, archival tumour analysis and optional fresh biopsies. 68 patients (pts) with metastatic small bowel cancer (SBC) or colorectal cancer (CRC) were recruited to TARGET between May 2015 and May 2018. Archival biopsies were analysed using a 24 gene NGS panel (Qiagen GeneRead DNAseq Targeted Panel V2) and a 322 gene NGS panel (Foundation Medicine). Upon recruitment pts underwent further testing with a plasma-based ctDNA NGS assay examining 653 genes.

Results: The median age of pts was 56 yrs. 48% (33/68) were colon tumours, 34% (23/68) were rectal, 9% (6/68) were rectosigmoid and 7% (5/68) small bowel. Pts had failed an average of 3 lines (range 1–5) of treatment prior to recruitment. The average time between archival biopsy and liquid biopsy was 927 days. 74% (50/68) of patients had both sufficient tumour DNA in their plasma, and archival biopsies for analysis. Sequencing of ctDNA detected all mutations reported in tumour in 76% (38/50) of patients. Additional mutations were picked up in the ctDNA in 30% (15/50) of pts. Neither the interval between collection of archival biopsies and blood tests ($p = 0.300$), nor lines of previous treatment ($p = 0.844$), significantly affected detection of new mutations. 19 pts were treated with anti-EGFR therapy following their tumour biopsy, 31% (6/19) of these cases developed recognised resistance mutations (*KRAS* and *EGFR*) in ctDNA on serial analysis. *TP53* mutations were commonly detected in the ctDNA (60%), followed by *KRAS* (51%), *PIK3CA* (15%) and *PTEN* (3%). Other mutated genes included *CTNNB1*, *BRAF*, *FGFR3* and *ERRB2*. 25% (17/68) of pts were enrolled on a phase I trial following recruitment to TARGET, however only 6% of pts were enrolled on a matched trial using a rationale based upon the results of molecular testing.

Conclusion: ctDNA may be used for routine molecular characterisation of metastatic SBC/CRC and results can be analysed to track the development of resistance. Novel therapeutic options are needed as there are limited treatment options for these pts. The TARGET trial continues to recruit patients from all cancer types, allocating patients to clinical trials based on ctDNA and tumour profiling.

No conflict of interest

286 (PB-047) Poster

The relation between the quantum entanglement in theoretical physics as a new insight into the cancer biology

S. Niknamian[1], S. Zaminpira[1]. [1]*Cellular and Molecular Biology, University of Cambridge, United Kingdom, Biology, Tehran, Iran*

Quantum entanglement is a phenomenon in theoretical physics that happens when pairs or groups of particles are generated in ways that the quantum state of each particle cannot be described independently of the others, even when the particles are separated by a large distance. Instead, a quantum state must be described for the system as a whole. Based on the introduction of cancer as an evolutionary metabolic disease (EMHC), each cancerous cell are eukaryotic cells with different metabolic rate from healthy cells due to the damaged or shut down mitochondria in them. Assuming each human

eukaryotic cell as a particle and the whole body as a quantum entangled system, is a new perspective into the description of the cancer disease and this link between theoretical physics and biological sciences in the field of cancer therapies can be a new insight into the cause, prevention and treatment of cancer. Additionally, this perspective admits the Lamarckian evolution in the understanding of the mentioned disease. We have introduced each human eukaryotic cell containing mitochondria as a QES, also, the whole body containing healthy and normal cells as a QES as well. The difference between the entropy of the healthy and cancer cells has been mentioned in this research as well.

No conflict of interest

287 (PB-048) Poster

Extracellular vesicle analysis as non-invasive tool to predict and follow treatment response in bladder cancer

P. Hååg[1], C.H. Sha[1], J. Farnebo[2], P. Hydbring[1], V. Arapi[1], R. Lewensohn[3], K. Viktorsson[1], A. Ullen[3]. *[1]Karolinska Institutet, Oncology/Pathology, Stockholm, Sweden; [2]Karolinska Institutet, Physiology and Pharmacology, Stockholm, Sweden; [3]Theme Cancer, Karolinska University Hospital, Stockholm, Sweden*

Background: Urothelial cancer of the bladder is the most common cancer of the urinary tract and about 3000 patients is diagnosed each year in Sweden. About 20–30% of these patients develop muscle invasive or metastatic disease which requires platinum-based chemotherapy. However, progress and development of resistant disease is common. Effective second line treatment is a challenge and there is unmet medical need in platinum-progressive metastatic urothelial cancer due to few treatment options and short median overall survival.

Within the international, multicentre prospective Phase I trial (VINSOR trial), we have studied the standard second-line chemotherapy drug vinflunine in combination with the targeted compound sorafenib. In this study we have explored the biomarker potential of tumor-derived extracellular vesicles (EVs) in plasma from a subset of the patients with different response profiles.

Material and Methods: Plasma and urine samples were collected prior to treatment and at day 8 and prior to cycle two in the VINSOR trial and have been stored at −80C. Plasma from patients with different length of overall survival and progression free survival was analysed for EVs and their biomarkers. For EVs/exosomes isolation plasma was filtered through a 0.22 μm filter, and around 500 μl was loaded on IZON exosome isolation columns. 500 μl elution fractions were collected and analyzed for size and concentration of EVs/exosomes using nanoparticle tracking analysis (NTA). EV/exosome specific markers (CD9, CD63) as well as urothelial carcinoma surface proteins (VEGFR2; FGFR3, PDGFR, Ephrin A1, EphA2 were profiled using western blotting. The miRNA expression pattern of EVs was analyzed by NanoString and further processed using Ingenuity Pathway Analyses.

Results: EVs including exosomes were efficiently isolated from plasma samples from the VINSOR-trial. Their amount and size varied among the patients both at baseline and during the treatment course and are currently explored in context of treatment response and outcome. Western blot analyzes revealed expression of the exosome marker CD9 and variances among urothelial proteins prior and post treatment. RNA was isolated from fraction 8 with the most concentrated and pure exosomes and miRNA pattern was analysed using NanoString. Differences in miRNA profiles were observed both between patients and prior and post treatment indicting a putative biomarker potential of EV/exosome derived miRNA profiling.

Conclusions: EVs were efficiently isolated from plasma of urothelial carcinoma patients. Clear differences in amount and size of EVs/exosomes, protein surface markers and miRNA expression pattern is observed during treatment. Thus analyses of EVs/exosome may hold biomarker potential to predict and follow treatment response in patients with advanced urothelial cancer treated by targeted combination chemotherapy.

No conflict of interest

288 (PB-049) Poster

Clinical significance of circulating microRNA-200c expression in breast cancer

P. Yadav[1], M. Mirza[2], K. Nandi[2], S.K. Jain[3], R. Kaza[3], P. Sharma[1], A. Saxena[2]. *[1]All India Institute of Medical Sciences, Department of Biochemistry, Jodhpur, India; [2]Maulana Azad Medical College, Department of Biochemistry, New Delhi, India; [3]Maulana Azad Medical College, Department of Surgery, New Delhi, India*

Background: Breast cancer is the leading cause of cancer-related death in the female population. MicroRNA-200c (miR-200c), a non-coding RNA, is important in the epithelial to mesenchymal transition (EMT) and metastasis in breast cancer. The present study was focused on diagnostic and prognostic role of serum miR-200c expression in breast cancer patients.

Materials and Methods: Study includes 75 histopathologically confirmed, newly diagnosed breast cancer patients and 75 healthy controls. Serum samples were collected before and after treatment from each patient. Total RNA from serum was isolated by using Trizol reagent. Total RNA was polyadenylated and reverse transcribed into cDNA. The expression level of miR-200c was detected by using miRNA qRT-PCR. Relative expression was analyzed using U6 snRNA as a reference. Total follow up period was 41 months and mean follow up period was 28 months. Kaplan-Meier survival analysis was performed for overall survival of breast cancer patients. The present study was conducted at Maulana Azad Medical College and Associated Hospitals, New Delhi, India. The Ethical approval was obtained from the Institutional Ethics Committee of Maulana Azad Medical College, New Delhi, India.

Results: In cases, more than 8 mean fold increased microRNA-200c expression was observed compared to the healthy controls. On ROC analysis for diagnosis, An AUC of 0.743 with 69.7% sensitivity and 85.33% was observed. Level of miR-200c expression was significantly decreased (5.6 mean fold) after treatment. There was a significant association seen between miR-200c expression with TNM stage ($p = <0.0001$), histological grade (0.04), lymph node status ($p = 0.003$) and distant metastasis ($p = 0.001$) of breast cancer patients. ROC analysis for prognosis yielded significant AUC values (0.817 and 0.866 respectively) for early vs advanced stage and distant metastasis. There was a significant association ($p = 0.006$) found between overall survival and expression of miR-200c in breast cancer patients.

Conclusion: Our results suggest that circulating miR-200c overexpression might be a useful non-invasive, diagnostic and prognostic indicator for breast cancer patients. A large pool study will be required to confirm our findings.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Preclinical Models

289 (PB-050) Poster

Spontaneous metastasis of prostate cancer: dynamics of dissemination, dormancy and growth

J. van Dorp[1], C. de Ridder[1], W. Teubel[1], M. van Royen[2], G. Jenster[1], W. van Weerden[1]. *[1]ErasmusMC, JNI, Urology, Rotterdam, Netherlands; [2]ErasmusMC, JNI, Pathology, Rotterdam, Netherlands*

Background: With improvements in diagnostic modalities such as functional imaging, (oligo)metastatic prostate cancer is being diagnosed with greater frequency. Understanding its biology will help to identify novel targets to block crucial steps in the development and progression of metastasis. We have established a patient-derived xenograft (PDX) model of prostate cancer with spontaneous metastatic outgrowth in liver and shedding to lymph node, lung and bone. This study aims to unravel the metastatic process and assess effects of different therapeutic options on heterogeneity and clonality of primary tumor, circulating tumor cells (CTC's), dormant cells and micro- and macro-metastases.

Material and method: The PC339C-M21-L is a spontaneous liver metastasis model from the human prostate PC339 PDX model originating from TURP material from a CRPC patient. PC339-derived cell line, PC339C was transduced to produce luc2-eGFP fused protein (M21-lentiviral) for imaging using bioluminescence (PC339C-M21). Additionally, PC339C cells were transduced (lentiviral) to produce three endogenous fluorescent proteins Cerulean, Venus and mCherry (PC339C-CVM) to identify a wide spectrum of different color combinations using fluorescent microscopy. Immunodeficient male NOG mice (Taconic, Ry, Denmark) were subcutaneously inoculated with PC339C-M21-L or PC339C-CVM cells. To allow metastatic outgrowth, the primary tumor was removed and mice were monitored weekly by IVIS and/or MRI to assess development of liver metastases. At sacrifice liver and other organs were sampled and blood was drawn for isolation of CTC's.

Results: Subcutaneous PC339C-M21 tumors induced shedding of cells to various organs and outgrowth of macrometastases in liver. Accumulation of viable cancer cells could be identified by IVIS in various tissues, which we were able to subculture. One day after tumorectomy, such accumulated disseminated cells had disappeared, suggestive of their transient nature.

Once macro-metastases in the liver had developed, foci of accumulated disseminated cells re-appeared. PC339-CVM cultures remained stable in the proportion of color combinations upon long-term culturing. Subcutaneous injection resulted in a primary tumor with a patchy fluorescent expression pattern indicating all labeled clones to be represented. Upon removal of the primary tumor, multiple visible (macro)metastases appeared spontaneous in the liver with each single lesion showing the same color expression, indicating a monoclonal origin. In addition, single and very small groups of fluorescently labeled cells could be identified in the lung, indicative of dormant cells and monoclonal micro-metastases.

Conclusions: This preclinical metastatic liver model is a novel tool to trace the lineage of (metastatic) clones and to assess the impact of therapeutic interventions on metastatic PCa.

No conflict of interest

290 (PB-051) Poster

Susceptibility of BRCA associated PDAC to immunotherapy

C. Stossel[1,2,3], D. Atias[1], Y. Glick-Gorman[1], S. Halperin[1], M. Raitses Gurevitch[1], M. Schvimer[4], R. Denroche[5], S. Gallinger[6], G. Markel[2,3], R. Ortenberg[3], R. Berger[1,2], T. Golan[1,2]. *[1]Sheba Medical Center, Oncology institute, Tel Hashomer, Israel; [2]Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel; [3]Sheba Medical Center, Ella Lemelbaum Institute for Immuno-Oncology, Tel Hashomer, Israel; [4]Sheba Medical Center, Pathology, Tel Hashomer, Israel; [5]Ontario Institute for Cancer Research, Transnational Research Initiative in Pancreatic Cancer, Toronto, Canada; [6]University Health Network, Surgery, Toronto, Canada*

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. Germline-BRCA1/2 mutation carriers are the most well defined DNA damage repair deficient subgroup and present up-to ~15% of PDAC in selected populations. DDR-deficient cells display susceptibility to DNA-damaging agents and PARP inhibition (PARPi). However, durable responses are limited and resistance evolves. Immunotherapy strategies have not shown significant clinical benefit in PDAC. DDR-deficient PDAC features increased mutational burden, which may confer sensitivity to checkpoint inhibition. Compared to the general PDAC population, BRCA1/BRCA2-mutated cases have higher incidence of PD-1 and PD-L1 expression, respectively. We hypothesize that immuno-modulating strategies may have therapeutic value in BRCAmut PDAC, due to the higher, neo-antigen-encoding mutational burden.

We have developed unique patient-derived xenograft (PDX) models from metastatic PDAC patients (n = 45). These models resemble the morphologic and genomic characteristics of primary PDAC (Golan;Oncotarget;2017). 13/42 PDX models were established from germline BRCA-mutated patients obtained at distinct time points; before treatment and at progression. Correlation between disease course at tissue acquisition and response to PARPi/platinum was demonstrated in PDXs in-vivo (Golan;IJC;2018). 4/6 models were classified as "unstable"/"SDBR" by whole genome sequencing.

Hematopoietic-engrafted PDAC PDX model: 3–4 week old NSG mice underwent sub-lethal irradiation and transplanted with CD34+ stem cells purified from umbilical–cord-blood. Human cell engraftment (hCD45) was detected from week 12 onwards. On week 18, cryopreserved tumor chunks from a PDX model established from a germline BRCA2 6174delT PDAC patient with high mutational (>32,000 SNVs) and neoantigene load was subcutaneously transplanted to engrafted and non-engrafted mice (control) and thereafter treated with pembrolizumab (10 mg/kg;q3-4) for 5 weeks(n = 5–6/group). Weight and tumor volume ((length*width^2)/2) were measured bi-weekly. Peripheral-blood was obtained every ~2–3-weeks. An attenuation of tumor growth was demonstrated in the hCD45-engrafted pembrolizumab treated mice compared to control, with one mice demonstrating complete response. T and B cells were detected in splenocytes, bone-marrow and blood of engrafted mice. A trend of correlation between hCD45 engraftment and tumor growth inhibition was observed and IHC analysis demonstrated tumor T cells infiltration.

DDR-deficient PDAC tumors display increased mutational load and may serve as a favorable subgroup for development of effective (immuno)therapy strategies. Preliminary data demonstrated checkpoint inhibition efficacy in a humanized BRCAmut PDAC PDX model. The humanized model may serve as a platform for development of immunotherapy combinations for BRCAmut PDAC patients.

No conflict of interest

291 (PB-052) Poster

SRA737, a novel Chk1 inhibitor, shows efficacy in CCNE1-amplified and MYCN-overexpressing high-grade serous ovarian cancer patient-derived xenograft models

G.Y. Ho[1,2,3], H. Barker[1], C. Vanderberg[1], O. Kondrashova[1], E. Kyran[1], E. Lieschke[1], O. McNally[2], A. Hamilton[2,3], V. Heong[1], R. Hansen[4], S. Milutinovic[4], B. Strouse[4], M. Hedrick[4], C. Hassig[4], D. Bowtell[3], W. Matthew[1], C. Scott[1,3], AOCS. *[1]Walter and Eliza Hall Institute for Medical Research, Stem Cells and Cancer, Melbourne, Australia; [2]Royal Women Hospital, Oncology, Melbourne, Australia; [3]Peter MacCallum Cancer Centre, Oncology, Melbourne, Australia; [4]Sierra Oncology, Sierra, Vancouver, Canada*

Background: Oncogene-driven high-grade serous ovarian cancers (HGSOC) with *CCNE1* or *MYCN* pathway activation exhibit defective cell cycle checkpoint control and replicative stress (RS). The DNA damage response effector kinase, Chk1, modulates the cellular response to RS and has been shown to be upregulated in these subtypes of HGSOC. SRA737 is a novel, potent, highly selective and orally available Chk1 inhibitor that is currently under clinical investigation in high-RS cancers including HGSOC. We explored the efficacy of single agent SRA737 at three doses compared to olaparib, a PARP inhibitor recently approved for HGSOC, in our preclinical HGSOC models.

Methods: Two HGSOC patient derived xenografts (PDX) with defined clinicopathologic and molecular characteristics hypothesized to have high intrinsic RS were selected for *in vivo* drug testing. PDX #111 was generated from a HGSOC with 59-fold amplification of *CCNE1* while PDX #29 has a 9-fold amplification of *CCNE1* and over-expression of the *MYCN* pathway. Both HGSOCs were refractory to standard platinum-based therapy and were WT for *BRCA1/2* and related gene mutations. Upon reaching treatment size (180–300 mm^3), mice bearing PDX tumors were randomized to daily treatment for 21 days with SRA737 (100 mg/kg, 50 mg/kg, or 25 mg/kg), olaparib (100 mg/kg) or vehicle. Tumor volume was monitored until the experimental endpoint of 700 mm^3, or 120 days post completion of therapy was reached. Short term harvest (STH) experiments were also performed following a single dose of SRA737 of 100 mg/kg or 50 mg/kg. Tumors were collected at 12 hours following treatment to determine on-target drug effects.

Results: Treatment of PDX #111 with SRA737 at 100 mg/kg resulted in significant stabilization of disease, leading to prolonged median time to harvest (TTH) of 78 days for SRA737 vs. 43 days for vehicle (p value <0.001). As expected, olaparib treatment was less effective and inferior than SRA737 at 100 mg/kg (TTH = 46 days; p value <0.001) given the absence of *BRCA1/2* mutations in this model. Impressively, in PDX #29, SRA737 at 100 mg/kg resulted in tumor regression leading to improved median TTH and outperforming olaparib (median TTH of 81 days for SRA737 100 mg/kg, 46 days for olaparib and 39 days for vehicle; p value <0.05 for both). Analysis of cell cycle and RS biomarkers in the STH samples is underway.

Conclusion: Chk1 inhibition by SRA737 shows promising efficacy in *CCNE1*-amplified and *MYCN*-overexpressing preclinical PDX models of HGSOC, where other targeted therapies such as PARP inhibitors show limited activity. These *in vivo* data support the ongoing monotherapy clinical trial of SRA737, which includes the prospective enrollment of patients with these HGSOC subtypes.

Conflict of interest: Corporate-sponsored Research: Sierra Oncology, Inc provided financial support for this project. Dr. Ryan Hansen, Dr. Snezana Milutinovic, Mr. Bryan Strouse, Mr. Michael Hedrick and Dr. Christian Hassig are employees of Sierra Oncology, Inc.

292 (PB-053) Poster

Precision cut cancer tissue slices derived from cancer patients as a tool for the investigation of immune-modulatory compounds

K. Bernoth[1], M. Piller[1], M. Petersen[1], O. Timm[1], J. Krüger[1], H. Juhl[2], K. David[3], N. Grabinski[1]. *[1]Indivumed GmbH, Research Services, Hamburg, Germany; [2]Indivumed GmbH, Chief Executive Officer, Hamburg, Germany; [3]Indivumed GmbH, Head of Research and Development, Hamburg, Germany*

Background: The goal of personalized medicine is to provide individual patients with the most appropriate treatment. This approach strongly depends on extensive characterization of individual tumors and their sensitivity to therapeutics. We previously have shown that our drug testing platform based on precision cut cancer tissue slices (PCCTS) is applicable to analyze individual responses of patients to defined compounds. In the context of immunotherapy, information on the abundance and activation of immune cells within individual tumors and the testing of immune-modulating compounds such as immune checkpoint modulators or bispecific antibodies gained in importance.

Material and Methods: Solid tumors were freshly collected and cut using a Krumdieck tissue slicer. PCCTS were cultured in 24-well plates with and without treatment. Patient blood was collected prior to surgery and patient derived, viable PBMCs were isolated. After 24 h, PCCTS were used for preparation of single cell suspension using the Miltenyi GentleMacs. Subsequently, Flow cytometry was performed on the Cyflow space instrument. In addition, proteins isolated from fresh frozen PCCTS were used for protein expression analysis by Simple Western Size (SWS). Supernatants were analyzed for cytokines by MSD technology.

Results: The PCCTS platform enabled the examination of effects of immune-modulatory compounds in a fully human, patient derived model as well as the immunological interaction with autologous, viable PBMCs. After treatment, the PCCTS used within the drug testing platform, exhibited distinct populations of immune cells analyzed by flow cytometry using a multiplex panel i.e. for Tregs (CD127, CD25, FoxP3). In the individual cases, different numbers of Tregs were identified within the immune cell populations depending on treatment. For the analysis of cytokines in supernatants, the pro-inflammatory panel of MSD had been used. Treated PCCTS showed increasing release of cytokines such as IFN-γ, TNF-α and IL-2 compared to untreated controls. Overall viability of the PCCTS was influenced by the co-cultivation of PCCTS with autologous PBMCs. Moreover, modulation of downstream signaling of therapeutic targets, including Ras/MAPK and PI3K/AKT pathways were observed by quantifying these targets using SWS technology.

Conclusions: Indivumed's PCCTS platform represents a unique opportunity to test immune-modulatory compounds in a fully human, patient derived model that is close to the *in vivo* situation. The PCCTS system conserves the cellular architecture and tumor microenvironment. Furthermore, this platform enables the investigation of effects and immunological interaction after addition of patient-derived PBMCs. In summary, Indivumed's PCCTS drug profiling platform is a useful tool for the preclinical investigation of immune-modulatory compounds, such as bispecific antibodies.

No conflict of interest

293 (PB-054) Poster

Establishment of ImmunoGraft® with human immune system (HIS) and PDX co-engraftment using NOG-EXL mice to evaluate checkpoint inhibitors

B. Verma[1], M. Mancini[1], A. Davies[1], D. Sidransky[2], A. Wesa[1], N. Goodwin[1]. *[1]Champions Oncology, Research and Development, Hackensack, USA; [2]Johns Hopkins University, Otolaryngology, Head and Neck Surgery, Baltimore, USA*

Background: The clinical success of immune checkpoint modulators and development of next-generation immune-oncology (IO) agents underscores the need for robust patient-derived models to evaluate novel therapeutics. The Champions ImmunoGraft® model utilizing humanized NOG mice is an innovative pre-clinical model for assessing the efficacy of IO agents against solid tumors. Improved immunodeficient mouse strains, such as triple transgenic NOG-EXL mice expressing huIL-3 and huGM-CSF, allows for superior HIS development. In this study, we compared human immune lineage development, tumor infiltrating leukocytes, as well as an assessment of tumor response to checkpoint inhibitor in this humanized mouse platform.

Materials and Methods: NOG and NOG-EXL mice were humanized by intravenous transplantation of cord blood (CB) CD34+ hematopoietic stem cells (HSC). Establishment of human immune components in peripheral blood was evaluated by flow cytometry across 9 CB donors. Next, NOG-EXL mice were humanized with CB-HSC from 2 donors, monitored for engraftment then implanted with a patient-derived xenograft (PDX) tissue from a non-small cell lung carcinoma (NSCLC) patient. Immune cell populations (T cells, macrophages, myeloid-derived suppressor cells (MDSC) and dendritic cells (DC)) were evaluated by flow cytometry at 4 and 6 weeks post-tumor implantation in various tissues. For nivolumab (a-PD-1; 10 mg/kg) treatment, dosing was initiated at a tumor volume of 80–150 mm^3. Responses were determined as changes in tumor volume.

Results: NOG-EXL mice engrafted more readily than NOG (1000% versus 80%, respectively), with greater than 25% huCD45+ cells in the periphery. Some donor to donor variability was observed in HIS engraftment in both mouse strains, although both strains permitted T cell, B cell and some myeloid cell development. T cell lineage development was equivalent in both strains at 12-weeks post–HSC transplantation. Improved myeloid lineage (CD33+) development was found in NOG-EXL animals. Macrophage, MDSC, as well as T cells were found in tumor infiltrates. Evaluation of PD-1 blockade in NSCLC PDX ImmunoGraft® in NOG-EXL mice indicated HIS donor variability impacted treatment efficacy in vivo.

Conclusions: While the ImmunoGraft® platform is reflective of human tumor microenvironment (both immune and tumor cells), mouse strains with improved HIS development promises to further enhance its value for screening IO therapies. We demonstrated that NOG-EXL mice allow better engraftment and HIS development compared to NOG. Evaluation of nivolumab efficacy in NSCLC PDX model in this enhanced ImmunoGraft® indicates that PD-1 blockade is feasible, and offers an opportunity to evaluate therapeutics targeting myeloid populations. The ImmunoGraft® has the potential to advance translational IO drug discovery and development.

Conflict of interest: Advisory Board: Neal Goodwin, David Sidransky. Board of Directors: David Sidransky.

294 (PB-055) Poster

Characterization of tumor infiltrating lymphocytes in a panel of patient-derived xenografts propagated in different humanized mouse models

E. Oswald[1], D. Lenhard[1], S. Schmitt[1], A. Edinger[1], A. Loehr[1], A.L. Peille[1], A. Behnke[1], V. Knauff[1], J. Schueler[1]. *[1]Charles River Discovery Services, Freiburg, Germany*

1: The field of cancer immunology is rapidly moving towards innovative therapeutic strategies. As a consequence, the need for robust and predictive preclinical platforms arises. The current project characterizes a panel of >20 patient-derived xenografts (PDX) in different humanized mouse models. This approach elucidates the advantages of PDX models in the presence of human immune cells. New insights of immune cell infiltration in the different tumor models and entities will help to identify the optimal pre-clinical model for specific therapies in the immune-oncology field.

2: >20 different PDX models were implanted subcutaneously (s.c.) into both flanks of 1-2 immune-deficient or humanized mice of different age, respectively. For immune cell substitution, T cells were expanded and T cells or fresh PBMCs were injected i.v. into tumor bearing mice. Body weights and tumor volumes were determined twice weekly. At termination immune cell infiltration was confirmed in peripheral blood, spleen, bone marrow and tumors by flow cytometry. Human immune cell subsets in spleen, liver and tumor tissue were assed via Immunohistochemistry. Human and mouse cytokines in serum of tumor-bearing mice were determined at the end of the experiment.

3: In the present study we compared tumor growth of our PDX model in the presence or absence of immune cells. The existence of immune cells, irrespective of mouse or human, had an impact on tumor growth behavior. In most models, the tumor growth was delayed in the presence of immune cells. Of note, a subset of models showed tumor growth inhibition after >20 days of human immune cell transfer. To clarify the role of different immune cell subtypes, we correlated immune cell infiltration in hematopoietic organs and tumor tissue with tumor take rate. The latter correlated negatively with the percentage of human and mouse immune cells in the tumor as well as peripheral blood. In line with this observation, cytokines indicating activation of the immune cells were upregulated in tumor-bearing immune-deficient mice, substituted with human PBMCs or expanded T cells. Of note, mice injected with human PBMCs showed the onset of GvHD earlier in the course of the experiment as mice engrafted with expanded T cells.

4: In conclusion, our study validates the PDX-based humanized mouse model more in detail. The different players within a tumor have significant impact on tumor growth behavior and biology. The design of future preclinical studies must take those characteristics into account by adapting the read-outs and the necessary control groups. In summary, the platform enables the interaction of tumor cells with human immune cells in a tumor microenvironment. This preclinical PDX based in vivo platform provides an innovative tool to support the development of new drugs targeting the host immune response.

No conflict of interest

295 (PB-056) Poster

Identification of the predictive biomarker signatures for TAK-931, a CDC7 inhibitor, in a preclinical phase 2-like study

H. Shin[1], E. Koenig[2], T. Nambu[3], J. Yu[4], M. Zhang[4], K. Kannan[5], E. Lightcap[6], A. Ohashi[7], H. Niu[8]. *[1]Millennium Pharmaceuticals, Inc., Data Science Institute, Cambridge, MA, USA; [2]Millennium Pharmaceuticals, Inc., Department of Oncology, Cambridge, MA, USA; [3]Takeda Pharmaceutical Co., Ltd., Oncology Drug Discovery Unit, Fujisawa, Kanagawa, Japan; [4]Millennium Pharmaceuticals, Inc., IO Biology, Cambridge, MA, USA; [5]Millennium Pharmaceuticals, Inc., Oncology Therapeutic Area Unit, Cambridge, MA, USA; [6]Millennium Pharmaceuticals, Inc., Oncology Drug Discovery Unit, Translational Sciences, Cambridge, MA, USA; [7]Millennium Pharmaceuticals, Inc., Oncology Drug Discovery Unit, Cambridge, MA, USA; [8]Millennium Pharmaceuticals, Inc., Translational and Clinical Biomarker, Cambridge, MA, USA*

Background: TAK-931, a highly specific CDC7 kinase inhibitor, is an investigational drug under clinical development in Ph1/2 studies for treatment of advanced cancers. To identify potential predictive biomarkers for patient stratification, we conducted a preclinical phase 2-like study in various patient-derived xenograft (PDX) models including colon (n = 40), pancreas (n = 24), lung (n = 23), and ovary (n = 3).

Material and Methods: Genomic and transcriptomic profiles of these human tumors were generated via next-generation sequencing. Antitumor efficacy of TAK-931 was evaluated in these PDX models. Tumor growth inhibition and mice survival were measured upon TAK-931 treatment. A correlative study was conducted to identify tumor genetic mutations associated with the antitumor efficacy of TAK-931.

Results: From the unbiased association analysis, specific oncogenic gene mutations were found to be significantly correlated with response to TAK-931. For example, *TP53* mutations showed significant correlation in all tumor models analyzed ($p \leq 0.0009$). To identify which cancer indications are enriched with these biomarker signatures, we performed a genome-wide association analysis using the patient genomic data in The Cancer Genome Atlas (TCGA). Specific tumor types were found enriched with these biomarkers in >50% patients, which guides the cancer indication selection for clinical investigation of TAK-931.

Conclusions: The identification of predictive genomic biomarkers in preclinical PDX followed by *in silico* interrogation of public TCGA database may allow enrichment for patients who would most likely benefit from TAK-931 treatment and allow for an expedited path to early clinical proof of concept.

Conflict of interest: Other Substantive Relationships: Hyunjin Shin, Erik Koenig, Jie Yu, Mengkun Zhang, Karuppiah Kannan, Eric Lightcap, Akihiro Ohashi, and Huifeng Niu disclose that they are employees of Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. Tadahiro Nambu is an employee of Takeda Pharmaceutical Co., Ltd.

296 (PB-057) Poster

Novel prostate cancer models for immuno-oncology and immunotherapy research

R. Barbosa Marques[1], M. van Gelder[1], P. van Duijn[1], C. de Ridder[1], J. Trapman[2], R. Debets[3], W. van Weerden[1]. [1]*ErasmusMC, JNI, Urology, Rotterdam, Netherlands;* [2]*ErasmusMC, JNI, Pathology, Rotterdam, Netherlands;* [3]*ErasmusMC, JNI, Medical Oncology, Rotterdam, Netherlands*

Background: Immunotherapy is emerging as a promising treatment option in oncology. So far, clinical trials have shown significant responses in a subset of prostate cancer patients. To improve success rates, current research focusses on combination therapies, novel immune targets, as well as potential biomarkers that can help in patient selection. However, appropriate in vivo models that represent anti-tumor immune responses in prostate cancer are scarce.

Material and method: To investigate the mechanisms of tumor growth and test prostate cancer targeted therapies in the context of a functional immune system we established two novel animal model systems. The syngeneic Pten knockout mouse model of prostate cancer (MuCaP) consists of four syngeneic cell lines, injected subcutaneously in immunocompetent FVB male mice. Tumor take and growth was followed over time with calipers. Tumors and blood were collected at sacrifice. CD3-positive cells were counted by immunohistochemistry and T-cell profile was assessed by qPCR. To develop a humanized prostate cancer model, human immune reconstituted mice (HuNOG, Taconic, Denmark) were inoculated subcutaneously with PC339 and PC346C patient-derived xenograft (PDX) lines. Androgen deprivation was performed through surgical castration, tumor growth was monitored by calipers and blood was sampled at various time points for immune cell profiling. Blood and tumor immunoprofiles were analyzed by flow cytometry and qPCR.

Results: Two of the syngeneic MuCaP tumor lines showed aggressive tumor growth, while the two other tumor lines hardly grew and could be considered as indolent. There was a clear association between tumor aggressiveness and involvement of cytotoxic T-cells: aggressive tumors showed low T-cell infiltration, whereas indolent tumors showed increased T-cell infiltration with a characteristic gene signature of effector cytotoxic T-cells (CD8+/PD1+/IFNγ+). To establish a humanized model for the testing of immunomodulatory therapies, we transplanted human immune reconstituted HuNOG mice with 2 prostate cancer PDX lines, which resulted in >90% take rate and consistent tumor growth, comparable to that observed in athymic nude mice. Flow cytometry of peripheral blood revealed significant reconstitution with human immune cells (>80% hCD45+ cells), including CD20+ B-cells, CD4+ and CD8+ T-cells. QPCR analysis showed expression of these markers in the tumor tissue, which were increased after castration, indicative of tumor infiltration by the human immune cells.

Conclusions: Here we present two novel in vivo models that allow evaluation of novel immune-targeted therapies in prostate cancer.

No conflict of interest

297 (PB-058) Poster

DNA repair deficiency sensitizes lung cancer cells to NAD+ biosynthesis blockade

M. Touat[1], T. Sourisseau[1], N. Dorvault[2], R.M. Chabanon[2], M. Garrido[2], D. Morel[2], D. Krastev[3], L. Bigot[1], J. Adam[1], J. Frankum[3], A. Sarasin[4], K. Olaussen[1], L. Friboulet[1], F. Bouillaud[5], G. Pierron[6], A. Ashworth[7], A. Lombès[5], C. Lord[3], J.C. Soria[1], S. Postel-Vinay[2]. [1]*Gustave Roussy, Inserm U981, Villejuif, France;* [2]*Gustave Roussy, Inserm U981 & ATIP-Avenir Team, Villejuif, France;* [3]*The Institute of Cancer Research, The CRUK Gene Function Laboratory and Breast Cancer Now Research Centre, London, United Kingdom;* [4]*Gustave Roussy, CNRS UMR-8200, Laboratory of Genetic Stability and Oncogenesis, Villejuif, France;* [5]*Institut Cochin, Inserm U1016, CNRS UMR 8104, Paris, France;* [6]*Gustave Roussy, CNRS UMR-9196, Functional Organization of the Cell, Villejuif, France;* [7]*UCSF, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, USA*

Background: Synthetic lethality is a potent mechanism-based approach exploiting specific genetic vulnerabilities of cancer cells. ERCC1, a key protein of the nucleotide excision repair pathway, is frequently deficient in non-small cell lung cancers (NSCLC), thereby representing an attractive target for synthetic lethal approaches in this disease.

Material and Methods: Using large-scale proteomic (SILAC) and metabolomic (LC/GC-MS, and LC-QTOF) profiling, we compared in-house generated isogenic models of ERCC1-proficient versus ERCC1-deficient NSCLC. Significant hits were revalidated using complementary assays including western blotting, gene expression profiling and immuno-histochemistry (IHC) on tumor series. Sensitivity to selective nicotinamide phosphoribosyltransferase (NAMPT) inhibitors (FK866, GNE-617) was assessed both in vitro and in vivo. Mitochondrial structure and function were characterized using electron microscopy, high-resolution respirometry, and respiratory chain spectrophotometric assays. Effects of acute and long-term ERCC1 silencing on DNA repair capabilities and NAMPT expression were evaluated using ERCC1 siRNA and shRNA silencing.

Results: We found marked metabolic rewiring of ERCC1-deficient populations, including decreased NAD+ levels, reduced expression of the NAD+ biosynthetic enzyme NAMPT and of the mitochondrial respiratory chain cytochrome c oxidase subunits COX4I1, COX5B, and COX6C. NAMPT protein decrease, a potentially targetable node of ERCC1-deficient cells, was validated by IHC in an independent set of ERCC1-deficient human NSCLC. Exposure of ERCC1-deficient cells to NAMPT inhibitors showed profound and selective sensitivity *in vitro* and *in vivo*, which was rescued by the addition of nicotinamide mononucleotide (the direct product of NAMPT enzymatic activity) or by re-introducing the functional ERCC1 isoform. Further metabolomic characterization showed significant mitochondrial defects in ERCC1-deficient populations, including abnormal mitochondrial structure, decreased respiratory capacity with mitochondrial respiratory chain complex IV deficiency, and alterations in the TCA cycle and NAD+ biosynthesis pathway. In functional studies evaluating the consequences of a gradual loss of ERCC1 over time, acute ERCC1 defect lead to increased ADP-ribosylation activity and NAMPT levels, whereas chronic ERCC1 defect resulted in decreased NAD+ levels, NAMPT expression and ADP-ribosylation capacities. These findings suggest a model for ERCC1-deficient NSCLC sensitivity to NAMPT inhibitors in which NAD+ is the central sensor of fitness.

Conclusions: This study opens novel therapeutic opportunities that exploit a yet undescribed nuclear – mitochondrial synthetic lethal relationship in NSCLC cells. Our findings provide preclinical rationale for the clinical evaluation of novel NAMPT inhibitors in patients with ERCC1-deficient NSCLC.

No conflict of interest

299 (PB-060) Poster

Density-dependent regulation of cell growth by KIF5B-RET fusion gene

M. Lee[1], J.Y. Shin[1], J.O. Kim[1], M.Y. Kim[1], S.R. Kim[2], J.H. Kang[2]. [1]*Cancer Research Institute, College of Medicine, The Catholic University of Korea, Laboratory of Medical Oncology, Seoul, South Korea;* [2]*Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea 222 Banpodaero Seochogu, Division of Medical Oncology, Department of Internal Medicine, Seoul, South Korea*

Background: RET rearrangement is detected with 1–2% of frequency in lung adenocarcinoma. The KIF5B-RET fusion gene has poor response to

RET tyrosine kinase inhibitors. Hippo and TGF-β pathways are involved in signaling process which is related to cancer cell density. They also affect the regulation of cell growth and proliferation and tumor suppression. We investigated the role of TGF-β on cell growth and proliferation in KIF5B-RET transfected cells.

Methods: We evaluated anti-proliferative activity of TGF-β/smad inhibitor in HEK293T cells transfected with KIF5B-RET fusion gene and empty vector using CCK8 assay and colony formation assay. We investigated protein expressions of signal molecules of hippo and TGF-β pathway with cell density using western blot; the signal molecules were LATS1, pYAP, MST1/2, SAV1, YAP/TAZ, MOB1, TGFβ, Smad2/3 and Smad4.

Results: The cell growth of KIF5B-RET transfected cells was delayed compared to the empty vector transfected cells. The TGF-β protein expression was increased in the KIF5B-RET transfected cells.

In the KIF5B-RET transfected cells, TGF-β expression in the total protein was decreased with high cell density, but not changed in the nuclear protein. The expression of Smad2/3 and smad4 proteins was higher than that of empty vector cells, and increased in both total and nuclear proteins with high cell density. The expressions of hippo signal molecules were mostly expressed in nuclear proteins. With low cell density, LATS1 was highly expressed in cytoplasm and nuclear protein. YAP/TAZ expression was observed in cytoplasmic protein with low cell density and in nuclear protein with high cell density.

The anti-proliferative activity of TGF-β/smad inhibitor was similar between empty vector cells (61.5 ± 1.2 μM) and KIF5B-RET transfected cells (68.0 ± 3.2 μM). The colony numbers of empty vector cells was increased between 2 days to 7 days of incubation with TGF-β/smad inhibitor (145.3 ± 0.4 vs. 162.6 ± 0.3 colonies/well, $P < 0.05$). However, in KIF5B-RET transfected cells, no more increase of colony numbers was observed in the period of 7 days treatment of TGF-β/smad inhibitor (137.7 ± 0.7 colonies/well).

Conclusion: In KIF5B-RET transfected cells, TGF-β expression was increased and increased expressions of smad2/3 and smad4 in nucleus were consistently observed as cell density increased. With high cell density, YAP/TAZ affecting cell growth and proliferation migrated from cytoplasm into the nucleus. A TGF-β/smad inhibitor significantly delayed the cell growth compared to empty vector cells.

No conflict of interest

300 (PB-061) Poster

Preclinal models of patient-derived xenografts on humanized mice for translational immuno-oncology research

M. Stecklum[1], A. Wulf-Goldenberg[1], M. Paterka[1], B. Brzezicha[1], I. Fichtner[1], J. Hoffmann[1]. [1]*EPO -Experimental Pharmacology & Oncology Berlin-Buch GmbH, Humanized Mice, Berlin, Germany*

Background: The preclinical evaluation of novel immune checkpoint modulators is dependent on models with functional human immune cells. In previous experiments, we have demonstrated, that hematopoietic stem cells (HSC) can proliferate and differentiate in vivo to form a functional humanized immune system with T cells, B cells, NK cells, monocytes, and dendritic cells. Furthermore, we determined PD-L1 expression as a predictive marker and target for immunotherapy on different patient-derived xenografts (PDX). By transplantation of PDX we successfully generated a fully human tumor-immune-cell model in mice. Finally, we evaluated the functionality of the model by the treatment with the checkpoint inhibitors like Ipilimumab and Nivolumab. In parallel we investigated the functionality of the human immune cells and evaluated concepts for combination therapies i.e. with chemotherapy or radiation.

Methods: HSC were transplanted i.v. into immunodeficient mice. Engraftment was monitored by FACS analysis for human immune cells in the blood. PD-L1 expression was determined by FACS and immunohistochemistry (IHC). PDX from over 10 different entities (i.e. melanoma, neuroblastoma, head&neck, colon, or renal cancer) were transplanted on humanized mice and treated with Ipilimumab and Nivolumab alone or in combination with radiation. Blood and tumor samples were analysed by FACS and IHC for immune cell infiltration and activation.

Results: The transplanted HSC showed engraftment in mice with proliferation and differentiation and established a functional human immune system. 14 weeks after HSC inoculation up to 20% of the human immune cells in the blood were T-cells, characterized by a high PD-1 expression. So far we have transplanted more than 40 different PDX from 10 different tumor entities on humanized mice. Most of investigated PDX (>70%) successfully engrafted on humanized mice and showed no significant difference in tumor growth compared to growth on non-humanized mice. However for some PDX we observed a delayed tumor growth or a complete rejection. Engraftment delay seems to correlate with the PD-L1 expression of PDX (the higher PD-L1, then the higher growth delay). Furthermore, these results demonstrate the functionality of the engrafted human immune cells against some PDX. Treatment with Ipilimumab or Nivolumab alone or in combination led to a minor tumor growth delay and an increased percentage of T-cells in the blood and in the tumor. Response to checkpoint inhibitors showed a correlation to innate immune response and PD-L1 expression of PDX and could further increased by combination with radiotherapy.

Conclusions: Our humanized immune-PDX models enable appropriate preclinical translational research on tumor immune biology and the evaluation of new therapies and combinations, as well as the identification and validation of biomarkers for immune therapy.

No conflict of interest

301 (PB-062) Poster

Preclinical evaluation of novel treatment strategies in patient-derived xenograft (PDX) models of pancreatic cancer

D. Behrens[1], R.T. Lawlor[2], C. Heeschen[3], M. Buchholz[4], J. Siveke[5], A. Wengner[6], A. Saluja[7], B. Buettner[8], J. Hoffmann[8]. [1]*EPO, Experimental Pharmacology & Oncology Berlin-Buch GmbH, D82, Berlin, Germany;* [2]*ARC-NET University of Verona, Policlinico G.B. Rossi, Verona, Italy;* [3]*QMUL, Barts Cancer Institute, Centre for Stem Cells in Cancer & Ageing, London, United Kingdom;* [4]*Philipps University Marburg, Center for Tumor Biology and Immunology, Marburg, Germany;* [5]*DKFZ, University Hospital Essen, West German Cancer Center, Essen, Germany;* [6]*Bayer AG, Oncology Research, Berlin, Germany;* [7]*University of Miami, Sylvester Pancreatic Research Institute and Department of Surgery, Coral Gables, USA;* [8]*EPO, Experimental Pharmacology & Oncology Berlin-Buch GmbH, Building H82, Berlin, Germany*

Background: Pancreatic cancer (PC) remains a lethal disease with only 3–8% of patients surviving 5 years after diagnosis of the tumor (WHO, 2012). Within the EU project "CAM-PaC" a comprehensive panel of thirty patient-derived PC xenografts (PDX) was established and used for the efficacy screening of new therapeutic options. Within this study, responders to the MPS-1 inhibitor BAY1161909, the Super Enhancer disrupting agent Minnelide and the MEK inhibitor Trametinib were identified and analyzed for potential biomarkers.

Methods: Patient tumors were collected during surgery and circulating tumorigenic cancer stem cells were isolated from the peripheral blood using VAR2CSA-coated magnetic beads. Both were transplanted subcutaneously into NOD/SCID/IL2y^{-} mice and propagated in NMRI:nu/nu mice after engraftment. These were morphologically and molecularly characterized by histopathological revision and with NGS panels, designed based on pathway aggregated genes identified with the International Cancer consortium (described by Bailey et al., Nature 531, 2016). Standard drugs were applied using clinically relevant dosages and schedule. MPS-1 inhibitor BAY1161909 was given in monotherapy and in combination with Abraxane. Minnelide (MTD) was applied second line after three cycles of chemotherapy (Cisplatin, Abraxane, Gemcitabine) and Trametinib was tested as monotherapy.

Results: All PDX correlated with histopathological and molecular characteristics of patient tumours. BAY1161909 monotherapy showed moderate anti-tumor efficacy with an average tumor growth inhibition of 30% ($p > 0.05$). However, tumor relapse after the end of chemotherapy was delayed in mice treated with the combination of BAY1161909 and Abraxane compared to Abraxane alone. 14 out of 28 PDX models tested to date were identified as responders (tumor growth inhibition >50%) to Minnelide and 5 out of 16 to Trametinib. While Minnelide induced tumor growth inhibition above 80% in 32% of the models, Trametinib achieved the same efficacy in only 6% of the tested PDX models.

Conclusion: The described PDX panel clearly reflects clinical situation of pancreatic cancer due to their histologic growth and detection of inherent and acquired treatment resistance as well as recurrent disease. In a few cases, the tested drugs induced complete remissions. We are currently analyzing the molecular data to determine response markers. Our approach may offer personalized treatment options for PC patients.

No conflict of interest

302 (PB-063) Poster

Systemic and local syngeneic bone metastasis models for immuno-oncology drug development

T. Kähkönen[1], M.I. Suominen[1], J. Mäki-Jouppila[1], J.M. Halleen[1], A. Scholz[2], J. Bernoulli[1]. [1]*Pharmatest Services, Oncology, Turku, Finland;* [2]*Bayer AG, Bayer, Berlin, Germany*

Bone metastases count 30–70% of metastases in the most common cancers including breast, lung and bladder cancer, and multiple myeloma. Despite recent progress in cancer treatment bone metastases remain incurable.

However, novel therapies including immunotherapies have potential to cure bone metastatic disease. The aim of this study was to establish novel syngeneic models with a focus on bone metastasis that could be used in preclinical efficacy studies.

Syngeneic models were established for breast (4T1-GFP), bladder (MBT-2) and lung cancer (KLN-205), and multiple myeloma (5TGM1). The cells were inoculated into systemic circulation (4T1 intracardially or 5TGM1 into the tail vein) or into the bone marrow (MBT-2 and KLN-205 intratibially). In the 4T1 model, tumor growth was followed by GFP imaging *ex vivo* and in the 5TGM1 model by measuring serum IgG2b paraprotein and TRACP5b (marker of bone-resorbing osteoclasts) levels during the study. Tumor-induced bone changes were followed by X-ray imaging. At sacrifice, hind limbs were collected and analyzed by histology. The effects of standard-of-care (SOC) compounds were assessed in the 4T1 (cyclophosphamide, 100 mg/kg or zoledronic acid, 0.1 mg/kg) and 5TGM1 (bortezomib, 1 mg/kg) models. Effect of anti-PD-1 (200 μg/dose) was evaluated in the MBT-2 model.

In the 4T1 model, osteolytic bone lesions were formed within 13 days. The lesions were imaged by X-ray, and tumor burden by GFP. About 50% of the mice had metastases in lungs, ovaries, kidneys and adrenal glands based on GFP imaging. Cyclophosphamide decreased tumor burden and the area of osteolytic bone lesions. Zoledronic acid decreased osteolytic lesion area but had no effect on tumor burden. Osteolytic lesions were observed in the 5TGM1 model, and the study was ended at day 35. Soft tissue metastases were observed in ovaries, kidneys and adrenal glands in about 30% of the mice. Bortezomib decreased serum paraprotein and TRACP5b levels compared to the control group. In both 4T1 and 5TGM1 models, cachexia and paraplegia were occasionally observed. In the intratibial MBT-2 and KLN-205 models, large osteolytic lesions were observed within 28 days from cancer cell inoculation, and in the KLN-205 model also lung metastasis were observed. Anti-PD-1 treatment decreased osteolytic tumor area in the MBT-2 model. Histological tumors were confirmed in the bone marrow of hind limbs in all models.

A high incidence of bone metastases was achieved in all models. The use of systemic models allows studying the effects of test compounds in prevention or treatment of metastasis. Intratibial models can be used when the primary interest is in cancer-induced changes in bone. Mimicking the clinical situation, none of the SOC compounds could prevent tumor growth completely, and therefore combination therapies are warranted for better overall efficacy.

No conflict of interest

304 (PB-065) Poster

HuPharm: a standardized interactive data analysis and reporting platform for preclinical cancer pharmacology

B. Mao[1], J. Li[1], S. Guo[1], H. Li[2]. [1]*Crown Bioscience Inc., Systems Biology, Taicang, China;* [2]*Crown Bioscience Inc., Global Scientific Research and Innovations, San Diego, USA*

Background: One of the essential components of any anti-cancer drug development is preclinical cancer pharmacology study that is commonly consisted of design, planning, execution, data analysis and reporting. The reliability of the scientific conclusions based on proper design/data integrity is the foundation to the decision to advance agent, or not, to next step human clinical trials. In addition, a standard data analysis platform not only saves labor and reduces errors, but also helps to achieve objective interpretations and comparisons for data generated from different laboratories and/or study directors. This report aims at developing a web platform (HuPharm™) that automates statistical pipelines and report generation for standard preclinical anti-cancer pharmacology, with intuitive and interactive interface that enables cancer pharmacologists, often non-statisticians, effectively handling data validation, exploratory data analysis and statistical analysis.

Material and Methods: We used RStudio's Shiny package to build an interactive web app (HuPharm™), and we used Knitr and Rmarkdown packages to generate a highly informative study report. Parametric and non-parametric statistics is used to analyze pharmacology data from two or more groups by a fully automated workflow.

Results: Based upon user inputs, HuPharm™ can dynamically generate a series of tables and figures to facilitate preclinical data analysis, such as tumor volume summary statistic table and plots, tumor growth curves, omnibus group comparison plots, all pairwise group comparison table and post hoc analysis results table. Data, tables and figures can be downloaded in multiple formats. In addition, HuPharm™ can generate an all-in-one study report including all figures and tables.

Conclusions: HuPharm™ is a convenient tool to aid researchers in the field of preclinical tumor trials for study design and data analysis. It likely adds rigor and objectivity into preclinical cancer pharmacology studies, while saving time and labor.

No conflict of interest

305 (PB-066) Poster

Evaluation of categorical response methods in predicting drug responses in transplanted tumor models

X. Jiang[1], H. Li[2], S. Guo[1]. [1]*Crown Bioscience Inc., Systems Biology, Taicang, China;* [2]*Crown Bioscience Inc., Global Scientific Research and Innovations, San Diego, USA*

Background: Transplanted tumor mouse models are widely used in preclinical oncology studies to evaluate efficacy of therapeutics. Recently, prediction drug accuracy using a single mouse per group in population based studies has been systematically assessed, but only on patient-derived xenograft models (PDXs). Systematic research of the same on cell line derived xenografts (CDXs) and syngeneic tumor models (homografts) are lacking.

Material and Methods: We evaluated three methods that all classify drug response into several categories for their accuracy in predicting drug response among one or multiple mice for all three transplanted tumor models (PDX, CDX and syngeneic) using tumor volume data for >30,000 mice. These methods classified the drug responses into three, four, or six categories. We collected studies with eight or more mice and assigned each mouse a categorical response. A most frequent response was designated as the "Majority Response." We then counted the number of mice whose response agreed with or deviated from the Majority Response. The response concordance/deviation profiles for all studies were then used to quantify the prediction accuracy of those methods. To investigate how the addition of mice improves prediction accuracy, we randomly sampled n (n = 1 to 7) mice from a study and obtained the median response by ranking the n responses, which was then compared to the Majority Response of the full study, concordance/deviation was then computed. The analysis was applied to PDXs, CDXs, and syngeneic models to obtain and compare their response and mouse number patterns.

Results: By examining the very large efficacy datasets we have accumulated over the decade, we found that when the Majority Response is at the two ends of efficacy spectrum, being complete response (CR) and progressive disease (PD), a single mouse response agrees more often with the Majority Response than responses in between (*e.g.*, stable disease or SD, partial response PR) for all three methods. Such agreement varies by tumor model platforms, where syngeneic models exhibit larger variations in their drug response. Further, increasing mouse numbers improves prediction accuracy but such accuracy varies greatly among response categories. Quantitative results are presented in graphs and tables to illustrate the above observations.

Conclusions: Large preclinical anti-cancer pharmacology datasets can be used to derive the technical parameters that help to guide study design and data analysis for better assessing drug efficacy.

No conflict of interest

306 (PB-067) Poster

Cathepsins K and X as possible biomarkers in glioblastoma

B. Breznik[1], A. Porčnik[2], M. Koprivnikar Krajnc[1], J. Kos[3], C.J.F. Van Noorden[1], T.T. Lah[1]. [1]*National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia;* [2]*University Medical Centre Ljubljana, Department of Neurosurgery, Ljubljana, Slovenia;* [3]*Faculty of Pharmacy, University of Ljubljana, Department of Pharmaceutical Biology, Ljubljana, Slovenia*

Background: Glioblastoma, the most malignant brain tumor, is characterized by the single cell invasion of cancer cells into brain parenchyma, preventing complete tumor resection by surgery. Cysteine proteases cathepsins B, S and L, which are involved in malignant progression, have been found to be overexpressed in glioblastoma. Here, we focused on cathepsins K and X, which have been poorly investigated in glioblastoma progression, but have an important role in other types of cancers.

Material and Methods: Protein expression of cathepsins K and X in glioblastoma samples was localized and semi-quantified using immunohistochemistry. Kaplan-Maier survival analysis was performed to evaluate the predictive value of cathepsin K and X expression at the protein levels. On the other hand, gene expression of cathepsins K and X were correlated with glioma grade, glioblastoma subtype and overall survival of glioblastoma patients using publically-available transcriptomic datasets.

Results: With respect to localization, we have shown that cathepsin K and X proteins were clustered in CD133-, SDF-1α- and CD68-positive glioblastoma stem cell niche regions around arterioles and gene expression of cathepsins K and X correlated with gene expression of niche markers. Glioblastoma stem cell niches are the regions within GBM tissue, where the therapy-resistant and highly-malignant glioblastoma stem cells are protected from therapy and immune system. Protein expression of cathepsins K and X

was highly and heterogeneously expressed in the glioblastoma samples. Gene expression of cathepsins K and X increased with glioma grade, in particular in the mesenchymal glioblastoma subtype. High cathepsin X, mRNA expression but not protein expression correlated with poor patient survival. However, cathepsin K expression at mRNA or protein level and glioblastoma patient survival were not found.

Conclusions: Presence of both cathepsins in glioblastoma stem cell niche regions indicates specific roles in glioblastoma stem cell regulation. Cathepsins K and X are highly expressed in glioblastoma tissue and cathepsin X gene expression level has predictive value for patients with glioblastoma. The results have to be confirmed in further prospective studies.

No conflict of interest

307 (PB-068) Poster

Transmembrane protein CD9 as a biomarker in different subtypes of glioblastoma

M. Novak[1], B. Breznik[1], B. Majc[1], N. Podergajs[1], M. Nikšić Žakelj[2], A. Porčnik[3], J. Mlakar[4], T.T. Lah[1]. *[1]National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia; [2]Institute of Oncology Ljubljana, Department of Experimental Oncology, Ljubljana, Slovenia; [3]University Clinical Centre Ljubljana, Department of Neurosurgery, Ljubljana, Slovenia; [4]Institute of Pathology, Faculty of Medicine, Ljubljana, Slovenia*

Background: The tetraspanin CD9 has been shown to be involved in various cellular activities, including tumor cell invasion, apoptosis and resistance to chemotherapy. Based on public REMBRANDT database for brain tumors, CD9 high expression was linked with shorter patient survival. Glioblastoma (GBM) consists of heterogeneous cell types including a subset of stem cell-like cells (GSC) presumably sustaining tumor growth. Silencing of CD9 in glioblastoma cell lines led to decreased cell proliferation, survival, invasion and self-renewal ability. It was enriched across a larger set of GSCs, but not in the normal brain counterparts, thus CD9 may be evaluated as a GSC-biomarker.

Material and Methods: Primary GBM and GSC cell lines were established from a set of patient GBM tumors of different subtypes, from a joint bio-bank of TRANS-GLIOMA project partners (Slovenia-Italy) called GLIOBANK. Quiagen extraction kit was used to isolate mRNA and proteins from GBM tumors, GBM and GSC cell lines. GBM subtypes (proneural (PN), mesenchymal (MES), calssical and neural subtype), were defined using 12 fingerprint signature genes (*COL1A2*, *COL1A1*, *TGFBI*, *THBS1*, *DAB2*, *S100A4*, *P2RX7*, *STMN4*, *SOX10*, *ERBB3*, *ACSBG1* and *KCNF1*) using RT-qPCR according to *Behnan* (*2017*) and *Breznik* (*2017*). GSC cells were irradiated (IR), using Gulmay 225 X-ray system. Lentiviral shRNA method was used to silenced CD9. Clonogenic assay and immunohistochemistry were done according to the standard protocol.

Results: CD9 is differently expressed in a cohort of tumor tissue samples of different subtypes and in GBM and GSC cell lines. It is up-regulated in GSC cell lines isolated from tumor tissue samples and in GBM MES subtype. When CD9 was silenced in GSC cells, the one that were irradiated with lower doses had bigger effect on plating efficiency. When using higher irradiation doses, CD9 seemed to act as a tumor suppressor.

Conclusions: Although molecular mechanism of CD9 activity were proposed (*Shih, 2017*), its role in GSC radioresistance remains elusive. Screening for CD9 expression in cohort of patient tumor samples and cell lines, shows different expression pattern in different GBM subytypes. Data provides an opportunity to explore/identify CD9 as a novel, precision biomarker/therapeutics for GBM.

No conflict of interest

308 (PB-069) Poster

Exploiting the spectrum of BRCA-associated Pancreatic Ductal Adenocarcinoma

M. Raitses[1], C. Stossel[1], D. Atias[1], S. Halperin[1], Y. Glick-Gorman[1], J.M. Wilson[2], R.E. Denroche[2], I. Lungu[2], S. Gallinger[3], T. Golan[1]. *[1]Sheba Medical Center, Oncology Institute, Ramat Gan, Israel; [2]Ontario Institute for Cancer Research, PanCuRx, Translational Research Initiative in Pancreatic Cancer, Toronto, Canada; [3]Ontario Institute for Cancer Research, Department of Surgery, University Health Network, Toronto, Canada*

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. BRCA-associated PDAC comprises a clinically relevant subtype, as it is a most studied PDAC subpopulation. A portion of these patients is highly susceptible to DNA damaging therapeutics (DDR) including PARP inhibition (PARPi), however, responses are heterogeneous and clinical resistance evolves. This differential response among germline (GL) BRCA mutations carriers is suggestive of additional features underlying the nature of response/resistance to treatment. In the whole genome sequence (WGS) analysis on patient-derived xenografts (PDX) established from BRCA-associated PDAC patients, we have shown correlation between genomic subtype and response to treatment in pre-clinical model and patients' clinical scenario. Recent studies in ovarian carcinoma patients have suggested that the absence of locus-specific loss of heterozygosity (LOH) may be a biomarker of primary resistance to DDR/PARPi.

The goal of this study was to verify the established genomic classification of BRCA-associated PDAC tumors and to investigate additional genomic features predictive of response.

Methods: We have performed an analysis on clinical data of PDAC patients with GL/somatic mutations in BRCA1/2 genes diagnosed at Sheba (Israel) and OICR (Canada) in conjunction with comprehensive WGS analysis of corresponding tumor/PDX samples. We analyzed both primary resected tumors along with PDXs established from primary/metastatic lesions and performed classification into genomic subtypes. Additionally, loss of heterozygosity (LOH) was tested.

Results: Overall, 63 cases of BRCA-associated PDAC were identified in both institutions (July 2008 – Feb 2018). Sixty-two were carriers of GL BRCA1/2 mutations and one harbored somatic mutation in BRCA2. Stage at diagnosis was I/II (n = 16) and III/IV (n = 47); a recurrence of disease was observed in the majority (10/16) of patients with early stage. More than 30% are alive with disease and responding to platinum/PARPi for >12 months, three patients demonstrate a complete response >3 years. A subset of patients (~25%) demonstrated resistance and limited response and died $\leq$9 month from diagnosis/progression.

Preliminary WGS data analysis of 5 PDXs and 13 primary resected tumors showed strong correlation between genomic subtype and overall survival. LOH status showed correlation with unstable genomes in 9/12 cases. Additionally, 4/6 stage I/II patients who did not recur, display the unstable genome subtype.

Conclusion: Analysis of 18 BRCA-associated PDAC cases analyzed by WGS presented here, demonstrated correlation between unstable genomic subtype and response to treatment with DDR agents/PARPi. Genomic data to be extracted from WGS and LOH analyses of additional samples will provide more insight into understanding the spectrum of response to treatment.

No conflict of interest

309 (PB-070) Poster

The in vivo screen: a format allowing the identification of sensitive PDX models

N. Caushaj[1], T. Metz[1], J. Schmollinger[1], A.L. Peille[1], S. Gorynia[1]. *[1]Charles River Discovery Research Services, GmbH, Freiburg, Germany*

Background: Patient-derived tumor xenografts (PDXs) represent the gold standard for preclinical efficacy testing of anti-cancer agents. Two main assay formats are commonly used for in vivo efficacy tests: the standard format with group sizes of 6 to 12 mice and the single mouse trial (SMT) format with one mouse per model per treatment. The former approach is typically used for tests with a small number of tumor models whereas the latter is used for large numbers of PDX models. While the standard PDX format can demonstrate statistically significant efficacy of a given treatment in a pre-selected PDX model, the single mouse trial allows determination of response rates at the population level. The disadvantages of both approaches are that the former, because of the required resources, is not suitable for testing a larger number of models while the latter is not fully reliable when it comes to the sensitivity of individual tumor models. We developed an alternative third test format, the in vivo screen, with a group size of three tumor-bearing mice. This group size is still small enough to allow the screening of a large number of models but at the same time increases the confidence in the results obtained for individual models.

Material and Methods: For in vivo screen experiments immunocompromised mice (NMRI nu/nu) bearing a subcutaneous tumor of 50–250 mm^3 were used. Per treatment arm three animals were dosed with either the vehicle only or an investigative compound. Tumor volumes were determined twice weekly by caliper measurements. Tumor models used for the in vivo screen were either chosen randomly or pre-selected, e.g. based on specific molecular characteristics.

Results: Our results suggest that three animals per treatment arm are sufficient to accurately identify drug sensitive models. Compared with the single mouse trial, which focuses on a population response, the in vivo screen serves as a rapid and reliable tool to identify responsive models within a large PDX collection. The data show that with a heterogeneously growing model the risk of one tumor exhibiting aberrant growth is comparatively high. Using three tumor-bearing animals per group reduces the impact of aberrant tumor growth and minimizes the risk of false positive or negative results.

Conclusions: Our findings demonstrate that the in vivo screen is a cost- and time-effective approach to identify sensitive models within large tumor panels. The identified sensitive models can be used for follow-up experiments using the standard in vivo format and the data generated can also be used for biomarker identification.

No conflict of interest

310 (PB-071) Poster

Development of a disseminated AML mouse model to evaluate the therapeutic activity of immune checkpoint inhibitors using bioluminescence imaging

V. Mahajan[1], T. Bui[1], I. Belle[1], L. Usher[1], E. Rainbolt[1], S. Kidder[1], D. Harris[1]. [1]*Charles River Laboratories, Discovery Services, Morrisville, USA*

Acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) are among the most common leukemia subtypes diagnosed in adults. Treatment options include chemotherapy, stem cell transplantation and more recently the use of checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4 to enhance antitumor responses. In this study we developed a disseminated AML disease model using PD-L1$^+$, C1498 tumor cells to evaluate the therapeutic response to immune checkpoint inhibitors, PD-1, PD-L1 and CTLA-4 and to examine the effect of treatment on infiltration of immune cell populations in selected tissues. C1498 cells were transduced with a lentivirus vector construct containing luciferase and GFP reporter genes. Upon establishment of a clonal population with stable reporter expression, C1498-luc3-gfp expressing cells were implanted intravenously into syngeneic albino C57BL/6 mice and growth of the leukemic tumor cells was monitored via in vivo bioluminescence imaging (BLI) using IVIS Spectrum CT®. In vehicle-treated animals tumor growth was progressive and most animals reached endpoint within 30 days. Luciferase expression was widespread with metastatic development in peripheral organs and tissues. An efficacious response to therapy directed against the PD-1/PD-L1 axis was observed, with anti-PD-L1 showing greater overall efficacy than anti-PD-1. However, combination of anti-PD-1 and not anti-PDL-1 with anti-CTLA-4 further improved the therapeutic efficacy as measured by prolonged survival and reduced tumor growth. To understand how these immunotherapies function in suppressing tumor growth we are analyzing the composition and phenotype of tumors and immune cell infiltrates by flow cytometry, histology, and mRNA transcriptome array analysis. In summary, comprehensive immunological characterization of the C1498 model revealed the importance of PD1/PD-L1 pathway in immune evasion and provides a rationale for therapeutic intervention with checkpoint blockade.

No conflict of interest

311 (PB-072) Poster

Predicting PARPi sensitivity in patient derived ex vivo 3D tumour cultures

S. Basten[1], J. Overkamp[1], B. Herpers[1], K. Yan[1], L. Price[1], W. Vader[2]. [1]*OcellO, OcellO BV, Leiden, Netherlands;* [2]*Vitroscan, Predictive Diagnostics, Leiden, Netherlands*

Background: Poly(ADP-ribose) polymerase (PARP) inhibitors are a new class of anticancer drugs designed to target BRCA1/2-associated hereditary cancer and sporadic tumors with defects in homologous recombination (HR). Several PARP inhibitors (PARPi) have been approved as maintenance therapy for women with platinum-sensitive epithelial ovarian cancer. However, a significant proportion of patients do not respond to PARPi therapy and the treatment comes at high cost. To date it is not possible to predict which patients will respond to treatment. Tools to improve stratification for PARPi treatment in the clinic would be of great value.

We have initiated clinical trials to validate our drug response platform for treatment response prediction for cancer patients, including ovarian cancer. Our technology, which is based on image analysis of 3D tumour cultures, accommodates accurate evaluation of drug sensitivity with small amounts of heterogeneous tumour material (tumour, ascites). Based on the results from these trials we are developing diagnostics to predict drug responses for cancer patients.

Methods: 3D cultures embedded in a protein-rich hydrogel are generated from tumour biopsies, and exposed to standard-of-care therapies, targeted therapies and drug combinations. An automated high content screening platform measures cell and tissue morphology, and reports responses such as tumour cell killing, growth arrest and local invasion. Per tumour type and drug, morphological features are selected as standard read-outs for the response. We correlate clinical response on standard of care drugs (e.g. carboplatin, paclitaxel, olaparib, niraparib) with drug response of patients' tumour cultures, and associated genetic defects (BRCA1/2, HR). Our x-omics approach has an integrated database and biobank for testing novel molecules to benchmark these against standard of care drugs.

Results: We present first results of drug sensitivity in patient derived ex vivo 3D tumour cultures of fresh and cryopreserved tumour material. Standard-of-care therapies were tested and results are compared with clinical response. Differentiated drug responses are identified for treatment schedules including platinum-based drugs, taxanes, PARPi's.

Conclusion: Our technology enables drug sensitivity testing in ex vivo 3D cultures from patients. This allows evaluation of patient-specific treatment responses to novel and standard-of-care drugs. Ongoing trials will reveal the correlation of our in vitro test with treatment responses and relevant diagnostics parameters in the clinic.

Conflict of interest: Ownership: Leo Price is a shareholder of OcellO B.V. and VitroScan B.V. Board of Directors: Willemijn Vader is a director of VitroScan. Leo Price is a director of VitroScan and OcellO.

312 (PB-073) Poster

Genomic characterization of Chinese kidney cancer revealed novel prognostic difference across populations

Q. Zhao[1], J. Xue[2], W. Qian[2], T. Liu[3], B. Fan[4], J. Cai[4], Y. Li[1], J. Liu[1], Y. Yang[1], H. Li[5], S. Guo[2], N. Zhang[1]. [1]*Beijing Cancer Hospital, Department of Urology, Beijing, China;* [2]*Crown Bioscience Inc., Systems Biology, Taicang, China;* [3]*Daqing Oilfield General Hospital, Department of Urology, Daqing, China;* [4]*Crown Bioscience Inc., Genomics and Biomarker, Beijing, China;* [5]*Crown Bioscience Inc., Global Scientific Research and Innovations, San Diego, USA*

Background: Kidney cancer causes increasing mortality worldwide and in the Chinese patient population. Large scale omics collections like the Cancer Atlas Genome (TCGA) have been released and analyzed for hundreds of kidney cancer patients, mostly North American origins, but the data focusing on cases in the Chinese kidney cancer (CKC) patient population have yet to be reported for comparison.

Material and Methods: We performed transcriptomic profiling and genomic analysis of 65 tumor tissues and 12 matched normal tissues from CKC patients, of which clear cell renal cell carcinoma (ccRCC) is the vast majority (84.6%), followed by papillary (7.7%), chromophobe (3.1%) and medullary (3.1%) RCC.

Results: Transcriptiome sequence revealed that PBRM1 mutates with a frequency of 11% in the Chinese ccRCC patients, much lower than that in the TCGA Caucasians (34%), which is confirmed by RT-PCR of the targeted sequencing. We identified 34 gene fusion events including 5 recurrent ones, many are associated with apoptosis, cancer suppression and metastasis. We classified the Chinese ccRCC patients into three classes by gene expression. Class 1 shows significantly elevated gene expression in the VEGF pathway, while Class 3 is comparably depleted. Class 2 is characterized by increased expression of extracellular matrix organization genes and is strongly associated with high-grade tumors. We applied the expression signature to TCGA ccRCC patients and found that it better distinguishes tumor prognosis than reported ones[1]. Class 2 shows worst survival and Class 3 is a rare subtype ccRCC in the TCGA cohort. Computational analysis on the immune microenvironment of CKC identified immune-active tumors with remarkably elevated CD8 positive T cells, thus may benefit from immunotherapies. We also found that resting B cells and activation of NK cells are important factors affecting tumor suppression.

Conclusions: CKC patients have distinct genomic profiles that can help cancer prognosis and treatment.

No conflict of interest

313 (PB-074) Poster

Orthotropic homograft tumors (syngeneic) display distinct tumor infiltrate immune cells patterns from that of subcutaneous counterparts

X. An[1], X. Tu[1], M. Zipeto[2], X. Feng[1], C. Jiang[1], H. Li[1]. [1]*Crown Bioscience Inc., Scientific Research Innovation, Taicang, China;* [2]*Crown Bioscience Inc., Scientific Operation, San Diego, USA*

Background: Subcutaneous homograft, including syngeneic cell derived mouse tumors are the most commonly used preclinical pharmacology models for immune-oncology (I/O) research[1, 2]. The subcutaneous tumors are readily transplanted and monitored. On the other hand, more difficult orthotopically implanted tumors may be more biologically and pharmacological relevant to true human cancer for growth in more relevant environments. It is widely accepted that tumor microenvironment (TME), including tumor infiltrate immune cells (TILs) play significant role in tumor biology as well as pharmacology, particularly in response to I/O treatment,

which maybe better maintained in orthotropic model as compared to subcutaneous models, leading to different properties between the two types of transplantations.

Materials and Methods: We compared histopathology (HE staining) of tumor and immunohistochemistry (IHC) staining of TILs of both syngeneic cancer cell line derived tumors model and homograft of primary mouse tumors model (MuPrime^{TM}, most derived from tumorigenic GEMM) between subcutaneous and orthotropic transplants (pancreatic models were used in this study), in order to assess whether there are difference in TILs between the two transplantations.

Results: Our examinations documented several distinct features of TILs. First, TILs, including T cells and macrophages, are highly concentrated along the edge of the tumors, in contrast to that the TILs are significantly more infiltrated into inside of the tumors in orthotropic tumors (*e.g.* pancreatic models was used in this study). Second, in subcutaneous tumors, the I/O treatments, *e.g.* anti-PD1, anti-CTLA4 antibody or the combination resulted in further infiltration of TILs from tumor margin into tumor center. Third, overall the larger tumors have fewer TILs than smaller ones, regardless of transplantation types.

Conclusions: In general, orthotropic homograft mouse tumors demonstrated distinct TIL pattern from those of subcutaneous counterparts, which may contribute to different tumor biology and pharmacology (I/O).

No conflict of interest

314 (PB-075) Poster

Image-based analysis of the myeloid cell landscape in the 3D co-culture with tumor cells

G. Goverse[1], K. Yan[1], L. Guelen[2], P. Vink[2], L. Price[1], L. Daszkiewicz[1]. *[1]OcellO, Immuno-Oncology, Leiden, Netherlands; [2]Aduro Biotech, Inc., Drug Discovery, Oss, Netherlands*

Background: The myeloid cell compartment plays an important role in anti-tumor immune responses and represents a heterogeneous population with both cancer-promoting and cancer-restraining actions. Unleashing the full potential of cancer immunotherapies requires an understanding of the cellular mechanisms that govern these opposite actions. To date, high throughput relevant preclinical models for dissecting the interactions between different cellular players in the tumor microenvironment are lacking. Previously we have shown that our 3D image-based co-culture system allows assessing efficacy of immune modulators to enhance PBMC infiltration and tumoroid killing. Our main goal was to improve this model by incorporating a more complete human immune system. To do that we first generated diverse myeloid populations in a 3D environment and then used our image-based platform to describe the different subsets. The image analysis software was trained on a set of features that reproducibly allowed discrimination between undifferentiated monocytes, M1 and M2 macrophages and dendritic cells. The different myeloid subsets were next co-cultured with tumor cells to analyze the complex cellular interplay of the TME.

Material and Methods: Different myeloid populations were generated in 3D from monocytes derived from healthy donors PBMCs. Polarized M1 and M2 macrophages, DCs and undifferentiated monocytes were then co-cultured in 3D with SKBR3 tumor cells or 3D tumoroids derived from this cell line. The cellular interactions were visualized using high-content microscopy and quantified with multiparametric morphometric analysis with OMiner^{TM} software.

Results: 3D image analysis enabled the discrimination of immune-tumor cell interactions and revealed the effect of myeloid cells on tumor growth in co-culture. Our approach also enables the analysis of how tumor-driven mechanisms regulate myeloid cell differentiation and contribute to the immunosuppressive microenvironment. These results provide a means to elucidate the bi-directional interplay between tumor and immune cells and allows for analysis of functional reprograming of the suppressive population towards a M1 phenotype induced by drug candidates.

Conclusions: The 3D assay presented here enables visualization and measurement of effects of immunotherapies on cells that engage in a more physiologically relevant spatial setting than when culturing them in traditional 2D cultures. Using morphological measurements different myeloid cell subsets can be distinguished, which offers a very attractive alternative for complex and labor-intensive phenotyping based on markers expression and cytokine release profiling. The ultimate goal is to develop a highly sophisticated platform for testing cancer immunotherapies that combines the complexity of the TME and the robustness of a high throughput screening platform.

Conflict of interest: Corporate-sponsored Research: Gera Goverse, Kuan Yan, Leo Price, Lidia Daszkiewicz are full time employees of OcellO, B.V. Lars Guelen, Paul Vink are full time employees of Aduro Biotech, Inc. Both companies co-sponsored this research.

315 (PB-076) Poster

Assays for measurement of functional signal transduction pathway activity as readout in preclinical stem cell-based model systems of healthy and diseased tissue

L. Holtzer[1], H. van Ooijen[1], W. Verhaegh[1], A. van de Stolpe[1]. *[1]Philips Research, Precision and Decentralized Diagnostics, Eindhoven, Netherlands*

Background: Experimental disease model systems, like cell and tissue culture, aim at mimicking tissue (patho)physiology in vitro, for example for (targeted) drug development. This requires comparison between the cell or tissue model and actual pathology in a patient, and quantitative readout of drug efficacy and toxicity. When using stem cell derived disease models (e.g. induced pluripotent stem cells), it is important to confirm that the required cell differentiation state has been achieved. For this, 10–15 signal transduction pathways are important that govern major involved cellular processes. We developed a new diagnostic approach to measure functional activity status of these signal transduction pathways in individual cell and tissue samples, based on Bayesian computational model inference of pathway activity from measurements of *mRNA levels of target genes of the transcription factor* associated with the respective signalling pathway (Verhaegh et al., Cancer Res 2014:2936–45).

Method: Assays have been developed for androgen (AR) and estrogen receptor (ER), Hedgehog (HH), Wnt, TGFbeta, Notch, NFkB, and FOXO-PI3K pathways, and provide a quantitative pathway activity score. After calibration on samples with known ground-truth pathway activity, the models were frozen and biologically validated on different healthy and diseased cell and tissue types, including drug-treated samples, using Affymetrix HG-U133Plus2.0 microarrays (multiple public datasets from the Gene Expression Omnibus). Subsequently, pathway analysis was performed on Affymetrix expression microarray data from public datasets containing samples from stem cells cultured under different conditions and differentiated to various mesodermal and endodermal cell types.

Results: Measured pathway activities clearly changed during the differentiation process of stem cell based model systems, which allows the assessment of cell differentiation/maturation status. In human embryonic stem cells, HH was found active and TGFbeta, Notch and PI3K pathway activity depended on culture conditions (GSE19902). Differentiation of mesenchymal stem cells to intestinal cells (GSE52658) inhibited TGFbeta and induced Wnt activity.

Conclusion: Our mRNA-based pathway assays can measure signal transduction pathway activity across cell and tissue types, and can be used as readout for stem cell derived culture models, to confirm required cell differentiation state of stem cell derivatives before further experimenting. Furthermore, the assays can be used to compare cultured disease tissue to actual pathology in a patient, and to assess drug efficacy on a disease model and assessment of toxicity on a healthy cell/tissue model. Assays have been adapted to qPCR, enabling use on FFPE tissue and small samples.

No conflict of interest

316 (PB-077) Poster

Translational imaging to monitor syngeneic oncological mouse models

S. Gorynia[1], J. Schüler[1], A. Shatillo[2], J. Rytkönen[2], K. Lehtimäki[2], P. Poutiainen[3], N. Zanella[1], A. Nurmi[2], T. Huhtala[2]. *[1]Charles River, Discovery Services, Freiburg, Germany; [2]Charles River, Discovery Services, Kuopio, Finland; [3]Kuopio University Hospital, PET Radiopharmacy, Kuopio, Finland*

Background: Experimental tumors in rodents represent an important preclinical tool to develop innovative anticancer compounds. Imaging has become important part of the research. It enables monitoring of e.g. metabolic activity, hypoxic areas, volumetry and biodistribution within same individual over time. Magnetic resonance imaging (MRI) and positron emission tomography (PET) are widely used for detection of the tumors, evaluation of therapeutic response and induced changes, with longitudinal follow-up for early detection of possible tumor recurrence. The purpose of this work was to illustrate the value of MRI, MRS and PET imaging in oncological research.

Material and Methods: Total of 14 mice were implanted with breast cancer cell line 4T1 into mammary fat pad. MRI experiments were performed at days 12 and 19 post-implantation using 11.7 T small animal scanner. Experimental protocol consisted sequence for absolute T2 mapping, anatomical images, spin-echo diffusion maps, localized 1H MRS experiment and dynamic gadolinium enhancement. For the hypoxia imaging at days 13 and 20, mice were dosed with ^{18}F-FMISO simultaneously with PET scan. For metabolism on days 15 and 22, mice were dosed with ^{18}F-FDG prior scanning.

Results: From anatomical coronal MR images necrotic area within tumor was clearly visible. Also, increased diffusion coefficient values were seen over tumor progression, most likely originating from dominant necrotic core. In DCE-MRI dynamic gadolinium enhancing experiment different perfusion rate was seen in total, outer area and core part of the tumor. Rapid tumor growth often leads to inadequate angiogenesis resulting to hypoxic and even necrotic areas in tumor mass, which was visualized and quantified using corresponding tracers for metabolism and hypoxia in PET imaging. The images showed clearly both the metabolically active and hypoxic regions within the tumor. Standard uptake values (SUV) of hypoxia and metabolic activity tracers were analysed. These values can be used to evaluate treatment response to tumor growth.

Conclusions: In this study various changes related to tumor progression were studied using MRI and PET. In MRI necrotic areas within tumor, diffusion, perfusion rate in different parts of the tumor as well metabolites indicating tumor status were followed. From the PET data hypoxic and metabolic profiling were assessed. As a summary, *in vivo* imaging studies in animal models provides a powerful and translational research tool for comprehensive evaluation of neoplastic disease progression and treatment efficacy.

No conflict of interest

317 (PB-078) Poster

Anti-podoplanin cancer-specific antibody is advantageous for antitumor activities and a prognostic marker of oral cancer

Y. Kato[1], M. Kaneko[1], S. Yamada[1], Y. Sawa[2]. *[1]Tohoku University Graduate School of Medicine, Department of Antibody Drug Development, Sendai, Japan; [2]Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Deparment of Oral Function & Anatomy, Okayama, Japan*

Background: Podoplanin/PDPN, a ligand of CLEC-2, is involved in platelet aggregation and cancer metastasis. The physiological function of PDPN has been reported to be very important in many normal tissues such as type I alveolar cells of lung, podocytes of kidney, and lymphatic endothelial cells. In this study, we aimed to produce cancer-specific anti-PDPN monoclonal antibodies (mAbs). Furthermore, we investigated whether the reactivity of cancer-specific anti-PDPN mAbs might be a prognostic marker of oral cancer.

Material and Methods: We immunized mice with LN229/PDPN cells (PDPN-transfected glioblastoma cell line), and selected mAbs, which react with PDPN-expressing cancer cell lines such as LN319 (glioblastoma cell line) and PC-10 (lung squamous cell carcinoma cell line) and do not react with PDPN-expressing normal cells such as primary lymphatic endothelial cells (LECs) and HEK-293T (renal epithelial cell line) in flow cytometry. We further investigated the reactivity of anti-PDPN mAbs using immunohistochemistry (IHC) against oral cancers. The binding epitope of anti-PDPN mAbs was analyzed using alanine scanning method. Antibody-dependent cellular cytotoxicity (ADCC) and anti-tumor activities were examined against oral cancer cells. Finally, we compared the reactivity of well-known anti-PDPN mAbs, D2-40 and a newly established cancer-specific anti-PDPN mAb using IHC about 50 oral cancer patients. The association between the reactivity of anti-PDPN mAbs and clinical/pathological features were analyzed.

Results: We established a cancer-specific anti-PDPN mAb (clone: LpMab-23). LpMab-23 reacted with PDPN-expressing cancer cell lines whereas it weakly recognized PDPN-expressing normal cells in flow cytometry. LpMab-23 reacted only with PDPN- expressing cancer cells, not with LECs in oral cancer tissues using IHC although LECs were detected by D2-40 in all oral cancer tissues. Furthermore, D2-40 and LpMab-23 showed different reactions to cancer cells in oral cancer tissues. The epitope mapping of anti-PDPN mAbs revealed that LpMab-23 recognized a cancer-specific glycopeptide including Thr55/Ser56; in contrast, the epitope of D2-40 was independent of glycan. LpMab-23 revealed high ADCC and anti-tumor activities against oral cancers. The Kaplan-Meier curves of the five-year new metastasis-free survival rate (nMFS) were significantly lower in LpMab-23-positive patients than in the patients with LpMab-23-negative ones.

Conclusions: A cancer-specific anti-PDPN mAb LpMab-23 was successfully established. LpMab-23 is advantageous in anti-tumor activities. LpMab-23-positive cases could be a useful predictor of poor prognosis for oral cancer.

No conflict of interest

318 (PB-079) Poster

The metabolic landscape browser: a novel tool for understanding pan-cancer metabolic processes through variance in gene expression data

V. Leeuwenburgh[1,2], A. Bhattacharya[1], C. Urzua[1], M. Walvoort[2], H. Jalving[1], S. de Jong[1], R. Fehrmann[1]. *[1]University Medical Center Groningen, Department of Medical Oncology, Groningen, Netherlands; [2]University of Groningen, Stratingh Institute for Chemistry, Department of Chemical Biology, Groningen, Netherlands*

Background: Altered metabolism is a hallmark of cancer. To study metabolic processes in (cancer) cells we have developed an open-access web-based tool based on patient-derived tumor and healthy tissue samples as well as cell line data.

Materials and Methods: A total of 34,494 samples were obtained from the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), Genomics of Drugs Sensitivity in Cancer (GDSC) and the Cancer Cell Line Encyclopedia (CCLE), representing 108 tissue (sub)types. This included 28,200 patient-derived tumor samples. We applied independent component analysis (ICA), which is a computational method to separate multivariate signals (gene expression profiles) into additive subcomponents, so-called transcriptional components (TCs). To characterize the biology of these TCs, gene set enrichment analysis (GSEA) was applied, utilizing 671 gene sets describing metabolic processes from 5 different gene set databases. Subsequently, the TCs were filtered based on high enrichments for gene sets related to metabolic processes. In addition, ICA also provided the "activity" of these metabolic TCs for each individual sample present in our data set.

Results: ICA on 34,494 samples resulted in 132, 151, 136 and 137 metabolic TCs for GEO, TCGA, CCLE and GDSC, respectively. These metabolic TCs were used to define the metabolic landscape in 88 different tumor (sub)types. The tool will be accessible through an open access, web-based portal that will be made available soon. To demonstrate the potential of this tool, we used these metabolic TCs to find genes previously not known to be involved in metabolism that coregulate with known metabolic genes. Furthermore, based on the activity of metabolic TCs in each individual sample, we were able to identify metabolic patterns specific for certain types of (tumor) cells. Known metabolic processes, such as melanin metabolism in melanoma and heme metabolism in haematopoietic cells could readily be identified. In addition, we were able to identify transcription factors that show high correlation with several metabolic TCs, such as the well-known metabolism-associated HIF1-, NRF2- and CHOP/ATF4 transcription factors. Furthermore, we assessed the association of these metabolic TCs with drug sensitivity (defined by IC50 values) utilizing the CCLE and GDSC cell line compendiums.

Conclusions: Our metabolic landscape in a large set of tumor, healthy tissue and cell line samples provides researchers with a comprehensive resource to gain more insight into metabolic processes relevant in different cancer types. This might lead to new therapeutic strategies directed at modulating specific metabolic pathways relevant for tumor behavior or treatment response.

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No conflict of interest

319 (PB-080) Poster

Radiotherapy in combination with immunotherapy in a variety of syngeneic mouse models of cancer

A. Wulf-Goldenberg[1], M. Stecklum[1], M. Paterka[1], I. Fichtner[1], J. Hoffmann[1]. *[1]EPO, Experimental Pharmacology & Oncology Berlin-Buch GmbH, Immuno-Oncology, Berlin, Germany*

Background: The recent clinical success of immune checkpoint modulators has stimulated immune-oncology research leading to the identification of new tumor immunology targets. However both, target validation and drug development need highly characterized preclinical immune oncology models for identification of clinically relevant biomarkers and defining rational combination strategies. Tumor expression of PD-L1 has recently been approved as first predictive biomarker for immunotherapy with the PD1/PD-L1 checkpoint inhibitors.

Results: We screened our syngeneic mouse models for PD-L1 expression to identify such a correlation. PD-L1 positive and negative murine tumor models were transplanted on C57Bl/6 or Balb/c mice. Most of them showed no difference in tumor growth comparing PD-L1 antibody treated with untreated mice (= fully immune resistant). However, P388 and some others showed a delayed growth (= partly immune resistant), whereas no tumor model where fully growth inhibited (= immune sensitive).

Furthermore, we were interested, whether the antitumoral treatment effects of checkpoint inhibitors can be increased by combination with radiation. For the evaluation of local tumor radiation in combination with checkpoint inhibitor we optimized multiple parameters, including radiation dose and frequency, drug treatment sequence and duration.

We tested a broad panel of syngeneic tumor models towards their response towards the combination with local tumor radiation and PD-L1 antibodies. The studies were accompanied by measurement of tumor infiltrating immune cells.

Increased efficacy of the combination compared to single treatment was observed in several models as P388. PD-L1 treatment of P388 bearing mice resulted in 19% tumor growth inhibition, radiation alone in 40% tumor growth inhibition, but the combination of checkpoint inhibitor and radiation resulted in 71% tumor growth inhibition. However, frequently models as lewis lung showed only a slight tumor growth delay. Treatment resistance seemed to correlate with a low percentage of immune cells in the tumor (cold tumors). The evaluation of the PD-L1 expression in the tumors revealed, that radiation can induce PD-L1 expression.

Conclusion: Our results are demonstrating, that syngeneic tumor models in mice can be used to evaluate strategies for the combination of immunotherapies with radiation.

No conflict of interest

320 (PB-081) Poster

TLR2 inhibition reprograms the tumor microenvironment and delays progression of recurrent ovarian cancer in vivo

A. Alvero[1], M. Pitruzzello[1], B. Keogh[2], P. Mcguirk[2], M. Reilly[2], G. Mor[1]. [1]*Yale University, Ob-Gyn, New Haven, Connecticut, USA;* [2]*Opsona Therapeutics Ltd, In Vivo Pharmacology, Dublin, Ireland*

Background: Primary ovarian cancer is highly responsive to standard of care comprised of combination chemotherapy and surgery. In contrast, recurrent ovarian cancer presents as an extremely chemoresistant and widely metastatic disease thus limiting the value of chemotherapy and surgery. More than 80% of patients that initially respond to 1st-line treatment experience recurrent ovarian cancer and eventually succumb to the disease.

We showed in previous studies the importance of the TLR2 signaling pathway during ovarian cancer recurrence. TLR2 signaling is able to promote tumor repair pathways and hence facilitates the development of recurrent disease.

Using a xenograft model of recurrent ovarian cancer, we evaluated the ability of a humanized anti-TLR2 (OPN-305) antibody to modulate the tumor microenvironment and prevent recurrence.

Materials and Methods: mCherry-positive human ovarian cancer cells were injected intra-peritoneally (i.p.) in athymic nude mice. The growth of i.p. tumors were measured using the In Vivo FX system. Mice received four doses of Paclitaxel (12 mg/kg) or vehicle control to treat primary disease. Afterwards, mice were re-randomized to receive maintenance therapy with either saline (n = 7) or OPN-305 (n = 11). Tumor burden was quantified using mCherry fluorescence area. Protein levels were determined by western blot analysis and immunephenotyping determined by FACS analysis.

Results: 1st-line Paclitaxel induced tumor regression in all treated animals. When these mice were observed through the maintenance treatment, animals maintained on OPN-305 had significantly slower tumor kinetics (p = 0.0001) and significantly less tumor burden (p = 0.0082) compared to mice maintained on saline. Maintenance therapy with OPN-305 had a beneficial effect on progression-free interval (p = 0.0407, compared to those maintained on saline).

Molecular analysis of tumors isolated from mice that received OPN-305 showed lower abundance of the mesenchymal gene Twist1, a known driver of chemoresistance and metastasis. Finally, maintenance therapy with OPN-305 had a significant effect on the intra-tumoral immune infiltrate and lead to a decrease in the number of CD11b+/Gr-1+ myeloid derived suppressor cells (MDSC) (p = 0.0327, compared to saline group).

Conclusion: We demonstrate for the first time that maintenance therapy with a humanized anti-TLR2 antibody OPN-305 is able to modify the molecular and immune-phenotype of the tumor microenvironment and delay the progression of recurrent ovarian cancer. Our data suggest that by inhibiting the TLR2 pathway, we can block the capacity of the tumor microenvironment to recruit MDSC, which are critical in the process of tumor repair and are major inhibitors of anti-tumoral responses. These results suggest the value of OPN-305 as part of maintenance therapy in ovarian cancer.

Conflict of interest: Other Substantive Relationships: Brian Keogh, Peter McGuirk, and Mary Reilly are employed by Opsona Therapeutics.

321 (PB-082) Poster

Selection of suitable PDX models for the investigation of novel anti-cancer molecules using a comprehensive database

H. Klett[1], A.L. Peille[1], A. Maier[1], G. Kelter[1], K. Klinger[1], D. Bug[2], J. Schueler[1]. [1]*Charles River Discovery Research Services, Charles River Freiburg, Freiburg, Germany;* [2]*RWTH Aachen University, Institute for Imaging and Computer Vision, Aachen, Germany*

The Charles River compendium comprises more than 700 tumor models including patient derived xenografts (PDX), cell lines, cell line derived xenografts, syngeneic and genetically engineered mouse models. These models have been extensively profiled for histological features, molecular data, and sensitivity to standard-of-care compounds, allowing a precise selection of suitable tumor models for preclinical anti-cancer agent testing.

Here, we present a potential workflow for the investigation of novel anti-cancer molecules using the example of EGFR targeting small molecules as well as antibodies evaluated on PDX – *in vitro* and *in vivo* – pre-clinical platforms. In a first step, suitable models were selected from the Charles River Compendium by means of EGFR expression. The selection included several high expressing models as well as low expressers for proof of principle. Subsequently, EGFR expression was confirmed on protein level by immunohistochemistry using a tissue micro array and quantified by the OSANO software for the selected PDX models. A screen on 25 EGFR-targeting small molecules in 74 PDX derived cell lines in 2D revealed 27 highly sensitive and 25 resistant models. Among the sensitive models, gastric cancer and melanoma were statistically over-represented. A subsequent *ex vivo* 3D screen on 296 PDX models using the 10 most active EGFR-targeting compounds confirmed and extended the data-set of the 2D screen. Again, gastric cancer but as well non-small cell lung and renal cancer were highly sensitive towards treatment. The predictivity of the *in vitro* platforms was proven by *in vivo* characterization of Cetuximab, Gefitinib and Erlotinib in 23 PDX models. The predictivity of the 3D clonogenic assay for the PDX in vivo assay was precise for Gefitinib and Erlotinib. For Cetuximab the 3D assay was under-predictive as the antibody was much more active *in vivo* as *in vitro*. Furthermore, we could identify a predictive biomarker for model sensitivity towards an EGFR-targeting drug treatment using both in vitro and in vivo data. Combining those datasets with clinical data will give us the possibility to validate the biomarkers towards their translational value.

Taken together, the compendium-based model selection helps to test potential drug treatments in suitable *in vitro* and *in vivo* cancer models and thereby proofs to be a reliable tool in the drug development pipeline, including sensitivity, predictive biomarker, and validation analysis.

No conflict of interest

322 (PB-083) Poster

Determination of the starting dose in the first-in-human clinical trial of ERY974, a novel T cell–redirecting bispecific antibody targeting glypican-3, from MABEL and NOAEL approaches

Y. Sano[1], S.I. Komatsu[1], W. Frings[1], A. Kaneko[1], I. Takahiro[1], Y. Kayukawa[1], N. Ono[1], K. Sakata[1], T. Fujii[1], Y. Miyazaki[1], Y. Kinoshita[1], Y. Azuma[1], T. Tsunenari[1], S. Kishishita[1], M. Endo[1], J. Nezu[1]. [1]*Chugai Pharmaceutical Co., Ltd., Research Division, Kamakura, Japan*

Background: ERY974 is a humanized IgG4 bispecific T cell–redirecting antibody currently in Phase 1 clinical trial (NCT02748837). ERY974 consists of a common light chain and two different heavy chains that respectively recognize glypican-3 and CD3. In general, to determine the first-in-human (FIH) dose for monoclonal antibodies (mAb) with low toxicity, the no observed adverse effect level (NOAEL) is used. However, after the FIH clinical trial of an anti-CD28 agonist mAb, which resulted in severe life-threatening adverse events directly related to the pharmacology of the anti-CD28 mAb, a guideline created by the regulatory agencies recommended the minimal anticipated biological effect level (MABEL) approach when determining a FIH dose for high-risk drugs. Accordingly, we determined the FIH dose based on both toxicological and pharmacological studies, with the NOAEL based on a toxicological study in cynomolgus monkeys, and the MABEL based on an *in vitro* cytotoxicity assay.

Materials and Methods: To determine the NOAEL, we conducted a single-dose study in cynomolgus monkeys with slow intravenous infusion (0.1, 1, and 10 μg/kg over 30 min), in which we observed general conditions and examined cytokine levels in blood, and also conducted a pathological analysis. To determine the MABEL, we selected the cytotoxic assay as the most sensitive *in vitro* assay method and huH-1 as the most sensitive cell-line. Therefore, we used the EC10 value in a cytotoxic assay in huH-1 to calculate the MABEL.

Results and conclusion: In the cynomolgus monkey study, a transient increase in blood cytokines was observed from 1 μg/kg, with IL-6 being the

most prominent cytokine. Signs of a deteriorating general condition, such as red skin, reduced food consumption, and body weight loss, were also noted in a dose-dependent manner. Histopathological findings such as decreased lymphocytes in the thymus and increased immune cell infiltration in multiple tissues were also seen at the highest dose. From these results, we determined the lowest dose 0.1 μg/kg as the NOAEL, and calculated a starting dose of 3.2 ng/kg, considering body surface area and species difference. On the other hand, the starting dose from the MABEL approach was calculated to be 4.9 ng/kg, at which C_{max} after 4 hours IV infusion is predicted to be equal to the EC10 value of the cytotoxic assay. By combining the NOAEL and MABEL approaches, we selected 3.0 ng/kg as a FIH dose for the Phase 1 clinical trial (NCT02748837).

Conflict of interest: Ownership: Chugai Pharmaceutical Co., Ltd.

324 (PB-085) Poster

Cross-talk between macrophages and thyroid cells in early and late thyroid tumor stages: in vitro studies

G. Mauro[1], M. Mazzoni[1], M. Erreni[2], P. Romeo[1], M.C. Anania[1], R. Avigni[2], M.G. Rizzetti[1], M.G. Borrello[1], P. Allavena[2], A. Greco[1]. [1]*IRCCS Foundation, Istituto Nazionale dei Tumori, Milan, Reasearch Department, Milano, Italy;* [2]*IRCCS Clinical and Research Institute Humanitas, Rozzano, Department of Immunology, Milano, Italy*

Background: Inflammation plays a critical role in many tumor types, including thyroid cancer. Conditions predisposing to cancer, as well as causative genetic events, contribute to the construction of an inflammatory microenvironment that facilitates thyroid tumor progression by limiting immune surveillance and therapy response.

Thyroid carcinoma, the most frequent endocrine neoplasia, includes several variants characterized by different biological and clinical features: from the early indolent stage papillary thyroid microcarcinoma to undifferentiated and aggressive anaplastic carcinoma.

Recent studies have documented the occurrence in thyroid cancer of oncogene induced senescence, a mechanism acting as a barrier in cancer progression, tightly connected with inflammation, as it triggers a complex proinflammatory programme known as the "senescence associated secretory phenotype." Recently, we have proposed senescent thyrocytes as an *in vitro* model of early stage of thyroid cancer.

Material and Methods: Here we characterized the *in vitro* interplay between macrophages and cells representative of early and late thyroid tumor stages, modeled by senescent thyrocytes and tumor cell lines, respectively. Purified peripheral blood-derived human monocytes were exposed to thyroid cell-derived conditioned medium (CM), and were assessed for their phenotype by flow cytometry, ELISA and gene expression analysis.

Results: We found that, upon exposure to CM derived from both senescent thyrocytes and thyroid tumor cell lines, human monocytes undergo M2-like polarization, as shown by high level of CD206 and low level of MHC II surface markers, and by the upregulation of CCL17 secretion. We also observed the upregulation of several genes, including: M-CSF (CSF1), genes coding for chemokines (CCL20, CCL3, CCL4, CXCL1, CXCL8) and cytokines (IL-6, IL-23, IL-24), Osteopontin, VEGF and the prostaglandin-endoperoxide synthase enzyme (PTGS2), known also as COX-2, involved in the convertion of arachidonic acid to prostaglandins (e.g. PGE2). Interestingly, we found that PTGS2-PGE2 axis exerts an important role on M2-like polarization. Indeed, the monocytes exposure to CM from thyroid cell treated with the specific PTGS2 inhibitor Celecoxib impaired the M2-like polarization, as demonstrated by a clear reduction of CD206 expression and of CCL17 secretion.

Conclusions: Overall, our results demonstrate that both senescent thyrocytes and thyroid tumor cell lines trigger M2-like macrophage polarization, thus suggesting that the interaction with microenvironment already occurs in the early stage of thyroid tumor development.

No conflict of interest

325 (PB-086) Poster

Optimisation of protocols for the establishment of pancreatic cancer organoids and cell lines from patient derived xenografts (PDX) tumour models

S. Nelson[1], S. Roche[1], F. O'neill[1], A. Larkin[1], J. Crown[2], N. Walsh[1]. [1]*Dublin City University, National Institute for Cellular Biotechnology, Dublin 9, Ireland;* [2]*St. Vincent's University Hospital, Dept. of Medical Oncology, Dublin, Ireland*

Background: With a five-year survival rate of 8%, pancreatic cancer is the deadliest of all cancers. The lethality of this cancer is due to its rapid progression, ability to metastasise and develop resistance to chemotherapeutics at an early stage. Research into this devastating disease has done little to increase survival rates, this may be because the methods which are currently used to grow cancer cells *in vitro* do not represent the original tumour. Recent advances in cell culture have allowed for the production of organoids. Organoids are miniature tumour models cultured in a dish, which have the same three-dimensional architecture, and are phenotypically to tumours *in vivo*. Organoids can also be used to represent the different stages of disease. We have developed a protocol to establish pancreatic tumour organoids and primary cell lines from patient derived xenografts (PDX) using both fresh and cryopreserved tumour samples.

Materials and Methods: Tumour samples were dissociated into single cells, and infiltrating mouse cells removed. The single cells were resuspended in Matrigel and plated in conditioned media (CM) containing Wnt3a, R-spondin 3 and Noggin. Organoids were passaged every 7–10 days, cell lines passaged once cells became adherent and began to proliferate.

Results: We developed a workflow capable of establishing organoid and primary cell cultures from both fresh and cryopreserved PDX pancreatic tumours. This rapid and multipurpose workflow enables highly stable standardised protocol for the simultaneous establishment of organoids and primary cell lines enabling more flexible downstream *in vitro* modelling of pancreatic cancer. The protocol involves the optimisation of media, dissociation of the tumour, removal of infiltrating mouse cells, and optimising volume of Matrigel for correct cell density. We are currently undertaking functional and molecular characterisations of the organoids and cell lines; and are comparing these primary cell cultures to the PDX tumour and original patient tumour. The organoids and primary cell lines which have been established will also be used to test clinical treatments for pancreatic cancer, and for the identification of genetic changes resulting in pancreatic cancer.

Conclusions: The protocol which has been optimised has allowed for the successful establishment of organoids and primary cell lines, using both fresh and cryopreserved PDX tumour samples. The successful establishment of these primary cultures using cryopreserved tumour samples gives access to a biobank of samples. The use of these models in pancreatic cancer research will give an insight into the changes which occur from original tumour to established cell lines and will allow for the identification of new treatments, as well as the causative mutations of this devastating disease.

No conflict of interest

326 (PB-087) Poster

Chemotherapeutic response of primary cultures models established from pancreatic cancer patient derived xenografts

S. Roche[1], F. O'neill[1], E. Welsch[1], V. Guinot[1], N. Swan[2], N.L. Straubinger[3], J. Murphy[2], K. Conlon[4], R. Mcdermott[5], N. Conlon[1], J. Meiller[1], G. Justin[4], M. Moriarty[1], R.M. Straubinger[3], M. Clynes[1]. [1]*Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland;* [2]*St Vincent's University Hospital, Department of Pathology and Laboratory Medicine, Dublin 4, Ireland;* [3]*University at Buffalo, Department of Pharmaceutical Sciences, Buffalo, USA;* [4]*St Vincent's University Hospital, Department of Surgery, Dublin 4, Ireland;* [5]*St Vincent's University Hospital, Department Of Medical Oncology, Dublin 4, Ireland*

Background: Pancreatic cancer survival rate is poor, with a 5 year survival rate of 7% in the Republic of Ireland. Over the last 40 years, pancreatic cancer is one of the only solid tumours to have minimal improvement in patient outcome. In Ireland pancreatic cancer surgical resection is limited to two hospitals, Cork University Hospital and St Vincent's University Hospital (SVUH), Dublin. In collaboration with SVUH, we have established Ireland's only pancreatic cancer patient derived xenograft (PDX) bio-bank program, including the establishment of primary cell-lines when possible. Chemotherapy options for pancreatic adenocarcinomas (PDAC) patients are usually to Gemcitabine/Abraxane or FOLFIRINOX, depending on ECOG status.

Methods: Tumour material was obtained from candidate patients following surgical resection with curative intent. After initial macroscopic pathological confirmation, material surplus to diagnostic sampling was cold transferred and implanted sub-cutaneous into CB17/Icr-Prkdc^{scid} mice, with an approximate mean transfer time of 80 minutes from surgery to implantation.

A biobank was created of patient tumours, PDX tumours, and cryopreserved tumour lines. Following one passage *in vivo,* using a cold digestion with hyaluronidase collagenase, primary cell lines were derived.

Primary culture cells and established cell lines were examined in a 2D and 3D *in vitro* model for the response to standard of care chemotherapeutic agents.

Results: To date, 20 PDAC tumours have been expanded as patient derived xenograft (PDX) models, with a take rate of 71%. These PDX tumours

have been histopathologically confirmed to maintain the same morphology as the original patient tumours.

From these PDAC PDXs, primary cell-lines were isolated and developed. Using established and primary pancreatic cancer cell-lines we have investigated the effect of standard of cancer chemotherapeutics in 2D and 3D. In 2D triple combination of 5-FU, Oxaliplatin and the Irinotecan metabolite, SN-38, displayed similar potency across the cell lines, with the primary cell lines being somewhat more resistant. Both established and primary cells were significantly more resistant to the triple chemotherapeutic combination when analysed in a 3D *in vitro* model.

Conclusion: This research demonstrates the generation of low passage PDX models are a scientifically valuable resource. The pancreatic PDX biobank represents a versatile and expandable patient cohort for pre-clinical investigation. The establishment of primary cell lines provides a "closer-to-patient" platform for the investigation of rational combination therapies *in vitro* – a valuable asset for therapeutic screening prior to pre-clinical investigation.

The assessment of rational, clinically relevant chemotherapeutic combinations in a 3D preclinical *in vitro* model demonstrates a clearer reality of *in vivo* applicability.

No conflict of interest

327 (PB-088) Poster

Evaluation of anti-androgen therapy in a panel of prostate patient-derived xenograft models

J. Davies[1], N. Papadopoulou[1], L. Wainwright[1], A. Oakden[1], C. Roberts[1], J. Wrigley[1], J. King[1], W. Qian[2], L. Zhang[2], B. Fan[2], Y. Yin[1], A. Collins[3], J. Cai[2], D. Ouyang[2], J. Kumari[1]. *[1]Crown Bioscience UK Ltd., Operations, Loughborough, Leicestershire, United Kingdom; [2]Crown Bioscience Inc, Pharmacology, Beijing, China; [3]University of York, York, United Kingdom*

Background: Prostate cancer is the most common cancer in men in the UK and the second most common cancer worldwide and the fourth most common cancer overall, with more than 1 million new cases diagnosed each year. Understanding the biology of prostate cancer has improved and resulted in the development of new targeted therapies such as abiraterone (Zytiga®) and enzalutamide (Xtandi®), however the lack or preclinical models that adequately represent the spectrum of benign, latent, aggressive, and metastatic forms of the human disease has hampered research. Patient derived xenograft (PDX) have been reported to be more clinically relevant that cell lines and as a result an increase in the generation of PDX have provided a breadth of new models for different cancer types. Here we report the establishment and validation of a panel of prostate PDX models and their utilization in preclinical studies.

Material and Methods: Primary prostate cancer samples obtained from patients in the UK undergoing radical prostatectomy were collected with ethical consent, disaggregated and established subcutaneously in $Rag2^{-/-}gC^{-/-}$ mice (Jackson Laboratory) to generate PDX models. Subcutaneous tumor growth was evaluated 3 times a week by electronic callipers and volume estimated using the formula 0.5 (LxW^2). Once established, tumours were expanded in NSG mice (Jackson Laboratory) and tissue collected at termination for RNA sequencing and immunohistochemistry (IHC) for prostate markers. PDX were tested for sensitivity to docetaxel (Taxotere®), abiraterone and enzalutamide in vivo in mice bearing subcutaneous tumours which had reached a mean tumour volume of 100 mm^3. Tumours were samples pre and post dosing as well as blood samples collected.

Results: A panel of prostate PDX models have been established and expanded. Two models were developed from patients diagnosed with castrate resistant prostate cancer (CRPC) and two models from patients who showed hormone sensitivity. Histologically the structure of the original patient sample was retained by the PDX models. In addition, these models showed high KLK3 (PSA) expression levels by RNA sequencing and IHC staining as well as androgen receptor expression. One of the CRPC models showed a TMPRSS-ETS fusion, partial response to docetaxel ($p < 0.0001$, Two way ANOVA) in vivo when compared to vehicle group and poor response to abiraterone and enzalutamide, whereas the second CRPC model showed no response to any of the agents tested.

Conclusions: We have established and characterised a panel of prostate PDX models which provide unique and clinically relevant models for preclinical drug evaluation for prostate cancer.

No conflict of interest

328 (PB-089) Poster

Functional protein and pathway profiling of patient-derived tumor models for drug testing and precision medicine applications using Reverse Phase Protein Arrays (RPPA)

B. Gierke[1], M. Bodenhöfer[2], C. Schmees[2], M. Pawlak[1]. *[1]NMI, Protein Profiling, Reutlingen, Germany; [2]NMI, Tumor Biology, Reutlingen, Germany*

Background: Molecular profiling of patient tumors today uses high throughput genome and transcriptome technologies, yet proteins and especially their functions, on which drugs can act, are poorly predicted. Proteomic immunoassay approaches like Reverse Phase Protein Arrays (RPPA) have demonstrated that signaling pathway profiling of up to hundreds of samples and proteins in parallel can add valuable information (i) providing multiple marker patterns from small amounts of tissue specimen, and (ii) providing biological answers based on a functional protein level: Are pathways active downstream of a driver mutation? Will drug treatment be effective? What are the underlying mechanisms? Can we identify predictive marker proteins? We combine RPPA with cell line and patient-derived model systems as efficient "close-to-in-vivo" in vitro systems to generate phenotype/pathway signature information to be used for drug testing and personalized medicine applications.

Material and Methods: Protein lysates were prepared and analyzed from various tumor model systems treated with different chemotherapeutic and targeted drugs and compounds. Sample sources included patient-derived Xenograft models, homo and heterotypic 3D Insight™ tumor microtissues (Insphero) and 3D microtumor cultures derived from fresh patient tumor material, treated at different doses and times. Protein lysates were analyzed via RPPA applying sensitive fluorescence immunoassays with up-front well-validated antibodies against 100+ focused protein markers (total, phospho forms) of key oncology pathways (e.g. MAPK, PI3K/Akt/mTOR).

Results: Phenotype/pathway signatures were generated from the RPPA data. Changes upon treatment of the individual pathways – their activities and associate protein markers – are used for correlations to mechanisms of drug action e.g. efficacy/resistance, and for treatment response prediction. Treatment responses of 3D tumor models were benchmarked with conventional 2D cell culture models as controls.

Conclusion: Multiplex protein and pathway profiling using RPPA can provide meaningful biological information from functional protein and pathway signatures generated from of up to hundreds of proteins and samples – measured in parallel and from minute amounts of starting materials. Leveraging several advanced patient-derived tumor models, our results demonstrate the power of phenotype profiling for gaining conclusive drug mode-of-action information, identification of early response treatment markers and development of precision therapies.

No conflict of interest

329 (PB-090) Poster

Recapitulating the orthotopic tumour microenvironment using bioluminescent syngeneic models in immune competent mice for checkpoint inhibitor interrogation

N. Papadopoulou[1], J. Davies[1], L. Wainwright[1], J. Wrigley[1], S. Jiang[1], J. King[1], Y. Yin[1], J. Kumari[1]. *[1]Crown Bioscience UK Ltd., Oncology, Loughborough, Leicestershire, United Kingdom*

Background: Checkpoint inhibitors are widely being evaluated in numerous cancer types, but suitable preclinical models are required to effectively evaluate the therapeutic response as well as the opportunities for combination treatments. Subcutaneous syngeneic models are routinely used to evaluate the impact of immunotherapies on tumour growth and tumour invading leucocytes (TILs). However, the tumour microenvironment of orthotopic models is more comparable to the patient due to the organ-specific location of the tumour which facilitates metastatic spread and also recapitulates the immune and stromal component interactions with the tumour. Bioluminescent imaging (BLI) enables non-invasive longitudinal monitoring of orthotopic tumour burden and end stage quantification of tumour spread. Here we report the generation of a panel of bioluminescent syngeneic cell lines for orthotopic and metastatic modelling with an aim to assess the impact of standard of care agents, immune checkpoint therapies and combinations in a more clinically relevant environment.

Methods: Bioluminescent variants of syngeneic cell lines were established by lentiviral transduction. Orthotopic models were established via direct implantation into a number of different organs e.g. liver, breast, pancreas, bladder and tumour growth assessed by BLI twice weekly and at end stage (Spectrum CT; PerkinElmer). Response to standard of care agents such as sorafenib, gemcitabine, docetaxel and immune checkpoint therapy (anti-CTLA-4, anti-PD-1) was also evaluated and TIL infiltration was assessed by FACs analysis and IHC.

Results: The success rate of tumour transplantation into different organs ranged from 60 to 100% as confirmed by both in-life imaging and ex vivo imaging at termination. For example, Hepa 1–6 appeared to grow within the parenchyma of the liver whereas 4T1 metastasized to the lungs and bone from the mammary fat pad, which was easily quantified by BLI. Real-time quantification of tumour size could be correlated with biomarkers and end stage assessment, for example, treatment with Sorafenib and check point inhibitors was correlated with alpha-fetoprotein (AFP) and end stage tumour burden, showing significant response in the orthotopic setting ($p < 0.001$, Two way ANOVA). Differences in TIL infiltration as detected by FACs and IHC were observed as well as stromal and blood capillary infiltration when compared to the subcutaneous site.

Conclusions: Bioluminescent syngeneic models enables clinically relevant interrogation of standard of care agents, immunotherapies and combination which is distinct from the subcutaneous setting. The orthotopic microenvironment influences the tumour growth and TIL infiltration which requires characterisation by both FACS and IHC. These models also enable the assessment of disease progression and modelling the metastatic environment.

No conflict of interest

331 (PB-092) Poster

Identification of predictive biomarkers in novel patient-derived xenograft (PDX) models of peritoneal metastasis from colorectal cancer

E. Pachmayr[1], B. Brzezicha[2], B. Büttner[2], B. Rau[3], U. Stein[1], W. Walther[4]. [1]*ECRC, Charité and Max-Delbrück-Center Berlin, Translational Oncology of Solid Tumors, Berlin, Germany;* [2]*Epo GmbH Berlin, Experimental Pharmacology, Berlin, Germany;* [3]*Charité, Surgical Clinic, Berlin, Germany;* [4]*Charité, Universitätsmedizin Berlin, Campus Berlin-Buch, Translational Oncology of Solid Tumors, Berlin, Germany*

Background: One terminal stage of colorectal cancer (CRC) is represented by the peritoneal metastasis (PM) with only limited therapy options. To improve therapeutic outcome, identification of clinically relevant prognostic and predictive markers is essential. In this context patient derived xenograft (PDX) models represent a useful platform for molecular as well as response analyses. Access to in vivo models of PM will improve the evaluation of chemosensitivity as well as identification of novel biomarkers and therapeutic targets. Here we generated the first patient PDX models of PM from CRC to test chemotherapy response and to analyze predictive biomarkers, such as the Wnt signaling target S100A4.

Material and Methods: For PDX establishment surgical tumor specimens of 48 patients were transplanted subcutaneously onto NOG mice and later transferred to NMRI nu/nu mice for further passaging. Of those, 23 PDX have stably engrafted. Thirteen PDX models thereof were characterized regarding histology (immunohistochemistry; IHC), chemosensitivity (in vivo drug treatment) and biomarker expression (IHC, real-time RT-PCR) in more detail. Chemosensitivity of PDX models was tested towards a panel of conventional and targeted drugs. Biomarker expression was analyzed in PDX tumor tissues at mRNA and protein level and correlated to treatment response of the PDX.

Results: All PDX retained the histological appearance of original patient tumor samples. They further showed individual growth characteristics reflected by range of tumor doubling times. PDX of PM showed replacement of human stroma by mouse stroma already in early passages. In chemosensitivity testing the PM PDX responded to different degrees to the tested drugs. Overall, for conventional drug 5-fluorouracil and oxaliplatin only limited response was seen. Best response was determined for irinotecan. Targeted drug cetuximab, bevacizumab and erlotinib generated only limited response. Correlation analyses of the biomarkers revealed, that expression of the metastasis-promoting gene S100A4 correlated best to chemotherapy response of the PDX.

Conclusions: The novel PDX models of PM from CRC reflect key characteristics of the primary tumors. They provide a useful platform for identification and validation of novel predictive biomarkers, such as S100A4 and chemosensitivity testing for improved therapy of patients suffering from CRC-derived PM.

No conflict of interest

332 (PB-093) Poster

Antiproliferative effects of metronomic combination of Vinorelbine and 5-Fluorouracil on triple-negative breast cancer cells is due to induction of autophagy and senescence rather than apoptosis

M.G. Cerrito[1], S.M. Bonomno[2], M. De Giorgi[2], A. Scagliotti[2], D. Pelizzoni[2], E. Grassilli[2], B. Paolo[2], G. Roberto[2], M. Lavitrano[1], M.E. Cazzaniga[2]. [1]*Università Milano-Bicocca, School of Medicine and Surgery, Monza MB, Italy;* [2]*University of Milano-Bicocca, Medicine and Surgery, Monza, Italy*

Background: Triple-negative breast cancer (TNBC) is an aggressive tumor with limited treatment options and very poor prognosis following progression after standard chemotherapeutic regimens. One of the emerging strategy to achieve disease control while reducing toxicity is metronomic chemotherapy (mCHT) which inhibits the tumor growth. In the recent clinical study, Victor-2, we evaluated a new metronomic combination (mCHT) of Capecitabine (CAPE) and Vinorelbine (VNR) in breast cancer patients showing a disease control rate with a median Progression-Free Survival (PFS) of 4.7 months in 28 TNBC patients.

In the *in vitro* study, named Victor-0, we examined the effect of metronomic (mCHT) *vs* standard (STD) schedule of administration of different combinations of 5-Fluorouracil (5FU), the active metabolite of CAPE, and VNR in TNBC cell lines MDA-MB-231 and BT-549.

Methods: TNBC cell lines MDA-MB-231 and BT-549 were exposed to different concentration of VNR alone or with 5-FU for 4 and 96 h. To simulate the metronomic dosing schedule, we replaced the drug-enriched medium every 24 h, while to simulate the conventional administration protocol cells were exposed to VNR alone or with 5-FU for 4 h, then the medium was changed and replaced with fresh medium without drug every 24 h. The IC_{50} was calculated by non-linear regression fit of the mean values of data obtained in triplicate experiments. Cell viability/cytotoxicity assays was evaluated by MTT assay and senescence by S-b-gal staining. Cell cycle was analyzed by FACS of PI-stained cells. Modulation of apoptotic/autophagic markers was assessed by western blot analysis and immunofluorescence.

Results: A significant anti-proliferative activity was observed in cells treated with mCHT *vs* STD administration of 5FU or VNR alone. Combination of the two drugs showed an additive inhibitor effect on cell growth in both cell lines. After exposure of cells to 5FU and VNR under mCHT *vs* conventional schedule of administration we observed a different modulation of chemoresistance factor Bcl-2, the pro-apoptotic protein Bax, cleaved effector caspase-3 and expression of LC3A/B autophagy protein indicating that autophagy and cellular senescence contribute more than apoptosis to the growth suppressive effect triggered by metronomic therapy.

In conclusion, our data give novel insights and help to understand which molecular mechanism involved in the cell death of TNBC are triggered by the different chemotherapeutic treatments and/or schedules. In particular, our data indicate that the efficacy of the metronomic schedule is due to the the combined triggering of authophagy and cellular senescence in TNBC cells.

No conflict of interest

333 (PB-094) Poster

Eliminating tumor-initiating cells in an ovarian cancer relapse model

M. Ozaki[1], C. House[1], K. Mazan-Mamczarz[2], M. Lal-Nag[2], C. Thomas[2], C. Annunziata[1]. [1]*National Cancer Institute, Women's Malignancies Branch, Bethesda, USA;* [2]*National Institutes of Health, National Center for Advancing Translational Sciences, Rockville, USA*

Background: Tumor-initiating cells (TICs) are a subpopulation of cells that have been found to be chemotherapy resistant, and contribute to cancer relapse. Ovarian cancer has an extremely high rate of relapse, with over 70% of advanced stage cases relapsing within 2 years. Based on our previously published work, we hypothesize that we can target the NF-kappaB pathway to eliminate TIC populations.

Methods: We performed both siRNA screen and high throughput drug screen to identify pathways critical to ovarian cancer cell growth in spheroid conditions. NF-kB reporter assay was used to measure changes in NF-kappaB signaling by RNAi or drug exposure. CD133 expression and ALDH activity assay measured changes in the frequency of stem-like cells. Spheroid formation and spheroid viability assays measured the effects of pathway interruption on ovarian cancer cell biology. We investigated two mouse models – survival and relapse – to pinpoint the role of these signaling events in the course of ovarian cancer pathogenesis.

Results: Our siRNA screen showed that NF-kappaB genes NXF1, RAC2, CDC42, BCL2L1, and SERPINB3 were essential for TICs. Using a GFP NF-kappaB reporter, cells grown in TIC conditions had higher NF-kappaB signaling than cells grown in adherent conditions. Our drug screen identified a cohort of drugs toxic to TICs: bardoxolone methyl, salinomycin, disulfiram, and elesclomol. Addition of the drugs to cells grown in TIC conditions

decreased NF-kappaB signaling after 48 hours of drug exposure. These four drugs were able to inhibit cancer spheroid formation, decreasing both size and number of spheroids with increasing concentration. Spheroids are a heterogenous collection of cells, so it was unclear if these drugs were targeting TIC cells or other cells within the spheroid. To address this, we looked at markers for TICs: high CD133 expression and high ALDH activity. Exposure to bardoxolone methyl, disulfiram, and elesclomol decreased cell populations with these markers, indicating that these drugs are affecting TICs. In an *in vitro* model of relapse, we treated cells with chemotherapy followed by treatment with these four drugs and showed that these drugs decreased TIC marker-expressing cells. Our mouse survival model is ongoing, testing the drugs bardoxolone methyl and disulfiram given after chemotherapy, to measure if these drugs will increase overall survival of the mice. Correlative studies will analyze liver cells to confirm on-target drug activity. Following the survival study, we inoculate tumors into mouse ovarian bursa, and use ultrasound to measure the time to relapse after mice are given three cycles of chemotherapy, followed by treatment with vehicle, bardoxolone methyl or disulfiram.

Conclusion: Ovarian cancer tumor initiating cells depend on NF-kappaB signaling. This pathway may become an important target to prevent ovarian cancer relapse.

No conflict of interest

334 (PB-095) Poster

The EurOPDX EDIReX project: towards a European Research Infrastructure on patient-derived cancer models

E. Vinolo[1], J.P. Morris[1], A. Bruna[2], A.T. Byrne[3], R.B. Clarke[4], S. de Jong[5], J. Jonkers[6], A. Krenek[7], G.M. Maelandsmo[8], M.T. Mayrhofer[9], T.F. Meehan[10], P.G. Pelicci[11], A. Piris-Gimenez[12], L. Price[13], L. Rizzotto[14], S. Roman-Roman[15], F. Sarno[16], L. Vezzadini[17], A. Villanueva[18], E. Medico[19]. *[1]Seeding Science SPRL, Limelette, Belgium; [2]Cancer Research UK Cambridge Institute, Cambridge Cancer Centre, University of Cambridge, Cambridge, United Kingdom; [3]Royal College of Surgeons in Ireland, Physiology & Medical Physics Dept, Dublin, Ireland; [4]Manchester Cancer Research Centre, University of Manchester, Breast Biology Group, Division of Cancer Sciences, Manchester, United Kingdom; [5]University Medical Centre Groningen, University of Groningen, Medical Oncology, Cancer Research Centre Groningen, Groningen, Netherlands; [6]The Netherlands Cancer Institute, Division of Molecular Pathology, Oncode Institute, Amsterdam, Netherlands; [7]Masarykova Univerzita, Institute of Computer Science, Brno, Czech Republic; [8]Oslo University Hospital, Institute for Cancer Research, Oslo, Norway; [9]Biobanking and BioMolecular resources Research Infrastructure, European Research Infrastructure Consortium, Graz, Austria; [10]European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom; [11]European Institute of Oncology, Department of Experimental Oncology, Milan, Italy; [12]Vall d'Hebron Institute of Oncology and CIBERONC, Barcelona, Spain; [13]Ocello bv, Leiden, Netherlands; [14]Katholieke Universiteit Leuven, TRACE PDTX Platform, Leuven, Belgium; [15]Institut Curie, PSL Research University, Department of Translational Research, Paris, France; [16]Hospital de Fuenlabrada, Department of Medical Oncology, Fuenlabrada, Spain; [17]Kairos3D srl, Turin, Italy; [18]Catalan Institute of Oncology ICO, Bellvitge Biomedical Research Institute IDIBELL, Chemoresistance and Predictive Factors Laboratory, ProCURE, L'Hospitalet de Llobregat, Barcelona, Spain; [19]University of Torino, Candiolo Cancer Institute IRCCS and Department of Oncology, Turin, Italy*

Background: Counteracting high failure rates in the development of new treatment strategies in oncology and improving therapeutic management of cancer patients requires preclinical models that can account for the complexity and heterogeneity of human tumours. Patient-derived cancer xenografts (PDXs) are developed by transplanting tumour fragments into immunodeficient mice. They **maintain histopathological features and genetic profiles of the original patient tumours and** are increasingly recognised as reliable models to predict treatment efficacy and discover sensitivity and resistance biomarkers with immediate clinical relevance.

Materials and Methods: To effectively recapitulate and therapeutically interrogate the heterogeneity of human cancer, a dozen European cancer centres and university hospitals involved in translational oncology joined forces in 2013 to start EurOPDX, an academic research consortium that now gathers 18 institutions throughout Europe and in the US (www.europdx.eu). The goal of the Consortium is to maximize exploitation of PDXs and other patient-derived models for cancer research by: (i) integrating institutional collections into a multicentre repository now reaching more than 1500 models for 30+ different cancer types; (ii) defining common standards to improve the quality and reproducibility of oncology preclinical data; (iii) sharing models within and outside the consortium to perform collaborative precision oncology "xenopatient" trials. Building on its first successes, EurOPDX is now teaming up with other key academic and SME partners in a four-year project to build the "EurOPDX Distributed Infrastructure for Research on patient-derived Xenografts" (EDIReX project funded under EU's H2020 research and innovation programme, grant no. 731105).

Results: This new cutting-edge European infrastructure will offer access to PDX resources for academic and industrial cancer researchers through 6 state-of-the-art installations or "nodes." We will present the specific objectives of the project, including our work towards standardisation and optimisation of biobanking, quality control and data tracking, and the performance of *in vivo* drug efficacy experiments. Access to the resource, including the distribution of cryopreserved samples from established models, the structured biobanking of user-developed models and the performance of drug efficacy studies, will be offered through a grant application system to open in October 2018. Selection of the models by users will be made possible thanks to the newly-developed EurOPDX Data Portal, of which a prototype will be presented.

Conclusion: Through the EDIReX project we aim to improve preclinical and translational cancer research and promote innovation in oncology by integrating a European PDX repository and facilitating access to this much-needed resource for European and worldwide researchers.

No conflict of interest

335 (PB-096) Poster

3D multi-culture model reveals CXCR4/CXCL12 role in Lung cancer

M. Papadimitriou[1], D. Ntanovasilis[1], I. Papasotiriou[1]. *[1]Research Genetic Cancer Centre S.A., Research and Development, Florina, Greece*

Background: Tumor microenvironment plays a significant role to tumor survival, proliferation and metastasis. It consists of both cellular components and ECM. This study examines the role of cellular components in lung cancer concerning the expression of several markers including hypoxia markers and cytokines, as well as markers usually found in immune cells, using a 3D multi-culture model.

Materials and Methods: The lung cancer cell line CORL105 was cultured alone and with endothelial cell line HUVEC, in co-culture, in hanging drop; monocytes isolated from healthy donor were added to the mono and co-culture, in order to determine their effect on CD163, CD206 and CD64 marker expression. Moreover, the expression of HIF-1a, CXCR4 and CXCL12 was examined both in mono, co and tri-cultures. Marker expression was measured via flow cytometry.

Results: CORL105 spheroids are larger than the ones consisting of both cancer cells and endothelial cells, or cancer cells, endothelial and monocytes. Moreover, spheroids that include endothelial cells are formed faster than the mono-culture ones. Marker HIF-1a expression is higher in mono-culture spheroids, but tri-culture spheroids exhibit higher CD14, CD163 and CD206 expression than the spheroids consisting of CORL105 and monocytes. CXCR4 and HIF-1a co-express higher in tri-cultures in contrast to mono and co-cultures, whereas HIF-1a and CXCL12 exhibit similar expression pattern in co and tri-culture spheroids. Concerning CXCR4 and CXCL12 simultaneous expression, it seems to be higher in tri-culture spheroids.

Discussion: Clearly, cancer manipulates its environment and affects the expression levels of cytokines and markers that are involved in macrophage polarization. The results above indicate that immune cells could be involved in higher cytokine production, aided by hypoxia. Nevertheless, in order to establish their actual role in lung cancer more studies are required.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Radiation Interactive Agents

336 (PB-097) Poster

Targeting checkpoint kinases for the more effective treatment of radioresistant aggressive breast cancers

B. Chandler[1], S. Nyati[1], C. Ritter[1], L. Moubadder[1], E. Olsen[1], A. Michmerhuizen[1], A. Pesch[1], K. Wilder-Romans[1], L. Pierce[1], A. Chinnaiyan[2], C. Speers[1]. [1]*University of Michigan, Department of Radiation Oncology, Ann Arbor, USA;* [2]*University of Michigan, Department of Pathology, Michigan Center for Translational Pathology, Ann Arbor, USA*

Background: Increased rates of locoregional recurrence leading to poorer clinical outcomes have been observed in triple-negative breast cancer (TNBC) despite the use of radiation therapy (RT), therefore approaches that result in radiosensitizaton in TNBC are critically needed. Our previous work identified a group of cell cycle kinases overexpressed in TNBC. Here we described the impact of elevated expression of two of these identified kinases, MELK and TTK, on radiation response and patient outcomes in TNBC.

Methods: TCGA breast cancer datasets were used to determine MELK and TTK expression in the intrinsic subtypes of BC. Clonogenic survival assays were used to determine the radiosensitization in cell lines after pharmacologic MELK or TTK inhibition. DNA damage was quantified using gH2AX staining in TNBC cell lines. NHEJ and HR stable reporter systems were used. Kaplan-Meier analysis was used to determine the impact of MELK or TTK expression on locoregional recurrence (LRR) and overall survival (OS). A Cox proportional hazards model was constructed to identify potential factors of LRR-free survival in univariate (UVA) and multivariable analyses (MVA).

Results: Our radiosensitizer screen nominated two cell cycle checkpoint kinases, MELK and TTK, as the kinases most highly expressed in radioresistant breast tumors. MELK and TTK expression is significantly elevated in breast cancer tissue compared to normal breast tissue (Q-VAL: 8.7 E – 291) and are most highly expressed in basal-like tumors compared to other subtypes (N = 2,900 samples) and TNBC cell lines (N = 51, $p <$ 0.0001). MELK and TTK expression is significantly correlated with intrinsic radioresistance in a panel of 23 BC cell lines (R = 0.58–0.65, *p*-value = <0.001). Genomic (siRNA knockdown) or pharmacologic inhibition of MELK and TTK increased radiosensitivity *in vitro* in 4 different TNBC cell lines (rER 1.38–1.72). Mechanistically, MELK inhibition led to impaired NHEJ repair after RT, and TTK inhibition inhibited HR repair. MELK or TTK inhibition or knockdown significantly radiosensitized TNBC xenografts and PDXs and markedly delayed tumor doubling time and growth (median tumor doubling 7.95 (RT alone) vs. 29.1 days (combination), 8.5 (RT alone) vs. 24.5 days (combination); *p*-value <0.0001). Clinically, patients treated with breast conserving surgery and RT with lower than median MELK or TTK expression had worse local-recurrence free (LRF) survival and overall survival (HR for local recurrence 1.7–3.1 as continuous variable, p-value <0.001) compared to patients with higher than median expression. In MVA only MELK expression, TTK expression, and grade were associated with worse LRF survival in 3 independent datasets.

Conclusion: Our results support the rationale for clinical development of cell cycle checkpoint kinases inhibitors (MELK and TTK) as a novel radiosensitizing strategy in TNBC.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Regulatory Affairs

337 (PB-098) Poster

Utilization, safety, and efficacy of the FDA compassionate use program

N. Feit[1,2], E. Smith[3], R. Cambria[4], J. Anopa[4], J. Deighan[4], E. Pamer[1], R. Wilson[5], J. Greenfield[6], O. Zivanovic[3], D. Hyman[1]. [1]*Memorial Sloan Kettering Cancer Center, Early Drug Development Service, New York, USA;* [2]*Weill Cornell Medical College, Weill Cornell Medicine, New York, USA;* [3]*Memorial Sloan Kettering Cancer Center, Gynecology Service, New York, USA;* [4]*Memorial Sloan Kettering Cancer Center, Department of Research and Technology Management, New York, USA;* [5]*Memorial Sloan Kettering Cancer Center, Department of Surgery, New York, USA;* [6]*Weill Cornell Medicine, Department of Neurological Surgery, New York, USA*

Background: Little is known about the utilization and outcomes of the FDA's Expanded Access ("Compassionate Use") program, which permits access to investigational drugs outside the context of clinical trials. Recent national passage of "Right to Try" legislation in the US promises to further expand this type of investigational drug use outside of formal clinical trials. We therefore aimed to evaluate the efficacy and safety of this policy mechanism as employed at a large comprehensive cancer center.

Materials and Methods: All compassionate use applications for cancer products submitted between January 1, 2012 and January 1, 2018, the data cutoff date, to the Memorial Sloan Kettering IRB were reviewed. The medical record as well as all associated regulatory submissions were abstracted for each patient. Responses were assessed by the treating physician at ≥6 weeks into therapy. Descriptive statistics were used to report patient demographic, clinical, pathologic, and other characteristics.

Results: We identified 219 unique single-patient use (SPU) compassionate use applications in which the requested agent was ultimately administered in 193 cases. Median age of patients treated was 41 years (absolute range 3–88, interquartile range 13.5–60). 57 unique cancer types were treated, with the most common being hematologic (40.6%), peripheral nervous system (14.6%), lung (7.3%), and primary CNS (7.3%). In total, 69 unique investigational agents were requested. At the time of SPU submission, the most advanced clinical development status of each agent was as follows: preclinical (1.8%), Phase I (22.8%), Phase II (33.3%), Phase III (37.0%), and post-marketing (4.6%). Comparing the regulatory status of each agent at the time of SPU initiation to its status at the data cutoff date, the percentage of FDA-approved agents increased from 4.6% to 26.0%. The most frequently requested agents were receptor- or signal-transduction agents (42.0%) and immunomodulators (38.4%). 81.9% (158/193) of patients were evaluable for response and of these, 20.3% responded and an additional 25.3% achieved stable disease. Response rates were numerically higher in patients treated with marketed agents (40.0%) compared to those without marketing authorization (mean 18.9%, range 13.0–23.1%). Drug-related severe adverse events (SAEs) of grade ≥3 occurred in 26.5% of cases. A total of 1 drug-related grade 5 SAE occurred.

Conclusion: These data suggest that compassionate use SPUs are being utilized at all stages of clinical development in a wide range of cancer types and patient ages. A subset of patients derive clinical benefit in the form of objective responses but serious toxicity was also observed. More detailed utilization and clinical outcomes data will be presented.

No conflict of interest

Friday, 16 November 2018

POSTER SESSION

Drug Resistance and Modifiers

340 (PB-003) Poster

Discovery of promising anti-cancer drug combination using YAP-TEAD interaction inhibitors with standards of care in mesothelioma and NSCLC cells

A. Soude[1], M. Barth[2], S. Delaporte[1], F. Chirade[1], S. Contal[2], C. Valaire[1], I. Konstantinova[1], J.L. Junien[3], P. Broqua[3]. [1]*Inventiva, Biology, Daix, France;* [2]*Inventiva, Chemistry, Daix, France;* [3]*Inventiva, Management, Daix, France*

The Hippo pathway is a critical player in several processes involved in cancer progression, including cell proliferation and EMT. YAP mediates the downstream effects of Hippo signaling and high nuclear expression of YAP has been documented in many tumor types. YAP translocates to the nucleus, binds to TEAD transcription factors and drives the expression of target genes involved in cell proliferation, migration and survival. In addition YAP-TEAD interaction has been suggested to induce resistance of cancer cells to many anti-cancer agents, such as RAF, MEK, and EGFR-TK inhibitors as well as several standards of care like cisplatin and taxol (Lee 2015, 2016, Hsu 2016). Pleural mesothelioma, which is extremely aggressive with limited treatment options and no cure, is inherently chemo-resistant with only 50% of patients responding to the standard of care treatment and consequently it has a very poor prognosis.

We have recently reported the discovery of YAP-TEAD interaction inhibitors that disrupt the YAP-TEAD complex and block proliferation of tumor cells. These compounds represent promising new treatment modalities for mesothelioma where the Hippo pathway is highly deregulated and YAP mainly localized in the nucleus (AACR 2016).

The aim of this study was to investigate the potential of our YAP-TEAD interaction inhibitors to alleviate the resistance to standard of care agents of mesothelioma and NSCLC cell lines. The effect of our compounds alone and in combination with Pemetrexed, a folate antimetabolite drug were investigated in H2052 and A549, mesothelioma and NSCLC cancer cells respectively. To assess the efficacy of Pemetrexed+/−Inventiva compounds, the cell lines in 2D or in 3D cultures were exposed to single agents and combinations in a dose response for 4 days or 15 days. ATP CellTiterGlo or EDU incorporation assays were used to measure cell growth.

We found a synergistic effect between the YAP-TEAD interaction inhibitors and Pemetrexed, leading to a drastic inhibition of cancer cell proliferation and an increase of cytotoxicity. This suggests that YAP-TEAD interaction inhibitors used in combination with existing chemotherapeutics could be used to attenuate multidrug resistance and re-sensitize chemo-resistant cancer cells.

No conflict of interest

343 (PB-006) Poster

Mechanisms of tumor cell invasion in a pancreatic neuroendocrine tumor before and after the anti-angiogenic treatment

J. Pinto[1], I. Zuazo[1], A. Martínez-López[1], O. Casanovas[1]. *[1]Catalan Institute of Oncology, IDIBELL, Program Against Cancer Therapeutic Resistance, Barcelona, Spain*

Introduction: Local invasion is a key cellular cell-biological event in the metastatic cascade. In response to a changing microenvironment, cancer cells may act using two main strategies of invasion: single cell invasion and collective invasion. Results from our group demonstrated an irreversible increase in the incidence of invasive tumors during anti-angiogenic treatment in the RIP-TAG2 mouse model. Determining how tumor cells initiate and sustain invasive behavior may help improve the development of new intervention modalities.

Materials and Methods: The RIP1-Tag2 is a transgenic mouse model of pancreatic islet cell tumorigenesis (PNETs). In order to study invasion mechanisms in these tumors, they were molecularly characterized regarding their invasion capacity before and after the anti-angiogenic treatment (sunitinib). Furthermore, using βTC4 cells from RIP1-Tag2 we developed a three-dimensional (3D) tumor spheroid model to the evaluate invasion pattern in 3D culture *in vitr*o.

Results: Deeply studying tumor invasion in RIP1-Tag2 model, we observed that it does not follow a classical epithelial-mesenchymal transition, but it rather involves mechanisms of collective tumor cell invasion. In these tumors, molecular signatures were identified implicated in cell-cell adhesion, specifically on cadherin and claudin families. We also found a correlation between the expression of these targets and a high tumor invasion capacity. Moreover, these data were also confirmed in a 3D tumor spheroid model. Finally, E-cadherin and Claudin1/4 expression were significantly correlated in neuroendocrine primary tumors and metastases from patients.

Conclusions: Using two different approaches, we have verified that members of cadherin and claudin families might be interesting targets implicated in invasiveness in pancreatic neuroendocrine tumors before and after anti-angiogenic treatment. The analysis of clinical samples supports our preclinical data suggesting the integrative action of E-cadherin and Claudin1/4 in pancreatic neuroendocrine tumors from patients.

This project has the support of a National Council for Scientific and Technological Development (CNPq) from Science Without Borders, Brazil.

No conflict of interest

344 (PB-007) Poster

Development and characterization of CV6-168, a novel and selective dUTPase inhibitor that enhances the antitumour efficacy of TS-targeted therapies

P. Wilson[1], K.A. Mulligan[1], K.A. Mclaughlin[1], V. Prise[1], M.L. Wilson[2], P.G. Johnston[2], R.D. Ladner[1]. *[1]CV6 Therapeutics NI Ltd., Research and Development, Belfast, United Kingdom; [2]Queen's University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom*

Background: Thymidylate synthase (TS) inhibitors are standard-of-care therapies for the majority of high incidence cancers. Deoxyuridine triphosphate nucleotidohydrolase (dUTPase) is a gatekeeper enzyme that protects cancer cell DNA from the misincorporation of uracil that would otherwise occur during treatment with TS-targeted therapies such as 5-FU, capecitabine, S-1, pemetrexed and methotrexate. Uracil misincorporation induced by inhibition of dUTPase causes extensive and irreparable DNA damage during treatment with TS-targeted therapies and strongly enhances cancer cell death. dUTPase is highly-expressed across many cancer types and is associated with resistance to TS-targeted therapies. Here we report the development and characterization of CV6-168, a novel and selective inhibitor of dUTPase that significantly enhances the efficacy of TS-targeted therapies against a broad spectrum of human cancer models.

Materials and Methods: The mode of inhibition of dUTPase by CV6-168 was determined against recombination human dUTPase in a fluorescence-based assay developed in our laboratory. *In vitro* cell growth inhibition was determined by CellTiter-Glo in 22 human cancer cell lines spanning 13 cancer types. Cell viability was measured by clonogenic cell survival assay in 46 human cancer cell lines spanning 18 cancer types. DNA damage was assessed by flow cytometry by measuring H2A.X and ATM activation. Imbalances in nucleotide pools were measured using the aforementioned fluorescence-based assay. The *in vivo* antitumour activity of CV6-168 was evaluated in cancer xenograft models.

Results: CV6-168 inhibited dUTPase in a competitive and reversible mode with a K_i of 251 nmol/L. CV6-168 exhibited no intrinsic cytotoxicity as a single agent but significantly enhanced cancer cell death in clonogenic assays when combined with FUdR (5-FU active metabolite) in all cell lines across all cancer types tested, including an HCT-116 5-FU-resistant colon cancer model. CV6-168 significantly enhanced the growth inhibition induced by FUdR in 21 out of 22 cancer cell lines analysed. The mean sensitization factor was 23.7 and median 11.7 (range 1.5 – 119). Mechanistic analyses demonstrated that this enhancement was accompanied with a significant increase in dUTP pool expansion and DNA damage. CV6-168 significantly increased the antitumour activity of 5-FU in both the HCT116 and LoVo colon xenograft cancer models with no evidence of increased toxicity.

Conclusions: CV6-168 is a potent and selective dUTPase inhibitor that significantly enhances the anticancer activity of 5-FU as demonstrated in both *in vitro* and *in vivo* models. CV6-168 represents a promising new therapeutic agent with the potential to significantly improve the clinical efficacy of TS-targeted therapies across multiple cancer types. CV6-168 will be evaluated in an upcoming Phase I clinical trial.

Conflict of interest: Ownership: Robert D Ladner, Karl A Mulligan, Peter M Wilson, Kylie McLaughlin, Vivien Prise. Advisory Board: Melissa L Wilson. Board of Directors: Robert D Ladner, Karl A Mulligan.

345 (PB-008) Poster

Therapeutic resistance of 3D pancreatic cancer tumorspheres involves differential cancer stem cell response

N. Walsh[1], S. Nelson[1], S. Roche[1], F. O'neill[1], J. Crown[2]. *[1]Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland; [2]St Vincent's University Hospital, Department of Medical Oncology, Dublin, Ireland*

Background: Pancreatic cancer is a highly lethal disease. Despite improvements in diagnosis, treatment and surgical care, the overall 5-year survival is 8%. Therapeutic resistance to chemotherapy is a major cause of treatment failure and recurrence in pancreatic cancer. Cancer Stem Cells (CSCs) are a small population of cells responsible for tumour initiation, progression, metastasis and chemo-resistance. CSCs also have been considered as the main cause of cancer recurrence. Therefore, targeting CSCs could be an effective strategy to improve the survival outcomes of pancreatic cancer patients.

Material and Methods: 3D pancreatic cancer tumorspheres were developed in established and primary pancreatic cancer cultures and resistance generated to a range of chemotherapeutic drugs (Gemcitabine, 5-FU, Paclitaxel and Cisplatin). Tumorsphere culture was used to analyse the self-renewal capability of CSCs, and to enrich these cells from bulk cancer cells. CSC sub-populations were enriched for using $CD133^+/CD44^+/ESA^+$ populations from therapeutic resistant tumorspheres.

Results: Not all cell lines formed primary tumorspheres however, therapeutic resistance of 3D tumorspheres was highly increased compared to 2D monolayer. Therapeutic resistance of the 3D tumorspheres was verified by applying a secondary tumorsphere formation with dissociated single-cell suspension from primary tumorspheres. HPAC resistant tumorspheres exhibited the most robust response to chemotherapy treatment and possessed a high differential CSC content. Profiling of the therapeutic resistant 3D pancreatic cancer tumorspheres revealed increased invasive phenotype, expression of self-renewal related genes and epithelial-mesenchymal transition.

Conclusion: Development of therapeutic resistant 3D pancreatic cancer tumorspheres allowed for the characterisation of the CSC-associated

phenotype in response to chemotherapy which more accurately reflects the cellular architecture and heterogeneity *in vivo*.

No conflict of interest

346 (PB-009) Poster

AZD3229, a KIT/PDGFRA inhibitor with best in class potential for the treatment of gastrointestinal stromal tumors (GIST)

R. Anjum[1], J. Kettle[2], M. Grondine[1], E. Anderson[1], D. Bhavsar[1], V.P. Reddy[2], J.M. Smith[3], S.E. Pearson[3], M.J. Packer[2], A.R. Harmer[4], T. Collins[4], J. Mettetal[5], C. Brown[1], R. Overman[6], W. Nissink[3], H. Wang[1], E. Barry[1], R.D. Jones[2], W. Shao[1], S. Guichard[1]. *[1]AstraZeneca, Oncology, IMED Biotech Unit, Boston, USA; [2]AstraZeneca, Oncology, IMED Biotech Unit, Cambridge, United Kingdom; [3]AstraZeneca, Oncology, IMED Biotech Unit, Cambridge, USA; [4]AstraZeneca, Drug Safety and Metabolism, IMED Biotech Unit, Cambridge, United Kingdom; [5]AstraZeneca, Drug Safety and Metabolism, IMED Biotech Unit, Boston, USA; [6]AstraZeneca, Discovery Sciences, IMED Biotech Unit, Cambridge, United Kingdom*

Background: GIST is the most common human sarcoma driven by gain of function mutations in either KIT or PDGFRA. Although first line imatinib has revolutionized the treatment of GIST, drug resistance caused by secondary KIT or PDGFRA mutations develops in over 80% of patients. Sunitinib (2nd line) and regorafenib (3rd line) do not have activity against the full spectrum of KIT/PDGFRA mutations and show an overall median time to tumor progression of only 5–6 months. Potent inhibition of KDR in these agents is associated with significant high-grade hypertension, amongst other significant toxicities. A well tolerated drug that targets the broad spectrum of KIT/PDGFRA mutations is therefore an unmet need for the treatment of GIST patients.

Materials and Methods: AZD3229 cellular potency was assessed in GIST 430 cells (Ex11 del/V654A) and Ba/F3 cell lines driven by primary and primary/secondary mutations of KIT or PDGFRA and KDR in a 3-day growth assay. AZD3229 was dosed *in vivo* in xenograft models with primary and primary/secondary KIT mutations and evaluated for pKIT signaling and tumor growth inhibition (TGI). Change in arterial blood pressure was measured using telemetry in rats dosed with AZD3229.

Results: AZD3229, identified using structure-based drug design, potently inhibits growth of a wide spectrum of mutant KIT and PDGFRA-driven Ba/F3 cell lines (∼25 cell lines tested; GI50 range: 1–50 nM) with over 50-fold selectivity vs KDR (Table 1). At a dose of 20 mg/kg, AZD3229 causes extensive and durable inhibition of pKIT (90% inhibition for ∼12 h) in xenograft models with primary ex11 del mutation and primary/secondary ATP-binding pocket mutations (GIST 430 and PDX model of KIT V654A) and A-loop mutations (PDX model of KIT Y823D and Ba/F3 KIT D816H). Furthermore, in these models, AZD3229 leads to extensive tumor regressions (ranging from –65% to –90% TGI) when dosed orally at 20 mg/kg BID for 4–6 weeks. In a rat telemetry study, no change in arterial blood pressure was observed at a dose predicted to give equivalent target engagement.

Conclusions: AZD3229 leads to extensive and durable pKIT inhibition in multiple preclinical models driven by KIT mutations leading to regressions with a margin to KDR-driven blood pressure changes. AZD3229 therefore has potential as a best in class mutant KIT/PDGFRA inhibitor leading to durable responses as a 2nd line therapy in advanced GIST with an ambition to move into 1st line GIST without risk of hypertension.

Conflict of interest: Other Substantive Relationships: We are employees and shareholders of AstraZeneca Pharmaceuticals.

Table 1. Effect of AZD3229 and standard of care agents on growth of Ba/F3 cells with representative KIT and PDGFRA mutations and KDR respectively (GI50 : µM)

ID	KIT Ex11 del	KIT Ex 11 del/ V654A	KIT Ex11 del/ D816H	KIT Ex11 del/ T670I	PDGFRA V561D/ D842V	KDR
AZD3229	0.001	0.003	0.009	0.016	0.022	1.378
Imatinib	0.017	0.393	0.535	>10	0.567	>10
Sunitinib	0.004	0.006	0.398	0.005	0.631	0.033
Regorafenib	0.021	0.231	0.29	0.033	0.522	0.114

347 (PB-010) Poster

Understanding acquired resistance to CDK9 inhibition in hematologic tumor models

C. Andersen[1], T. Proia[1], J. Cidado[1], S. Criscione[1], S. Boiko[1], G. Mills[2], P. Janne[3], L. Drew[1]. *[1]AstraZeneca, Bioscience Oncology IMED Biotech Unit, Waltham, USA; [2]The University of Texas MD Anderson Cancer Center, Department of Systems Biology, Houston, USA; [3]Dana-Farber Cancer Institute, Lowe Center for Thoracic Oncology, Boston, USA*

Background: Transient inhibition of the transcriptional regulator cyclin-dependent kinase 9 (CDK9) leads to downregulation of short half-life genes, including the pro-survival gene MCL1. Consequently, MCL1-addicted cancers undergo rapid apoptosis upon CDK9 inhibition (CDK9i) *in vitro* and *in vivo*. This observation has prompted entry of several CDK9i agents into the clinic. Clinically, as with many targeted therapies, emergence of acquired CDK9i resistance is anticipated. However, mechanisms of CDK9i resistance are poorly understood.

Materials and Methods: We generated *in vivo* models of CDK9i resistance through repeated, intermittent dosing of haematologic cancer xenografts with AZ5576, a potent and selective CDK9 inhibitor. MV411 (leukemia) and MOLP8 (myeloma) cells were implanted subcutaneously in SCID mice and dosed twice weekly with 60 mg/kg AZ5576 until tumors were actively growing through treatment. Resistant tumors were then passaged into naïve mice and further characterized by evaluating efficacy of other CDK9i compounds and BH3 mimetics. Molecular changes between resistant and parental tumors were profiled using RNA sequencing (RNAseq) and reverse phase protein array (RPPA) analysis; hits were confirmed using western blot and PCR analyses.

Results: AZ5576 treatment initially produced robust antitumor effects; 90% tumor growth inhibition (TGI) for MV411 and >100% (regressions) for MOLP8. However, resistant tumors began to emerge after 5–6 weeks of treatment. AZ5576-resistant (5576R) tumors displayed cross-resistance to other CDK9 inhibitors in clinical development including dinaciclib and AZD4573. MOLP8 5576R xenografts were also less responsive to direct MCL1 inhibition relative to parental (66% vs >100% TGI, respectively). Transcriptomics and proteomics identified alterations in several signaling pathways in MV411 and MOLP8 5576R tumors including the apoptosis pathway. Specifically, 5576R tumors showed decreased MCL1, BIM, and BAK, as well as upregulation of BCL2. Neither model was sensitive to single agent BCL2 inhibition. However, combining BCL2 with CDK9 inhibition in MOLP8 5576R tumors led to disease stabilization (90% TGI). Additional altered signaling pathways in 5576R tumors include AKT and MAPK. However, perturbation of these pathways did not impact CDK9i sensitivity.

Conclusions: Hematologic cancer xenografts develop CDK9i resistance *in vivo* after extended treatment with AZ5576. AZ5576-resistant tumors have significant changes at the transcriptional and proteomic level including upregulation of BCL2. Combining BCL2 inhibition with CDK9i partially overcomes resistance, leading to tumor stasis in 5576R xenografts. Understanding CDK9i resistance preclinically will identify strategies to overcome and potentially prevent it from developing in patients.

Conflict of interest: Advisory Board: G. Mills is a Scientific Advisory Board (SAB) member/consultant with AstraZeneca. Corporate-sponsored Research: C. Andersen, T. Proia, J. Cidado, S. Criscione, S. Boiko, and L. Drew are current employees of AstraZeneca. Other Substantive Relationships: P. Janne has served as a consultant for and has received research funding from AstraZeneca.

348 (PB-011) Poster

Heat shock protein 90 inhibitors overcome the resistance to various Fms-like tyrosine kinase 3 inhibitors

K. Katayama[1], K. Noguchi[1], Y. Sugimoto[1]. *[1]Keio University, Faculty of Pharmacy, Division of Chemotherapy, Tokyo, Japan*

Background: *Fms-like tyrosine kinase 3* (*FLT3*) mutation, *FLT3*-ITD, is a driver oncogene and recognized as a molecular target in acute myeloid leukemia (AML). Three FLT3 inhibitors, midostaurin, quizartinib, and gilteritinib, are currently under consideration for the treatment of FLT3-ITD-positive AML. However, several resistant mutations (N676, F691, D835, and Y842 mutations) in tyrosine kinase domain (TKD) of FLT3-ITD have been reported to confer the resistance to quizartinib and/or midostaurin. This study aimed to explore new effective inhibitors to overcome the resistance.

Materials and Methods: Ba/F3-ITD, Ba/F3-ITD+N676K (N676K), Ba/F3-ITD+F691L (F691L), Ba/F3-ITD+D835V (D835V), and Ba/F3-ITD+Y842C (Y842C) cells were established by transfection with the corresponding *FLT3*-ITD plasmids by retroviral infection method. Two quizartinib-resistant cell lines, QR1 and QR2, were generated by exposing FLT3-ITD-positive AML MV4-11 cells to the increasing concentrations of quizartinib for 6 months.

Growth inhibition assays were performed to assess sensitivity to drugs. Protein expression and phosphorylation were examined by immunoblotting, and cell cycle and apoptosis analyses were by flow cytometry.

Results: Growth inhibition assays revealed that N676K cells showed resistance to midostaurin, and D835V and Y842C cells were resistant to quizartinib, as reported previously. Interestingly, F691L cells showed hyper resistance not only to quizartinib and midostaurin but also to gilteritinib. We then screened 50 small molecule inhibitors for overcoming the resistance to FLT3 inhibitors in Ba/F3 transfectants and found that heat shock protein 90 (HSP90) inhibitors, 17-AAG, 17-DMAG, retaspimycin, and luminespid, suppressed proliferation of the transfectants, accompanied with the down-regulation of STAT5, AKT, and MAPK pathways. 17-AAG induced G1 arrest followed by increasing sub-G1 fraction in all transfectants. The effect of HSP90 inhibitors on D835V cells was superior to the other cell lines. Similar results were obtained in QR1 and QR2 cells that harbor D835H and D835V mutations, respectively, in *FLT3*-ITD gene. QR1 and QR2 cells showed resistance to quizartinib but not to gilteritinib and midostaurin; however, they were more sensitive to HSP90 inhibitors than MV4-11 cells. HSP90 inhibitors well-downregulated STAT5, AKT, ERK, cyclins, and phosphorylated RB, and induced caspase-dependent apoptosis in QR1 and QR2 cells. Finally, 17-AAG significantly enhanced the sensitivity of QR1 and QR2 cells to daunorubicin.

Conclusions: HSP90 inhibitors are effective against FLT3 inhibitors-resistant cells harboring various TKD mutations in FLT3-ITD.

No conflict of interest

349 (PB-012) Poster

Poly (ADP-ribose) polymerase1 deficiency is not correlated with rucaparib and veliparib sensitivity in A2780 cells

Y. Nonomiya[1], K. Noguchi[1], K. Katayama[1], Y. Sugimoto[1]. [1]*Faculty of Pharmacy, Keio University, Division of Chemotherapy, Tokyo, Japan*

Background: Poly (ADP-ribose) polymerase (PARP) inhibitors have high anti-tumor activity against BRCA1/2-mutated ovarian and breast cancer. In Japan, olaparib, a PARP1/2 inhibitor has been approved for the maintenance treatment of platinum-sensitive recurrent ovarian cancer. In this study, we have isolated three olaparib-resistant cells lines (ola-R cl.3, cl.10 and cl.15) from human ovarian cancer A2780 cells, and explored the mechanism for the sensitivity to PARP inhibitors.

Material and Methods: Sensitivity to the PARP inhibitors, olaparib, rucaparib and veliparib, was evaluated by the cell growth inhibition assay and the colony formation assays. The expression of PARP1 and PARP2 was confirmed by western blotting. Poly-ADP-ribosylation of cellular proteins was also evaluated by western blotting. PARP inhibitor-induced γH2AX foci were visualized with Immunofluorescence confocal microscopy. The number of γH2AX foci with a diameter of 0.5 μm or more in each cell was counted. At least 100 cells were used in each experiment.

Results: Ola-R cl.3, cl.10 and cl.15 cells showed 17–30-fold higher resistance to olaparib than the parental A2780 cells. These resistant cells showed only marginal levels of cross-resistance to other PARP inhibitors, rucaparib and veliparib. In addition, the resistant cells showed 2–5-fold higher collateral sensitivity to other cytotoxic agents, cisplatin and SN-38. The resistant cells did not express the drug efflux transporters, P-GP/ABCB1, BCRP/ABCG2, MRP1/ABCC1, MRP2/ABCC2 and MRP3/ABCC3. The numbers of olaparib-induced γH2AX foci of the resistant cells were significantly lower than those of the parental A2780 cells. However, the numbers of rucaparib- and veliparib-induced γH2AX foci of the resistant cells were similar to those of the parental A2780 cells. PARP1 expression was detected in A2780 cells, but not in the resistant cells. A2780 and the resistant cells expressed similar levels of PARP2. Similar results were obtained in the RT-PCR experiments for *PARP1* and *PARP2*. In the resistant cells, the cellular content of poly-ADP-ribosylated proteins detected by anti-poly-ADP-ribose antibody, was lower than that in the parental A2780 cells. Cell growth inhibition and colony formation assays showed that PARP1 knockdown conferred resistance to olaparib, but not to rucaparib and veliparib.

Conclusions: Our results suggest that PARP1 expression is one of the sensitivity factors of olaparib, but not of rucaparib and veliparib in human ovarian cancer A2780 cells.

No conflict of interest

350 (PB-013) Poster

Pre-clinical evaluation of targeting autophagy for the treatment of Oral Squamous Cell Carcinoma

S. Magnano[1], J. O'sullivan[2], D. Zisterer[1]. [1]*Trinity College Dublin, School of Biochemistry and Immunology, Dublin, Ireland;* [2]*Trinity College Dublin, School of Dental Science, Dublin, Ireland*

Background: Oral Squamous Cell Carcinoma (OSCC) is the sixth most common form of cancer worldwide. The 5-year survival rate in oral cancer is only 50% mostly due to chemoresistance and late diagnosis. There is a compelling demand for improved therapeutic options for OSCC. It has been demonstrated that a cell survival pathway known as autophagy is frequently activated in tumour cells treated with chemotherapeutics. The role of autophagy in OSCC remains poorly understood and further study is required to clarify whether it plays a role in chemoresistance and in tumour progression. The goal of this study is to investigate cell death mechanisms induced in oral cancer cells in response to standard OSCC chemotherapeutics (e.g. cisplatin) and to examine the potential role of autophagy in chemoresistance. Specifically this project aims to determine whether a combination of chemotherapy and autophagy inhibition represents a novel treatment strategy to bypass drug resistance in OSCC.

Methods: The effect of cisplatin on the viability of the OSCC cell lines was evaluated by the Alamar Blue® assay. Apoptosis was examined through flow cytometric analysis of Annexin V/PI stained cells and by immunoblotting of caspase 3 cleavage products. Autophagy was investigated by the Cyto-ID® Autophagy Detection Kit and immunoblotting of LC3-II and p62 proteins.

Results: Cisplatin potently reduced the viability of OSCC cells and induced apoptosis and autophagy in a dose- and time-dependent manner. Additionally, pretreatment of OSCC cells with autophagy inhibitors (chloroquine and bafilomycin A1) enhances cisplatin-induced apoptosis.

Conclusions: Cisplatin induces both apoptosis and autophagy in OSCC cells. The autophagy inhibitors chloroquine and bafilomycin A1 sensitize OSCC cells to cisplatin-induced apoptosis, suggesting that autophagy may play an important role in chemoresistance. Targeting autophagy may open new avenues for developing new treatment strategies to improve clinical outcome in OSCC patients.

No conflict of interest

351 (PB-014) Poster

SPRY1 is a candidate oncogene in braf-mutant cutaneous melanoma

B. Montico[1], G. Giurato[2], F. Colizzi[1], E. Comaro[1], E. Pivetta[3], D. Benedetti[4], A. Weisz[2], A. Steffan[1], L. Sigalotti[5], E. Fratta[1]. [1]*IRCCS-National Cancer Institute, Aviano, Immunopathology and Cancer Biomarkers, Aviano, PN, Italy;* [2]*University of Salerno, Department of Medicine, Baronissi SA, Italy;* [3]*IRCCS-National Cancer Institute, Aviano, Molecular Oncology, Aviano, PN, Italy;* [4]*IRCCS-National Cancer Institute, Aviano, Clinical and Experimental Onco-Hematology Unit, Aviano, PN, Italy;* [5]*University Hospital of Udine, Institute of Clinical Pathology, Udine, Italy*

Background: About 50% of cutaneous melanoma (CM) harbor BRAF mutations that constitutively activate MAPK pathway. Targeted therapy with BRAF inhibitors (BRAFi) has proven efficacy in BRAF-mutant metastatic CM, but patients almost invariantly develop an early resistance. The underlying mechanisms are various and heterogeneous, but resistance usually associates with an altered signaling. Sprouty 1 (SPRY1) is an upstream mediator of MAPK signaling pathway, and despite it has been found to be implicated in different biological processes, its specific role in BRAF-mutant CM has not been completely elucidated.

Material and Methods: The expression of SPRY1 was evaluated by quantitative real-time PCR (qRT-PCR) and Western blot analyses in BRAF-mutant CM cell lines established in our Institution. SPRY1 gene was knock out using CRISPR based-strategy. Cell proliferation was measured in parental cells and SPRY1 knock-out ($SPRY1^{KO}$) clones using xCELLigence instrument. RNA-seq identified mRNAs modulated in $SPRY1^{KO}$ clones respect to parental cells. Modulation of signaling pathways was evaluated by qRT-PCR and Western blotting, whereas ROS induction was assessed by flow cytometry. Cell viabilities before and after BRAFi treatment were evaluated using xCELLigence instrument and by clonogenic assays. Apoptosis was assessed by Annexin V/PI staining.

Results: SPRY1 was found high expressed in a panel of BRAF-mutant CM cell lines both at mRNA and protein level. To investigate the effects of SPRY1 knockdown in BRAF-mutant CM, SPRY1 expression was silenced by using the CRISPR/CAS9 technology. Transfected cells were sorted into single cells and subsequently expanded to generate $SPRY1^{KO}$ clones. Cell proliferation was reduced in $SPRY1^{KO}$ clones in a time-dependent manner relative to parental cells. RNA-seq identified a large number of mRNAs significantly differentially expressed in $SPRY1^{KO}$clones, thus indicating a

difference in the mRNA expression profiles respect to parental cells. Gene ontology analysis indicated that the top of the neighbor coding gene function of differentially expressed mRNAs were involved in cell proliferation and cell death. Consistent with these data, SPRY1 inactivation was associated with: i) decreased phosphorylation of p38; ii) enhanced activation of p53; iii) decreased protein levels of several anti-apoptotic proteins. Furthermore, BRAFi treatment was more effective in SPRY1^{KO} clones which also exhibited higher basal ROS levels respect to parental cells.

Conclusions: Altogether, our findings reveal important insights into SPRY1 function in BRAF-mutant CM, and suggest its potential involvement in response to BRAFi treatment.

No conflict of interest

352 (PB-015) Poster

The synergistic effect of melphalan and XPO1 inhibition in preclinical models of multiple myeloma

J. Turner[1], A. Bauer[1], J. Dawson[1], J. Gomez[1], T. Nishihori[2], D. Sullivan[3]. *[1]H Lee Moffitt Cancer Center, Experimental Therapeutics, Tampa, USA; [2]H Lee Moffitt Cancer Center, Hematogical Oncology/BMT, Tampa, USA; [3]H Lee Moffitt Cancer Center, Clinical Science, Tampa, USA*

Background: Multiple myeloma (MM) accounts for approximately 10% of all hematologic malignancies, with 30,770 new cases (16,400 men and 14,370 women) and 12,770 related deaths (6,830 men and 5,940 women) estimated to occur in the US in 2018. Significant increases in response/survival rates have been seen over the past several years, although MM remains incurable and treated patients ultimately die from progressive disease refractory to anti-myeloma therapy. Melphalan, an alkylating agent that produces DNA interstrand crosslinks, is one of the most widely used and effective drugs for the treatment of MM. However, acquired melphalan resistance is a major obstacle to the improvement of outcomes in MM therapy.

Materials and Methods: In this study, we show that the exportin 1 inhibitors (XPO1i's) selinexor, eltanexor, and KOS-2464 sensitize human MM to melphalan. We demonstrate this antitumor effect in both parental and melphalan-resistant human MM cell lines *in vitro*, in NOD-SCID-g mouse models *in vivo*, and in patient-derived MM cells *ex vivo*. Mechanistic studies include g-H2AX and alkaline comet assays for DNA damage and repair and ELISA, PAGE, and intracellular proximity ligation assays for monoubiquitinated FANCD2 activation.

Results: Human RPMI8226 (8226) (P = 0.0003), H929 (P < 0.0001), and U266 (P < 0.0001) MM cell lines and melphalan-resistant 8226/LR5 (P < 0.0001) and U266/LR6 (P < 0.0001) cell lines were highly sensitized to melphalan by XPO1i. CD138+/light chain+ MM cells from newly diagnosed and relapsed/refractory MM patients were significantly sensitized by XPO1 inhibition to melphalan (P < 0.0001). XPO1i/melphalan combination treatment demonstrated a strong synergistic antitumor effect when compared to single-agent melphalan in NOD-SCID-g mice challenged with both parental U266 (P < 0.002) MM and melphalan-resistant U266/LR6 (P < 0.005) MM tumors, with little toxicity (less than 10% weight loss). The XPO1i/melphalan drug combination increased DNA damage in a dose-dependent manner more than single-agent melphalan or XPO1i alone, as shown by alkaline comet assay (P = 0.0011) and g-H2AX expression (P = 0.0008). Western blot (P < 0.025) and proximity ligation assay (P = 0.00037) showed that the addition of XPO1i decreased monoubiquitinated FANCD2 protein expression in MM cells as compared to melphalan alone, indicating that XPO1is inhibits DNA repair. Therefore XPO1i/melphalan synergistic cell kill may be due to increased XPO1i/melphalan-induced DNA damage and decreased repair.

Conclusions: We found that combination therapies using the investigational compounds selinexor or eltanexor with melphalan may have the potential to significantly improve MM treatment outcomes. The combination of selinexor and melphalan is currently being investigated in the context of high-dose chemotherapy and autologous transplant.

No conflict of interest

353 (PB-016) Poster

Modeling mechanisms of acquired ralaniten resistance to promote drug discovery and optimize clinical responses

J. Obst[1], J. Wang[1], K. Jian[2], D. Williams[2], A.H. Tien[1], N.R. Mawji[1], T. Tam[1], Y.C. Yang[1], R.J. Andersen[2], K.N. Chi[3], B. Montgomery[4], M. Sadar[1]. *[1]BC Cancer, Genome Sciences Centre, Vancouver, Canada; [2]University of British Columbia, Chemistry and Earth, Ocean and Atmospheric Sciences, Vancouver, Canada; [3]BC Cancer Agency, Medical Oncology, Vancouver, Canada; [4]University of Washington, School of Medicine, Seattle, USA*

Background: Inhibition of the androgen receptor (AR) is the mainstay treatment for advanced prostate cancer. While initially effective, the disease ultimately progresses to metastatic castration-resistant prostate cancer (mCRPC), which is lethal. Ralaniten (formally EPI-002) specifically targets the AR N-terminal domain (NTD). This allows for sustained inhibition in the context of constitutively active AR-splice variants and mutations which often drive resistance to current therapies. Ralaniten acetate (EPI-506) is the pro-drug of ralaniten, and the first AR-NTD inhibitor to enter clinical trials (NCT02606123, ESSA Pharma, Inc.). Here we have generated and characterized a model of acquired ralaniten resistance to aid in the development of next generation AR-NTD inhibitors.

Methods: A resistant cell line (LNCaP-RAL^R) was created by serially passaging parental LNCaP cells in the presence of ralaniten. *In vitro* and *in vivo* studies using both cell lines were employed to confirm biological resistance. A human Affymetrix microarray identified increased expression of UGT2B genes involved in phase II metabolism (glucuronidation) in the resistant line, and was validated using qRT-PCR, western blot and functional studies. Serum samples collected from patients treated in the clinical trial were used to identify ralaniten metabolites. EPI-045 is an analog of ralaniten and was shown to have reduced rates of UGT2B mediated glucuronidation by HPLC. Sensitivity of LNCaP-RAL^R cells to EPI-045 was confirmed *in vitro* and *in vivo*.

Results: Ralaniten displayed antitumor activity in LNCaP but not LNCaP-RAL^R xenografts; a function of its impaired ability to inhibit AR transcriptional activity. Strikingly, LNCaP-RAL^R cells retained sensitivity to antiandrogens and AR knockdown by targeted siRNA, implying growth remains driven by AR signalling. Interrogation of microarray data revealed candidate genes (UGT2B family) whose expression and activity was associated with ralaniten resistance in additional samples. Knockdown of UGT2B isoforms was sufficient to restore sensitivity to ralaniten in resistant cells. LC/MS and ion spectra data using clinical samples, revealed that ralaniten is glucuronidated in humans. EPI-045 (which is resistant to glucuronidation) significantly inhibited AR mediated transcription and proliferation in LNCaP-RAL^R cells – both *in vitro* and *in vivo*.

Conclusions: We have generated a model of acquired ralaniten resistance, and demonstrated that selective modification of ralaniten can improve drug stability by reducing its metabolism by glucuronidation. LNCaP-RAL^R cells remain dependent upon AR signalling, and are sensitive to both EPI-045 and antiandrogens used clinically. This work highlights the potential for combination or sequential therapy following ralaniten resistance, and will hopefully drive the discovery of additional AR-NTD inhibitors.

Conflict of interest: Ownership: JO, JW, KJ, DW, AHT, NRM, YCY, RJA, MDS are inventors of licensed technology to ESSA Pharma, Inc. JW, DW, NRM, RJA, MDS have stock equity in ESSA Pharma, Inc. Board of Directors: MDS and RJA are consultants, Directors and Officers of ESSA Pharma Inc.

354 (PB-017) Poster

Downregulation of Cyclin B1 as a potential mechanism of resistance to the cell cycle checkpoint kinase 1 (CHK1) inhibitor, Prexasertib (Prex)

J. Nair[1], B. Haynes[1], T.T. Huang[1], J.M. Lee[1]. *[1]National Cancer Institute, Women's Malignancies Branch, Bethesda, USA*

Background: High grade serous ovarian cancer (HGSOC) is the most lethal gynecologic malignancy in the United States. Most HGSOCs have a loss of G1 checkpoint due to p53 inactivation and rely heavily on CHK1-mediated G2 cell cycle arrest for survival. We recently showed early clinical activity of CHK1 inhibitor (CHK1i) Prex monotherapy in heavily pretreated HGSOC patients (pts). But, most pts ultimately develop resistance to Prex. Alterations of cell cycle is one of the major mechanisms of resistance to DNA repair inhibitors. Thus, we hypothesized that a CHK1-independent delay in G2/M phase might give HGSOC cells sufficient time to repair DNA damage, making cells resistance to Prex treatment.

Methods: We developed Prex-resistant (PrexR) cell lines with OVCAR5 and OVCAR8 (both *BRCA* wild type, platinum-resistant HGSOC cell lines) by culturing them in progressively increasing concentrations of Prex over 3–4 months. Immunofluorescence (IF) for γ-H2AX staining, cell cycle analysis, and immunoblot were performed to identify and characterize key proteins of cell cycle and DNA damage response pathways. Experiments were repeated at least twice. Statistical analysis was done by Student's T test. Cell growth assays are shown as mean + SD. Transcriptome profiles of PrexR and parental cells were generated using RNA-seq. Aberrant pathways were identified through GSEA analysis for Prex-resistance.

Results: Cell growth assays using XTT showed >1000-fold increase in viability to Prex treatment in Prex-R cells compared to parental cells. An increase in γ-H2AX staining (>3 fold over untreated), indicative of DNA damage, was observed in parental cells when treated with 20 nM Prex overnight, while no changes were seen in PrexR cells. Cell cycle analysis showed a ~2-fold increase in G2 population (28–34%) in PrexR relative to parental cells (12–20%). Prex treatment (20 nM overnight) did not affect this G2 delay in PrexR while parental cells show markedly increased S

phase (42%) relative to untreated (7–10%). Sustained loss of active form of CHK1-S296 expression and lower levels of S216 phosphorylation on its substrate CDC25C were also seen in PrexR cells but not in parental cells upon Prex treatment. Immunoblotting and cell cycle analysis demonstrated that this G2 delay in PrexR cells was via constitutive downregulation of Cyclin B1 while not affecting levels of its partner CDK1. Transient silencing of Cyclin B1 attenuated cytotoxicity of Prex in parental cells (1.7–1.9 fold of control, $p < 0.05$). Gene expression studies are under analysis.

Conclusion: Our studies suggest that CHK1-independent G2 delay in PrexR cells is likely due to downregulation of Cyclin B1, a key limiting factor of mitotic entry, which may be a potential mechanism of Prex-resistance in HGSOC.

No conflict of interest

355 (PB-018) Poster

Novel RET+ patient derived cell lines reveal unique signaling dynamics and dependencies

L. Schubert[1], A. Le[1], M. Yoo[1], F. Kinose[2], A. Doak[1], S. Nelson-Taylor[1], U. Rix[2], A.C. Tan[1], R. Doebele[1]. *[1]University of Colorado School of Medicine, Department of Medicine, Division of Medical Oncology, Aurora, USA; [2]Moffitt Cancer Center, Department of Drug Discovery, Tampa, USA*

Background: Approximately 1–2% of NSCLCs harbor chromosomal rearrangements of the ret proto-oncogene (*RET*). *RET* rearrangements result in the aberrant expression and constitutive, ligand-independent activation of the RET kinase, which promotes cancer cell growth, proliferation and survival. *RET* is fused to *KIF5B* in ~70% of *RET+* lung cancers, but can have other 5' partners such as *CCDC6*, *NCOA, TRIM33* and *TRIM24*. Older multi-kinase RET inhibitors have historically had low response rates, however, newer, selective RET inhibitors are currently being tested in clinical trials.

Materials and Methods: In order to determine mechanisms of intrinsic resistance to RET inhibitors we developed three novel, patient-derived *RET+* cell lines: CUTO22 (*KIF5B-RET*), CUTO32 (*KIF5B-RET*) and CUTO42 (*EML4-RET*). We performed MTS proliferations assays and western blots to assess the efficacy and signaling dynamics of RET inhibition. To isolate the role of the 5' partners in response to RET inhibitors we have also employed CRISPR/Cas9 technology to model endogenous *Ret* rearrangements in Ba/F3 cells. RNA sequencing was completed for the CUTO22, CUTO32 and LC-2/Ad (commercial *CCDC6-RET+* cell line) cell lines +/−RXDX-105. Additionally, we subjected the LC-2/Ad, CUTO22 and CUTO32 cell lines to a comprehensive drug screening assay+/−RXDX-105.

Results: We discovered that the CUTO22 and CUTO42 cell lines were sensitive to RET inhibition, while the CUTO32 cells were resistant to multiple RET inhibitors. We determined that phospho-RET (pRET) was successfully inhibited by ponatinib and RXDX-105 in all three cell lines, however, downstream inhibition varied across cell lines. The two non-*KIF5B* cell lines, LC-2/Ad and CUTO42, had more dramatic inhibition of pAKT than the *KIF5B-RET+* cell lines following RET inhibition. Ba/F3 cells harboring CRISPR/Cas9 generated *Kif5b-Ret* and *Trim24-Ret* rearrangements were equally sensitive to multiple RET inhibitors. In order to address possible bypass pathways that may account for resistance to RET inhibitors in the CUTO32 cell line we found that inhibition of Src sensitized the cells to RET inhibition. In order to determine other pathways *RET+* cells may be dependent on we performed GSEA analysis of RNAseq data which showed that PI3K signaling, EMT and angiogenesis were enriched in CUTO22 and CUTO32 cells compared to LC-2/Ad. Drug screening data similarly revealed unique vulnerabilities; the CUTO32 cell line was uniquely sensitive to two PLK1 inhibitors, volasertib and ON-01910. CUTO22 and CUTO32 cells both demonstrated sensitivity to several AURORA kinase inhibitors in the drug screen.

Conclusions: These data suggest that lack of response to RET inhibitors, particularly in the case of *KIF5B-RET,* is not due to poor drug binding. Overall, RET+ lung cancers may have heterogeneous cellular signaling and pathway reliance.

Conflict of interest: Ownership: RCD, stock ownership: Rain Therapeutics. Advisory Board: RCD, Advisory board/consulting: Ignyta, AstraZeneca, Bayer, Takeda. Other Substantive Relationships: RCD, Licensed Patents: Abbott Molecular, Rain Therapeutics. RCD, Licensed Biologic Materials: Ignyta. ATL, Licensing fees: Abbott Molecular.

356 (PB-019) Poster

The impact of tryptase, TGF-beta1 and selective cytokines on the response to gemcitabine/nabpaclitaxel-based chemotherapy in pancreatic ductal adenocarcinoma

L. Porcelli[1], R.M. Iacobazzi[1], R. Di Fonte[1], S. Serrati[2], A. Argentiero[3], O. Brunetti[4], N. Silvestris[3], A. Azzariti[1]. *[1]IRCCS-Istituto Tumori "Giovanni Paolo II", Experimental Pharmacology, Bari, Italy; [2]IRCCS-Istituto Tumori "Giovanni Paolo II", Nanothechnology Lab, Bari, Italy; [3]IRCCS-Istituto Tumori "Giovanni Paolo II", Medical Oncology Unit, Bari, Italy; [4]Ospedale Mons. R. Dimiccoli, Medical Oncology Unit, Barletta, Italy*

Background: Pancreatic ductal adenocarcinoma (PDAC), one of the most aggressive malignancies, has up to 80% of scar tissue surrounding the malignant cells which contributes to tumor growth and dissemination. The two chemotherapy regimens utilized in an advanced pancreatic cancer setting are FOLFIRINOX and gemcitabine/nabpaclitaxel. The role of scar tissue in chemoresistance is almost completely unknown. Therefore, we investigated the role that mast cells and cancer associated fibroblast (CAFs) play in decreasing the response to gemcitabine and nabpaclitaxel combination in a panel of PDAC cell models. In addition, we provide a proof of concept of their impact on the outcome of gemcitabine/nabpaclitaxel systemic chemotherapy in PDAC patients.

Materials and Methods: Cytotoxicity was tested by MTT assay. Cell cycle progression and apoptosis were determined by flow cytometry and FITC Annexin V Apoptosis Detection Kit, respectively. Tumor metastatis were determined by Boyden invasion assay and tumor angiogenesis by in vitro capillary morphogenesis assay. Tryptase and TGF-b1 release was quantified by Elisa kit. The release of cytokines was determined by Bio-PlexPro™ Human Chemokine assay. Cell targets were determined by Western Blotting. All experiments were performed with or without conditioned media from mast cells and CAFs.

Results: We demonstrated that CAFs and mast cells decreased the response to the combination of gemcitabine and nabpaclitaxel in PDAC models through the tryptase/PAR-2 and TGF-b1 pathways. The change in cytokines (MIF, IL-6, IL-8, TNF-alpha and SDF-1 levels) and the response to gemcitabine/nabpaclitaxel treatment in PDAC models and in PDAC patients were strictly correlated.

Conclusions: We conclude that monitoring the levels of selected cytokines together with that of the tryptase and of the TGF-b1 in blood samples of PDAC patients, after the beginning of gemcitabine/nabpaclitaxel therapy, could predict the response to the treatment.

No conflict of interest

357 (PB-020) Poster

Afatinib plus dasatinib causes non-apoptotic cell death in afatinib-resistant HER2-positive breast cancer cells

N. Conlon[1], A. Canonici[1], C. Morgan[2], M. Cremona[2], B. Hennessy[2], A. Eustace[1], J. Crown[3], D. Collins[1], N. O'donovan[1]. *[1]Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland; [2]Royal College of Surgeons in Ireland, Department of Molecular Medicine, Dublin, Ireland; [3]St. Vincent's University Hospital, Department of Medical Oncology, Dublin, Ireland*

Background: Afatinib is an irreversible, small molecule pan-HER family inhibitor, which is approved for the treatment of non-small cell lung cancer (NSCLC). We have previously shown that HER2-positive breast cancer cell lines are sensitive to afatinib treatment. The mechanisms of resistance to afatinib have been examined in NSCLC; however, resistance in HER2-positive breast cancer has not been investigated. In order to determine the mechanisms of resistance to afatinib in HER2-positive breast cancer, a HER2-positive breast cancer cell line, SKBR3, was exposed to afatinib for six months and examined for druggable alterations that may overcome afatinib resistance. The aims of this study were then to assess the potential of one these druggable targets, Src, and examine the mechanism of cell death with its inhibition.

Material and Methods: SKBR3 cells were treated with 150 nM afatinib twice-weekly for 6 months. Growth response to afatinib and dasatinib was assessed by acid phosphatase assay. Reverse phase protein array (RPPA) was used to elucidate the changes in key signalling pathways, which confer the resistance phenotype. In order to determine the mechanism of cell death due to afatinib plus dasatinib, SKBR3-A cells treated with afatinib plus dasatinib were examined by caspase 3/7 activation assay, fluorescence microscopy for autophagic activity, propidium iodide-based cell cycle analysis, and Western blotting of excreted HMGB1 levels.

Results: After 6 months of exposure to afatinib, the SKBR3-A cells were more resistant to afatinib compared to parental cells (IC_{50}SKBR3-A 284 ±

28.2 nM vs SKBR3-Par 10.9 ± 3.4 nM). RPPA interrogation of the SKBR3-A cells showed alterations in several pathways, including significantly increased levels of p-Src (Y416). SKBR3-A cells were more sensitive to Src inhibition with dasatinib compared to SKBR3-Par cells and the combination of afatinib and dasatinib was highly synergistic in SKBR3-A cells (CI value = 0.09 ± 0.06). Combination treatment did not cause significant induction of G1 cell cycle arrest (p = 0.36), apoptosis (p = 0.3) or autophagy (p = 0.21). However, afatinib plus dasatinib stimulated a significant increase in secreted HMGB1, a marker of necrosis induction.

Conclusions: Src represents a potential therapeutic target to overcome resistance to afatinib in HER2-positive breast cancer. The effect of afatinib and dasatinib is apoptosis-independent and, instead, stimulated necrotic cell death in SKBR3-A cells.

Conflict of interest: Corporate-sponsored Research: Alexandra Canonici, Norma O'Donovan, John Crown – Boehringer Ingelheim.

358 (PB-021) Poster

Preliminary results MATCH-R trial, a prospective trial to study acquired resistance of tumors from patients treated with molecular targeted agents or immunotherapy

B. Besse[1], L. Lacroix[2], S. Michiels[3], L. Friboulet[4], E. Rouleau[2], N. Auger[2], J. Adam[2], C. Nicotra[5], L. Bigot[4], J.G. Judde[6], E. Angevin[5], D. Planchard[1], Y. Loriot[4], L. Tselikas[7], F. Andre[4], J.C. Soria[5], E. Solary[8], G. Vassal[9], C. Massard[5]. [1]*Gustave Roussy, Cancer Medicine, Villejuif, France;* [2]*Gustave Roussy, Biopathology, Villejuif, France;* [3]*Gustave Roussy, Statistics, Villejuif, France;* [4]*Gustave Roussy, Unit 981, Villejuif, France;* [5]*Gustave Roussy, Durg Development, Villejuif, France;* [6]*Xentech, Head, Evry, France;* [7]*Gustave Roussy, Radiology, Villejuif, France;* [8]*Gustave Roussy, Haematology, Villejuif, France;* [9]*Gustave Roussy, Pediatric, Villejuif, France*

Background: It is increasingly important to understand the molecular mechanisms underpinning primary or acquired resistance to targeted therapies and immunotherapies as it can point to the use of specific drug combinations that can prevent or delay resistance.

Material and Methods: MATCH-R () is a prospective trial to identify molecular mechanisms of primary or acquired resistance to targeted therapies and immunotherapies in patients with unresectable or metastatic cancer (NCT02517892). Clinical characteristics are recorded, a biopsy tissue is performed (and rebiopsy is required in selected cohorts) and serial blood samples are collected. Targeted NGS, SNP array, WES and RNAseq are performed on the tissue biopsies, and PDX are established (in collaboration with Xentech) for selected drugs/target. Five cohorts are open. Cohort 1 includes patients with drug's acquired resistance, defined as a PD after a PR/CR or a SD for at least 6 months. Cohorts 2–5 mandate pre and post drug biopsies, for selected population based on tumor localization (prostate, bladder), drug category (immunotherapy) or presence of a target (ALK, EGFR, etc.). We report preliminary results of the feasibility of MATCH-R.

Results: From June 29th 2015 and as of June 15th 2018, 309 patients were included. Primary tumors were mainly lung (n = 142), Prostate (n = 86), urothelial (n = 24). Tumor cells content was ≤10% in 28 patients (9%), median was 50% (0–95) for all samples, 50% for the liver (n = 50), nodes (n = 46), or lung (n = 96) biopsies, 40% for prostate (n = 54) biopsies. There were no grade 5 toxicities. Analysis failed in 56 (19%) patients, are pending in 10. WES was successfully performed in 236 (78%) patients, RNAseq in 212 (71%), both in 205 (69%). Out of 93 patients treated with a kinase inhibitor, a resistance mechanism was successfully identified in 70 patients (75%): secondary mutations in 41% of the cases, bypass mechanisms in 36% and non genetic mechanisms in 23%. Out of 154 tumors grafted, 48 (31%) PDX models were established from 15 lung tumors, 12 prostate tumors, 9 bladder tumors, 3 bellini tumors and 9 other tumo types. 12 PDXs were tested for their drug's resistance and 11 matched the patient's tumor resistance to FGFR, EGFR, ALK, MEK, MDM2 or NOTCH1 inhibitors.

Conclusions: MATCH-R successfully delivers large molecular characterizations, that allow to identify resistance mechanisms. MATCH-R generated PDXs models that mimic the patient's tumor resistance. Our data sharing policy allow partnering with academic or industrial entities. This ongoing trial will be amended to better suit potential future collaboration.

No conflict of interest

360 (PB-023) Poster

Integrated pharmacodynamic analysis identifies two metabolic adaption pathways to metformin in breast cancer

S. Lord[1], W.C. Cheng[1], D. Liu[1], E. Gaude[2], S. Haider[3], N. Patel[1], E. Teoh[1], F. Gleeson[1], D. Mcgowan[1], A. Thompson[4], M.L. Ah-See[5], C. Snell[6], L. Ayers[7], F. Karpe[8], M. Pollak[9], C. Frezza[2], J. Fenwick[10], F. Buffa[1], A. Harris[1]. [1]*University of Oxford, Department of Oncology, Oxford, United Kingdom;* [2]*University of Cambridge, MRC Cancer Unit, Cambridge, United Kingdom;* [3]*The Institute of Cancer Research, Breast Cancer Now Toby Robins Research Centre, London, United Kingdom;* [4]*MD Anderson Cancer Centre, Breast Surgical Oncology, Houston, USA;* [5]*Luton and Dunstable University Hospital NHS Foundation Trust, Department of Oncology, Luton, United Kingdom;* [6]*Mater Research Institute, Department of Anatomical Pathology, Brisbane, Australia;* [7]*Oxford University Hospitals NHS Foundation Trust, Department of Cellular Pathology, Oxford, United Kingdom;* [8]*University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, United Kingdom;* [9]*McGill University, Department of Oncology, Montreal, Canada;* [10]*University of Liverpool, Department of Molecular and Clinical Cancer Medicine, Liverpool, United Kingdom*

Background: Epidemiological studies have shown that treatment of type 2 diabetes with metformin is associated with reduced cancer risk and phase 3 trials investigating metformin as a cancer therapy are underway. However, there remains controversy as to the mode of action of metformin in tumours at clinical doses. To investigate this we conducted a clinical study that integrated measurement of markers of systemic metabolism, dynamic FDG-PET-CT, transcriptomics and metabolomics at paired time points to profile the bioactivity of metformin in primary breast cancer.

Materials and Methods: 40 non-diabetic patients were recruited with primary breast cancer to a neoadjuvant window trial. Patients received an escalating dose of metformin to 1500 mg for 2 weeks with pre- and post-metformin dynamic 18F-FDG PET-CT scans, serum metabolic markers, and tumour biopsies for whole transcriptome RNASeq, tumour metabolomics and immunohistochemistry.

Results: Assessment of tumour FDG kinetics using a classic 2-tissue compartment model with three rate constants showed a 1.3 fold change (FC) post-metformin in the composite 18F-FDG flux constant, K_{flux} (p = 0.041, paired t-test). Mass spectrometry metabolic analysis revealed a decrease in intratumoral levels of propionylcarnitine (FC -0.50, p = 0.039) and acetylcarnitine (FC –0.40, p = 0.046) (wilcoxon rank test). RNASeq revealed metformin activates multiple mitochondrial metabolic pathways. Two tumour groups were identified with distinct metabolic responses, an OXPHOS transcriptional response group (OTR) for which there is an increase in OXPHOS gene transcription and an FDG response group (FR) with increased 18-FDG uptake. Increase in proliferation, as measured by a validated proliferation signature, suggested patients in the OTR group were resistant to metformin treatment. No correlation existed between metformin's effects on host metabolism and its tumour specific effects.

Conclusions: This data provides strong evidence that metformin has a direct effect on breast cancer metabolism at clinical doses. We conclude that mitochondrial response to metformin in primary breast cancer is likely to define anti-tumour effect.

No conflict of interest

Friday, 16 November 2018

POSTER SESSION

Epigenetic modulators

361 (PB-024) Poster

Expanding the potential for epigenetic therapies in combination with current standard-of-care therapy for prostate cancer

J. Manley[1], G. Gregg[1], S. Mcdade[1], I. Mills[1], D. Waugh[1], M. LaBonte Wilson[1]. [1]*Queen's University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom*

Background: Prostate cancer (CaP) is the 2nd leading cause of male cancer in the Western world and therefore significant health burden worldwide. First line Standard-of-care (SOC) therapies for organ confined disease are surgery and radiation. Radiation, although highly effective, results in relapse in over 30% of patients within 3 years and resistance to subsequent hormonal therapies brings about progression to incurable castrate-resistant disease.

Epigenetic silencing of cancer-related genes has been shown to be highly influential in CaP disease progression. Epigenetic regulators, including histone deacetylases (HDAC), are commonly mutated and highly expressed in cancer, representing attractive clinical targets. Although five HDAC inhibitors are FDA-approved for myelodysplastic syndromes, pan-HDAC inhibitors (HDACi) have not been effective to date in CaP clinical trials.

As class 1 HDACs are highly expressed in CaP, we hypothesise that class 1 HDAC inhibition by Entinostat is sufficient to induce anti-tumour effects and synergise with the current SOC therapies, radiation and androgen deprivation, demonstrating its potential to be integrated as a novel treatment strategy for CaP.

Material and Methods: PC3, LNCaP and C4-2B cell lines were treated with Entinostat as a single agent and in combination with Radiation, Enzalutamide or Abiraterone. Synergistic responses were determined through growth inhibition and colony formation assays with transcriptome and proteome analysis to understand the mechanism of action *in vitro* and the determination of optimum treatment scheduling for combination therapy. Caspase-Glo 3/7 assay and FACS analysis were performed to measure apoptotic activation.

Results: Entinostat was assessed as a single agent across a panel of CaP cell lines and was active with a median IC50 of 1.08 μM (range, 0.586–1.871 μM). A combination of Entinostat with either radiation or androgen deprivation therapy resulted in a synergistic reduction in cell proliferation and survival with increased apoptotic population and caspase-3/7 activation. Molecular analyses showed a decrease in androgen receptor in C4-2B cells treated with Entinostat compared with vehicle control, accompanied by a reduction in expression of AR-regulated gene *KLK3* encoding prostate-specific antigen which was further reduced in response to combination with androgen deprivation.

Conclusions: HDACi have shown limited success in clinical trials for solid tumours including CaP, however, there is great potential for the use of class-1 specific HDACi Entinostat in combination with current SOC therapies. Determining the mechanism of action of these synergistic effects of HDACi in CaP will improve clinical application and determination of patient stratification through better understanding of predictive biomarkers.

No conflict of interest

364 (PB-027) Poster

Enzalutamide and decitabine in combination inhibits growth of castration resistant prostate cancer cells with a greater response than enzalutamide alone

J. Gaddy[1], D. Goodrich[2], G. Chatta[3], K. Wadosky[2]. *[1]University at Buffalo, Internal Medicine, Buffalo, USA; [2]Roswell Park Comprehensive Cancer Center, Pharmacology and Therapeutics, Buffalo, USA; [3]Roswell Park Comprehensive Cancer Center, Department of Medicine, Buffalo, USA*

Background: Metastatic castrate resistant prostate cancer (mCRPC) remains a fatal disease. It has contributed to high mortality and morbidity. Until 2011, limited treatment options were available. Despite the availability of potent AR targeted agents such as enzalutamide emergence of resistance is inevitable. We have demonstrated that in part, enzalutamide-resistance is due to reversible epigenetic changes. Treatment of prostate cancer (PCa) cell lines with inhibitors of Ezh2 histone methyltransferase can restore enzalutamide sensitivity. While in clinical development, Ezh2 inhibitors are not yet approved. We hypothesized that enzalutamide resistance may also be reversible *via* epigenetic modulation using the DNA hypomethylating agent decitabine, an inhibitor of DNA methyltransferase (DNMT).

Methods: We treated human CRPC cell lines with decitabine as a single agent or in combination with enzalutamide. Each line was treated with decitabine at 10 nM or 100 nM when used in combination or as a single agent. Enzalutamide was used at 10 μM. Our endpoint was cell number.

Results: Our data show that enzalutamide and decitabine in combination is more effective compared to enzalutamide alone. In 22Rv1 cells, an established mCRPC cell line, decitabine with enzalutamide was significantly more effective in reducing cell number compared to decitabine alone, with a decrease of 50.6% vs. 28.7%. In addition, 10 nM decitabine was more effective in combination with enzalutamide than 100 nM, with a 5.96% greater decrease in cell number. These data are consistent with known clinically effective doses, where lower doses of decitabine have been shown to decrease disease burden while reducing the cytotoxic effects of DMNT inhibition.

Conclusions: In human CRPC cell lines, enzalutamide and decitabine are more effective in combination than enzalutamide alone. These data suggest that decitabine can improve the efficacy of enzalutamide and support rationale for pursuing a clinical trial for enzalutamide and decitabine for the treatment of mCRPC.

No conflict of interest

365 (PB-028) Poster

Aristoyagonine, a naturally occurring alkaloid, reveals anticancer activity by inhibiting the bromodomain-containing protein 4 (Brd4)

S.U. Choi[1], H.K. Lee[1], C.O. Lee[1], C.H. Park[1]. *[1]Korea Research Institute of Chemical Technology, Drug Discovery Division, Daejeon, South Korea*

Background: Bromodomain-containing protein 4 (BRD4) is known to play a key role in tumorigenesis. It binds acetylated histones to regulate the expression of numerous genes. Because of the importance of brd4 in tumorigenesis, much research has been undertaken to develop brd4 inhibitors with therapeutic potential. As a result, various scaffolds for bromodomain inhibitors have been identified.

Material and Methods: To search for novel BRD4 inhibitors with new scaffolds, we performed mid-throughput screening using two different enzyme assays, alpha-screen and ELISA. In addition, we measured the c-Myc level in cancer cells to confirm the BRD4 inhibiting activity of the hit compounds. Then, we tested anticancer activity in vivo by using Ty82 cancer cell xenograft assay.

Results: We found several BRD4 inhibitory hit compounds including the arsitoyagonine. Arystoyagonine is a natural compound and is reported its isolation from Sarcoccapnos plants in 1984, firstly. The 50% inhibitory concentrations (IC50) of the aristoyagonine are 0.8 μM in BRD4 inhibitory alpha-screen assay and 0.75 μM in TY82 cancer cell cytotoxicity assay. It also reduced the cancer volume about 50% in vivo assay by using the Ty82-xenograft mouse model. In addition, we have tested hERG pach clamp assay, and it revealed the IC50 value about 80 μM. Then, we tested the cytotoxicity of the compound against human gastric cancer cells in comparison with the I-BAT 762, a BRD4 inhibitory standard drug, and we found it revealed comparable cytotoxicity to I-BAT 762 in some cell lines.

Conclusions: We found aristoyagonine as a novel BRD4 inhibitor, and it revealed anticancer activity in vivo cancer xenograft system. This is the first report to describe a natural compound as a Brd4 inhibitor, and the structural characteristics of aristoyagonine could be helpful for designing more effective BRD4 inhibitors.

No conflict of interest

366 (PB-029) Poster

HDAC inhibition: transcriptional impact of resminostat on disease-related processes in CTCL

A.C. Bretz[1], U. Parnitzke[1], M. Borgmann[2], S. Hamm[1]. *[1]4SC Aktiengesellschaft, Translational Pharmacology, München/Martinsried, Germany; [2]4SC Aktiengesellschaft, Product Development, München/ Martinsried, Germany*

Background: Cutaneous T-cell lymphoma (CTCL) is a non-Hodgkin lymphoma characterized by clonal proliferation of malignant, skin-homing T cells. Epigenetic alterations are known to play a key role in CTCL pathogenesis.

Resminostat is an orally available inhibitor of HDAC enzymes in phase II clinical development. It modifies histone acetylation, induces gene expression changes resulting in cell growth inhibition, modified cell differentiation and enhanced tumor immunogenicity.

Here, we present data elucidating the effects of resminostat in CTCL cell lines using a genome-wide approach.

Materials and Methods: Resminostat-mediated effects on gene expression were examined in vitro in CTCL cell lines My-La, HH, and HuT78 using RNA-seq analysis. H3K27 histone acetylation was analyzed employing ChIP-seq analysis.

Results: Resminostat induced lysine acetylation in CTCL cell lines in a dose-dependent manner including a genome-wide accumulation of the epigenetic activation mark histone H3K27ac. RNA-seq analysis revealed extensive transcriptional changes upon resminostat treatment including both gene induction and repression. Interestingly, a significant part of a gene set associated with disease progression (Litvinov et al. 2017) was down-regulated. Regulation of genes involved in Th1/Th2 differentiation indicated that resminostat favors a Th1-phenotype implying an effect on the Th1/Th2 balance. Further, skin-homing receptors and the pruritus mediator IL-31 were downregulated suggesting a potential impact on disease manifestation and symptoms.

To broaden our understanding of resminostat's effects, confirmatory experiments including other HDACi are currently under evaluation.

Conclusions: Resminostat affects the transcriptional regulation of marker genes associated with disease-related processes like skin-homing, Th1/Th2 differentiation, disease progression and pruritus. These data strongly suggest that resminostat has the potential to delay or even prevent disease progression in CTCL.

Resminostat is currently under evaluation in a randomized phase II trial in patients with advanced stage (IIB-IVB) mycosis fungoides or Sézary Syndrome with disease control after prior systemic (RESMAIN, NCT02953301).

Conflict of interest: Corporate-sponsored Research: All authors are employees of 4SC AG.

367 (PB-030) Poster

Mutations in epigenetic and chromatin remodeling genes in metastatic solid tumors: results from the prospective molecular profiling MOSCATO and MATCH-R trials

P. Martin Romano[1], M. Daphne[2], V. Loic[2], E.D. Yolla[1], B. Capucine[1], V. Andrea[1], B. Rastilav[1], G. Anas[1], M. Aurelien[1], C. Stephan[1], A. Nathalie[3], S. Jean Yves[4], S. Eric[5], H. Antoine[1], B. Benjamin[6], L. Ludovic[3], R. Etienne[3], S. Jean Charles[1], M. Christophe[1], P.V. Sophie[1]. *[1]Gustave Roussy Cancer Campus Grand Paris, DITEP, Drug Development Department, Villejuif, France; [2]Université Paris Saclay, Inserm Unit U981, Villejuif, France; [3]Gustave Roussy, Department of Medical Biology and Pathology, Villejuif, France; [4]Gustave Roussy, Department of Pathology, Villejuif, France; [5]Gustave Roussy, Department of Hematology, Villejuif, France; [6]Gustave Roussy Cancer Campus Grand Paris, Department of Medical Oncology, Villejuif, France*

Background: The role of mutations in genes involved in epigenetic and chromatin remodeling processes has been well studied in tumorigenesis, but their prevalence in metastatic solid tumors is unknown. We aimed at: (i) performing a systematic description of the presence of mutations in genes involved in epigenetic processes in solid tumors and (ii) evaluating their relationship with clinical characteristics and outcome in patients (pts) included in two prospective molecular profiling trials at Gustave Roussy.

Methods: Pts with metastatic solid tumors enrolled in MOSCATO (NCT01566019) and MATCHR (NCT02517892) trials underwent an on-purpose tumor biopsy. Molecular profiling was performed using Whole Exome Sequencing (WES). Alterations – copy number alterations, pathogenic variants (PV) or variants of unknown pathogenicity (VUP) – in 129 genes encoding chromatin remodelers, erasers, writers, readers and histones were assessed by a molecular tumor board. Clinical characteristics and outcome were collected.

Results: Between Dec 2011 and Oct 2016, molecular data from successful WES from 301 tumors were evaluated; 414 mutations in epigenetic genes – 134 (32%) PV and 280 (68%) VUP – were identified in 167 pts (55.5%). Median age of these pts was 59 y/o (range, 4–83); 46% were male. Twenty different tumor types were represented, including non-small cell lung cancer (NSCLC; 35 pts, 21%), breast cancer (20 pts, 12%) and HNSCC (17 pts, 10%); 93 tumor samples (56%) presented more than one mutation in epigenetic genes. Most frequent altered genes were: *KMT2D* (17.8%), *KMT2C*(14%),*KMT2A*(10%) and *ARID1A* (10%). These alterations were more frequent in NSCLC 94 (23%), urothelial cancer 59 (14%) and breast cancer 49 (12%). Median number of previous treatment lines was 3 (range, 1–11) including chemotherapy (160 pts; 95.8%), targeted therapy (96 pts; 57.5%) and immunotherapy (20 pts; 12%). Correlation between mutations in epigenetic genes and clinical characteristics as well as outcome on therapy will be presented at the meeting.

Conclusions: Mutations in epigenetic genes occur in more than half of the pts with metastatic solid tumors. With the current development of novel epigenetic-modifying drugs and synthetic lethal strategies for epigenetic deficiencies, these might soon represent druggable targets in a selective and targeted fashion. The functional significance of most variants is still unknown: functional studies and correlation with clinical outcome will help deciphering their druggability, role in disease progression and treatment resistance.

Conflict of interest: Other Substantive Relationships: Pr. JC SORIA is full time employee of Medimmune since sept 2017. All remaining authors have declared no conflicts of interest for this work.

368 (PB-031) Poster

Novel, small molecule protein arginine methyl transferase 5 (PRMT5) inhibitors for treatment of glioblastoma

D. Sivanandhan[1], S. Garapaty[1], G.P. Seerapu[1], R. Das[1], N. Kapoor[1], R. Kar[1], A.K. Singh[1], S. Vadivelu[2], R. Gangaiah[3], Z. Mohd[3], N. Tamizharasan[2], I. Swamy[2], K. Subramaniam[2], J. Tibhe[2], K. Vaidyalingam[1], S. Birudukota[1], D. Thiagaraj[1], R.R. Burri[2], S. Rajagopal[2], S. Rajagopal[1]. *[1]Jubilant Biosys Ltd, Biology, Bangalore, India; [2]Jubilant Biosys Ltd, Medicinal Chemistry, Bangalore, India; [3]Jubilant Biosys Ltd, ADME/Tox, Bangalore, India*

Background: PRMT5, an epigenetic modulator is highly expressed in several cancers, including lymphoma and glioblastoma. PRMT5 over-expression is thought to be an important factor in its tumorigenicity due to its repressive function on the expression of tumor suppressor genes. Recently, specific novel role of PRMT5 has been reported in glioblastoma (GBM), where cancer cells with altered splicing are addicted to PRMT5 for their survival. Therefore, PRMT5 inhibition could be a novel and effective approach for GBM and PRMT5 inhibitors with brain penetration could be valuable to treat this cancer.

Materials and Methods: Structure based drug design were used to identify novel PRMT5 inhibitors. FlashPlate® methylation assay was used to assess *in vitro* potency. Cell based activity of these inhibitors was assessed by measuring the symmetrical dimethylation of known cellular protein SmD3. Long term cell proliferation assays were used to assess the functional effect of PRMT5 inhibition. Orthotopic models were used to assess tumor growth inhibition *in vivo*.

Results: A number of compounds from two different series showed strong *in vitro* potency against PRMT5, which were comparable to the clinical molecule GSK-3326595. Multiple co-crystal structures have been solved in-house and are extensively used in optimization of these novel scaffolds. Their cell based potency, as measured by proliferation assay in multiple haematological and solid tumor cell lines was comparable to biochemical potency. Jubilant PRMT5i (JPRMT5i), from one of the series showed an *in vitro* potency of 0.0.045 μM and 0.005 μM in inhibiting SmD3 dimethylation. JPRMT5i exhibited an GI_{50} of 0.02 to 1.5 μM in inhibiting proliferation of GBM cell lines. JPRMT5i showed good *in vitro* ADME properties in terms of aqueous solubility and metabolic stability and reasonable oral bioavailability and brain exposure in mouse pharmacokinetics studies. In GBM orthotopic model, oral administration of JPRMT5i at 50 mg/kg resulted in statistically significant increase in survival, which was accompanied by strong biomarker modulation. Treatment was tolerated well as observed by body weight changes.

Conclusions: Additional back-up molecules have been identified in both series that show comparable potency with better PK properties, including brain exposure. Few of these NCEs are being tested as standalone therapy and in combination with other standard of care agents in GBM models. Data from these studies will be presented in this meeting.

No conflict of interest

369 (PB-032) Poster

EZH2 inhibition as an effective treatment for androgen-dependent prostate cancer

B. Bradley[1], J. Mcgrath[1], C.C. Yuan[1], F. Zhao[1], P. Sawant[1], C. Hatton[2], X. Han[2], B. Bryant[2], A. Conery[2], P. Trojer[1]. *[1]Constellation Pharmaceuticals, Translational Sciences, Cambridge, USA; [2]Constellation Pharmaceuticals, Functional Genomics, Cambridge, USA*

The histone lysine methyltransferase EZH2 is the catalytic subunit of the PRC2 complex, and methylates histone H3 on lysine 27, a mark associated with gene repression. EZH2 is recurrently mutated in cancers, such as non-Hodgkins lymphoma (NHL), resulting in repression of genes involved in apoptosis and differentiation. EZH2 is over-expressed in metastatic cancers, including castration-resistant prostate cancer (mCRPC) when compared to primary PC. Elevated EZH2 expression and concomitant reduced expression of PRC2 target genes correlate with poor prognosis and survival in PC. Additionally, EZH2 amplification is observed in >25% of neuroendocrine PCs. In aggregate, there is ample evidence that EZH2 plays an important role in the progression of cancer via its ability to transcriptionally reprogram cancer cells.

CPI-1205 is a potent, reversible, SAM-competitive, small molecule inhibitor of EZH2. CPI-1205 was well tolerated in a phase 1 clinical trial performed in patients with NHL, and is currently being evaluated for safety and efficacy in a phase 1b/2 trial in patients with mCRPC in combination with the androgen receptor (AR) inhibitor enzalutamide, or the CYP17 inhibitor abiraterone. Many mCRPC tumors remain dependent on AR signaling, and while initially responsive to enzalutamide or abiraterone, the tumors develop resistance and progress. Several mechanisms of resistance to anti-androgens involve AR gene alterations, including AR amplification or recurrent mutations in AR enhancers resulting in increased expression, and/or acquisition of secondary mutations in AR that alter ligand binding. Additionally, the expression of AR splice variants lacking their ligand binding domain are often observed, resulting in constitutive AR signaling.

To better understand the role EZH2 plays in PC, we treated cell lines with CPI-1205 alone, and in combination with enzalutamide or abiraterone. Cell lines dependent on AR signaling are uniquely sensitive to EZH2 inhibition as a single agent, whereas cell lines that lack detectable levels of AR protein remain insensitive. We show that in vitro, EZH2 inhibition overcomes several mechanisms of resistance to anti-androgens, including expression of the

AR-V7 splice variant or AR over-expression. CPI-1205 cooperates with both enzalutamide or abiraterone resulting in synergistic cell growth inhibition, and enhanced efficacy in mouse xenograft models of PC. Transcriptomic approaches revealed that CPI-1205 inhibits cell growth via alteration of lineage and differentiation transcriptional programs independent of the AR pathway. When combined with AR inhibitors this results in changes in lineage specific genes, as well as an enhanced downregulation of the AR pathway. Thus, EZH2 inhibition represents a novel therapeutic approach for mCRPC patients that remain dependent on AR signaling, yet no longer respond to anti-androgens.

Conflict of interest: Ownership: Constellation Pharmaceuticals.

370 (PB-033) Poster

Functional annotation of SWI/SNF complex protein Arid1a in pancreatic cancer

S. Gupta[1], S. Ferri-Borgogno[1], T. Griffiths[1]. [1]*The UT M.D. Anderson Cancer Center, Translational Molecular Pathology, Houston, USA*

Background: The genetic complexity of pancreatic adenocarcinoma (PDAC), revealed by exome analyses of patient tumors, demands a personalized molecular-targeted approach to treatment. While it has been difficult to successfully target the top four mutant genes, *Kras*, *Tp53*, *Cdkn2a* and *Smad4*, found in most patient samples; there is a defined subset of patients with deleterious mutations in genes encoding SWI/SNF complex, mainly in AT-rich interactive domain 1A (*Arid1a*).

Material and Methods: To understand functional role of Arid1a in PDAC development and malignancy, we generated a genetically engineered mouse model (GEMM) of PDAC driven by mutant Kras (G12D) either with ("KAC") or without Arid1a-deletion ("KC"). These mice were necropsied at regular time intervals to study the spontaneous disease progression or allowed to get morbid to generate K-M survival curve. Upon necropsy, pancreas and other organs were collected for formalin fixation to allow histochemical analysis. Some live tumors were also collected to derive cell lines, which were further analyzed by various functional assays and transcriptome analyses. Such lines were also subjected to high throughput drug screens targeting mouse kinome and clinically actionable drugs to discover therapeutic vulnerability of Arid1a-deficient PDAC tumors.

Results: Littermate comparison of KAC with KC mice revealed faster disease development in absence of Arid1s expression and shorter survival of these mice. Transcriptome analysis on KAC cells showed enrichment of epithelial-to-mesenchymal transition (EMT) associated genes and other oncogenic pathways.

Conclusions: Based on our observations, we conclude that early loss of Arid1a cooperate with mutant Kras signaling in faster disease progression in a genetically engineered mouse model (GEMM) of PDAC.

No conflict of interest

Friday, 16 November 2018

POSTER SESSION

Molecular Targeted Agents – PART II

371 (PB-034) Poster

Combination of the AKT inhibitor ARQ 751 with Immune Checkpoint Inhibitor and Other Therapeutic Agents

Y. Yu[1], R.E. Savage[1], S. Eathiraj[1], T. Hall[1], B. Schwartz[1]. [1]*ArQule Inc, Clinical Development, Burlington, USA*

Background: Dysregulation of the PI3K-AKT signaling pathway is associated with many cancers. There are multiple mechanisms of AKT dysregulation in cancer including activation of receptor tyrosine kinases, gain-of-function mutations of PIK3CA, PTEN deficiency, AKT amplification and activating mutations in AKT such as AKT1-E17K. ARQ 751 is a second-generation AKT inhibitor, which has distinct physico-chemical properties compared to ArQule's first-generation inhibitor, miransertib (ARQ 092). ARQ 751 is currently in a dose-escalation phase 1 clinical study in molecularly defined cancer patients.

Methods: The biochemical IC_{50} for ARQ 751 against AKT 1/2/3 was determined. The binding of ARQ 751 to both wild-type AKT1 and AKT1E17K was assessed using intrinsic tryptophan fluorescence quench. In vitro combination studies were performed using an anti-proliferative MTS assay as either single agents or in combination. *In vivo* efficacy was tested in patient-derived tumors with endometrial cancer cells bearing AKT1E17K mutations. A syngeneic mouse colon CT-26 model was used to test the combined effect of ARQ 751 with an anti-PD-1 antibody.

Results: ARQ 751 inhibited AKT1/2/3 activity with IC_{50} values of 0.54, 0.79, and 1.3 nM, respectively. In binding studies, ARQ 751 exhibited K_d values of 1.2 nM for wild-type AKT1 and 8.6 nM for the AKT1E17K mutant. Endometrial PDX models harboring AKT1E17K showed tumor growth inhibition of 68, 78 and 98% at ARQ 751 dose levels of 25, 50 and 75 mg/kg, respectively. The combination of ARQ 751 with either an ER antagonist or aromatase inhibitor exerted a higher anti-proliferative effect in ER+ and PIK3CA mutant endometrial cancer cells compared to single agent. Combination studies were also performed with ARQ 751 and enzalutamide in LNCaP prostate cancer cells (PTEN null). In this study, pathway analysis showed that inhibition of AR by enzalutamide elevated pAKT while inhibition of AKT by ARQ 751 increased AR protein expression, demonstrating that the combination of ARQ 751 and enzalutamide blocked both AR and AKT pathways. Additionally, combination studies with ARQ 751 and ARQ 531 (reversible BTK inhibitor) were performed in 1 CLL and 3 MCL cell lines and showed either synergistic or additive effects. Similar results were obtained when ARQ 751 was combined with the BTK inhibitor ibrutinib. *In vivo* combination of ARQ 751 with anti-PD-1 antibody in a syngeneic mouse colon CT-26 model exhibited superior anti-tumor activity compared to the single agents.

Conclusions: ARQ 751, a potent, selective, next generation AKT inhibitor, can be combined with multiple therapeutic agents including immune checkpoint inhibitors, ER antagonists, AR antagonists and BTK inhibitors. These data provide the rationale for testing combinations of ARQ 751 with these therapeutic agents clinically.

Conflict of interest: Corporate-sponsored Research: Yi Yu, Ronald E. Savage, Sudhardshan Eathiraj, Terence Hall, Brian Schwartz are the employees of ArQule, Inc.

372 (PB-035) Poster

Coccoquinones, new anthraquinone derivateives, suppress p53-dependent growth of cancer cells

D. Tatsuda[1], I. Momose[2], M. Amemiya[1], K. Sumiyoshi[3], T. Watanabe[1], M. Kawada[1], M. Shibasaki[1]. [1]*Institute of Microbial Chemistry, Tokyo, Tokyo, Japan;* [2]*Institute of Microbial Chemistry, Numazu, Shizuoka, Japan;* [3]*Meiji Seika Pharma Co., Ltd., Bioscience Laboratories, Kanagawa, Japan*

Background: Transcription factor p53 is a negative regulator of cell growth in eukaryotic cells. p53 induces cell cycle arrest or apoptosis in response to damages induced by ultraviolet light, infrared light, and chemical agents. In cancer cells, p53 activity is usually suppressed by binding to Mdm2, a ubiquitin E3 ligase. Mdm2 ubiquitinates p53 and induces proteasome degradation of p53. Because p53 activation can induce p53-dependent cell cycle arrest and cell death in cancer cells, activation of the p53-dependent signaling pathway is an attractive target for the development of anti-cancer drugs.

Material and Methods: We established a cell-based assay using human glioblastoma LNZTA3 cells in which p53 expression can be regulated by tetracycline. To search for a new activator of the p53-dependent signaling pathway, we screened microbial metabolites. Human glioblastoma LNZTA3 cells (tetracycline-regulated p53 expression), human gastric cancer MKN45 cells (p53 wild-type), and MKN7 cells (p53 mutant-type) were incubated with the metabolites for 3 days, and cell viability was measured by MTT assay.

Results: As a result of screening, we identified coccoquinones A and B, novel anthraquinone derivatives, from *Staphylotrichum coccosporum* PF1460. *Staphylotrichum coccosporum* PF1460 was isolated from a soil sample collected in Ishigaki Island, Okinawa prefecture, Japan. The 28S rRNA-D1/D2 and ITS-5.8S rRNA sequences of strain PF1640 were identical to those of *Staphylotrichum coccosporum* NBRC33272 (100%). Coccoquinones isolated as a red amorphous solid. The UV spectrum of coccoquinones was similar to that of quinofuracins. The structures of coccoquinones were closely related to versicolorone and paeciloquinones. Coccoquinones induced p53-dependent growth suppression and the accumulation of p53, p21, and PUMA; and promoted the degradation of PARP in p53-expressing LNZTA3 cells.

Conclusions: These data suggest that coccoquinones activate p53 and induce cell death *in vitro*.

No conflict of interest

373 (PB-036) Poster

In vitro and in vivo activity of a HER2-targeted thorium-227 conjugate (HER2-TTC) in HER2 low expressing and T-DM1/trastuzumab resistant preclinical mouse models

J. Karlsson[1], V. Cruciani[1], D. Grant[1], C. Ellingsen[1], G. Petros[1], A. Kristian[1], U. Hagemann[2], C. Schatz[2], R. Bjerke[1], O. Ryan[1], D. Mumberg[2], A. Cuthbertson[1]. [1]*Bayer AS, Thorium Conjugate Research, Oslo, Norway;* [2]*Bayer AG, Oncology, Berlin, Germany*

HER2 is overexpressed in several cancers and is a highly validated target for the treatment of breast and gastric cancer also serving as a prognostic and predictive biomarker. Several HER2-targeting antibodies are approved or are in late phase clinical development. Current HER2-targeted therapies are effective in treating cancers overexpressing HER2, but find utility amongst a limited population of patients with breast or gastric cancer (∼20%). During treatment, many patients become resistant or are simply not eligible for these therapies, due to low expression levels of the target (∼55%). Therefore there exists a high unmet medical need for new drugs with alternative mechanisms of action targeting HER2.

Targeted alpha therapy (TAT) has become an established modality in the treatment of metastatic castration-resistant prostate cancer following the approval of radium-223 dichloride. TATs are highly cytotoxic due to the high linear energy transfer of the alpha-particle emitting radionuclide which induces complex DNA double-strand breaks in the targeted tumor cell. Thorium-227 has a half-life of 18.7 days, decaying to radium-223 via alpha-particle emission. We describe herein an antibody conjugate capable of delivering the payload thorium-227 (^{227}Th) to cancer cells expressing the human epidermal growth factor receptor 2 (HER2).

The HER2 targeted thorium-227 conjugate (HER2-TTC) comprises a humanized HER2 targeting IgG1 antibody covalently linked via an amide bond to a chelator moiety (3,2-HOPO) enabling room temperature radiolabeling with the alpha particle emitter ^{227}Th. The preclinical activity of HER2-TTC was characterized in low HER2 expressing as well as in T-DM1/trastuzumab resistant models.

HER2-TTC was prepared at high radiochemical yield and purity. Binding of HER2-TTC to recombinant HER2 was shown to be comparable to trastuzumab. *In vitro*, pM cytotoxicity and specificity of HER2-TTC was shown on HER2 positive cancer cell lines. *In vivo,* specific tumor accumulation and anti-tumor activity of HER2-TTC was observed following i.v. injection of 125–500 kBq/kg at a protein dose of 0.18 mg/kg in mice bearing s.c. breast and gastric cancer patient derived xenograft models. These models were chosen as they were known to be resistant to T-DM1/trastuzumab and had a range of HER2 expression as evidenced from IHC (1+ to 3+).

The results support the development of a HER2-TTC for the treatment of T-DM1/trastuzumab resistant disease and offer potential for the population of patients with low HER2 expression.

No conflict of interest

374 (PB-037) Poster

Development of PROTACs for targeted degradation of MALT1

D. Jones[1], W. Gibbs[1], M. Ambler[2], C. Crompton[2], B. Saxty[1], M. Newman[1], K. Ansell[1]. [1]*LifeArc, Biology, Stevenage, United Kingdom;* [2]*LifeArc, Chemistry, Stevenage, United Kingdom*

Background: Proteolysis Targeting Chimeras (PROTACs) are heterobifunctional molecules that facilitate degradation of targeted proteins in the cell via the ubiquitin proteasome system. PROTACs are comprised of a ligand for the protein of interest, and a ligand for an E3 ligase (typically VHL or Cereblon) bound by a linker. Recruitment of the E3 ligase to the protein of interest elicits polyubiquitination of the target protein resulting in its proteasomal degradation. In contrast to traditional occupancy-driven pharmacology, PROTACs work catalytically to degrade their targets and in so doing elimate scaffolding as well as biochemical functions of the target.

Results: We have synthesised multiple PROTACs that are effective at knocking-down proteins of interest, incorporating VHL or Cereblon E3 ligase ligands. By using general proteasome inhibitors we have verified that degradation occurs through the proteasome. Experiments have shown that degradation is both concentration and time dependent, beginning at around 4 hours with maximal effects observed by 24–48 hrs.

One such target is the paracaspase MALT1, a cysteine-dependent, arginine-directed protease that is essential for the initiation of adaptive immune responses. MALT1 acts as a scaffold protein to promote NFκB activation, but is also a protease that cleaves a number of substrates, including RelB, CylD and A20. A PROTAC approach thus offers a novel modality to knock down MALT-1, affecting both its catalytic and scaffold functions.

Conflict of interest: Other Substantive Relationships: All authors are employees of LifeArc.

375 (PB-038) Poster

Role of MALT1 in cell cycle control: scaffold vs protease activity

D. Jones[1], W. Gibbs[1], M. Newman[1], A. Levy[1], M. Ambler[2], K. Ansell[1], B. Saxty[1]. [1]*LifeArc, Biology, Stevenage, United Kingdom;* [2]*LifeArc, Chemistry, Stevenage, United Kingdom*

Introduction: The paracaspase MALT1 is a cysteine-dependent, arginine-directed protease that is essential for the initiation of adaptive immune responses. Oncogenic, constitutive activation of MALT1 has been associated with the formation of diffuse large B-cell lymphomas of the activated B-cell subtype (ABC DLBCL), a highly aggressive human B-cell lymphoma characterized by poor patient outcome.

As a scaffold protein, MALT1 promotes TRAF6-dependent IKK activation, which triggers a rapid but transient NFκB activation. MALT1 also has a protease activity and cleaves a number of substrates, including RelB, CylD and A20. This has been reported to result in a variety of cell responses including cytokine production and proliferation.

Results: A high throughput screening programme performed at LifeArc identified compounds which biochemically inhibit MALT1 protease activity. We also developed a set of MALT1-targeting PROTAC molecules (see accompanying poster) which efficiently knock down MALT1 in a dose and time related manner. Differential effects of inhibition and knock-down were observed on NFκB signalling in Jurkat cells.

MALT1 is constitutively active in the B-cell lymphoma cell line OCI-Ly3. In this cell line, enzymatic activity of MALT1 appeared key for the observed reduction in cell proliferation following compound exposure. Overt cell kill was not observed however. The mechanism by which MALT1 inhibitors mediate a cell cycle arrest was investigated and the AP-1 family of transcriptional regulators were implicated in this activity, with up-regulation of the cyclin dependent kinase inhibitor p21.

Conclusions: These approaches to elucidate the role of MALT1 in specific cell backgrounds is driving new combination approaches for development of an oncology therapeutic, while the PROTAC knock-down offers an alternative modality with different impact on downstream signalling pathways.

Conflict of interest: Other Substantive Relationships: All authors are employees of LifeArc.

376 (PB-039) Poster

Integrated pharmacokinetic-pharmacodynamic (PK-PD) and exposure-response (E-R) analyses to support the first-in-human (FIH) Phase I/II study of FGF401

Y. Gu[1], M. Wilbaux[2], S. Yang[3], D. Demanse[4], A. Jullion[4], C. Meille[2], R. Siddani[3], D. Graus Porta[5], A. Myers[6]. [1]*Novartis, Pharmacokinetic Sciences, Shanghai, China;* [2]*Novartis, Pharmacometrics, Basel, Switzerland;* [3]*Novartis, Pharmacometrics, East Hanover, USA;* [4]*Novartis, Biostatistics, Basel, Switzerland;* [5]*Novartis, Oncology Translational Research, Basel, Switzerland;* [6]*Novartis, Translational Clinical Oncology, Shanghai, China*

Background: The fibroblast growth factor 19 (FGF19)–fibroblast growth factor receptor 4 (FGFR4)–b Klotho (KLB) signaling pathway regulates bile acid synthesis and is also a key driver in certain forms of hepatocellular carcinoma (HCC), a deadly disease with limited treatment options. FGF401, a highly potent and selective FGFR4 inhibitor, is being evaluated in a FIH Phase I/II study (NCT02325739) in patients with HCC or solid tumors characterized by FGFR4/KLB expression. The aim of this work was to utilize modeling approaches to establish the exposure-response (E-R) relationships of FGF401 with various efficacy, safety and biomarker endpoints, and to characterize the selected dose in this trial.

Material and Methods: All data were extracted from the ongoing study. Concentration-time profiles in man were compared to the preclinical IC90 for pFGFR4 inhibition (∼26 ng/mL) to evaluate the target coverage. Primary PK parameters and dose proportionality were assessed using non-compartmental analysis. A population (pop) PK model was developed to further characterize FGF401 exposures and derive PK metrics. To investigate exposure-efficacy relationship, a popPKPD model was developed to describe the tumor growth inhibition. In addition, time to progression (TTP) was analyzed by Kaplan-Meier method, and stratified by PK metrics quartiles. To evaluate exposure-safety relationship, discrete longitudinal analyses were performed on the occurrence of common adverse events related to FGFR4 pathway inhibition (AST↑ and diarrhea). A popPKPD model was also developed to describe the evolution of ALT over time. To evaluate the E-R of PD biomarkers, circulating FGF19 and 7-alpha-hydroxy-4-cholesten-3-1 (C4) were analyzed with popPKPD models. Appropriate covariates were evaluated in all modeling analyses.

Results: FGF401 shows favorable PK in man. Exposure increases almost dose-proportionally. Of the evaluated PK metrics, Ctrough best correlates with TTP. Ctrough >28 ng/mL was associated with a significant prolonged TTP, consistent with the preclinical observation that Ctrough >IC90 drives the antitumor efficacy. The majority of individuals maintained the efficacy-required Ctrough level at 120 mg QD. Simulations of the popPKPD tumor growth inhibition model also favored the dose of 120 mg QD. No relationship between FGF401 exposure and AST↑ or diarrhea was identified within the observed dose range. The popPKPD ALT model also showed consistent findings. Both FGF19 and C4 popPKPD models indicate that the maximal PD effect reached at clinical tested dose range.

Conclusions: This integrated analysis, covering PK, efficacy, safety and biomarkers, provides insights into the E-R relationships of FGF401, and demonstrates the utility of this methodology in early clinical development. These data support the FGF401 120 mg QD dose regimen.

Conflict of interest: Corporate-sponsored Research: All the authors are employees of Novartis. NCT02325739 is a Novartis-sponsored clinical trial.

378 (PB-041) Poster

Leveraging the pentarin platform to selectively deliver PI3 K inhibitors to solid tumors leading to superior efficacy in preclinical models

S. Perino[1], B. Moreau[1], J. Quinn[1], A. Cirello[1], T. Cipriani[1], G. Sharma[1], K. Kriksciukaite[1], B. White[1], K. Whalen[1], M. Bilodeau[1], R. Wooster[1]. [1]*Tarveda Therapeutics, Biology, Watertown, USA*

HSP90 is a chaperone protein that is overexpressed and activated in the harsh environment of many tumors. Due to this overexpression and activation in cancer cells, small molecule ligands of HSP90 are retained in tumors for as much as 20 times longer than in blood or normal tissue and have been used to image tumors in patients. Tarveda Therapeutics has leveraged this preferential accumulation of HSP90-targeting ligands in tumors to selectively accumulate and release anti-cancer payloads, such as phosphoinositide 3-kinase (PI3K) inhibitors.

The PI3K pathway is an intracellular signaling pathway that regulates cell survival, proliferation, and differentiation. In cancer, there is frequent activation of the PI3K pathway and its critical role in cell growth and survival has made it an attractive target for pharmacologic intervention. To date, while PI3K inhibitors are approved for the treatment of leukemia and lymphoma, only allosteric mTOR inhibitors, such as everolimus and temsirolimus, are approved for clinical use for the treatment of solid tumors with PI3K/AKT/mTOR pathway activation. In addition, there is increasing evidence that PI3K pathway inhibitors are insufficient at achieving deep inhibition of the pathway at doses that can be tolerated by patients. We hypothesize, by leveraging the preferential accumulation of HSP90-targeting ligands in tumors, and by masking of the PI3K inhibitor active site though conjugation to HSP90 targeting ligands, HSP90-PI3K conjugates could selectively accumulate and be retained in tumors leading to deep pathway inhibition over time with an overall decrease in toxicity.

To test this hypothesis, we have generated HSP90-PI3K conjugates and evaluated them in preclinical models of human cancer. We demonstrated superior tumor accumulation, in comparison to the PI3K inhibitor alone, with multiple conjugates in multiple xenograft models. Human tumor xenograft studies in nude mice resulted in greater efficacy than that achieved with the PI3K inhibitor alone. In addition, since a known and potentially dose limiting side effect of inhibitors targeting these pathways is hyperglycemia, glucose levels were monitored post PI3K inhibitor and HSP90-PI3K conjugate dosing. Selective delivery of our HSP90-PI3K conjugate was able to mitigate the increase in glucose levels observed post dosing with the PI3K inhibitor alone, demonstrating that selective delivery may be able to increase the therapeutic window in comparison to PI3K inhibitors alone. These data demonstrate that by leveraging the preferential accumulation of HSP90-targeting ligands in tumors, we can selectively deliver PI3K inhibitors to achieve deep pathway inhibition leading to efficacy in multiple tumor models without hyperglycemia induction in mice.

No conflict of interest

379 (PB-042) Poster

Comparison of the efficacy and safety of transarterial chemoembolization with and without apatinib for the treatment of BCLC Stage C hepatocellular carcinoma

S. Chen[1]. [1]*Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Department of Interventional Oncology, Fuzhou, China*

Background: Hepatocellular carcinoma (HCC) is among the 5 most-common cancers worldwide and has a poor prognosis.[1] Most patients are diagnosed at advanced stages and are only eligible for palliative therapy. The phase II clinical trial of apatinib in the treatment of BCLC stage B or C HCC indicated that apatinib was effective, well tolerated and effective in the treatment of intermediate and advanced HCC.[2–3] This study aimed to evaluate the safety and efficacy of transcatheter arterial chemoembolization (TACE) combined with apatinib (TACE-apatinib) treatment and TACE-alone treatment for Barcelona Clinic Liver Cancer stage C HCC.

Materials and Methods: We retrospectively reviewed 80 consecutive patients with BCLC stage C HCC who received TACE-apatinib or TACE-alone as the initial treatment. We compared the clinical and laboratory outcomes, imaging findings at 1 and 3 months after TACE, tumor response, time to progression (TTP), overall survival (OS), and adverse events between both groups.

Results: The overall response rate was higher in the TACE-apatinib group than in the TACE-alone group at 1 and 3 months after treatment (66.7% vs 39.6%, respectively, P = 0.020; 45.8% vs 17.6%, respectively, P = 0.021). The median TTP and OS in the TACE-apatinib group were longer than those of the TACE-alone group (TTP: 6.3 months vs 3.5 months, respectively, P = 0.002; OS: 13.0 months vs 9.9 months, respectively, P = 0.041). Apatinib-associated side effects such as hypertension, hand-foot syndrome, oral ulcers, proteinuria, and diarrhea were more prevalent in the TACE-apatinib group than in TACE-alone group (P < 0.05).

Conclusion: Compared to TACE-alone treatment, TACE-apatinib increased the TTP, OS, and tumor-response rate at 1 and 3 months after treatment of BCLC stage C HCC without any significant increase in severe adverse events.

No conflict of interest

Table 1 (abstract 379 PB-042): In the two groups were followed up for 1 and 3 months objective response

	Treatment group	CR	PR	SD	PD	ORR(%)	X^2	P
1 month	TACE-apatinib	0	18	6	3	18/27(66.7)	5.236	0.020
	TACE alone	0	21	18	14	21/53(39.6)		
3 months	TACE-apatinib	0	11	3	10	11/24(45.8)	5.395	0.021
	TACE alone	0	6	5	23	6/34(17.6)		

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; TACE, transarterial chemoembolization.

380 (PB-043) Poster

Molecular profiling of a case of cervical cancer with progression of 16 years

P. Moreno-Acosta[1], N. Vial[2], M. Molano[3], D. Mayorga[4], A. Huertas[5], A. Romero-Rojas[6], S. Carrillo[5], J. Acosta[7], A. Vallard[2], N. Magne[2]. [1]*Instituto Nacional de Cancerologia, Bogotá D.C., Colombia;* [2]*Institut de cancérologie de la Loire-Lucien Neuwirth, Department of Radiation Oncology, Saint-Priest en Jarez, France;* [3]*The Royal Women's Hospital, Microbiology and Infection Diseases, Melbourne, Australia;* [4]*National Cancer Institute, Group in Radiobiology Clinical, Molecular and Celular, Branch Research, Bogota, Colombia;* [5]*National Cancer Institute, Biobank, Bogota, Colombia;* [6]*National Cancer Institute, Group of Pathology Oncology, Bogota, Colombia;* [7]*National University of Colombia, Pathology Group, Bogota, Colombia*

Background: Cervical cancer is one of the most prevalent malignancy and of higher mortality in the world, and is considered a marker of underdevelopment. Its detection, diagnosis and precursor lesions, called low-grade and high-grade lesions or cervical intraepithelial lesions, are the main purpose to reduce the morbidity and mortality of this disease. In most cases of precursor lesions, these resolve spontaneously. Only a small part progresses to high-grade lesions and invasive cancer. The aim of this study was to describe and analyze the molecular profile in samples of high-grade intraepithelial lesion and locally advanced cancer of a case of cervical cancer with progression of 16 years.

Methods and Materials: 1986's of high-grade intraepithelial lesion and 2002's uterine cervical carcinoma samples were retrospectively compared in a single patient. Proteins expressions (IGF-IR α and β, Survivin, GLUT1,

CAIX and hTERT) were assessed with immunochemistry. HPV was detected and typed. Polymorphism for the p53 gene codon 72 was analyzed.

Results: In the locally advanced cancer sample an increase in the expression of IGF-IRα, IGF-IRβ, hTERT and decrease in the expression of CAIX, GLUT1 and Survivin was observed, whereas in high-grade intraepithelial lesion sample was observed an increase in the expression of CAIX, GLUT1, hTERT and decreased IGF-IRα, IGF -IRβ. A HPV co-infection with the same variants and a p53 comparable "Arginine/Proline" genotype was reported both on 1986's and 2002's samples.

Conclusions: The variations of protein expressions suggest that these biomarkers not contribute in the same proportion at each stage of cancer development. CAIX, GLUT1, Survivin and hTERT seem to play a key role in the evolution from high-grade intraepithelial lesion to cervical carcinoma. IGF-1R might also play a key role in cancer appearance and its local and distant promotion. The persistence of HPV infection, all the more for co-infection, suggests its clinical impact in tumoral transformation and the heterozygous genotype containing Proline might explain the surprisingly long time (more than 16 years) between diagnosis of pre-cancerous lesions and of cervical cancer without any treatment, with a probably resistant to HPV-induced mutation p53 genotype. Thus, the biological and molecular profiling might be valuable biomarkers, reflecting the future or current disease aggressiveness. If the present hypotheses are validated by further studies, such molecular profiling could guide the selection of the most appropriate targeted molecular compound or personalized therapy, as previously suggested in trials evaluating antiviral drugs in HPV-mediated cervical cancer treatments.

No conflict of interest

References

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381 (PB-044) Poster

Antitumor activity of novel STAT3 inhibitor YHO-1701 in combination with alectinib, an ALK inhibitor, in non-small cell lung cancer model with EML4-ALK fusion gene

F. Nishisaka[1], K. Taniguchi[1], M. Tsugane[1], H. Konishi[1], A. Yoshinaga[1], S. Ishii[2], H. Takahashi[2], T. Iijima[2], A. Asai[3], T. Matsuzaki[1], Y. Shishido[1]. *[1]Yakult Honsha Co., Ltd., Yakult Central Institute, Tokyo, Japan; [2]Yakult Honsha Co., Ltd., Pharmaceutical Department, Tokyo, Japan; [3]Graduate School of Pharmaceutical Sciences University of Shizuoka, Center for Drug Discovery, Shizuoka, Japan*

Background: Signal transducer and activator of transcription (STAT) 3 plays a critical role in regulating cell growth, metastasis, and survival. STAT3 signaling is constitutively activated in various types of cancers and hematological malignancies. YHO-1701 is an orally available STAT3 inhibitor. The objective of this study was to assess the efficacy of YHO-1701 either alone or in combination with molecular targeted agents on cell proliferation in vitro and tumor growth in a mouse xenograft model.

Material and Methods: The anti-proliferative effects of exposure to YHO-1701 and/or relevant molecular targeted agents were evaluated in a panel of 18 human leukemias, melanomas, and cancers of the lung, breast, colon, and thyroid by using WST-8 dye-based assay after 48 hours of administration. The median-effect method was used to analyze the combined drug effects. Immunoblot analysis was carried out to investigate change in levels of the key target molecules in each signal transduction pathway. The antitumor efficacy of YHO-1701 in combination with alectinib, an ALK inhibitor, was explored in an EML4-ALK fusion gene-positive NCI-H2228 xenograft mouse model. The test compounds were administered orally once a day for 5 consecutive weekdays followed by a 2-day rest at the weekend for 3 weeks.

Results: Additive or synergistic effects were observed in two thirds of the total number of combination treatments given. Of particular interest was the synergistic effect observed when YHO-1701 was combined with osimertinib (EGFR inhibitor), crizotinib, alectinib, ceritinib (ALK inhibitors), imatinib, or dasatinib (BCR-ABL inhibitors) over a wide range of drug concentration ratios. The results further revealed a close correlation between this synergistic effect and the cellular levels of the target molecules of YHO-1701 and each combination drug. In the NCI-H2228 xenograft mouse model, the tumor growth inhibitory rate of the YHO-1701/alectinib combination increased to 51.2%, whereas that of YHO-1701 or alectinib alone was 21.4% and 30.9%, respectively. The combination therapy was well tolerated in tumor-bearing nude mice.

Conclusions: The present study suggests that the combination of YHO-1701 and alectinib is worthy of further clinical investigation. In addition to ALK inhibitors, EGFR and BCR-ABL inhibitors also appear to be good candidates for combination with YHO-1701.

No conflict of interest

382 (PB-045) Poster

Sphingosine kinase 2 (SK2) targeting in the treatment of multiple myeloma: preclinical and phase I studies of opaganib, an SK 2 inhibitor, in multiple myeloma

Y. Kang[1], S. Fan[1], P. Sundaramoorthy[1], C. Gasparetto[1], G. Long[1], A. Garrett[1], E. Sellars[1], J. Mcintyre[1], L. Maines[2], V. Katz Ben-Yair[3], C. Smith[2], T. Plasse[3]. *[1]Duke University, Medicine, Durham, USA; [2]Apogee Biotechnology Corporation, Biotech, Hummelstown, USA; [3]RedHill Biopharma, Biopharma, Tel Aviv, Israel*

Background: Multiple myeloma (MM) remains an incurable disease; novel therapeutic agents not sharing similar mechanisms of action with existing drugs are needed. Sphingosine kinase 2 (SK2) plays a critical role in maintaining the ceramide:sphingosine-1 phosphate (S1P) rheostat and in the regulation of cell fate. We report preclinical studies and initial results in a phase I study of the orally available, SK2-specific inhibitor opaganib (ABC294640) in relapsed/refractory MM.

Materials and Methods: SK2 gene expression was measured by quantitative RT-PCR. SK2-specific shRNA was used to determine the role of SK2 in myeloma cell survival. The effectiveness of opaganib against myeloma was examined *in vitro* and *in vivo* in myeloma xenograft models. To determine the role of SK2 in microenvironment, VK*MYC myeloma cells were administered to $SK2^{-/-}$ knockout mice. We also conducted a phase I study of single agent opaganib in patients with relapsed/refractory MM previously treated with immunomodulatory agents and proteasome inhibitors (clinicaltrials.gov NCT01410981).

Results: SK2 is overexpressed in MM cell lines and primary human MM specimens. Inhibition of SK2 by RNA interference or treatment with opaganib induces apoptosis in MM cell lines and inhibits proliferation of primary human MM cells. Moreover, opaganib effectively inhibited myeloma tumor growth *in vivo* in mouse xenograft models.

Opaganib reduces the expression of Mcl-1 and c-Myc by inducing their proteasome degradation and exhibits synergistic anti-myeloma activity when combining with ABT-199 (venetoclax), a specific Bcl-2 inhibitor, in MM cells without a t(11;14) translocation *in vitro* and in mouse xenograft models.

In addition SK2 regulates mitophagy (i.e., autophagy of mitochondria) in myeloma cells. Furthermore, when VK*MYC myeloma cells were injected into WT recipients, $SK1^{-/-}$ knockout mice, or $SK2^{-/-}$ knockout, none of the $SK2^{-/-}$ recipient mice developed myeloma, whereas both WT and $SK1^{-/-}$ recipient mice developed myeloma, indicating a critical role of SK2 in the bone marrow microenvironment regulating myeloma pathogenesis.

Nine patients were enrolled in the clinical trial and were treated in continous 28-day cycles at the doses shown: 3 patients at 250 mg bid; 4 patients at 500 mg bid, and 2 patients at 750 mg bid. Average lines of prior treatment were 5. PK, PD and correlative studies were performed. No dose-limiting toxicities were observed. The peak plasma concentrations (C_{max}) of opaganib in patients receiving 500 mg bid were in the range expected to have therapeutic activity. Two patients had stable disease for about 4 months.

Conclusions: SK2 is an innovative molecular target for anti-myeloma therapy. Opaganib single agent or in combination has potential for treatment of relapsed/refractory MM patients previously treated with immunomodulatory agents and proteasome inhibitors.

Conflict of interest: Ownership: Dr. Charles Smith is the President and CEO of Apogee Biotechnology Corporation. Advisory Board: None. Board of Directors: None. Corporate-sponsored Research: RedHill Biopharma owns the right to opaganib and is the IND holder and one of the sponsors of the clinical study. Other Substantive Relationships: Dr. Terry Plasse and Ms. Vered Katz are employees of RedHill Biopharma, Ltd. The study was supported by NCI R01 CA197792 and NCI R44 CA199767 sub-awarded by Apogee Biotechnology Corporation.

383 (PB-046) Poster

TAS0728, a covalent binding, selective inhibitor of HER2, shows antitumor activity in HER2+ tumor models resistant to established anti-HER2 therapy

H. Irie[1], Y. Fujioka[1], K. Ito[1], K. Oguchi[1], H. Osawa[1], K. Funabashi[1], T. Shimamura[1], F. Nakagawa[1], R. Kawabata[1], H. Nagase[1], J. Uchida[1], S. Okubo[1], K. Matsuo[1], T. Utsugi[1], Y. Iwasawa[1]. *[1]Taiho Pharmaceutical Co. Ltd, Discovery and Preclinical Research Division, Tsukuba, Ibaraki, Japan*

Background: HER2 is a promising therapeutic target in various cancers. HER2-targeting antibodies (trastuzumab, pertuzumab) and a HER2-directed

antibody-drug conjugate (trastuzumab emtansine: T-DM1) are approved for the treatment of HER2 overexpressed breast cancer. However, there is still a medical need for patients refractory to anti-HER2 therapies. TAS0728 is a HER2-selective, covalent binding kinase inhibitor that has high kinase specificity excluding EGFR. In the present study, the antitumor activity of TAS0728 was evaluated in tumor models including NCI-N87 xenograft model refractory for T-DM1, and a patient-derived xenograft (PDX) model resistant to anti-HER2 therapies and other chemotherapies.

Material and Methods: Mixture with recombinant human HER2 cytoplasmic domain and TAS0728 or DMSO were injected into the liquid chromatography-mass spectrometry (LC-MS) to test for covalent adduct formation. Inhibitory effect of TAS0728 for kinase activity of various HER2 mutations was evaluated in series of MCF10 cell lines stably expressing various mutated HER2 genes, wild type HER2 or EGFR. For *in vivo* antitumor evaluation of TAS0728, NCI-N87 xenograft model or a PDX model were used. In the NCI-N87 xenograft model, T-DM1 was administered at 10 mg/kg (q3w, i.v.) until the tumor regression and the subsequent re-growth of tumor were observed despite continued T-DM1 treatment. The mice were divided into two groups. In continued treatment group, T-DM1 was administered at 10 mg/kg (q3w, i.v.), and in switched treatment group, TAS0728 was administered at 60 mg/kg for 42 days (q.d., p.o.). Furthermore, we evaluated TAS0728's antitumor effect in a PDX model derived from a HER2+ breast cancer patient who showed resistance to trastuzumab/pertuzumab-based therapy, T-DM1 and other chemotherapies. In the PDX model, TAS0728 was administered at 30 or 60 mg/kg for 27 days (q.d., p.o.).

Results: Covalent adduct formation of TAS0728 with human HER2 recombinant protein was confirmed in LC-MS analysis. In the MCF10A cells, TAS0728 had a potent inhibitory activity for phosphorylation of various HER2 mutations as well as wild type HER2 at 30–300 nM. In contrast, a higher concentration of TAS0728 (3,000 nM) was required for EGFR wild type inhibition. *In vivo*, TAS0728 induced tumor regression in the T-DM1 refractory NCI-N87 xenograft model, while T-DM1 was not effective. Moreover, in breast cancer PDX model refractory against anti-HER2 therapies, TAS0728 exerted the potent antitumor effect.

Conclusion: TAS0728 is a covalent binding, selective inhibitor of HER2 kinase. TAS0728 was effective in HER2+ tumor models resistant to anti-HER2 therapy. These results provide a rationale of therapy for patients refractory to established anti-HER2 therapy.

No conflict of interest

384 (PB-047) Poster

Comparison of pharmacokinetics and safety of reference bevacizumab (EU and US bevacizumab) and its biosimilar candidate CT-P16

J.G. Shin[1], J.L. Ghim[1], S.H. Cho[2], M.S. Nam[2], S. Han[3], S. Yu[4], T. Park[4], S. Kim[4], J. Bae[4]. [1]*Inje University Busan Paik Hospital, Department of Pharmacology and Clinical Pharmacology, Busan, South Korea;* [2]*School of Medicine INHA University, Department of Clinical Pharmacology, Incheon, South Korea;* [3]*The Catholic University of Korea Seoul St.Mary`s Hospital, Department of Clinical Pharmacology & Therapeutics, Seoul, South Korea;* [4]*CELLTRION Inc., Clinical Development, Incheon, South Korea*

Background: CT-P16 is on the development as a proposed biosimilar product of bevacizumab, a recombinant humanized monoclonal antibody that binds selectively to vascular endothelial growth factor-A. We conducted a phase I study to compare similarity in pharmacokinetic (PK), safety and immunogenicity between CT-P16 and 2 reference products, EU-approved bevacizumab (EU-B) and US-licensed bevacizumab (US-B).

Materials and Methods: In this double blind, randomized, three-arm, parallel-group, single-dose study, a total of 144 healthy male subjects were enrolled and 141 subjects received a single dose (5 mg/kg) of CT-P16 (n = 46), EU-B (n = 47) or US-B (n = 48) by intravenous infusion over 90 minutes on Day 1 and followed until Day 99 at 3 clinical sites in Republic of Korea. The primary endpoints were area under the concentration-time curve from time zero to infinity (AUC_{inf}), area under the concentration-time curve from time zero to the last quantifiable concentration (AUC_{last}), and maximum serum concentration (C_{max}). Serum blood samples for PK analysis were obtained for a total of 17 times. The safety and immunogenicity were monitored throughout the study.

Results: The 90% CI for the ratio of geometric means of all 3 comparison (CT-P16/EU-B, CT-P16/US-B, and EU-B/US-B) for primary PK endpoints were entirely contained within the predefined bioequivalence margin of 80 to 125%, indicating bioequivalence of all 3 study drugs (Table 1). The additional PK parameters including $\%AUC_{ext}$, T_{max}, $t_{1/2}$, λ_z, V_z, and CL were similar among 3 groups. From 141 subjects, infusion-related reactions were reported for 2 (4.3%), 2 (4.3%) and 4 (8.3%) subjects in CT-P16, EU-B, and US-B groups, respectively, and all cases were Grade 1 in intensity. After study drug administration, no proteinuria, hypertension, anemia, neutropenia or thrombocytopenia with higher than Grade 2 was reported. Post baseline anti-drug antibody positive results were reported from 2 (4.3%), 2 (4.3%) and 3 (6.3%) in CT-P16, EU-B, and US-B, respectively, however no subject reported neutralizing antibody positive result.

Conclusions: The bioequivalence of CT-P16 was demonstrated with the reference products, EU-B and US-B, as measured by primary PK endpoints (AUC_{inf}, AUC_{last}, and C_{max}) in healthy male subjects. The overall safety and immunogenicity profile of CT-P16 were comparable to those of reference products.

Conflict of interest: Other Substantive Relationships: SunYoung Yu, Taehong Park, Sinhye Kim, and Jihun Bae are employees of CELLTRION, Inc. Other authors have no potential conflict of interest to disclose.

Table 1 (abstract 384 PB-047): Statistical Analysis (ANCOVA) of PK Parameters: % Ratio (Test/Reference) [90% CI]

Parameter (unit)	CT-P16/EU-B	CT-P16/US-B	EU-B/US-B
AUC_{inf} (h·µg/mL)	103.88 [99.04, 108.96]	97.69 [93.14, 102.46]	94.04 [89.68, 98.61]
AUC_{last} (h·µg/mL)	104.32 [99.70, 109.15]	98.42 [93.99, 103.06]	94.34 [90.14, 98.74]
C_{max} (µg/mL)	102.98 [98.22, 107.97]	104.02 [99.24, 109.03]	101.01 [96.39, 105.85]

385 (PB-048) Poster

Hyperpolarization-activated cyclic nucleotide-gated channel (HCN) blocker, Ivabradine, a novel potential targeted treatment for triple-negative breast cancer

U.S. Khoo[1], K.C. Mok[1], H. Tsoi[1], E.P. Man[1], K.M. Chau[1], T.K. Lee[2], C. Gong[1]. [1]*The University of Hong Kong, Pathology, Hong Kong, Hong Kong;* [2]*The Hong Kong Polytechnic University, Department of Applied Biology & Chemical Technology, Hong Kong, Hong Kong*

Background: Triple-negative breast cancer patients who cannot benefit from targeted therapies, receive chemotherapeutic agents with attendant side effects. Ivabradine is an FDA approved Hyperpolarization-activated cyclic nucleotide-gated channels (HCN 1–4) targeting therapeutic agent, used clinically to treat chronic angina. We found HCN2 and HCN3 overexpressed in breast cancer compared with low expression in normal breast, both from TCGA data, cell line samples and clinical tissue samples. Treatment with Ivabradine significantly suppressed proliferation of breast cancer cells. We hypothesized that Ivabradine may be used to preferentially target breast cancer.

Materials and Methods: In-vitro functional assays using breast cancer cell-lines were used to characterize the mechanism of action of HCN channel inhibition on breast cancer cells. In-vivo mouse models using both breast cancer xenograft and patient derived tumor xenograft (PDTX) were also used to demonstrate the efficacy of Ivabradine treatment.

Results: Subcellular fractionation experiments confirmed HCN2 and HCN3 were largely localized to the cell membrane. MCF-10A, MDA-MB-231, MDA-MB-453 and BT-474 cell-lines were treated with 200 µM Ivabradine, 40 nM Paclitaxel or 625 nM Doxorubicin. All 3 drugs caused reduced cell viability and proliferation, and induced apoptosis of breast cancer cells. In contrast, Ivabradine did not significantly alter cell viability, proliferation nor apoptosis of the normal breast epithelial cell line MCF-10A. Mechanistically, we found that Ivabradine treatment could deplete intracellular calcium ion concentration, induce ER stress, caspase-mediated apoptosis, autophagy and cell senescence. Breast cancer xenografts MDA-MB-231 and MDA-MB-453 treated with Ivabradine showed decreased tumor growth compared with control group. Knockdown of HCN2 and HCN3 showed similar results as that with Ivabradine treatment in vitro as well as for in vivo xenografts. Ivabradine treatment also effectively reduced tumor growth in 6 out of 8 PDTX models established so far of triple negative breast cancer, with no discernable side effects on the treated mice.

Conclusions: Targeting HCN channels using Ivabradine can be an effective novel targeted therapy for triple negative breast cancer. Being a clinically approved drug, it can more readily pave the way for clinical trials. There is also potential to test the application of antibodies against HCN2 and HCN3 on PDTX and develop humanized antibodies for use as anti-cancer drugs.

No conflict of interest

386 (PB-049) Poster

Quantifying the effects of hepatic impairment on abemaciclib exposure to support dosing recommendations

S.W. van Beek[1], P.K. Turner[2], J.S. Van Der Walt[1]. [1]*Eli Lilly and Company Limited, PK/PD, Global PK/PD & Pharmacometrics, Windlesham, United Kingdom;* [2]*Eli Liily and Company, Global PK/PD & Pharmacometrics, Indianapolis, USA*

Background: Abemaciclib and its metabolites (M2, M18 and M20), which are formed in the liver, inhibit CDK4&6. The abemaciclib half-life doubles in severe hepatic impairment (HI), and the dose is adjusted from twice to once daily. The best measure of the active moiety is the potency-adjusted unbound area under the curve (AUC) of abemaciclib plus its metabolites. We assessed the adequacy of dose adjustment regimens on the predicted steady-state exposure.

Material and Methods: Weight, liver volume estimates and pharmacokinetic (PK) data after a single dose of 200 mg abemaciclib in healthy subjects (HS, n = 10), and subjects with mild (n = 9), moderate (n = 10) and severe (n = 6) HI based on Child-Pugh classification (NCT02387814) were analysed. A mechanistic population PK model (developed from 12 clinical trials in cancer patients and HS) was used to estimate changes in drug extraction and CYP3A4-mediated biotransformation. To evaluate dose adjustments, steady-state concentrations after once daily (OD) or twice daily (BD) doses of 50, 100, 150 and 200 mg in subjects with mild, moderate or severe HI were simulated. The abemaciclib, metabolites and active moiety AUC was compared to HS using ANOVA.

Results: Hepatic blood flow decreased 30% (mild), 44% (moderate), and 76% (severe). Metabolism of abemaciclib to M2 decreased 15% (mild), 23% (moderate) and 78% (severe). The fraction of M2 metabolized to M18 decreased 30% (mild), 50% (moderate) and 70% (severe). These changes resulted in increased abemaciclib and decreased M2, M20 and M18 exposure (Table 1). The active moiety increased 1.8 (95%CI 1.24–2.42) fold in severe HI, but was not different for mild and moderate HI from HS. At steady-state these changes resulted in a significant increase in active moiety exposure in severe HI (2.55 fold, 95%CI 1.25–3.85) but no difference was found for mild and moderate HI. A dose of 200 mg OD in severe HI provided similar exposure as 200 mg BD in HS (1.28 fold, 95%CI 0.00–2.58). Reduction to 100 mg BD maintained trough concentrations above 200 ng/mL (efficacy threshold in xenograft models) in severe HI.

Conclusions: Changes in liver blood-flow and reduced metabolism increased the total active moiety of abemaciclib in severe HI. At steady state, the recommended dose adjustment for severe HI to 200 mg OD would result in exposure of the active moiety comparable to healthy individuals and maintain abemaciclib concentrations above the clinical threshold.

Conflict of interest: Corporate-sponsored Research: Clinical trial NCT02387814 was sponsored by Eli Lilly and Company. PKT and JSW are employees of Eli Lilly and Company.

387 (PB-050) Poster

Studies of compound exerting synthetic lethality in β-catenin mutated tumor cells

H. Ikeda[1], E. Tashiro[1], M. Imoto[1]. [1]*Keio University, Biosciences & Informatics, Kanagawa, Japan*

Background: Wnt signaling pathway is known as a proliferation signaling which has crucial roles in the regulation of diverse processes, including embryonic development. β-Catenin is one of the major component of Wnt signaling pathway and plays a main role in this pathway. Mutation of the β-catenin gene, *CTNNB1*, would cause stabilization and nuclear accumulation of β-catenin protein, results in aberrant downstream transcriptional activity. β-Catenin is mutated in a wide variety of tumors, including 10% of all sporadic colon carcinomas and 20% of hepatocellular carcinomas, so, several small molecules targeting Wnt signaling pathway have been developed. However, there is still no effective therapeutic compound. Recent years, synthetic lethality is reported as a promising strategy for tumor treatment because of targeting respective gene mutation, and expected less side-effect for normal tissue. So, we searched for the compound which exerted synthetic lethality in β-catenin mutated tumors, in other words, induced cell death selectively in β-catenin mutated tumor cells.

Materials and Methods: We established the synthetic lethal screening assay system. Briefly, we treated the test samples to β-catenin mutated HCT116 cells and β-catenin wild type A375 cells, and determined cell death. In this assay, we screened more than 10,000 compounds from synthetic compound library.

Results: We found DS23280164 (DS23), which is provided by Daiichi-Sankyo Pharmaceutical Company, exhibited selective cell death against β-catenin mutated HCT116 cells. In order to confirm synthetic lethality with mutated β-catenin, we used β-catenin isogenic HCT116 cells. As mentioned above, DS23 induced cell death in β-catenin hetero-mutated parent HCT116 *CTNNB1* WT/Δ45 cells and wild type allele knockout *CTNNB1* Δ45/-cells. However, DS23 failed to induce cell death in mutant allele deficient HCT116 *CTNNB1* WT/-cells. Further analysis showed that DS23 induced reactive oxygen species (ROS) production, results in cell death.

Conclusions: It is suggested that DS23 exerts synthetic lethality in β-catenin mutated tumor cells. One of the cause of DS23-induced cell death might be ROS production.

No conflict of interest

388 (PB-051) Poster

Evaluation of antitumor activity of lenvatinib plus anti-PD-1 antibody combination in the hepatocellular carcinoma Hepa1-6 mouse syngeneic tumor model

T. Kimura[1], Y. Kato[1], Y. Ozawa[1], K. Kodama[1], J. Ito[1], K. Ichikawa[1], K. Yamada[1], Y. Hori[1], K. Tabata[1], K. Takase[1], J. Matsui[2], Y. Funahashi[1], K. Nomoto[2]. [1]*Eisai Co., Ltd., Tsukuba Research Laboratories, Tsukuba, Japan;* [2]*Eisai Inc., Oncology Business Group, Woodcliff Lake, USA*

Background: Lenvatinib (LEN) is a multitargeted tyrosine kinase inhibitor that selectively inhibits vascular endothelial growth factor receptor (VEGFR) 1–3, fibroblast growth factor receptor (FGFR)1–4, platelet derived growth factor receptor (PDGFR) α, RET and KIT. In a phase 3 clinical trial in unresectable hepatocellular carcinoma (HCC) (REFLECT study), LEN showed statistical non-inferiority of overall survival compared to sorafenib (SOR). Clinical study of LEN combined with an anti-PD-1 antibody (Ab) in HCC is in progress. Here, we investigated the effects of LEN on cancer immunity and evaluated antitumor activity with combination of anti-PD-1 Ab using the HCC Hepa1-6 mouse syngeneic tumor model.

Material and Methods: Immune cell populations in the Hepa1-6 syngeneic tumor model were analyzed by flow cytometry (FCM). Antitumor activities of LEN (10 mg/kg, p.o., q.d.) and SOR (30 mg/kg, p.o., q.d.) were tested in immunocompetent and immunodeficient mice bearing Hepa1-6 HCC tumors. $CD8^+$ T cell depletion by anti-mouse CD8α Ab in Hepa1-6 tumor model in immunocompetent mice was performed to assess contribution of $CD8^+$ T cells on antitumor activities with LEN and SOR treatments. Antitumor activities of LEN combined with anti-PD-1 Ab (200 μg/head, twice weekly) were evaluated in immunocompetent mice. Single-cell RNA sequencing (scRNA) analysis was conducted to investigate effects of LEN, anti-PD-1 Ab, and their combination on immune cell populations in tumor tissues.

Results: FCM analysis of immune cell populations in Hepa1-6 tumors showed high infiltration of activated $CD8^+$ cytotoxic T cells and indicated that Hepa1-6 tumor is a T cell-inflamed tumor. Although both LEN and SOR showed equivalent antitumor activities in immunodeficient mice, LEN showed greater antitumor activity compared to SOR in immunocompetent mice. With $CD8^+$ T cell depletion, antitumor activity of LEN was attenuated,

Table 1 (abstract 386 PB-049): Fold change of AUC_{last} of HI groups compared to HS

	Single dose						Steady-state
Child-Pugh group (n)	Abemaciclib	M2	M20	M18	Total active species	Adjusted Total active moiety	Adjusted Total active moiety$^+$
Mild (9)	1.06	0.75	0.70*	0.46	0.86	1.14	1.06
Moderate (10)	1.23	0.61*	0.51*	0.47	0.84	0.89	0.90
Severe (6)	2.02*	0.52*	0.31*	0.12	1.10	1.83*	2.55*

*p-value < 0.05, Dunnett's many-to-one test.
$^+AUC_{24}$.

but that of SOR was not. Combined treatment with LEN and anti-PD-1 Ab caused greater tumor regression and higher response rate compared to either agent alone in the Hepa1-6 syngeneic tumor model. scRNA analysis demonstrated that LEN treatment decreased the proportion of monocyte and macrophage populations and increased that of $CD8^+$ T cell populations, and those changes were enhanced with combination of anti-PD-1 Ab.

Conclusions: LEN showed greater antitumor activity with competent immune system compared to immune deficient system, and immune modulatory activities of LEN have a role in enhanced antitumor activity. Combinations of LEN with anti-PD-1 Ab enhanced antitumor activity in preclinical HCC models. Further investigation into immune modulatory activities of LEN to show combination activity with anti-PD-1 Ab is warranted.

Conflict of interest: Corporate-sponsored Research: This research was funded by Eisai Co., Ltd. Other Substantive Relationships: All authors are employees of Eisai Co., Ltd.

389 (PB-052) Poster

Diverse inter- and intra-patient circulating tumor cells (CTCs) phenotypic heterogeneity identified across multiple metastatic breast cancer (mBCa) cohorts

B. Hom[1], A. Jendrisak[1], J. Schonhoft[1], R. Graf[1], P. Ontiveros[1], M. Kearney[1], Y. Wang[1], M. Landers[1], R. Dittamore[1]. [1]*Epic Sciences, Translational Research, San Diego, USA*

Background: There is unmet need for biomarkers to guide Tx selections for mBCa pts among choice of hormone therapies, targeted therapies and chemotherapy. We previously developed phenotypic CTC heterogeneity measurements in mCRPC, and found that high heterogeneity pts are associated with relatively better overall survival with chemotherapy, while low heterogeneity pts have better survival with AR signaling inhibitors (Scher et al. 2017 Cancer Research). Here, the same methodology was applied to mBCa pt cohorts to ascertain the feasibility of CTC heterogeneity analysis in mBCa.

Material and Methods: 230 blood samples from mBCa patients were processed for CTC analysis utilizing the Epic Sciences platform. Following enumeration, multi-dimensional phenotypic characterization analysis was performed utilizing protein expression and digital pathology features. Features from each CTC (3760 CTCs from 165 pts, 85 HR+, 19 Her2+, 8 HR+/Her2+, 53 TNBC) were clustered using unsupervised approach (K-means) and the optimal number of clusters was determined using the elbow method with greater than 85% of variance taken into account. Shannon Index was used to assess the intra-patient CTC heterogeneity. 138 CTCs from various classified CTC subtypes (ranging from 11 to 30 CTCs per subtype) were single cell sequenced for copy number alterations.

Results: CTCs were detected in 77.4% (178/230) of mBCa patients and clustered into 7 phenotypic CTC subtypes. Subset of CTCs from TNBC pts had larger nuclear areas and higher CK expression compared to other mBCa pathological subtypes. A range of CTC phenotypic heterogeneity was observed across patients, with Shannon Index ranging from 0 (low heterogeneity) to 1.73 (high heterogeneity) and median of 0.95. Further, we observed a wide range of genomic instability scores and specific genomic alterations such as ERBB2 gain, FGFR1 gain, BRCA2 loss, and CDH1 loss across phenotypic CTC subtypes.

Conclusions: Diverse inter- and intra-patient phenotypic CTC heterogeneity is observed across multiple cohorts with specific genome profiles detected for different CTC subtypes. We seek to determine if patients with high heterogeneity might be better candidates for hormonal and targeted therapy. Studies linking heterogeneity to therapeutic efficacy and patient outcome are ongoing.

Conflict of interest: Other Substantive Relationships: We are employee of Epic Sciences.

390 (PB-053) Poster

Antitumor and antiangiogenic activities of lenvatinib (LEN) in mouse xenograft models of VEGF-induced hypervascular human hepatocellular carcinoma (HCC)

Y. Adachi[1], M. Matsuki[1], H. Watanabe[1], K. Takase[1], R. Dairiki[1], K. Tabata[1], K. Kodama[1], J. Matsui[1], Y. Funahashi[1], K. Nomoto[2]. [1]*Eisai Co., Ltd., Tsukuba Research Laboratories, Tsukuba, Japan;* [2]*Eisai Inc., Oncology Business Group, Woodcliff Lake, USA*

Background: HCC is known as a hypervascular tumor type, which provides the rationale for the current clinical testing of VEGF-targeted therapies in HCC. Lenvatinib (LEN) is a multi-targeted tyrosine kinase inhibitor that selectively inhibits VEGFR1–3, FGFR1–4, PDGFRα, RET and KIT. In a phase 3 clinical trial in unresectable HCC (REFLECT study, NCT01761266), LEN showed statistical non-inferiority of overall survival compared to sorafenib (SOR). Here, we investigated antitumor and antiangiogenic activities of LEN in VEGF-overexpressing human-HCC mouse xenograft models characterized by hypervascularization and aggressive tumor growth.

Material and Methods: Proliferation and tube formation of human umbilical vascular endothelial cells (HUVEC) were assessed in vitro. VEGF was exogenously overexpressed in HCC cell lines SNU-398 (SNU-398/VEGF) and SK-HEP-1 (SK-HEP-1/VEGF), with high VEGF expression confirmed by ELISA. Xenograft tumor models of these transfectants were established in immuno-deficient mice. Tumor microvessels were analyzed by immunohistochemical staining for endothelial cell marker CD31.

Results: LEN inhibited VEGF-induced HUVEC proliferation with IC_{50} values of 4.6 (for $VEGF_{121}$) or 4.4 nM (for $VEGF_{165}$), and IC_{50} values of SOR were 250 or 320 nM, respectively. The inhibitory activity of LEN at 10 nM against tube formation in VEGF plus FGF-induced tube formation assay was more potent than that of sorafenib at the same concentration (LEN, 98% inhibition; SOR, 22% inhibition). Ectopic VEGF overexpression in SNU-398 and SK-HEP-1 cell lines (with low VEGF-expression) led to hypervascularity and aggressive tumor growth of both transfectant xenografts. Both LEN (10 mg/kg) and SOR (30 mg/kg) inhibited tumor growth of the mock transfected SNU-398 model. However, only LEN showed clear antitumor activity in the hypervascular SNU-398/VEGF transfectant model. In the hypervascular SK-HEP-1/VEGF model, LEN led to nearly complete tumor stasis while SOR resulted in moderate inhibition of tumor growth rates. In all the VEGF-overexpressing models, antiangiogenic activity of LEN was significantly more potent than that of SOR.

Conclusion: LEN has potent inhibitory activity against VEGF signaling pathway, and this activity underlies robust antiangiogenic and antitumor activities of LEN in VEGF-overexpressing hypervascular HCC models.

Conflict of interest: Corporate-sponsored Research: This research was funded by Eisai Co., Ltd. Other Substantive Relationships: All authors are employees of Eisai Co., Ltd. and Eisai Inc.

391 (PB-054) Poster

Anagrelide, a novel anticancer compound for gastrointestinal stromal tumor

O.P. Pulkka[1], Y. Gebreyohannes[2], A. Wozniak[2], J.P. Mpindi[3], O. Tynninen[4], K. Icay[5], A. Cervera[5], S. Keskitalo[6], A. Murumägi[3], Y. Kulesskiy[3], K. Wennerberg[3], M. Varjosalo[6], P. Laakkonen[7], R. Lehtonen[5], S. Hautaniemi[5], O. Kallioniemi[3], P. Schöffski[2], H. Sihto[1], H. Joensuu[8]. [1]*University of Helsinki, Laboratory of Molecular Oncology, Translational Cancer Biology Research Program, Helsinki, Finland;* [2]*KU Leuven & University Hospitals Leuven, Deaprtment of Oncology & Department of General Medical Oncology, Leuven, Belgium;* [3]*University of Helsinki, Institute for Molecular Medicine Finland, Helsinki, Finland;* [4]*University of Helsinki and HUSLAB, Department of Pathology, Helsinki, Finland;* [5]*University of Helsinki, Genome-Scale Biology Research Program, Department of Biochemistry and Developmental Biology, Helsinki, Finland;* [6]*University of Helsinki, Institute of Biotechnology, Helsinki, Finland;* [7]*University of Helsinki, Translational Cancer Biology Research program, Helsinki, Finland;* [8]*University of Helsinki & Helsinki University Hospital, Laboratory of Molecular Oncology, Translational Cancer Biology Research program and Comprehensive Cancer Center, Helsinki, Finland*

Background: KIT and PDGFRA targeting tyrosine kinase inhibitors such as imatinib are highly effective in a therapy of gastrointestinal stromal tumor (GIST). However, advanced GISTs progress frequently on these therapies and there is dire need to find new therapeutic options for the patients. We investigated role of phosphodiesterase 3 family (PDE3) as a potential therapy target in GIST.

Material and Methods: PDE3A and PDE3B mRNAs were exceptionally highly expressed in GIST based on an *in silico* transcriptome database analysis of 9,783 human tissue and cancer samples. A high PDE3 expression was verified on protein level by using immunohistochemistry on tissue microarrays (TMAs) consisting of 630 human tumor samples. GIST882 and GIST48 cell lines were screened for sensitivity to 217 anticancer compounds, and the efficacy of PDE3-specific compounds was investigated further in GIST cell lines. The efficacy of anagrelide, the most potent PDE3-specific compound in GIST cell lines, was studied further in four GIST xenograft mouse models.

Results: A high PDE3 expression is frequent in GIST in comparison with other human tumor types. Anagrelide reduces tumor cell viability and promotes cell death by targeting PDE3s in GIST882 cell line. Anagrelide inhibits also tumor growth and reduces tumor volume in GIST xenograft mouse models.

Conclusion: PDE3A and PDE3B are expressed frequently in GIST and the PDE3-specific compound, anagrelide has an anticancer efficacy in the

GIST xenograft mouse models. Further testing of anagrelide in a clinical trial is warranted.

Conflict of interest: Ownership: O.P. Pulkka, O. Kallioniemi, H. Sihto, and H. Joensuu own stocks of Sartar Therapeutics Ltd. Other Substantive Relationships: H. Joensuu has a co-appointment at Orion Pharma.

393 (PB-056) Poster

Concurrent KIT and PI3 K signaling inhibition with imatinib and copanlisib as front-line treatment in gastrointestinal stromal tumors (GIST)

L.A. García Valverde[1], J. Rosell[1], G. Serna[2], C. Valverde[3], J. Carles[3], P. Nuciforo[2], J. Arribas[1,4], D. Zopf[5], O. Politz[5], C. Serrano[1,3]. *[1]Vall d'Hebron Institute of Oncology, Preclinical Research Program, Barcelona, Spain; [2]Vall d'Hebron Institute of Oncology, Molecular Oncology Group, Barcelona, Spain; [3]Vall d'Hebron Institute of Oncology, Medical Oncology, Barcelona, Spain; [4]Institució Catalana de Recerca i Estudis Avançats ICREA, Recerca, Barcelona, Spain; [5]Bayer AG, Therapeutic Research Groups Oncology, Berlin, Germany*

Background: Most GISTs depend upon continuous KIT oncogenic signaling. KIT-downstream PI3K/AKT pathway plays a critical role in GIST, thus emerging as an attractive target. However, PI3K inhibition has shown so far modest benefit in GIST patients. Here we evaluate preclinically copanlisib, a novel pan class-I PI3Kinhibitor with predominant activity on p110α and p110δ, in GIST as single-agent and in combination with first-line KIT inhibitor imatinib (IM).

Material and Methods: One IM-sensitive (GIST-T1) and 2 IM-resistant (GIST430/654, GIST-T1/670) cell lines were studied. Cells were treated with copanlisib alone or in combination with IM and studied for cell viability, proliferation (BrdU), pathway inhibition (immunoblotting) and apoptosis (caspase assay and immunoblotting). GIST-T1 and GIST-T1/670 xenografts were treated likewise and tumors analyzed for PI3K/AKT pathway inhibition by immunohistochemistry (IHC) at days 1 and 21.

Results: In cell viability assays copanlisib as single agent displayed IC50 values between 54.5 nM (GIST-T1) and 278.8 nM (GIST-T1/670). Immunoblots confirmed profound PI3K/AKT pathway inhibition. Immunoblots, proliferation and apoptosis assays revealed mild impact in proliferation and lack of pro-apoptotic activity, probably due to the unexpected cross-activation of MAPK signaling. Combined KIT and PI3K inhibition with IM and copanlisib, respectively, was synergistic, resulting in higher tumor proliferation impairment in both IM-sensitive and IM-resistant GIST cell lines, but only synergistic increase in apoptosis in GIST-T1.

GIST-T1 and GIST-T1/670 subcutaneous murine xenografts were treated for 21 days with IM and copanlisib both as single agents and in combination. Single-agent copanlisib significantly delayed tumor growth in both GIST-T1 and GIST-T1/670, and the combination showed a non-significant trend towards additive effect in GIST-T1, but not in GIST-T1/670. Al arms in the *in vivo* treatment were overall well tolerated.

Phospho-S6 was the pharmacodynamic biomarker that best correlated with PI3K/AKT pathway inhibition and antitumor treatment activity.

Conclusions: Copanlisib as single agent efficiently suppresses PI3K/AKT pathway in IM-sensitive and IM-resistant GIST, both *in vitro* and *in vivo*, resulting in delayed tumor growth. The addition of PI3K inhibition to KIT inhibition with IM is an effective therapeutic strategy in IM-sensitive GIST, but not in IM-resistant disease. Induction of apoptosis appears to be key in order to impact profoundly in treatment activity. Phospho-S6 assessment by IHC might be a reliable biomarker of tumor growth inhibition in GIST.

Conflict of interest: Corporate-sponsored Research: This project has been partially funded with a Bayer Research Grant.

394 (PB-057) Poster

A SMO inhibitor DCB-HDG2-411 with potent Hedgehog signaling pathway antagonist activity overcomes the Smo drug-resistant mutations

Y.S. Lee[1], M.Y. Kuo[2], C.W. Liu[1], Y.Y. Lu[2]. *[1]Development Center for Biotechnology, Institute of Pharmaceutics, Division of Bioactivity Identification, Taipei City, Taiwan; [2]Development Center for Biotechnology, Institute of Pharmaceutics, Division of Medicinal Chemistry, Taipei City, Taiwan*

The Hedgehog (Hh) signaling pathway is a critical regulator of embryonic patterning, and aberrant Hh pathway activation has been implicated in a diverse spectrum of cancers. Therefore, components of the Shh pathway (such as Shh, SMO, and GLI1/2) are viable therapeutic targets for anti-tumor strategy. Smo antagonists such as GDC-0449 and NVP-LDE225 have received FDA approval for treating basal cell carcinoma. However, primary and acquired SMO mutation-mediated resistance has emerged as a challenge to targeted therapeutics and may limit their anti-cancer efficacy. We report here the development of a potent Smo inhibitor designated DCB-HDG2-411 with potent Hh signaling pathway inhibition activity. DCB-HDG2-411 demonstrated Hh signaling pathway antagonist activity in a 293 cell-based Gli-luciferase inhibition assay upon agonist treatment (IC50 = 4.7 nM), and retains inhibition activity against the Smo wild-type and D473H mutant (this mutant is responsible for resistance to vismodegib in medulloblastoma patients) co-transfection, with IC50 of 1.6 and 8.1 nM, respectively. The inhibition activity of DCB-HDG2-411 toward other Smo drug-resistant mutations such as Smo-G497W and Smo-W535L is under investigation. DCB-HDG2-411 competed with BODIPY-cyclopamine binding using Smo-expressing Hela cells with IC50 of 5.2 nM, indicating the compound occupy the same binding site as cyclopamine. DCB-HDG2-411 can also inhibit Hedgehog signaling in human HEPM cells with IC50 of 1.4 nM in reducing the amount of Gli1 mRNA. Treatment of a $PTCH^{+/-}$ medulloblastoma allograft model with DCB-HDG2-411 (once-daily oral dosing at 10, 20, 40 mg/kg for 10 days) showed an effective dose-related antitumor activity mediated by inhibition of the Hh pathway. The DCB-HDG2-411 can therefore be evaluated further and serve as a potential drug development candidate for treatment of Hh pathway-driven cancers.

No conflict of interest

395 (PB-058) Poster

A phase 1 dose escalation study of ARQ 751 in adult patients with advanced solid tumors with AKT1, 2, 3 genetic alterations, activating PI3 K mutations, PTEN-null, or other known actionable PTEN mutations

S. Pant[1], V. Subbiah[1], J. Rodon[1], F. Janku[1], D. Hong[1], D. Karp[1], S. Piha-Paul[1], A.M. Tsimberidou[1], A. Naing[1], S. Fu[1], R.E. Savage[2], F. Chai[2], Y. Yu[2], K. Tith[2], R. Alfattal[1], B. Schwartz[2], F. Meric-Bernstam[1], T. Yap[1]. *[1]MD Anderson Cancer Center, Investigational Cancer Therapeutics, Houston, USA; [2]ArQule, Inc., Clinical Development, Burlington, USA*

Background: Dysregulation of the PI3K-AKT signaling pathway plays a critical role in cancer initiation and progression. AKT can be activated through activated RTKs, gain-of-function mutations of PIK3CA, PTEN deficiency, and AKT amplification or, activating mutations such as AKT1E17K. ARQ 751 potently inhibits AKT1, 2 and 3 with biochemical IC_{50} values of 0.55, 0.81 and 1.31 nM, respectively, with high selectivity. It does not inhibit any other kinase among the 245 tested by greater than 50% at 5 μM.

Material and Methods: This is a Phase 1 dose escalation study to assess the safety, tolerability, PK and preliminary anti-tumor activity of ARQ 751 in patients with advanced solid tumors with selected known genetic alterations. Treatment emerging adverse events (TEAE) were assessed per NCI CTCAE v. 4.03. Tumor response was evaluated per RECIST 1.1.

Results: 26 pts have been enrolled [81% female; median age 60 years; 89% White; 9 breast, 4 endometrial, 2 colon, 2 prostate and 9 others; median number of prior systemic therapies was 4.5]. Genetic alteration status at baseline: 12 activating PI3K mutations, 9 PTEN null/actionable mutations, 3 AKT1 mutations, 1 AKT3 mutation, 1 PI3K mutation +AKT3 amplication. Patients were treated at dose levels ranging from 5 mg QD to 100 mg QD.

Drug related TEAEs reported in $\geq$5% pts included nausea (19%), diarrhoea (15%), rash (12%), fatigue (8%), and mucosal inflammation (8%). There was 1 DLT (Grade 3 pruritic rash) reported at 100 mg QD. Additionally at the 100 mg QD dose, two Grade 2 rashes and one Grade 3 hyperglycemia were reported, therefore dose escalation above 100 mg QD was not initiated and an intermediate dose level of 75 mg QD is being explored.

In general, ARQ 751 increases in mean exposure were greater than dose proportional as the dose was increased from 10 to 50 mg QD; half-life ($t_{1/2}$) generally ranged from 16 to 22 h.

As of 15 Jun 2018, 6 of 9 breast cancer (BC) patients were evaluable for tumor responses including 4 ER+/PR+/Her2−, 1 triple negative, and 1 Her 2+. Among the 4 BC patients with ER+/PR+/Her2−, two achieved partial response (PR) (one with PTEN C296fs*2 mutation, and the other with PIK3CA H1047R mutation, both are currently on therapy), one achieved stable disease (SD) (PIK3CA c.3140A mutation), and one had progressive disease (PD) (PIK3CA H1047R mutation). The triple negative BC patient with PTEN L247fs*5 mutation achieved SD. The Her2+ patient with PI3K ESR1 mutation had PD.

Conclusions: ARQ 751 demonstrated a manageable safety profile to date. Preliminary anti-tumor activity was observed. Updated safety, PK, biomarker and efficacy data will be presented.

Conflict of interest: Corporate-sponsored Research: Brian Schwartz, Ronald E. Savage, Feng Chai, Yi Yu, Kate Tith are employee of the clinical trial sponsor, ArQule, Inc.

396 (PB-059) Poster

Cell-free DNA landscape of genomic alterations in over 3,000 patients with advanced breast cancer

L. Kiedrowski[1], A. Bardia[2], J. O'shaughnessy[3], B. Nagy[1], R. Lanman[1], M. Cristofanilli[4]. [1]*Guardant Health, Medical Affairs, Redwood City, USA;* [2]*Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, USA;* [3]*Baylor University Medical Center, Texas Oncology, US Oncology, Dallas, USA;* [4]*Northwestern University Feinberg School of Medicine, Robert H. Lurie Comprehensive Cancer Center, Chicago, USA*

Background: Plasma cell-free DNA (cfDNA) can provide somatic genomic information non-invasively and in real-time for patients (pts) with breast cancer. Current tissue-based genomic sequencing compendia typically includes early stage, relatively treatment-naïve cohorts. We performed descriptive analysis of genomic data from a large and generally pretreated pt cohort with stage III/IV breast cancer to better understand the landscape of cfDNA alterations in advanced breast cancer.

Materials and Methods: Deidentified aggregate genomic data from clinical samples submitted for cfDNA next-generation sequencing (NGS) analysis with Guardant360® (Guardant Health, Inc; Redwood City, CA, USA) between 11/7/2016 and 2/28/2018 with an indicated diagnosis of stage III/IV breast cancer was queried. Testing analyzed cfDNA for the presence of somatic single nucleotide variants (SNVs) in complete or critical exons of 73 genes as well as indels in 23 genes, copy number amplifications (CNAs) in 18 genes, and fusions in 6 genes.

Results: We identified a total of 3044 unique pts with advanced breast cancer who had 3695 consecutive test results, including serial specimens for some pts. The average pt age was 58 (range 18–95); 99% were female. 3244 tests (88%) had ≥1 somatic alteration (alt) detected; of these, a median of 4 alts were identified (range 1–85). The median mutant allele fraction (MAF) per alt was 0.50% (range 0.01–93.66%). Of the 2654 pts with ≥1 nonsynonymous (NS) alt detected, NS sequence alts were most frequently detected in *TP53* (56%), *PIK3CA* (37%), *ESR1* (24%), and *NF1* (14%); CNAs were most frequently detected in *MYC* (17%), *FGFR1* (16%), and *CCND1* (14%). Fusions were identified in 11 pts involving *FGFR3* (5), *ALK* (2), *NTRK1* (2), *RET* (1), and *ROS1* (1). Of the 649 pts with *ESR1* NS alts detected, 219 (34%) had multiple NS *ESR1* alts (range 2–9). *ERBB2* (HER2) CNAs and NS sequence alts were identified in 8% and 10% of pts, respectively. NS alterations were also identified in other genes with matched therapies, including *BRCA2* (9%), *BRCA1* (6%), *ATM* (4%), and *AKT1* (3%).

Conclusions: NGS of cfDNA identified multiple genomic alts in this large cohort of pts with advanced breast cancer, including those conferring acquired resistance to therapies, such as *ESR1* mutations, as well as those with potentially actionable matched therapies, such as *ERBB2* mutations/amplifications, *NTRK1* fusions, and *BRCA1*/*2* mutations. cfDNA analysis provides a noninvasive alternative to tissue biopsy to interrogate tumor genomics in pts with advanced breast cancer.

Conflict of interest: Ownership: LK, RN, and RL are shareholders of Guardant Health, Inc. Other Substantive Relationships: LK, RN, and RL are employees of Guardant Health, Inc.

398 (PB-061) Poster

Towards a therapeutically relevant subtyping scheme for triple-negative breast cancer (TNBC), profiling results from A Randomized, TNBC Enrolling trial to confirm Molecular profiling Improves Survival (ARTEMIS)

S. Seth[1], L. Huo[2], M. Gilcrease[2], H. Piwnica-Worms[3], R. Lau[2], B. Adrada[4], G. Rauch[4], G. Draetta[5], A. Futreal[6], W.F. Symmans[2], S. Moulder[7], J. Chang[8]. [1]*MD Anderson Cancer Center, Institute of Applied Cancer Science, Houston, USA;* [2]*MD Anderson Cancer Center, Department of Pathology, Houston, USA;* [3]*MD Anderson Cancer Center, Department of Experimental Radiation Oncology, Houston, USA;* [4]*MD Anderson Cancer Center, Department of Diagnostic Imaging, Houston, USA;* [5]*MD Anderson Cancer Center, Department of Therapeutics Discovery, Houston, USA;* [6]*MD Anderson Cancer Center, Department of Genomic Medicine, Houston, USA;* [7]*MD Anderson Cancer Center, Breast Medical Oncology, Houston, USA;* [8]*The University of Texas Health Science Center, Department of Integrative Biology and Pharmacology, Houston, USA*

Background: Approximately 50% of TNBC patients (pts) treated with standard neoadjuvant chemotherapy (NACT) will have extensive residual disease (Residual Cancer Burden II or III) which is associated with a 40–80% risk of recurrence and death. Pts with minimum to no residual disease (RCB 0-I) after NACT have <10% risk of recurrence. Thus, there is an urgent need to better understand targetable molecular mechanisms driving TNBC.

Methods: Samples from 220 TNBC pts treated with NACT +/– targeted therapy within the ARTEMIS trial were prioritized for transcriptomic and genomic profiling. Non-negative matrix factorization was used on array-based profiling, to identify six robust ARTEMIS subtypes, which were compared with Vanderbilt subtypes. CIBERSORT was performed to estimate relative immune-cell proportions and compared with pathologist's evaluations of tumor-infiltrating lymphocytes (TILs). Residual cancer burden (RCB), was assessed after surgery.

Results: Comparing ARTEMIS subtypes with Vanderbilt subtypes, revealed significant overlap with 4/6 clusters (Table). Logistic regression on ssGSEA scores vs subtypes revealed several pathways, selectively enriched specific subtypes. IM (Immune subtype), was enriched in INFg and INFa, while MYC/mTOR (CL2), showed enrichment of several proliferation-related pathways. In addition, pts classified as LAR and M, have high concordance across the two subtyping methods. However, two new subtypes did not associate significantly with any of the Vanderbilt subtypes: CL5 (ANGIO), was enriched in angiogenesis geneset, which includes targetable genes like, VEGF and FGFR and CL3 (MYO) with enrichment in genes related to myogenesis, and exhibits a gene-expression signature associated with down-regulation of KRAS.

	Vanderbilt							
ARTEMIS	BL1	BL2	IM	LAR	M	MSL	UNS	
IM (CL1)	0	3	23	0	0	2	3	Immune, INFa, INFg
MYC/MTOR (CL2)	28	2	11	0	1	0	12	mTOR, MYC, E2F
MYO (CL3)	5	0	5	0	6	3	5	KRAS down, Myogenesis
M (CL4)	6	0	4	0	22	1	5	Wnt, Notch
ANGIO (CL5)	2	11	1	0	9	8	6	Angiogenesis, Hypoxia
LAR (CL6)	0	5	1	17	1	0	1	Androgen Response

The ARTEMIS method was also able to classify 32 (15%) tumors classified as unknown (UNS) using Vanderbilt's method with noted enrichment in CL2 (MYC/mTORi).

Of note, TIL (tumor infiltrating lymphocytes) and LAR quantification using IHC were associated with respective ARTEMIS subtypes. Finally, the IM subtype was significantly associated with higher rates of RCB 0-I (p = 0.012) and the M subtype was associated with higher rates of RCB II-III (p = 0.028), irrespective of the neoadjuvant treatment regimen.

Conclusions: ARTEMIS subtypes reveal a therapeutically relevant classification system, which may be informative for targeted therapy selection in pts with chemotherapy-resistant disease. Further, we show a possibility to classify previously, un-classified (UNS) tumors which will be validated using additional cohorts (TCGA/METABRIC).

No conflict of interest

399 (PB-062) Poster

A biomarker study of the bispecific anti-DLL4/anti-VEGF antibody navicixizumab (OMP-305B83) in Phase 1a patients with previously treated solid tumors

S. Cai[1], A. Brunner[1], G. Argast[1], M. Wang[1], C. Zhang[1], A. Kapoun[1]. [1]*OncoMed Pharmaceuticals, Translational Medicine, Redwood City, USA*

Background: Inhibition of tumor angiogenesis has proven to be a successful approach to treating cancer and the two major ligands responsible for tumor angiogenesis are vascular endothelial growth factor (VEGF) and delta-like ligand 4 (DLL4). In addition, there is preclinical evidence that inhibition of DLL4 reduces tumorigenicity by reducing the number of tumor initiating cells. Navicixizumab (OMP-305B83) is an IgG2 humanized bispecific monoclonal antibody directed against both human DLL4 and VEGF.

Material and Methods: Exploratory biomarker analysis was performed on FFPE tumors, blood, and hair follicles from patients (pts) in a navicixizumab phase 1a study with a 3+3 dose escalation design (NCT02298387). Here we present biomarker analysis from the study. Blood and hair follicles were collected pre-dose and on days 28 to evaluate changes in Notch and VEGF-related gene expression. RNA expression was measured using Affymetrix Human GeneChip U133 plus 2 microarrays and genes significantly modulated between post-dose and pre-dose were identified. Gene Set Enrichment Analysis was performed to determine the biological processes affected by navicixizumab. In addition, FFPE samples from 43 subjects were analyzed by RNA-Seq. Univariate analysis was performed by correlating the expression of Notch and angiogenesis related genes with clinical outcome.

Results: Sixty six patients were treated in 8 dose-escalation cohorts (0.5, 1, 2.5, 3.5, 5, 7.5, 10, and 12.5 mg/kg) once every 3 weeks and an expansion cohort at 7.5 mg/kg. The trial assessed navicixizumab in refractory multiple solid tumor pts. The response rate in heavily pretreated ovarian cancer pts was 25% (3/12 PR). Pts with FFPE were ovarian (8), breast, uterine, endometrial (4 each) and other cancers. Expression levels of Notch, VEGF, and angiogenesis pathway genes in baseline FFPE samples were analyzed to investigate mechanism of action among the heterogeneous tumor types and explore trends with clinical response. Exploratory mutation analysis identified b-catenin mutations in several pts. Pharmacodynamic analysis in surrogate tissues demonstrated Notch and VEGF genes were significantly modulated in whole blood, including upregulation of *ANK1, FOXO3, BCL2L1* and downregulation of *HEY1*, consistent with engagement of both pathways by navicixizumab. Gene set enrichment analysis corroborated these results and showed that there was significant down-regulation of DLL4 and VEGF pathway genesets.

Conclusion: Biomarker analysis of pts from the Phase1a trial of navicixizumab in previously treated solid tumors demonstrated target engagement of both DLL4 and VEGF. Significant modulation of Notch and VEGF pathway genes by navicixizumab was observed in surrogate tissues. Updated biomarker results will be presented. Phase1b studies of navicixizumab plus chemotherapy are ongoing.

No conflict of interest

400 (PB-063) Poster

Super-enhancer landscapes of ovarian cancer reveal novel epigenomic subtypes and targets

M. Eaton[1], B. Johnston[1], K. Piotrowska[1], C. Collins[1], J. Lopez[1], S. Knutson[1], E. di Tomaso[1], J. Carulli[1], E. Olson[1]. [1]*Syros Pharmaceuticals, Inc., Cambridge, MA, USA*

Background: There is a critical unmet need for targeted therapies in ovarian cancer, especially high-grade serous ovarian cancer (HGSOC). We used enhancer mapping combined with transcriptomics and mutations to identify novel ovarian cancer subtypes and associated targets.

Material and Methods: With ChIP-seq for H3K27Ac, we profiled the enhancer landscape of 101 primary tumor samples from 7 ovarian cancer subtypes with a focus on HGSOC, 29 cell line models, 3 PDXs, and 8 non-cancerous samples of ovarian and fallopian tube tissue. We also profiled many of these samples through RNA-seq and a focused NGS-based mutational panel. We used matrix factorization methods to reveal novel subgroups of ovarian cancer patients and predicted their associated transcriptional circuitry.

Results: Through a computational deconvolution of enhancer maps, we identified novel enhancer-defined patient subtypes of ovarian cancer. While some known subtypes, such as granulosa cell, associated uniquely with their own enhancer profile, the majority of the primary tumor samples fell into 4 clusters that did not correlate with histological subtype or with known high-frequency ovarian cancer mutations. Each cluster was associated with its own unique super-enhancer (SE) signature, implying that each is driven by a unique transcriptional circuitry. There was a striking cluster-specific patterning of many known ovarian cancer related genes such as FOXM1, CD47, and MYC, and genes linked to pathways known to be dysregulated in ovarian cancer, including an SE linked to the RB pathway gene Cyclin E1. Furthermore, many additional cluster-specific SEs were discovered representing novel potential therapeutic targets. Interestingly, while we could assign ovarian cancer cell line models to these novel subtypes, many cell lines' enhancer landscapes appeared to be distinct from those of primary tumor cells.

Conclusions: Together, our results comprise the largest ovarian cancer enhancer mapping effort to date, and demonstrate how an integrated analysis of enhancers, transcriptomes, and genotypes together can yield transcriptional circuitry that reinforces the role of known pathways associated with ovarian cancer progression and treatment, can be used to select cell models that best recapitulate the enhancer landscape of primary tumors, and can be mined to identify novel targets and biomarkers. Cluster-specific SEs at MYC and CCNE1 suggest that some tumors have increased transcriptional dependency on these loci as well as the components of the corresponding transcriptional machinery. The role of one such component, CDK7, is currently being evaluated in ovarian cancer patients with SY-1365, a first-in-class selective CDK7 inhibitor in Phase 1 (NCT 03134638).

Conflict of interest: Ownership: All authors were shareholders and employees of Syros Pharmaceuticals at the time the work was conducted.

401 (PB-064) Poster

First-line treatment of metastatic non-small cell lung cancer (NSCLC): A randomized, double-blind, phase 2 trial of denosumab in combination with chemotherapy

R. de Boer[1], P. Pedrazzoli[2], B. Biesma[3], R. Natale[4], C.K. Lee[5], L. Zhu[6], A. Glennane[7]. [1]*The Royal Melbourne Hospital, Oncology, Parkville, Australia;* [2]*Policlinico San Matteo Pavia Fondazione, Oncology, Pavia, Italy;* [3]*Jeroen Bosch Ziekenhuis, Oncology, 's Hertogenbosch, Netherlands;* [4]*Cedars-Sinai Medical Center, Hematology/Oncology, Los Angeles, USA;* [5]*St. George Hospital, Oncology, Kogarah, Australia;* [6]*Amgen Inc., Biostatistics, Thousand Oaks, USA;* [7]*Amgen Inc., Medical Affairs, Thousand Oaks, USA*

Background: Lung cancer remains the leading cause of cancer death worldwide. The receptor activator of nuclear kappa-B ligand (RANKL) inhibitor denosumab (XGEVA®) reduces bone complication risk in patients with solid tumors. RANKL inhibition may also improve overall survival (OS) in NSCLC. This phase 2 double-blind randomized trial compared the effect of denosumab vs placebo on OS, in combination with first-line platinum-based chemotherapy in metastatic NSCLC with or without bone metastases (NCT01951586).

Methods: Adults with metastatic NSCLC were randomized 2:1 to receive denosumab 120 mg or placebo subcutaneously every 3–4 weeks plus a loading dose on study day 8 in combination with a first-line platinum doublet. Randomization was stratified by presence of bone metastasis, histology, and geographic region. Key criteria for patient eligibility were confirmed stage IV NSCLC, favorable ECOG status (0–1), radiographically evaluable disease, and adequate organ function. The primary study objective was to estimate the effect of denosumab plus standard of care (SOC) vs SOC alone on OS. Key secondary endpoints included correlation between tumor tissue RANK expression with objective response rate (ORR) or OS; ORR (complete or partial responses based on modified RECIST 1.1), progression-free survival (PFS), clinical benefit rate (patients with objective response plus those with stable disease or better for ≥16 weeks), and safety.

Results: A total of 226 patients were randomized to denosumab (n = 148) or to placebo (n = 78). Baseline demographics and disease characteristics were balanced between the two arms. Median OS was similar in the denosumab (10.7 months) and placebo arms (10.9 months; HR [95% CI] = 1.06 [0.75 –1.50]). No significant correlation was detected between tumor RANK/RANKL expression and relative benefit from denosumab plus SOC vs SOC alone on OS or ORR. Median PFS was similar in the denosumab (5.2 months) and placebo arms (5.7 months; HR [95% CI] = 1.05 [0.78–1.43]). The ORR was 36.8% for denosumab and 43.4% for placebo (odds ratio [95% CI] = 0.76 [0.43–1.35]). The clinical benefit rate was 47.9% for denosumab and 53.9% for placebo (odds ratio [95% CI] = 0.81 [0.46–1.43]). Overall incidences of AEs were similar between the two treatment arms. Hypocalcemia (18.6% vs 5.3%) and positively-adjudicated osteonecrosis of the jaw (2.8% vs 0%) were more frequently reported in denosumab vs placebo recipients.

Conclusions: The addition of denosumab to SOC showed no clinical benefit in patients with NSCLC. There was no correlation of improved treatment effect on OS with high RANK/RANKL expression levels. Overall safety results from this study were similar between the two treatment arms and comparable to those seen in other studies with denosumab 120-mg dosing. In this setting, denosumab should be used for management of bone metastases and their complications.

Conflict of interest: Other Substantive Relationships: Bonne Biesma reports no conflicts of interest. Richard de Boer has participated in speakers bureau with Amgen, Novartis, Merck, and Roche. Anthony Glennane and Li Zhu report employment and stock ownership from Amgen Inc. Chee Khoon Lee reports research grants from AstraZeneca and consulting fees from Roche, AstraZeneca, Takeda, and Novartis. Ronald Natale reports research grants from AbbVie, AstraZeneca, and Bristol-Myers Squibb. Paolo Pedrazzoli has received consulting fees from Baxter, and has participated in speakers bureau with Lilly and Baxter.

402 (PB-065) Poster

Post-transcriptional regulation of TSC1 in oral cancer

K. Mallela[1], A. Tiwari[1], S. Swamy[2], K.S. Gopinath[2], A. Kumar[1]. [1]*Indian Institute of Science, Department of Molecular Reproduction, Development and Genetics, Bangalore, India;* [2]*HCG-Bangalore Institute of Oncology, Surgical Oncology, Bangalore, India*

Background: Oral cancer is the most common form of Head and Neck cancer with a highest number (nearly 20%) of cases in India and ranks among the top three cancers. Role of the PI3K/AKT/mTOR pathway in the

pathogenesis of various cancers, including oral cancer, is well studied. A previous study from our group reported that the TSC proteins (TSC1/Hamartin and TSC2/Tuberin), key negative regulators of the mTOR signaling pathway, are down-regulated in oral cancer. It is well known that the expression of miRNAs is dysregulated in various cancers. These regulatory molecules act as either oncomirs or tumor suppressors, thereby serving as potential biomarkers for cancer diagnosis, prognosis and therapeutics. In the present study, we investigated if *miR-130a* targets hamartin/TSC1 and regulates the mTORC1 signalling.

Materials and Methods: Oral cancer cells were transfected with miR-130a overexpressing construct, mock or antagomir-130a and further analysis was done at RNA and protein levels to assess if miR-130a targets TSC1. Dual luciferase reporter assay was performed to validate the interaction between miR-130a and TSC1 in OSCC cells. Expression of TSC1 and miR-130a was done by real-time PCR in matched oral normal and tumor samples.

Results: Overexpression of miR-130a in oral cancer cells (SCC131 and SCC084) significantly reduced the levels of TSC1 and hence increased cell proliferation. Dual luciferase reporter assay confirmed the interaction of miR-130a with the 3′UTR of *TSC1*. We observed an inverse correlation between the expression levels of miR-130a and *TSC1* in oral tumor samples.

Conclusion: Upregulation of miR-130a led to a decreased level of TSC1 accompanied with a high mTORC1 activity and *vice versa*. Our study suggests that inhibition of miR-130a may suppress the tumorigenic potential through the regulation of TSC1/mTOR axis in oral cancer, and provide a novel therapeutic target.

No conflict of interest

403 (PB-066) Poster

Natural history, treatment (tx) patterns, and outcomes in MET dysregulated non-small cell lung cancer (NSCLC) patients (pts)

J. Wolf[1], C. Baik[2], R.S. Heist[3], J.W. Neal[4], A.S. Mansfield[5], R. Buettner[6], K.L. Davis[7], M. Giovannini[8], A. Mutebi[9], M.M. Awad[10]. [1]*University Hospital Cologne, Department I of Internal Medicine, Cologne, Germany;* [2]*University of Washington, Thoracic, Head and Neck Medical Oncology, Seattle, USA;* [3]*Massachusetts General Hospital, Cancer Center, Boston, USA;* [4]*Stanford Cancer Institute/Stanford University, Department of Medicine, Division of Oncology, Stanford, USA;* [5]*Mayo Clinic, Medical Oncology, Rochester, USA;* [6]*University Hospital Cologne, Institute for Pathology, Cologne, Germany;* [7]*RTI Health Solutions, Health Economics, Research Triangle Park, USA;* [8]*Novartis Pharmaceuticals Corporation, Global Clinical Development, East Hanover, USA;* [9]*Novartis Pharmaceuticals Corporation, US Health Economics and Outcomes Research, East Hanover, USA;* [10]*Dana-Farber Cancer Institute, Medical Oncology, Boston, USA*

Background: *MET* dysregulation through exon 14 skipping mutations, high-level gene amplification, or concurrent mutation with amplification occurs in ~3–4% of NSCLC and may be a negative prognostic factor. Currently, limited data are available to characterize the natural history of *MET*-dysregulated metastatic NSCLC pts. This study aims to characterize disease and pt characteristics, tx patterns, and outcomes in *MET*-dysregulated metastatic NSCLC.

Material and Methods: This is an ongoing multinational, retrospective, real-world medical record analysis of pts with advanced EGFRwt *MET*-dysregulated NSCLC, defined as *MET* mutated through exon 14 skipping mutations, *MET* amplification with gene copy number (GCN) $\geq$6 and/or gene/centromere ratio $\geq$2.2, or both mutation and amplification. Pts were included if they were $\geq$18 years old at diagnosis of metastatic NSCLC with $\geq$12 months of follow-up. Study measures include pt and disease characteristics at presentation, tx patterns, overall response rate to systemic therapy including chemotherapy, immunotherapy and MET inhibitors (METi), and Kaplan-Meier (KM) analyses of progression-free survival and overall survival (OS) across lines of therapy.

Results: This interim analysis included data from 131 pts (mutated [n = 70], amplified [n = 44], and concurrent mutated and amplified [n = 17]). Characteristics of mutated vs amplified vs concurrent mutation/amplification cancers are as follows: median age (yrs), 75 vs 64 vs 72; male, 47% vs 57% vs 59%; never smoker, 41% vs 7% vs 29%; squamous, 6% vs 2% vs 6%; median follow-up time from the first diagnosis of metastatic NSCLC (months), 12.1 vs 6.7 vs 13.4; stage IV at NSCLC diagnosis, 70% vs 68% vs 77%; brain metastases at metastatic NSCLC diagnosis, 20% vs 18% vs 47%; median number of systemic therapies, 2 vs 1 vs 3. METi were received by 37% vs 11% vs 59% pts, respectively. OS data are shown below (Table). Data on patterns of concurrent molecular alterations and additional tx specific efficacy data will be presented during the meeting.

Conclusions: *MET* exon 14 skipping mutations and high-level *MET* amplification define distinct NSCLC subgroups with poor prognosis. Tx with METi confers a survival benefit in both *MET* dysregulated groups. Further study of METi in these populations is warranted.

Conflict of interest: Ownership: Reinhard Buettner (co-founder and co-owner of Targos Molecular Pathology Inc, Kassel/Germany), Alex Mutebi (Novartis stockholder). Advisory Board: Juergen Wolf (Abbvie, AstraZeneca, BMS, Boehringer-Ingelheim, Chugai, Ignyta, Lilly, MSD, Novartis, Pfizer, Roche), Christina Baik (Astra Zeneca, Novartis), Rebecca S Heist (Tarveda), Joel W Neal (ARIAD/Takeda, AstraZeneca, Genentech/Roche, Eli Lilly), Aaron S Mansfield (Genentech, BMS), Mark M Awad (Bristol-Myers Squibb, Merck, Genentech, AstraZeneca, Pfizer, Nektar). Corporate-sponsored Research: Juergen Wolf (BMS, MSD, Novartis, Pfizer), Christina Baik (BluPrint, Novartis, Loxo, Pfizer, AstraZeneca, Spectrum, Celgene, Genentech/Roche), Rebecca S Heist (Genentech/Roche, Celgene, Mirati, Peregrine, Abbvie, Debiopharm, Millenium, Novartis, Daichii, Corvus, Exelixis), Joel W Neal (Genentech/Roche, Merck, Novartis, Boehringer Ingelheim, Exelixis, ARIAD/Takeda, Nektar Therapeutics), Aaron S Mansfield (Novartis), Keith L Davis (Novartis), Mark M Awad (Bristol-Myers Squibb). Other Substantive Relationships: Rebecca S Heist (Boehringer Ingelheim), Monica Giovannini (Novartis full time employee involved in clinical development of MET inhibitor), Alex Mutebi (Novartis employee).

Table (abstract 403 PB-066): KM Estimates of OS by Tx and Dysregulation Status

	All (n = 131)		Mutated (n = 70)		Amplified (n = 44)		Concurrently Mutated and Amplified (n = 17)	
From 1st diagnosis of metastatic NSCLC	Received METi (n = 41)	Not Received METi (n = 90)	Received METi (n = 26)	Not Received METi (n = 44)	Received METi (n = 5)	Not Received METi (n = 39)	Received METi (n = 10)	Not Received METi (n = 7)
n (%) of pts with event	25 (61)	73 (81)	13 (50)	30 (68)	5 (100)	37 (95)	7 (70)	6 (86)
Median OS, months (95% CI)	25.1 (17.8, 30.6)	7.9 (5.6, 9.9)	29.3 (12.5, 53.0)	11.9 (8.9, 18.7)	17.8 (7.2, 85.2)	6.1 (4.2, 7.9)	24.5 (9.2, 40.9)	7.4 (2.5, 10.7)

404 (PB-067) Poster

Ibrutinib in combination with sorafenib synergistically inhibits hepatocarcinogenesis by targeting EGFR signaling pathway

A. Noonan[1], C.H. Lin[1], K. Elkholy[1], N. Wani[2], D. Li[1], P. Hu[1], J.M. Barajas[1], L.B. Yu[3], X. Zhang[3], P. Maciej[3], S.T. Jacob[2], K. Ghoshal[1]. [1]*The Ohio State University, The James Comprehensive Cancer Center, Department of Pathology, Columbus, USA;* [2]*The Ohio State University, The James Comprehensive Cancer Center, Department of Cancer Biology and Genetics, Columbus, USA;* [3]*The Ohio State University, The James Comprehensive Cancer Center, Department of Biomedical Informatics, Columbus, USA*

Background: Hepatocellular carcinoma (HCC) is the second major cause of cancer related death worldwide because of limited therapeutic options. Our goal was to identify a novel combination therapy that can potentiate the action of sorafenib (SOR), currently the only FDA-approved first-line targeted therapy for advanced HCC.

Materials and Methods: Ibrutinib (IBT), approved for B cell malignancies, is an irreversible inhibitor of both TEC (BTK, ITK) and ERBB1-4 (EGFR, Her2) familiesof tyrosine kinases. SOR inhibits PDGFR and Raf family kinases. The efficacy of IBT and SOR combination therapy was evaluated in vitro and in vivo. HCC cell lines (HepG2, Hep3B, PLC/PRF/5, SNU-182, SNU-449, Huh-7, Hepa1-6 and HCCLM3 were used. NSG (NOD scid IL2R$\gamma^{-/-}$) mouse model with HCCLM3 xenografts and C56BL/6NCrlBR mouse orthotopic model with HCCLM3 and Hepa1-6 cells were used. Drug synergy was explicitly assessed for.

Results: IBT exhibits anti-HCC functions in vitro and ex vivo. More importantly, IBT–SOR co-treatment synergistically inhibited HCC cell proliferation and clonogenic survival by inducing apoptosis and suppressing tumor-sphere formation and expression of cancer stem cell markers. This combination therapy significantly suppressed the growth of highly aggressive HCCLM3 subcutaneous xenografts in immunodeficient mice, and increased survival of these mice bearing orthotopic HCCLM3 xenografts andimmuno-competent mice bearing orthotopic mouse Hepa1-6 tumors. Inactivation of EGFR and its downstream Akt and ERK signaling mediates anti-HCC effect of IBT. RNA-seq analysis showed the expression of several genes involved in cell proliferation, migration, anti-apoptosis, and stemness was down-regulated whereas genes promoting differentiation were upregulated by these tyrosine kinase inhibitors.

Conclusion: Our results in preclinical models demonstrate that the combination of IBT and SOR suppressed tumor growth in HCC cell lines and mouse models, and particularly in SOR-resistant models with activated EGFR/Akt/ERK signaling. A phase I/II clinical trial of ibrutinib-sorafenib combination in patients with HCC is planned to assess this hypothesis.

No conflict of interest

405 (PB-068) Poster

Targeting the TFE3 pathway in translocation renal cell carcinoma with the antibody-drug conjugate Glembatumumab vedotin

M. Lang[1], D. Wei[1], B.K. Gibbs[1], L. Ohanjanian[1], C.J. Ricketts[1], C. Sourbier[1], L.S. Schmidt[1,2], W.M. Linehan[1]. *[1]Urologic Oncology Branch, National Cancer Institute/NIH, Bethesda, USA; [2]Basic Science Program, Leidos Biomedical Research Inc., FNL, Frederick, USA*

Background: Renal cell carcinoma (RCC) consists of distinct subtypes with characteristic histologies, genetic mutations and clinical behaviors. The RCC subtype harboring an Xp11.2 chromosomal rearrangement (tRCC) is characterized by translocations of the TFE3 gene, a transcription factor that regulates cell growth and differentiation. This RCC subtype is mainly seen in young patients and can show aggressive behavior. The chromosomal rearrangements characterizing tRCC result in a gene-fusion product that leads to increased TFE3 expression, nuclear localization and transcriptional activity. Key signaling pathways in TFE3-fusion RCC are unknown and effective drug therapies are yet to be identified. Glycoprotein NMB (GPNMB) is a transcriptional target of TFE3 and a fully human antibody-drug conjugate (ADC) has been developed against GPNMB. This ADC, Glembatumumab vedotin (CDX-011), has shown pharmacologic effect against breast cancer and melanoma with high expression levels of GPNMB.

Material and Methods: GPNMB expression was validated at the RNA and protein level in tRCC tumor tissues and normal kidney tissues, tRCC-derived cell lines and cells with inducible expression of TFE3-fusion genes. Flow cytometry was used to show GPNMB localization to the extracellular membrane and a cytotoxicity assay was used to prove the targeted action of the ADC. Susceptibility of tRCC cells to CDX-011 was performed *in vitro* in 2D and 3D spheroid culture models and *in vivo* in a xenograft model.

Results: Previously, our lab has shown that TFE3 can directly regulate *GPNMB* expression and data provided by the TCGA project confirmed that kidney cancer samples with TFE3 translocations have high expression of *GPNMB*. Here we confirm that kidney tumors and cell lines carrying TFE3-fusions express high levels of *GPNMB*, while other kidney cancer cell lines are negative for the protein. We show that GPNMB localizes to the outer cellular membrane and is recognized by the human antibody used to produce the ADC. tRCC cells are sensitive to Monomethyl auristatin E, the tubulin inhibitor conjugated with the anti-GPNMB antibody. Unlike the non-conjugated antibody, CDX-011 is cytotoxic to GPNMB-expressing cells. The tRCC cell lines UOK120 and UOK124 show significant decrease of viability after treatment with 10 ug/ml CDX-011 in 2D and 3D spheroid culture. Tumor growth is significantly inhibited *in vivo* upon CDX-011 treatment of mice carrying UOK124 xenografts.

Conclusions: No drug has previously demonstrated convincing efficacy in *in vivo* models for tRCC. Here we show that TFE3-fusion kidney cancers express high levels of GPNMB and can therefore be specifically and directly targeted with the antibody-drug conjugate Glembatumumab vedotin. Our data therefore provide a promising therapeutic option for patients with tRCC with a drug that is currently undergoing stage 2 clinical trials.

No conflict of interest

406 (PB-069) Poster

Identifying predictive markers and novel combinations for TORC1/2 inhibition in ovarian and endometrial cancer

N. Grosenbacher[1], S. Crafton[2], J. David[1], C. Stets[1], B. Demoret[1], J. Chen[1], J. Hays[3]. *[1]The Ohio State University Comprehensive Cancer Center, Medical Oncology, Columbus, USA; [2]The Ohio State University Comprehensive Cancer Center, Gynecologic Oncology, Columbus, USA; [3]The Ohio State University Comprehensive Cancer Center, Medical and Gynecologic Oncology, Columbus Ohio, USA*

Background: In an effort to overcome the poor efficacy of targeted agents as monotherapy in endometrial and ovarian cancers, interest has turned to combination therapy. Significant fractions of endometrial and ovarian cancers have de-regulation of the PI3K/AKT/MTOR pathway due to inactivating mutations in PTEN or activating mutations in PIK3CA, AKT or MTOR. We examined a panel of endometrial and ovarian cell lines to determine response to TAK228, a dual TORC1/2 inhibitor and utilized mutational analysis and reverse phase protein arrays (RPPA) to identify possible mechanisms of resistance and synergy.

Material and Methods: We analyzed 5 endometrial (AN3CA, ECC, HEC1A, HEC1B, RL952) and 6 ovarian cancer cell lines (OV90, SKOV3, OVCAR3, OVCAR8, HEYA8, CAOV3) for sensitivity to AKT (MK2206) and MTOR inhibition (TAK228) utilizing XTT, scratch, and attachment independent growth assays. Co-operativity assays were performed according to the method of Chou-Talalay. Cell lysates before and after treatment were analyzed for protein expression of >300 proteins utilizing RPPAs and published mutational data was collected for each cell line.

Results: All cell lines showed sensitivity to both agents, with TAK228 being significantly more toxic in XTT and scratch assays than the AKT inhibitor MK2206 (mean IC50 20.2 nM vs 12 μM, respectively). TAK228 was also significantly more toxic than the TORC1 specific inhibitor rapamycin (mean IC50 32 nM vs 21 μM, respectively). Analysis of RPPA proteomics data identified VEGFR and SMAD1 pretreatment protein levels as being strongly correlated with sensitivity to TAK228. Also, a panel of growth stimulatory factors were associated with sensitivity in both pre-treatment protein samples and when looking at changes before and after treatment. Mutational analysis was not predictive of response. Modulation of the VEGF/VEGFR axis with antibody and small molecule inhibitors demonstrated synergy across multiple cell lines. Cell cycle modulation with CHK1/2 inhibitors also revealed marked synergy across multiple cell lines (mean CI <0.5).

Conclusions: Single agent targeted therapy in gynecologic malignancies has proven largely ineffective with the exception of PARP inhibition in select populations. Here we identify novel combinations with targeted agents in ovarian and endometrial cancer that can be quickly translated to clinical testing. A clinical trial with bevacizumab and TAK228 in recurrent ovarian and endometrial cancer is currently underway.

No conflict of interest

407 (PB-070) Poster

Discovery of a new small molecule with potent and selective in vitro and in vivo therapeutic activity in human lymphomas

E. Gaudio[1], F. Spriano[1], C. Tarantelli[1], M. Guala[2], E. Riveiro[3], G. Golino[4], A. Lupia[4], G. Costa[4], R. Rocca[4], L. Cascione[1], S. Alcaro[4], F. Paduano[4], F. Trapasso[4], E. Zucca[5], A. Stathis[5], N. Pazzi[2], F. Cavalli[5], F. Bertoni[1]. *[1]Università della Svizzera italiana, Institute of Oncology Research, Lymphoma & Genomics, Bellinzona, Switzerland; [2]Chimete, Research & Development, Tortona, Italy; [3]Early Drug Development Group, Research & Development, Paris, France; [4]University "Magna Græcia" of Catanzaro, Molecular Oncology, Catanzaro, Italy; [5]Ente Cantonale Ospedaliero, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland*

Introduction: We present a new small molecule, EG-011, with strong *in vitro* and *in vivo* anti-cancer activity in lymphoma models.

Materials and Methods: Lymphoma cell lines, including both B- and T-cells lymphomas, and solid tumor cell lines were exposed to a large range of concentrations of EG-011 as single agent for 72 h, followed by MTT proliferation assay. Apoptosis assay was performed in primary cells collected from two healthy donors by measuring the annexin V by FACS. Xenografts were established s.c. into the flanks of NOD-SCID mice; treatment with EG-011 (200 mg/kg, i.p. 5 days per week) started with already established tumors. Combinations were assessed in five cell lines (OCI-LY-1, OCI-LY-8, REC1, MINO, TMD8), exposed for 72 h to increasing doses of EG-011 alone and with increasing doses of FDA approved drugs. Synergy was assessed with Chou-Talalay combination index (CI): synergism (<0.9), additive (0.9-1.1), no benefit (>1.1).

Results: EG-011 presented a median IC50 of 2.25 mcM across 62 lymphoma cell lines (95% C.I. 1–5 mcM). A higher activity was observed in a group of 21 cell lines that had a median IC50 of 250 nM. This group comprised cell lines derived from germinal center B cell type diffuse large B cell lymphomas (GCB-DLBCL) (sensitive n = 11/21, resistant n = 9/41, P < 0.05), mantle cell lymphomas (sensitive n = 4/21, resistant n = 6/41, P n.s.), marginal zone lymphomas (sensitive n = 3/21, resistant n = 2/41, P n.s.), ABC-DLBCL, ALCL and canine DLBCL (one each). EG-011 did not show any anti-proliferative activity in a panel of 23 solid tumor cell lines (IC50s >10 mcM). Dose-depended increase of the cells in the sub-G0 phase (20–55%) was observed in OCI-LY-19 and REC1 cell lines after EG-011 exposure (at 500 nM and 2 mcM) for 72 h. Conversely, any cytotoxic effect in PBMCs from two healthy donors was observed after treatment at 1 and 10 mcM for 24 h and 48 h. In an *in vivo* xenograft experiment with the mantle cell lymphoma REC-1 cell line, EG-011 delayed tumor growth (P < 0.05) and tumor weight. At the end of the experiment EG-011-treated tumors were 2.2-fold smaller than controls (P < 0.001). When evaluated in combinations, EG-011 was synergistic with the anti-CD20 antibody rituximab in 5/5 cell lines. The combination with the Bcl2 inhibitor venetoclax was synergistic in 4/4 cell lines. The addition of the BTK inhibitor ibrutinib led to synergism in 2/2 ibrutinib sensitive cell lines, while the combination with the immunomodulant lenalidomide was beneficial in 3/3 lenalidomide sensitive cell lines (synergism in two and additivity in one). EG-011 and bendamustine were synergistic in 5/5 cell lines.

Conclusion: The selective anti-lymphoma activity, in both *in vitro* and *in vivo* models, and the observed *in vitro* synergisms with FDA approved targeted agents make EG-011 a novel intriguing drug candidate deserving further preclinical studies.

No conflict of interest

408 (PB-071) Poster

Epidermal growth factor receptor (EGFR) and Src as therapeutic targets in triple negative breast cancer (TNBC)

A. Canonici[1], A. Browne[1], M. Ibrahim[1], K. Fanning[1], S. Roche[1], N. Conlon[1], F. O'neill[1], J. Meiller[1], M. Cemona[2], C. Morgan[2], B. Hennessy[2], F. Solca[3], J. Crown[4], N. O'donovan[1], A. Eustace[1]. [1]*Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland;* [2]*Royal College of Surgeons in Ireland, Molecular Medicine, Dublin, Ireland;* [3]*Boehringer Ingelheim RCV GmbH & Co KG, Boehringer Ingelheim RCV GmbH Co KG, Vienna, Austria;* [4]*St. Vincents Hospital, Medical Oncology, Dublin, Ireland*

Introduction: TNBC comprises a group of heterogenous diseases, defined by the absence of the 3 principal breast cancer (BC) biomarkers; the oestrogen receptor, progesterone receptor and HER2. This lack of druggable targets renders TNBC difficult to treat, and the only established therapeutic option is chemotherapy. While the response rate to chemotherapy in early stage TNBC is high, the significant risk of relapse alongside the development of chemo-resistant tumours affords TNBC with its poor prognosis. There is therefore an urgent need for targeted therapeutic strategies in TNBC. EGFR is frequently overexpressed in TNBC and represents a viable therapeutic option. Unfortunately, anti-EGFR therapies in BC clinical trials have been disappointing, often due to the existence of compensatory pathways allowing continued cell survival.

Materials and Methods: In this study therefore, the effect of the protein tyrosine kinase inhibitor, afatinib, was tested alone and in combination with other promising targets (FGFR, Src, PI3K, mTOR, MET) in a panel of TNBC cell lines. The effect of treatment on cellular proliferation was assessed by acid phosphatase assay. Proteomic profiling by RPPA analysis was used to demonstrate the anti-tumour effect on signalling pathways, while PI uptake assessed the effects on cell cycle via flow cytometry. Finally, xenograft studies were utilised to examine the consequence of these treatments *in vivo*.

Results: All 14 cell lines responded to afatinib with IC_{50} values ranging from 0.008–5.0 μM. 3 were considered sensitive based on peak plasma concentration (80 nM; HDQP1, CAL851 and MDA-MB-468). We selected representative cell lines demonstrating afatinib sensitivity and resistance to investigate the added benefit of inhibiting the 5 additional molecular targets. In both cell lines, the addition of afatinib to all 5 inhibitors significantly decreased cellular proliferation compared to targeted treatment alone. However, the benefit of combined treatment over afatinib alone was more evident in the resistant cell line. Combining afatinib and the Src inhibitor, dasatinib had the most effective growth inhibition in both cell lines (>70%) and this effect was further investigated. In the panel of 14 TNBC cell lines, the 3 cell lines with greatest synergy between afatinib and dasatinib (A&D) were taken forward for RPPA analysis (BT20, HDQP1 & HCC1937). RPPA identified combined A&D treatment resulted in significant decreases in EGFR, ERK, Akt and Src signalling. A&D treatment induced significant increases in G1 cell cycle arrest which was further reflected in the RPPA which demonstrated a significant decrease in cyclin D1 and increase in p27. Finally, the combination of A&D demonstrated the greatest reduction in tumour volume *in vivo*.

Conclusion: We identified that the combination of A&D in the treatment of TNBC displays potent anti-cancer activity.

Conflict of interest: Corporate-sponsored Research: Alexandra Canonici, John Crown and Norma O'Donovan have received research funding from Boehringer Ingelheim (BI). Flavio Solca is a BI employee.

409 (PB-072) Poster

Conformation-selective inhibitors stabilize SRC in the globally closed conformation and are active in pre-clinical models of IDH mutant intrahepatic cholangiocarcinoma

J. Casaletto[1], J. Wilbur[2], B. Toure[3], B. Williams[1], M. Zubrowski[1], N. Gerami-Moayed[1], C. Klammt[2], L. Dipietro[3], R. Kipp[2], P. Fortin[2], J. Watters[1]. [1]*Relay Therapeutics, Biology, Cambridge, USA;* [2]*Relay Therapeutics, Biochemistry, Biophysics & Structural Biology, Cambridge, USA;* [3]*Relay Therapeutics, Chemistry, Cambridge, USA*

Background: SRC was recently identified as driver in IDH mutant Intrahepatic Cholangiocarcinoma (ICC). While SRC transitions between globally “open” (active) and globally “closed” (inactive) conformations, the structural mechanisms underlying conformational transitions are not well understood, and approved inhibitors stabilize SRC in the globally open conformation. We sought to develop inhibitors that stabilize SRC in the globally closed conformation, elucidate allosteric networks underlying conformational dynamics, and validate the SRC-dependency of IDH mutant ICC.

Materials and Methods: Small molecule inhibitors of SRC were identified through biochemical, biophysical, and virtual screening. Protein constructs including the SH2, SH3, and kinase domains of SRC were produced and co-crystallized with inhibitors. Compound-bound structures were determined using X-ray crystallography, and binding kinetics were evaluated using Surface Plasmon Resonance. RBE and SNU-1079 cell lines, derived from patients with IDH mutant ICC, were used for *in vitro* studies. Phosphorylation state of SRC (Y419) and S6 (S235/236) were used to monitor pathway inhibition, and Cell Titer-Glo was used to assess cellular viability. CRISPR/Cas9 was used to introduce the SRC gatekeeper mutation T341I into cells.

Results: We developed small molecule inhibitors that stabilize SRC in globally open or closed conformations. In the closed conformation, the SH2 and SH3 domains dock against the catalytic domain and decrease catalytic activity by a long-range allosteric mechanism. In the open conformation, the SH2 and SH3 domains release from the catalytic domain, the aC-helix moves in and a series of hydrophobic residues, referred to as the R-spine, align. These changes bring the catalytic machinery in close proximity, forming the fully activated state. A subset of molecules uncouple the global state from the local rearrangement of the catalytic machinery, allowing us to confirm Trp 263 as a central hub of allosteric information transfer. Subtle shifts in the R-spine residue Leu 328 and residue Tyr 385, outside of the R-spine, were found to have key interactions balancing the opening and closing mechanism. Treatment of IDH mutant ICC cells with molecules stabilizing the closed conformation inhibited pathway signaling and proliferation with IC_{50} < 500 nM. Introduction of the SRC (T341I) gatekeeper mutation rescued cells from the effects of tool compounds, confirming SRC as a critical target in IDH mutant ICC.

Conclusions: We developed small molecule inhibitors that stabilize SRC in the closed conformation, elucidated underlying allosteric networks, and confirmed the SRC dependence of IDH mutant ICC cells. Further evaluation of this mechanism for the treatment of patients with IDH mutant ICC is warranted.

Conflict of interest: Corporate-sponsored Research: All authors are full-time employees and shareholders of Relay Therapeutics.

410 (PB-073) Poster

MET mutant allele frequency (MAF) is correlated with glesatinib anti-tumor activity in patients with advanced non-small cell lung cancer (NSCLC) harboring MET alterations

H. Der-Torossian[1], G. Shapiro[2], P. Janne[3], M. Awad[3], K. Reckamp[4], W. Miller[5], D. Hong[6], M. Hussein[7], I. Percent[8], L. Bazenhova[9], A. Dowlati[10], K. Dong-Wan[11], B.C. Cho[12], K. Park[13], V. Tassell[14], R. Chao[1], D. Faltaos[15], J. Christensen[16]. [1]*Mirati Therapeutics, Medical, San Diego, USA;* [2]*Dana Farber Cancer Institute, Early Drug Development Center, Boston, USA;* [3]*Dana Farber Cancer Institute, Medical Oncology, Boston, USA;* [4]*City of Hope, Clinical Research Operations, Duarte, USA;* [5]*Jewish General Hospital, Department of Medicine, Department of Medicine, Canada;* [6]*MD Anderson, Department of Investigational Cancer Therapeutics – Division of*

Cancer Medicine, Houston, USA; [7]Florida Cancer Specialist, Medical Oncology, Saint Petersburg, USA; [8]Florida Cancer Specialists, Medical Oncology, Port Charlotte, USA; [9]University of California San Diego Health, Medical Oncology, San Diego, USA; [10]University Hospitals Cleveland Medical Center, Medical Oncology, Cleveland, USA; [11]Seoul National University, Department of Internal Medicine, Seoul, South Korea; [12]Severance Hospital, Yonsei University Health System, Medical Oncology, Seoul, South Korea; [13]Samsung Medical Center, Innovative Cancer Medicine Institute, Seoul, South Korea; [14]Mirati Therapeutics, Clinical Research, San Diego, USA; [15]Mirati Therapeutics, Pharmacology, San Diego, USA; [16]Mirati Therapeutics, Research, San Diego, USA

Background: Aberrant activation of MET as a result of METex14 mutations is an oncogenic mechanism that drives cancer development and progression in approximately 3% of NSCLC. In addition, *MET* amplification (METamp) has been reported as an adverse prognostic factor in patients with NSCLC. Glesatinib (MGCD265) is a potent, orally available, spectrum-selective MET inhibitor that has been studied in patients exhibiting METex14 mutations and/or METamp across Phase 1 and 2 studies. Here we evaluated whether high MAF of METex14 predicts tumor response to glesatinib.

Materials and Methods: NSCLC cancer patients harboring genetic alterations in *MET* were evaluated from Study 265–101, a Phase 1 study of glesatinib in patients with advanced solid tumors, and Study 265–109, a Phase 2 study of glesatinib in patients with locally-advanced or metastatic NSCLC with activating MET alterations. Qualifying mutations of METex14 were identified by RT-PCR (tissue) or NGS (tissue or blood). MAF was categorized as high/intermediate/low using cutoffs of dCT from RT-PCR ($\leq$−2/−2 to <1/$\geq$1) or MAF from tissue NGS ($\geq$0.6/0.2 to <0.6/$\leq$0.2). METex14 detected by blood NGS was categorized as high. Tumor activity was assessed in accordance with RECIST. Descriptive statistics were used.

Results: 43 evaluable patients with NSCLC harboring qualifying METex14 mutations were treated with glesatinib (32 in Phase 2/11 in Phase 1). Patients (26 women/17 men; median age 70 years – range 46–81; ECOG status 0–1/2 in 41/2) had advanced (40 metastatic/3 locally–advanced) NSCLC (37 adenocarcinoma/4 squamous/1 sarcomatoid/1 other) with a median of 2 therapies prior to receiving glesatinib (range 1–4). The overall objective response rate in this patient population was 33% (21% confirmed) and a clinical benefit rate of 86%. Of the 43 patients with METex14, 51/37/12% had high/intermediate/low MAF. With regards to anti-tumor activity, objective response was achieved in 41/25/14% (32/13/0% confirmed) of METex14 patients with high/intermediate/low MAF, respectively; and with mean best percent changes from baseline in sum of target lesions of –25/–19/–8%, respectively.

Conclusions: MET MAF may be a predictive factor for MET inhibitor anti-tumor activity in patients with advanced NSCLC. Further evaluation to optimize patient selection for treatment with MET inhibitors is warranted.

Conflict of interest: Ownership: Stocks/employment – Vanessa Tassell, Demiana Faltaos, James Christensen, Hirak Der-Torossian, Richard Chao. Advisory Board: Pasi Janne. Corporate-sponsored Research: Funding to all institutions for authors listed for participation in these clinical trials (Non-Mirati authors).

411 (PB-074) Poster

In vivo anti-tumor efficacy with a dual degrader of SMARCA2 and SMARCA4

G. Daginakatte[1], S. Sasmal[2], L.K. Satyam[1], M.K. Pothuganti[1], A. Ettam[2], S. Nunna[2], M.A. Shareef[3], S. Gopinath[3], K. Aithal[4], S. Mukherjee[2], M. Ramachandra[5], S. Samajdar[2]. [1]*Aurigene Discovery Technologies Limited, Molecular and Cell Biology, Bangalore, India;* [2]*Aurigene Discovery Technologies Limited, Medicinal Chemistry, Bangalore, India;* [3]*Aurigene Discovery Technologies Limited, Drug Metabolism, Bangalore, India;* [4]*Aurigene Discovery Technologies Limited, Pharmacology, Bangalore, India;* [5]*Aurigene Discovery Technologies Limited, Pre Clinical Biology, Bangalore, India*

SMARCA2/BRM and SMARCA4/BRG1 are the mutually exclusive DNA-dependent ATPases within the SWI/SNF complexes, which function in mobilizing nucleosomes to regulate transcription, DNA replication and repair, and higher-order chromosome dynamics. SMARCA4 is known to be mutated in a number of cancers which generally lack targetable oncogenes (such as mutant EGFR or ALK translocations). Based on the observation from genetic silencing studies that established a requirement of SMARCA2 for survival of tumor cells lacking SMARCA4, SMARCA2 has been proposed as a promising therapeutic target for the treatment SMARCA4-mutant cancers. SMARCA4 is highly expressed in certain tumor types without mutation but with overexpression resulting in increased proliferation and survival. SMARCA4 knockdown in these tumors lead to inhibition of proliferation, while increasing sensitivity to known chemotherapeutic agents, supporting the validity of targeting SMARCA4. Although genetic silencing of SMARCA2 leads to potent anti-proliferative activity in SMARCA4-deficient cancer cell lines, pharmacological studies with a probe capable of binding to SMARCA2 and SMARCA4 bromodomain have failed to show such an anti-proliferative phenotype. These findings triggered us to evaluate chemical degrader as an alternate approach to target SMARCA2/4 altered cancers.

Design and SAR-based optimization of bifunctional molecules with binding moieties for SMARCA2/4 and E3 ligase to induce proteasome-mediated degradation resulted in compounds that potently degraded both SMARCA2 and SMARCA4. Selective binding in biochemical assays and selective degradation of SMARCA2/4 over other bromodomain containing proteins such as BRD4 and CBP/p300 in western blot analysis were observed for these compounds.

Functional analysis of these compounds in a panel of cell lines indicated a potent anti-proliferative activity in selected cell lines that was not strictly dependent upon SMARCA4 status. Additionally, these compounds displayed acceptable drug-like properties including solubility, metabolic stability and pharmacokinetics in mice. In a syngeneic mouse model of lymphoma, thrice a week intravenous dosing of a SMARCA2/4 dual degrader showed dose-dependent tumor growth inhibition at well tolerated doses. Observed anti-tumor efficacy correlated with the target degradation in the tumor supporting the potential to further develop them for cancer therapy.

No conflict of interest

412 (PB-075) Poster

Spatial tumor heterogeneity drives mixed and site-specific progression patterns revealed by standardized radiological evaluations of patients treated on first-in-human trials

C. Pinto[1], C. Walmsley[1], D. Juric[1], M. Broudo[1], A. Muzikansky[1], D. Fitzgeral[1], T. Hong[1], J. Wolfgang[1]. [1]*Massachusetts General Hospital, Cancer Center, Boston, USA*

Background: Temporal and spatial heterogeneity of human tumors is a major impediment for development of effective targeted therapies. Intrinsic and acquired resistance associated with complex and continuously evolving genomic landscapes of human tumors results in lack of durable treatment responses, as well as mixed, discordant and disease site specific progression patterns.

Methods: We have performed standardized radiological assessments using Response Evaluation Criteria in Solid Tumors (RECIST) in 569 consecutive study participants across 53 treatment trials conducted in the Termeer Center for Targeted Therapies from October 1, 2010 to September 30, 2015. All imaging and response evaluation data were recorded in a centralized database linked to patient's treatment history, genomic profiles and clinical outcomes. Tumor response was determined at the level of each study participant as per RECIST, but we also assessed the response of each tumor lesion, recording all instances of mono-lesional progression (disease progression in a single lesion with all other lesions remaining stable or responding to therapy) or site-specific oligo-lesional progression (progression in one or more lesions in a single organ or disease site, with all other lesions outside that site remaining stable or responding to therapy). We also identified all instances of mixed responses to treatment, defined as presence of at least one responding lesion, with progression of all other lesions.

Results: Out of 569 study participants, we excluded 142 participants without at least two radiographic assessments at least 30 days apart and those who discontinued treatment due to adverse events. Among 427 eligible cases, 48.71% (208/427) experienced site-specific oligo-lesional progression. This occurred most often in the liver (62/208, 29.81%) followed by the lung (59/208, 28.37%), nodal (39/208, 18.75%), soft tissue (13/208, 6.25%), bone (12/208, 5.77%), and brain (9/208, 4.33%). Mono-lesional progression occurred in 24.12% (103/427) of cases, most often in the nodes (30/103, 29.13%) followed by the lung (23/103, 22.33%), liver (15/103, 14.56%), bone (10/103, 9.71%), soft tissue (9/103, 8.74%), and brain (6/103, 5.83%). 10.07% (43/427) of cases experienced mixed response, with at least one responding lesion, while simultaneously undergoing disease progression in other lesions.

Conclusion: Systematic analysis of disease progression patterns in patients treated with investigational agents reveals substantial proportion of treatment failures in a single lesion or disease site (potentially amenable to localized therapy). This finding may be due to lesion-specific genomic alterations or site specific factors in the tumor microenvironment, requiring further genomic and proteomic analyses currently underway in all cases with available biopsies or autopsy samples.

No conflict of interest

413 (PB-076) Poster

Evaluation of the pharmacology, pharmacodynamic activity and immune response modulation of the AXL kinase inhibitor TP-0903

S. Anthony[1], J. Sarantopoulos[2], M.S. Beg[3], F. Tsai[4], J. Taverna[2], S. Smith[1], H. Beever[1], S. Weitman[1], L. Mouritsen[5], W. Mark[5], Y. Matsumura[5], E. Tyagi[5], C. Whatcott[5], D. Bearss[5], S. Warner[5]. *[1]Tolero Pharmaceuticals, Inc., Clinical Development, Lehi, USA; [2]UT Health Science Center San Antonio, Mays Cancer Center, San Antonio, USA; [3]U of Texas SW Medical Center, Internal Medicine, Dallas, USA; [4]HonorHealth Research Institute, Virginia G. Piper Cancer Care Network, Scottsdale, USA; [5]Tolero Pharmaceuticals, Inc., Drug Discovery and Development, Lehi, USA*

Background: The receptor tyrosine kinase AXL, is overexpressed in many human cancers and its expression is associated with a poor prognostic outcome for patients. AXL is a member of the TAM family of kinases (AXL, MER, TYRO3), which are involved in multiple aspects of tumorigenesis. Increased expression of AXL is associated with increased oncogenic transformation, cell survival, proliferation, migration, angiogenesis, cellular adhesion and avoidance of the immune response. We discovered and are developing a small molecule AXL kinase inhibitor, TP-0903, and have shown the effectiveness of TP-0903 in cell-based and animal models of human cancers. TP-0903 has low nanomolar (IC50 = 12 nM) activity against the AXL kinase in biochemical assays. In further biochemical evaluation, TP-0903 is shown to inhibit the entire TAM family of kinases at nM potencies. Recent studies have indicated that AXL and other TAM family kinases are critical regulators of the innate immune response, and their activation may mediate immune-suppressive activity seen in many cancers.

Material and Methods: To explore the immune activating potential of TP-0903 we tested TP-0903 in several syngeneic mouse models of solid tumors. Markers of infiltrating immune cells in these models were assessed on both formalin-fixed and fresh tissues using standard immunohistochemical and real-time PCR techniques. Effects of TP-0903 on regulatory T-cells was also assessed in vitro using standard viability assays and multiplexed cytokine arrays.

Results: Inhibition of TAM kinase activity by TP-0903 was shown to enhance host immunity in these tumor models. The immune response affected by TP-0903 is associated with dose-related increases in the percent of tumor-infiltrating effector CD4+ and CD8+ T cells and enhanced therapy responses to immune checkpoint inhibitors. In addition, TP-0903 treatment results in an increase in activated dendritic cells and a reduction in immune-suppressive infiltrating neutrophils and regulatory T-cells. We are currently analyzing the effect of TP-0903 on markers of immune suppression in a multi-center, open-label, Phase 1/1b first-in-human study of TP–0903 which is underway in advanced solid tumors. Samples taken from patients are being analyzed for changes in immune cell presence and activity before and after treatment with TP-0903.

Conclusions: Taken together, these preclinical data support the potential therapeutic activity of TP-0903 as an immune modulating agent capable of enhancing tumor immune response in cancer patients as a single agent and when combined with therapies targeting immune checkpoints. Expansion cohorts from the current clinical study will further evaluate the potential of TP-0903 to enhance the activity of immune checkpoint inhibitors. Clinical trial information: NCT02729298.

Conflict of interest: Other Substantive Relationships: Employment.

414 (PB-077) Poster

Phase I trial of enzalutamide in combination with gemcitabine and nab-paclitaxel in the management of advanced pancreatic cancer

A. Kommalapati[1], R. Kim[2], T. Sri Harsha[3], B. Bertels[2], G. Wapinsky[2], N. Burke[2], A. Neuger[2], G. Springett[2], K. Almhanna[2], A. Mahipal[4]. *[1]University of South Carolina School of Medicine, Internal Medicine, Columbia, SC, USA; [2]Moffitt Cancer Center, Hematology/Oncology, Tampa, USA; [3]University of South Carolina Cancer Center, Internal Medicine, Columbia, USA; [4]Mayo Clinic, Hematology/Oncology, Rochester, MN, USA*

Background: Androgens were shown to play a key role in the growth and progression of pancreatic cancer. In this Phase I trial involving metastatic pancreatic cancer patients, we evaluated the safety and tolerability of the combination of enzalutamide, a novel androgen receptor (AR) antagonist with gemcitabine and nab-paclitaxel as a first-line treatment.

Methods: We used the standard 3+3 dose escalation design with cohort expansion to evaluate 2 dose levels of enzalutamide: 80 mg and 160 mg/day orally. In the expansion phase, AR+ was a pre-requisite criterion. Gemcitabine (1000 mg/m^2) and nab-paclitaxel (125 mg/m^2) was administered IV on days 1, 8 and 15 of a 28-day cycle. The dose-limiting toxicity (DLT) period was 28-days or until the beginning of the second cycle for the phase Ia part. In the phase 1 b portion, only patients with tumor-positive for AR were included. We evaluated the full pharmacokinetic (PK) profile for nab-paclitaxel after the initial dose through 48 hours post-dose while enzalutamide was tested on day 1 at 1 and 2 hours post-dose along with levels every 7 days to follow achievement of a steady state by day 29 (Cycle 2 Day 1).

Results: We enrolled 25 patients (16 males and 9 females) with metastatic pancreatic cancer, 12 patients at the first dose level and 13 patients at the second dose level. The median age was 68 years (32–84 years). No DLTs were observed. Grade 3/4 treatment related adverse events included neutropenia (44%), anemia (40%), leukopenia (24%), nausea and vomiting (20%), diarrhea (16%), infections (12%), thrombocytopenia (8%), thrombo-embolic event (8%), hypertension (8%), hypokalemia (8%), hyponatremia (8%), ALT elevation (8%), pneumonitis (4%), skin infection (4%) and hypoxia (5%). Median overall survival and progression-free survival was 9.73 [95%CI: 9.73–13.5] and 7.53 (95%CI: 6.05–12.8) months, respectively. PK analysis suggests that the combination therapy does not impact the kinetics of either drug evaluated. Enzalutamide reached steady-state levels between day 22 and 29 and the mean half-life of nab-paclitaxel was 19.6 ± 4.7 hours. All other PK parameters estimates are similar to historical data.

Conclusions: Enzalutamide 160 mg daily in combination with gemcitabine and nab-paclitaxel can be safely administered with no unexpected toxicities. We also noticed preliminary signals of efficacy with this combination. We continue to enroll patients with AR+ tumors in phase 1b part.

No conflict of interest

415 (PB-078) Poster

Assessing of Combretastatin A-4 Phosphate Activity for H1299 Lung Cancer

L. Liu[1], D. Yang[1], J. Chen[1], J. Campbell[1], S. Zhong[1], L. Zhang[2]. *[1]The University of Texas Southwestern Medical Center, Radiology, Dallas, USA; [2]The University of Texas at Dallas, Biological Sciences, Richardson, USA*

Background: Combretastatin A-4 phosphate (CA4P) (Millipore Sigma) is a well-characterized vascular disrupting agent that inhibits microtubule polymerization, which selectively induces the reduction in tumor blood flow, causing ischemia and cell death. CA4P is a water-soluble prodrug of the cis-stilbene CA4 originally isolated from the tree Combretum caffrum. Herein, we apply multimodality imaging to investigate the effect of CA4P on tumor oxygenation in human lung H1299-luc xenograft tumor model.

Material and Methods: 1 million H1299-luc cells were implanted subcutaneously in the right flank of female and male 8 week-old nude mice (n = 6). After 3–4 weeks, when the tumor size reaches 8–10 mm in diameter, at baseline, 1, 3, and 24 h after control saline or CA4P administration, bioluminescence imaging (BLI), power Doppler ultrasound, Multispectral optoacoustic tomography (MSOT) and oxygen-sensitive MRI were performed on separate tumor cohorts (n = 6 respectively). Specifically, MSOT analysis was performed with an MSOT InVision 256-TF device. Percentage of hemoglobin saturation (%SO2) was calculated. One day after MSOT, one mouse bearing tumor was selected and administered with pimonidazole intravenously to evaluate hypoxia. Two hours later Hoechst dye was administered and immediately sacrificed. IHC examined hypoxia (pimonidazole), vascular density (CD31), perfused vessels (Hoechst), together with hypoxia markers HIF-1, Glut1, and CA9.

Results: Three hours after 120 mg/kg CA4P administration, BLI showed very clearly reduced blood flow (indicating vascular shutdown) by 85% at 3 h, which partially recovered at 24 h. Power Doppler ultrasound failed to show blood flow due to lack of perfusion. Control tumors showed no significant change in any technique. MSOT showed that the administration of CA4P caused a significant reduction in vascular oxygen saturation at 1 and 3 hours after treatment. In reverse MRI response to oxygen breathing (R_2* increased instead of decreased) was observed at 3 h after CA4P administration, which returned to normal response (R_2* decreased) at 24 h. Baseline MRI T_2 maps revealed intra- and inter-tumoral heterogeneity, which accounts for the different patterns in tumor response to oxygen breathing at baseline. At 24 h after CA4P administration, new regions of necrosis (high T_2) occurred in the tumor center. No apparent necrosis development was observed for the control group.

Conclusions: Multimodality imaging characterized the effects of CA4P administration in a stepwise manner: disruption of the vasculature (3 h), reduced oxygenation (3 h), and cell death (24 h). This result illuminated the molecular events leading to tumor hypoxia and variations within one tumor and among different tumors.

No conflict of interest

417 (PB-080) Poster

Target expression/efficacy relationship of XMT-1522, a HER2-targeting Antibody Drug Conjugate (ADC), in an unselected series of Non-small Cell Lung Cancer (NSCLC) primary human carcinoma xenografts

R. Mosher[1], C. Vogel[2], P. Shaw[1], D. Zielinski[3], C. Boesler[3], A. Muhleisen[4], D. Frances[4], C. Schipper[4], M. Cyr[5], D. Jarlenski[5], B. Jasani[6], D. Mcgillicuddy[7], E. Hailman[8], H.U. Schildhaus[9], J. Ruschoff[10]. *[1]Mersana Therapeutics Inc., Translational Medicine, Cambridge, USA; [2]Targos Molecular Pathology, Project Management Department, Kassel, Germany; [3]Targos Molecular Pathology, Assay Development Department, Kassel, Germany; [4]Targos Molecular Pathology, Laboratory Services 1 Department, Kassel, Germany; [5]Mersana Therapeutics Inc., Clinical Operations, Cambridge, USA; [6]Targos Molecular Pathology, Pathology Department, Kassel, Germany; [7]Mersana Therapeutics Inc., Chemistry Manufacturing and Controls, Cambridge, USA; [8]Mersana Therapeutics Inc., Clinical, Cambridge, USA; [9]Institute of Pathology University Hospital, Pathology, Gottingen, Germany; [10]Targos Molecular Pathology, Management, Kassel, Germany*

Background: HER2/ERBB2 is a well validated target in tumors with HER2 gene amplification. Antibody-based therapies, trastuzumab and pertuzumab, and an ADC targeting HER2, ado-trastuzumab emtansine (T-DM1), are approved in HER2-positive breast cancer, and trastuzumab is also approved in HER2-positive gastric cancer. In NSCLC, HER2 amplification and mutation are infrequent events, each occurring in 1–2% of patients. Recent data suggested an effect of T-DM1 in NSCLC patients with HER2 activating mutations or amplification, but not in the broader patient population of HER2-expressing NSCLC (IHC 1+/2+ without amplification or mutation).

XMT-1522 is a novel antibody drug conjugate with ~12 Auristatin F-hydroxypropylamide (AF-HPA) payload molecules per antibody that binds a HER2 epitope distinct from the binding sites of trastuzumab/T-DM1 and pertuzumab. AF-HPA is capable of controlled bystander-effect killing, resulting in efficacy in tumors with heterogeneous antigen expression, and is metabolized intra-tumorally to an active non-permeable metabolite to enable greater systemic tolerability. Prior preclinical work has demonstrated *in vivo* efficacy of XMT-1522 in models of HER2 wild-type NSCLC. XMT-1522 has also been shown to induce immunogenic cell death *in vitro*, making it a candidate for combination studies with immune checkpoint inhibitors.

Materials and Methods: An ongoing series of NSCLC models (n = 3 animals/model) was treated with XMT-1522, 3 mg/kg q week × 3, with a subset of the models treated at 1 mg/kg q week × 3. Outcome data was expressed as median best response for each model. Vehicle treated blocks were obtained from each model, as available (currently 12/15 models), and evaluated by IHC (DAKO primary) and RNA expression methods. A series of 40 human lung NSCLC tumors, selected to represent tumors at a variety of expression values, was also evaluated by IHC (Ventana primary) and RNA expression methods. Protein/RNA/pre-clinical outcome correlations were examined.

Results: A median best response of >50% reduction was seen in 7/15 NSCLC models in the 3 mg/kg treated group and 3/10 NSCLC models in the 1 mg/kg treated group. An efficacy/outcome relationship was noted for RNA and protein expression. In the human tumor set, there was no apparent correlation between protein and RNA expression; in the xenograft set, higher RNA values were associated with higher protein scores. Tumor tissues classified as adenocarcinoma tended to have higher ERBB2 expression than tissues classified as squamous cell carcinoma.

Conclusion: In an unselected series of human primary xenografts, XMT-1522 yielded responses that related to HER2 protein and RNA expression levels. HER2 protein levels will be prospectively evaluated in the planned dose expansion groups of an ongoing early human clinical trial of XMT-1522 and RNA levels will be determined retrospectively.

Conflict of interest: Other Substantive Relationships: The Mersana co-authors are employees of Mersana Therapeutics. The Targos co-authors, and Dr. Schildhaus are employees of Targos Molecular Pathology. Dr. Ruschoff is also the head of Pathologie Nordhessen. The lab work described was contracted by Mersana to be performed at Targos.

418 (PB-081) Poster

BI 905711, a bi-specific molecule targeting both TRAILR2 and CDH17, induces effective TRAILR2 oligomerization and apoptosis specifically in CDH17 positive tumor cells

J.M. Garcia-Martinez[1], J. Ho[2], S. Wang[1], C. Weishaeupl[1], C. Giragossian[2], M.A. Impagnatiello[1], H. Lamche[3], J. Rinnenthal[1], S.M. Schmidbauer[1], N. Tamot[2], A. Wernitznig[1], K.P. Kuenkele[1]. *[1]Boehringer Ingelheim, Cancer Research Therapeutic Area, Vienna, Austria; [2]Boehringer Ingelheim, Biotherapeutics Discovery Research, Ridgefield, USA; [3]Boehringer Ingelheim, Biotherapeutics Discovery Research, Vienna, Austria*

TRAILR2 is a pro-apoptotic receptor widely expressed in cancers and its ligand-induced oligomerization results in the induction of apoptosis which is potentiated upon the formation of larger clusters. Several agonistic molecules have reached the clinic but were terminated due either to lack of efficacy or liver toxicity. CDH17 is a cell surface molecule that is absent in liver but co-expressed with TRAILR2 in several cancer types. BI 905711 is a tetravalent bispecific molecule targeting both TRAILR2 and CDH17, and is designed to selectively induce apoptosis in CDH17 expressing tumor cells via CDH17-dependent clustering of TRAILR2, while avoiding the hepatotoxicity associated with clustering of TRAILR2 in liver.

Here, we report the preclinical activity of BI 905711 using colorectal cancer (CRC)-derived cell lines both *in vitro* and *in vivo* as tumor xenografts implanted in nude mice.

We showed that, in a CDH17-dependent manner, BI 905711 triggered apoptosis in several CDH17 positive CRC tumor cells *in vitro*, but not in CDH17 negative/TRAIL sensitive liver-derived cells. BI 905711 *in vivo* efficacy was demonstrated using disease-related CRC xenograft models, where sustained tumor regressions were observed after administration of a single dose. Selective pathway engagement was assessed by measuring the downstream apoptosis biomarkers caspase 8 and 3/7. Significant activation of caspases was demonstrated not only in the tumor tissue, but also active caspases released from dying tumor cells could be detected in plasma samples.

In summary, we demonstrated that BI 905711 triggers apoptosis specifically in CDH17 positive tumor cells, decreasing the tumor burden in CDX CRC tumor models with the potential for a favorable safety and efficacy profile. BI 905711 represents a promising new approach for the treatment of CRC and additional CDH17 positive oncology indications.

Conflict of interest: Corporate-sponsored Research: All authors were employees of Boehringer Ingelheim at the time of data generation.

419 (PB-082) Poster

Synergistic anticancer activity of targeted anticancer agents with the RAD51 inhibitors IBR2 and IBR120

P. Ferguson[1], M. Vincent[2], J. Koropatnick[1]. *[1]London Regional Cancer Program, Cancer Research Laboratories, London, Canada; [2]London Regional Cancer Program, Oncology, London, Canada*

Background: Much of the reason for failure of anticancer chemotherapy is due to the outgrowth of cells rendered drug-resistant by one or more mutations to the target protein, by biochemical alterations not directly related to the target (such as proteins upstream or downstream of a signal cascade) and/or altered DNA repair. Reliance of tumor cells on these pathways because of “oncogene addiction,” non-oncogene dependency, and/or inherent genetic instability may contribute to higher selectivity of cytotoxic agents for tumor cells. To improve the antitumor activity of chemotherapy drugs, we demonstrated that IBR2, a new inhibitor of the DNA repair protein RAD51, enhanced cytotoxicity of various agents including inhibitors of tyrosine kinase receptors and microtubule function. We extended these studies to include combinations of IBR2 or another new RAD51 inhibitor [(R)-3-(2-(benzylsulfonyl)isoindolin-1-yl)-1h-indole (IBR120)] with several new drugs against a wider range of cell lines, in order to broaden their potential future clinical use.

Materials and Methods: Four-day drug exposures were conducted in 96-well plates. Relative cell density, determined using vital stains (alamarBlue©, neutral red), was reported as a percent of the fluorescence/absorbance of control cultures. Enhancement of the drug effect was reported as the degree by which IBR2 or IBR120 decreased the 50% inhibitory concentration of drug (IC50). Cell lines were tested from various tissue sources: breast (MCF-7, SK-BR-3), colon (HT-29), gastric (N87), non-small-cell lung (NSCLC) (A549b, H1650, H1975, osimertinib-resistant variants of H1650 and H1975), and melanoma (SK-MEL-5).

Results: IBR2 synergistically enhanced the antiproliferative activity of vemurafenib (B-raf inhibitor) by up to 80% against MCF-7 and HT-29, but had little effect against SK-BR-3, A549b, N87 or SK-MEL-5, demonstrating specificity of the enhancement. IBR2 also enhanced activity of regorafenib (multikinase inhibitor) against SK-BR-3 and SK-MEL-5, and afatinib (pan-HER inhibitor) against MCF-7. Of note, IBR2 strongly enhanced inhibition of MCF-7 and SK-BR-3 by 4-OH-tamoxifen. IBR120, which reportedly has selective inhibition of tumor cells, enhanced antiproliferative activity of osimertinib (EGFR inhibitor) by up to 80% against numerous cell lines, including several osimertinib-resistant NSCLC cell lines, as well as MCF-7, SK-BR-3, SK-MEL-5, and HT-29 (more than 90%). IBR120 also enhanced activity of regorafenib, gefitinib (EGFR inhibitor), imatinib (Bcr-Abl inhibitor), and vincristine (targets microtubules) against several other cell lines.

Conclusion: The ability of IBR2 and IBR120 to enhance antiproliferative activity of a wide variety of anticancer agents, and their potential selectivity for cancer cells, makes possible their future use as chemotherapy adjuvants to improve clinical outcomes.

Conflict of interest: Ownership: Drs. Koropatnick and Vincent are co-owners of Sarissa, Inc., which owns the license for IBR2 and IBR120.

420 (PB-083) Poster

Tipifarnib is highly active in HRAS-mutant HNSCC tumor models

M. Gilardi[1], Z. Wang[1], L. Kessler[2], M. Janes[3], Y. Chen[3], A. Gualberto[2], Y. Liu[4], S. Gutkind[1], F. Burrows[4]. [1]*University of California, San Diego, Pharmacology, San Diego, USA;* [2]*Kura Oncology, Clinical, San Diego, USA;* [3]*Wellspring Biosciences, Biology, San Diego, USA;* [4]*Kura Oncology, Translational Research, San Diego, USA*

Tipifarnib is a potent and highly selective inhibitor of farnesyltransferase (FT). FT catalyzes the post-translational attachment of farnesyl groups to signaling proteins that are requisite for localization to the inner cell membrane. While all RAS isoforms are FT substrates, only HRAS is exclusively dependent upon farnesylation for membrane localization and signaling activation, making HRAS mutant tumors uniquely susceptible to tipifarnib mediated inhibition of FT. Based upon this rationale, the efficacy and safety of tipifarnib in patients with HRAS mutant head and neck squamous cell carcinoma (HNSCC) is currently being evaluated in a multi-institutional, open-label Phase II trial (NCT02383927).

Herein we report the characterization of tipifarnib in preclinical models of HRAS mutant HNSCC. Tipifarnib was screened in a panel of 20 HNSCC cell lines, displaying potent inhibition of proliferation in 4/4 HRAS mutant lines tested in conventional monolayer assays, and the robustness and selectivity of the response was further enhanced in the 3D anchorage-independent format, as reported for other RAS inhibitors. Tipifarnib displaced HRAS, but not KRAS, from membranes and inhibited MAPK signaling in HNSCC cells in vitro.

Tipifarnib displayed robust antitumor activity in nine of ten cell line- and patient-derived xenograft (PDX) models of HRAS mutant HNSCC, including several that were resistant to chemotherapy and/or cetuximab, suggesting that tipifarnib may offer improved outcomes in this subset of HNSCC. Tipifarnib treatment in vivo resulted in downregulated MAPK kinase activity that was associated with reduced proliferation, activation of apoptosis and other phenotypic changes consistent with blockade of mutant HRAS signaling.

These preclinical findings compliment preliminary data from the ongoing Phase 2 clinical study of tipifarnib in which confirmed partial responses have been observed in HRAS mutant HNSCC patients who were relapsed and/or refractory to prior therapy, including platinum, immunotherapy and cetuximab +/– chemotherapy regimens. These data demonstrate that HRAS is a targetable oncogene in HNSCC through farnesyltransferase inhibition by tipifarnib.

Conflict of interest: Ownership: Kura Oncology, Inc., Wellspring Biosciences, Inc.

421 (PB-084) Poster

Targeting sorting nexins to treat ErbB-dependent breast cancer

B. Atwell[1]. [1]*University of Arizona, Molecular and Cellular Biology, Tucson, USA*

The Epidermal Growth Factor Receptor (EGFR) is a powerful driver of transformation and metastasis, being overexpressed and amplified in 60% of triple negative breast cancer. Yet, therapies designed to target EGFR have been successful in only a small subset of these cases. We and others have worked to understand why EGFR is so difficult to target in breast cancer, and have found that it is frequently mistrafficking during cancer progression and can function internally in a kinase-independent manner. Overexpression of adapter proteins (such as MUC1) drive EGFR off of the cell surface where it undergoes retrograde trafficking to locations including long-lived endosomes, the mitochondria and the nucleus. Once in these novel locations, EGFR drives migration, alters mitochondrial function and acts as a transcription co-factor for genes such as Cyclin D1. Blocking this retrograde trafficking results in the complete ablation of EGFR's ability to drive EGF-dependent migration. We are investigating the role of the Sorting Nexin (SNX) complexes to regulate EGFR trafficking, and have developed a peptide-based therapeutic to reintroduce normal trafficking of EGFR to the lysosome (Snx1.3). We have discovered that treatment of breast cancer cell lines with Snx1.3 results in a reduction of EGF-dependent migration. Furthermore, Snx1.3 treatment results in the degradation of EGFR and HER2, but not HER3, indicating a specificity in the interaction. We are currently working to better define the proteins driving the interactions between SNX and EGFR, stabilize the peptide and test its efficacy in mouse models of breast cancer.

No conflict of interest

422 (PB-085) Poster

The hypoxia-activated EGFR/HER2 inhibitor Tarloxotinib is activated by the plasma membrane reductase STEAP4

S. Silva[1], C. Guise[1], M. Bull[1], C. Print[1], J. Smaill[1], A. Patterson[1]. [1]*The University of Auckland, Faculty of Medical and Health Sciences, Auckland, New Zealand*

Background: Tarloxotinib is a clinical-stage prodrug that releases a potent, irreversible EGFR/HER2 inhibitor (Tarlox-TKI) selectively in severely hypoxic regions of tumours (<0.1% O_2). Mechanistic studies show that tarloxotinib is cell-excluded due to the positive-charge of the quaternary ammonium cation to which the 4-nitroimidazole trigger is appended. One-electron reduction leads to loss of the trigger moiety under oxygen-deficient conditions, releasing the neutral, diffusible "warhead" tarlox-TKI. Characterisation of oxidoreductases that facilitate one-electron reduction of tarloxotinib is critical for its clinical development.

Material and Methods: The rate of anoxic tarloxotinib metabolism (tarlox-TKI formation) was monitored by LC-MS/MS and compared with RNA transcript abundance (Affymetrix Primeview gene arrays) across a panel of human neoplastic cell lines. Plasmid-based cDNA expression and siRNA knockdown studies were employed to evaluate the role of putative oxidoreductases in tarloxotinib metabolism. CRISPR/cas9 mediated gene knockout was used to further interrogate the role of the predominant tarloxotinib reductase *in vitro* and *in viv*o.

Results: Amongst 36,000 transcripts and variants, the most highly correlated relationships to anoxic tarloxotinib metabolism were associated with *STEAP4* (Six-Transmembrane Epithelial Antigen of Prostate, family member 4). Gene overexpression in two low STEAP4 expressing cell lines (C33A and H1299) increased anoxic tarloxotinib metabolism by 28–59-fold relative to parental cells. siRNA knockdown of *STEAP4* suppressed tarloxotinib metabolism by >70% while knockdown of other putative oxidoreductases led to no changes in tarloxotinib metabolism. Gene disruption of *STEAP4* using a CRISPR/cas9 system indicated STEAP4 was the dominant tarloxotinib reductase *in vitro* and *in vivo*. STEAP4 is an NADPH-dependent, FAD and heme containing metalloreductase required for reduction of extracellular Fe3+ and Cu2+ to facilitate plasma membrane transport. Analysis of 163 databases from the Cancer Genome Atlas (TCGA) covering over 22,000 individual cancers indicates *STEAP4* mRNA is highly expressed in certain cancers including prostate, non-small cell lung, breast, esophageal, gastric and Head/Neck.

Conclusions: Identifying individual patients with elevated STEAP4 activity in their cancer may be important to guide patient selection during the clinical development of tarloxotinib. Tarloxotinib is currently under exclusive license to Rain Therapeutics Inc., Fremont, USA, and development is anticipated in various STEAP4-positive cancers.

Conflict of interest: Ownership: No conflicts. Advisory Board: Dr Adam Patterson is a member of the Scientific Advisory Board of Rain Therapeutics Inc. Dr Jeff Smaill is a member of the Scientific Advisory Board of Rain Therapeutics Inc. Board of Directors: No conflicts. Corporate-sponsored Research: No conflicts. Other Substantive Relationships: No conflicts.

423 (PB-086) Poster

In vitro and in vivo activity of TAS6417 against uncommon EGFR mutations

S. Hasako[1], N. Abe[1], T. Haruma[1], T. Komori[2], H. Ohsawa[1], K. Funabashi[1], K. Miyadera[1], T. Utsugi[1], Y. Iwasawa[1]. [1]*TAIHO Pharmaceutical Co., Ltd., Discovery and Preclinical Research Division, Tsukuba, Japan;* [2]*TAIHO Pharmaceutical Co., Ltd., Discovery and Preclinical Research Division, Tokushima, Japan*

Background: Somatic mutation of the EGFR gene, most of which are concentrated in the region of exon 18–21, is a major oncogenic driver of NSCLC. The most common mutations are deletion mutations in exon 19 and L858R substitution mutation in exon 21. They account for over 80% of activating mutations and the remaining 20% consists of other uncommon mutations comprising exon 20 insertions (ex20ins), G719X and L861Q. A number of clinical trials have been carried out to develop EGFR-TKI therapy for NSCLC patients with the common EGFR mutations, but there remain high medical needs for patients with uncommon EGFR mutations. While TAS6417 has been identified as a novel EGFR-TKI which possesses higher selectivity for ex20ins mutations over WT, but its potency and selectivity against other uncommon mutations are unknown.

Material and Methods: Human recombinant mutant EGFR (D770_N771insNPG) was used for MS analysis and biochemical assay. Cell viability assay were performed using CellTitier-Glo™. The protein and phospho-protein level were determined by Western blot or In-Cell Western analysis. Antitumor evaluation was conducted in athymic nude mice bearing allografts expressing mutant human EGFRs.

Results: Characterization of TAS6417 against uncommon mutations including ex20ins, G719X, and L861Q were performed. MS analysis and biochemical assay demonstrated that TAS6417 may exert inhibitory activity against EGFR D770_N771insNPG through covalent modification of the C797 residue. In cell viability assays, the GI_{50} values of TAS6417 for LXF 2478L cells harboring EGFR V769_D770insASV, NCI-H1975 with EGFR D770_N771insSVD cells, and human primary keratinocytes (NHEK-NEO), of which WT EGFR is implicated in the growth, were 86.5, 45.4, and 729 nM, respectively. Further analysis with In-Cell Western demonstrated that TAS6417 had potent inhibitory activity against EGFRs with other uncommon mutations such as G719A and L861Q mutations even if complexed with T790M acquired resistant mutation. Consistently, TAS6417 inhibited the proliferation of Ba/F3 cells expressing EGFR with G719A ± T790M or L861Q ± T790M with IC_{50} values ranging from 6.55 to 37.5 nM. In contrast, the IC_{50} value for WT EGFR was 676 nM. Selectivity for these mutations was superior to other representative EGFR-TKIs such as afatinib and osimertinib. Furthermore in an *in vivo* model using NIH/3T3 EGFR G719A cells, TAS6417 exerted marked antitumor efficacy at 50 mg/kg or more without severe body weight loss, comparable to efficacy shown in xenograft models of EGFR ex20ins mutations.

Conclusions: TAS6417 exerts mutant-selective inhibition against uncommon EGFR mutations including ex20ins, G719X, and L861Q while sparing WT. This mutation-selective characteristic led to a significant antitumor efficacy in mouse model, suggesting a promising therapeutic option for NSCLC patients with uncommon EGFR mutations.

No conflict of interest

425 (PB-088) Poster

Phloretin inhibits hepatic cancer via dowregulation of JAK2/STAT3 signaling and induction of apoptosis

S. Saraswati[1], A.A. Alhaider[1]. [1]*King Saud University, Pharmacology, Riyadh, Saudi Arabia*

Background: Phloretin possess numerous biological properties, including antidiabetic, anti-inflammatory, antiproliferative, and protein kinase C inhibition activity, but its effect of hepatic cancer is not explored yet.

Material and Methods: We investigated the anti-cancer effect of phloretin on an experimental carcinogenesis model of liver cancer by studying the antioxidant, anti-inflammatory, anti-proliferation, pro-apoptotic activities of phloretin in vivo. We evaluated the effects of phloretin on N-nitrosodiethylamine (DENA)-induced hepatocarcinogenesis in rats. We initiated hepatocarcinogenesis by intraperitoneal injection of diethylnitrosamine (DENA) followed by promotion with phenobarbital.

Results: Administration of phloretin at doses of 15, 30, and 50 mg/kg/day was started 4 weeks prior to the DENA injection and was continued for 22 weeks. Phloretin decreased the incidence, total number, multiplicity, size and volume of preneoplastic hepatic nodules in a dose-dependent manner. Furthermore, phloretin counteracted DEN-induced oxidative stress in rats as determined by restoration of superoxide dismutase, catalase, and glutathione-S-transferase levels and diminishing of myeloperoxidase activity, malondialdehyde and protein carbonyl formation in liver. Phloretin resulted in normal serum levels of IL-2, IFN-γ, AFP and AFU. We found that JAK2/STAT3 signaling was significantly up-regulated in DEN-treated group compared to that in control group. Western blot analysis showed that phloretin inhibited phosphorylation of STAT3 and its principal upstream kinase, Jak2. Further, it also decreased expression of cyclin D1 and Bcl-2 with activation of caspase-3 and increased expression of Bax. Immunohistochemical demonstrated the decreased expression of the PCNA, VEGF cyclooxygenase 2, iNOS, nuclear factor-kappa B p-65.

Conclusions: Phloretin exerts a significant chemopreventive effect against liver cancer via inhibition of cell proliferation and induction of apoptosis mediated by JAK2/STAT3 signaling pathway. This study also demonstrated that phloretin protects rat liver from cancer via modulating oxidative damage and suppressing inflammatory response.

No conflict of interest

426 (PB-089) Poster

A novel ERK inhibitor has potent activity in NRAS-mutant melanoma cancer models

J. Kucia-Tran[1], L. Bevan[1], R. Ferraldeschi[2], B. Kidane[3], J. Lyons[2], S. Rich[1], N. Wallis[1], J. Munck[2]. [1]*Astex Pharmaceuticals, Biology, Cambridge, United Kingdom;* [2]*Astex Pharmaceuticals, Translational Research & Development, Cambridge, United Kingdom;* [3]*Astex Pharmaceuticals, DMPK & Preclinical Development, Cambridge, United Kingdom*

Background: Currently there is no approved therapy for NRAS-mutant melanoma, an indication which is associated with aggressive clinical outcome and a poor prognosis. The NRAS mutation leads to constitutive activation of the ERK pathway, suggesting that the direct inhibition of ERK may provide an attractive therapeutic approach for the treatment of NRAS-mutant melanoma. As previously described, using fragment-based drug discovery we have identified a novel and selective inhibitor of ERK which inhibits both the catalytic activity of ERK as well as ERK phosphorylation. Here, we demonstrate the activity of this compound in models of NRAS-mutant melanoma.

Material and Methods: The anti-proliferative effect of the novel ERK inhibitor was studied in a panel of NRAS-mutant melanoma cell lines consisting of established cell lines and patient-derived cells. Inhibition of ERK catalytic activity and modulation of the mitogen activated protein kinase (MAPK) pathway was assessed by MSD, ELISA and Western Blotting. *In vivo,* a subcutaneous MA-MEL-28 xenograft model was employed to determine the compound's pharmacodynamic effects and anti-tumour activity.

Results: Eight out of ten established melanoma cell lines and patient-derived melanoma cells carrying an NRAS mutation showed sensitivity to the ERK inhibitor *in vitro*, with anti-proliferative IC_{50} values ranging from 95 to 420 nM. The compound inhibited ERK catalytic activity in NRAS-mutant melanoma cells, as indicated by a decrease in the phosphorylation of the ERK substrate, RSK. In addition, the compound also supressed the phosphorylation of ERK itself. Treatment with our compound also led to the induction of apoptosis, which was demonstrated by the induction of cleaved PARP and increase of the pro-apoptotic protein, Bim. Similarly, a strong decrease of phospho-RSK and phospho-ERK levels and activation of apoptosis was observed *in vivo* in MA-MEL-28 xenograft tumours, 2 hours after the oral administration of the ERK inhibitor. This corresponded to significant inhibition of tumour growth in this model following daily oral administration of the lead compound at 50 mg/kg, resulting in a maximal T/C response of 47% ($p < 0.0001$).

Conclusions: This work demonstrates the *in vitro* and *in vivo* activity of a novel, highly potent, selective ERK inhibitor in models of NRAS-mutant melanoma. These data support the clinical investigation of this series of compounds for the treatment of NRAS-mutant melanoma.

No conflict of interest

427 (PB-090) Poster

Effects of treatment regimens on antitumor activity of lenvatinib and anti-PD-1 antibody combinations in the CT26 mouse colon cancer syngeneic model

Y. Kato[1], T. Kimura[1], K. Tabata[1], Y. Hori[1], K. Yamada[1], T. Uehara[1], J. Ito[1], Y. Ozawa[1], K. Ichikawa[1], J. Matsui[2], Y. Funahashi[1], K. Nomoto[2]. [1]*Eisai Co., Ltd., Tsukuba Research Laboratories, Tsukuba, Japan;* [2]*Eisai Inc., Oncology Business Group, Woodcliff Lake, NJ, USA*

Background: Lenvatinib (LEN) is a multitargeted tyrosine kinase inhibitor that selectively inhibits vascular endothelial growth factor receptor (VEGFR) 1–3, fibroblast growth factor receptor (FGFR) 1–4, platelet derived growth factor receptor (PDGFR) α, RET and KIT. Phase 1b/2 clinical trials investigating combinations of LEN and anti-PD-1 antibodies are ongoing in selected cancer types including renal cell carcinoma, endometrial cancer and others. We previously reported inhibitory effects of LEN on tumor-associated macrophages and antitumor activities of LEN combined with anti-PD-1 antibody (Ab) in mouse syngeneic tumor models. In this study, treatment regimens and mechanisms of immune modulatory effects of the LEN plus anti-PD-1 Ab combination were investigated in CT26 mouse colon cancer syngeneic model.

Methods: Antitumor activities of monotherapy, sequential and combination treatments of LEN (10 mg/kg, qd) and anti-PD-1 Ab (200 μg/mouse, twice weekly) were examined in the CT26 colon cancer syngeneic model. Tumor-infiltrated lymphocytes were analyzed by flow cytometry to assess immune cell populations. Alterations in tumor immune responses were assessed by gene expression analyses using RNA sequencing and quantitative PCR.

Results: Combinations of LEN and anti-PD-1 Ab showed greater antitumor activity in the CT26 model compared with each monotherapy. In addition, prior treatment with LEN followed by anti-PD-1 Ab showed greater antitumor activity compared with anti-PD-1 Ab single agent treatment, particularly in mice whose tumor growth was strongly inhibited by LEN alone. Flow cytometric analysis showed that LEN or LEN plus anti-PD-1 Ab increased memory T cell populations compared with non-treatment. In addition, LEN or LEN plus anti-PD-1 Ab also increased Granzyme B-expressing $CD8^+$ T cells in tumor. Gene expression analyses of CT26 tumors treated with LEN plus anti-PD-1 Ab showed upregulation of cytotoxic molecules, Prf1 (perforin) and Gzmb (granzyme B), and genes related to memory T cells.

Conclusions: These results indicated greater antitumor activity of combination than each monotherapy and sequential treatments. The results from sequential treatment suggested that LEN may modify tumor microenvironment to lead to more sensitivity to anti-PD-1 Ab. The antitumor activity of the LEN plus anti-PD-1 Ab combination was accompanied by an immune modulatory response characterized by upregulation of cytotoxic molecules from T cells and memory T cells and genes related to memory T cells.

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428 (PB-091) Poster

Identification of robust E-cadherin-related synthetic lethal interactions in breast cancer

I. Bajrami[1], C. Lord[2]. [1]*Institute of Cancer Research, Breast Cancer Now, London, United Kingdom;* [2]*The Institute of Cancer Research, Breast Cancer Now, London, United Kingdom*

Background: The E-cadherin *(CDH1)* tumour suppressor gene encodes a calcium-dependent cell-cell adhesion glycoprotein, which has roles in maintaining cell polarity, differentiation, cell migration and survival. E-cadherin dysfunction is a feature common to many epithelial tumours, with the highest incidence occurring in diffuse gastric cancer (50%) and lobular breast cancer (63%) and can occur *via CDH1* mutation, deletion or epigenetic silencing. Although E-cadherin dysfunction is relatively common, precision medicine approaches that exploit this pathogenic alteration are not yet available.

Materials and Methods: We used a series of integrated approaches to identify synthetic lethal interactions that operate in a diverse set of models of E-cadherin deficiency in breast cancer. These included: (i) high-throughput *in vitro* genetic perturbation screens in isogenic and non-isogenic cell lines; (ii) analysis of *ex vivo* breast cancer explants; (iii) analysis of genetically engineered mouse models of invasive lobular carcinoma; (iv) analysis of mice bearing patient derived xenografted tumours.

Results: Perturbation screens in breast tumour cells with CRISPR/Cas9-engineered *CDH1* mutations identified a synthetic lethality between E-cadherin deficiency and inhibition of the receptor tyrosine kinase ROS1. Data from large-scale genetic screens in molecularly diverse breast tumour cell lines established that the E-cadherin/ROS1 synthetic lethality was not only robust in the face of considerable molecular heterogeneity but was also elicited with clinical ROS1 inhibitors, including foretinib and crizotinib; these effects also operated in *ex vivo* breast cancer explants, where crizotinib sensitivity was associated with loss of E-cadherin. ROS1 inhibitors induced mitotic abnormalities and multinucleation in E-cadherin-defective cells, phenotypes associated with a defect in cytokinesis and aberrant p120 catenin phosphorylation and localization. *In vivo*, ROS1 inhibitors produced profound antitumor effects in multiple models of E-cadherin-defective breast cancer. CRISPR-Cas9 mutagenesis screens are now underway to identify mechanisms that cause resistance to crizotinib in E-cadherin defective breast tumour cells.

Conclusions: These data provide the preclinical rationale for assessing ROS1 inhibitors, such as the licensed drug crizotinib, in appropriately stratified patients. The phase 2 ROLO clinical trial will now assess the effect of crizotinib treatment when combined with fulvestrant in patients with advanced, E-cadherin defective, breast cancer.

No conflict of interest

429 (PB-092) Poster

INC280 inhibits Wnt/β-catenin and EMT signaling pathways and its induce apoptosis in diffuse gastric cancer positive for c-MET amplification

S.H. Sohn[1], Y. Kim[1], B. Kim[1], H.J. Sul[1], H.S. Kim[2], H. Kim[3], J.B. Seo[4], Y. Koh[5], D.Y. Zang[1,2]. [1]*Hallym Translational Research Institute, Hallym University Medical Center Hallym University College of Medicine, Anyang-si, South Korea;* [2]*Department of Internal Medicine, Hallym University Medical Center Hallym University College of Medicine, Anyang-si, South Korea;* [3]*Department of Biological Sciences, Sungkyunkwan University, Suwon, South Korea;* [4]*Korea Basic Research Institute, Seoul Center, Seoul, South Korea;* [5]*Department of Bio-medical Gerontology, Ilsong Institute of Life Sciences, Hallym University, Anyang, South Korea*

Background: Intestinal-type gastric cancer is related to *Helicobacter pylori* infection whereas diffuse-type gastric cancer is more frequently related to genetic predisposition. Previous studies have shown that Runt-related transcription factor 3 (RUNX3) expression was significantly down-regulated in gastric cancer tissues compared with matched normal tissues. We showed that decreased levels of RUNX3 are significantly associated with hepatocyte growth factor receptor (MET) ($r = -0.4216$, $P = 0.0130$). In addition, c-MET is potentially a highly plausible target for gastric cancer therapy. Therefore, in the present study, the anti-cancer effects of the c-MET inhibitor on gastric cancer cells with or without c-MET amplification were evaluated.

Material and Methods: c-Met inhibitors such as crizotinib, foretinib, cabozantinib, tivantinib and tepotinib were purchased from Selleck Chemical and INC280 was gratefully supplied by Novartis. SNU620 and MKN45 were positive for c-Met amplification, but MKN28 was taken as a control. Each cell-line was treated with c-Met inhibitors. We measured cell viability, migration ability, apoptosis, mRNA expression and protein levels in each cell-line.

Results: INC280 treatment inhibits growth of a c-MET-amplified cell line. Dose-response non-linear regression analysis revealed that the half-maximal inhibitory concentration (IC50) of INC280 was 1.7 nM or 2.4 nM in the c-MET-amplified MKN45 and SNU620 (diffuse type) cell-line, respectively. Next, migration and apoptosis analysis demonstrated that INC280 showed the best inhibition and apoptotic rates with the smallest IC50s in MKN45 cells but not in c-MET-reduced MKN28 (intestinal type) cells. We also showed that INC280 inhibits the WNT signaling pathway and SNAIL expression in MKN45 cells.

Conclusions: INC280 shows significant inhibitory activity against c-MET-expressed diffuse gastric cancer. Our *in vitro* study strongly supports that INC280 could be used as therapeutic agents for diffuse gastric cancer with c-MET amplification.

No conflict of interest

430 (PB-093) Poster

TAS0612, a novel and highly potent RSK, AKT, and S6K inhibitor, exhibited strong antitumor effect in preclinical tumor models with deregulated RAS and PI3K pathway activities

K. Ichikawa[1], S. Ito[1], T. Machida[1], E. Kato[1], M. Yamada[1], R. Fujita[1], Y. Nakatsuru[1], H. Saito[2], G. Tanaka[2], K. Wakayama[1], H. Jona[1], T. Sugimoto[1], K. Matsuo[1], K. Miyadera[1], T. Utsugi[1], Y. Iwasawa[1]. [1]*Taiho Pharmaceutical Co., Ltd., Discovery and Preclinical Research Division, Tsukuba, Japan;* [2]*Taiho Pharmaceutical Co., Ltd., Discovery and Preclinical Research Division, Tokushima, Japan*

Background: The PI3K/AKT/mTOR pathway plays a crucial role in cancer cell growth and survival. Although molecular targeted drugs for PI3K, AKT and mTOR are under clinical development, it has been demonstrated that these drug show insufficient clinical response due to cancellation of negative feedback or the coexistence of mutations in driver genes such as KRAS and BRAF. TAS0612 was identified as a novel, highly potent, and orally bioavailable inhibitor of RSK, AKT, and S6K kinases. RSK has a key role in cell growth, migration, and transcription under the RAS pathway. Recent studies revealed that RSK is involved in the resistance to treatment of various cancers, e.g. breast, lung, and prostate cancer. Here we present a unique anticancer effect of TAS0612 in various cancer cells unresponsive to molecular targeted drugs and chemotherapy agents.

Material and Methods: The kinase selectivity profiling of TAS0612 was conducted in a kinase panel including more than 200 kinases. Cell growth inhibition was assayed by measuring cellular ATP. For pharmacodynamic marker inhibition and antitumor efficacy *in vivo*, human tumor cell lines were subcutaneously transplanted into the flank of nude mice. Dosing of compound was started when tumor size reached $\sim$150 mm^3. The total and phospho-protein level were determined by Western blot and ELISA analysis.

Results: TAS0612 inhibited enzymatic activity of AKT isoforms at a single digit nM level and inhibited RSK and S6K isoforms at sub nM level. Pharmacological inhibition of RSK, AKT, and S6K by TAS0612 was confirmed by the reduction of their phospho-substrates. TAS0612 showed stronger IC_{50} values than those of AKT, PI3K and PI3K/mTOR inhibitors regardless of whether cancer cell carried mutations in KRAS or BRAF gene. *In vitro*, PI3K, AKT, MEK, BRAF inhibitors couldn't inhibit downstream signals of cancer cells with complicated driver-gene mutations by a single or a combination treatment. However, TAS0612 clearly inhibited downstream signals and induced apoptosis. TAS0612 also demonstrated superior antitumor efficacy both in KRAS mutant (TOV21G and HEC-6) and BRAF mutant (MFE319) xenografts which were less sensitive to AKT inhibitors and PI3K/mTOR inhibitor. Furthermore, MFE319 xenograft tumor was refractory to platinum treatment (T/C% were about 60% to CDDP and CBDCA at their maximum tolerated dose). In the same model, TAS0612 showed significant efficacy around 5–20% of T/C%, suggesting the possibility for application in the treatment of cancer unresponsive to chemotherapy.

Conclusions: We identified a novel class and highly potent RSK/AKT/S6K inhibitor, TAS0612. TAS0612 would be efficacious for cancers in which PI3K/AKT inhibition is ineffective due to bypass growth and survival signal

with the RAS pathway. Further evaluations are currently ongoing to support clinical development.

Conflict of interest: Corporate-sponsored Research: Our work was sponsored by Taiho Pharmaceutical Co., Ltd. (All authors are employees of Taiho Pharmaceutical Co., Ltd.).

431 (PB-094) Poster

ET-D5, first-in-class synthetic Protein Phosphatase 1 (PP1) inhibitor for the treatment of aggressive tumors

A. Juhem[1], M. Pastor[1], D. Lecerclé[1], J. Deverchère[1], B. Bestgen[1], A. Popov[1]. [1]*Ecrins Therapeutics, R&D, La Tronche, France*

Background: ET-D5 is a new chemical entity and the first selective PP1 inhibitor to be evaluated in a clinical trial.

ET-D5 offers a comprehensive anti-cancer treatment, as the inhibition of PP1 produces anti-proliferative and anti-vascular activities. PP1 is highlighted as a major protein in control of cell cycle progression and the overexpression of PP1 is positively correlated with bad prognosis in different tumors types, such as sarcomas or lung adenocarcinomas.

Material and Methods: ET-D5 was discovered in a phenotypic screening based on the cell cycle perturbation. After cell phenotype characterization, different methods such as affinity chromatography, immunoblotting and siRNA were used for target identification and validation. We then evaluated ET-D5 in many *in vitro* and *in vivo* cancer models and refined its original mechanism-of-action. To develop this new chemical entity as an oral drug, we realized a formulation screening, and GLP regulatory toxicology studies were performed in two animal species, rats and dogs. ET-D5 efficacy is currently being evaluated in a comparative veterinary oncology Phase 1/2a clinical trial in companion dogs suffering from spontaneous sarcomas.

Results: Targeting the PP1, ET-D5 showed a strong *in vitro* (in a panel of different cancer cell lines) and *in vivo* anti-cancer activity in several mouse xenograft models (lung, thyroid, kidney, sarcomas …). MRI studies and dorsal window chamber experiments in mice demonstrated the anti-vascular activity of ET-D5. MR imaging can be used as the efficacy clinical biomarker. Nano-suspension formulation allowed us to increase the oral bioavailability from less than 5% to over 70%. GLP toxicology studies showed an excellent toxicology and safety profile.

Conclusions: Efficacy data, coupled with the successful completion of the regulatory toxicology studies provide a strong and relevant background for the development of ET-D5 as a first-in-class small molecule drug candidate in advanced cancer patients.

No conflict of interest

433 (PB-096) Poster

Overexpression of Aiolos promotes epithelial-mesenchymal transition and cancer stem cell-like properties in lung cancer cells

J.J. Hung[1], W.H. Hsu[1]. [1]*Taipei Veterans General Hospital, Department of Surgery, Taipei, Taiwan*

Background: Aiolos/Ikaros family zinc finger 3 (IKZF3), a member of the Ikaros family of lymphocyte maturation-driving transcription factors, is highly expressed in hematopoietic malignancies. However, its role in epithelial-mesenchymal transition (EMT) and cancer stem cell (CSC)-like properties in lung cancer remains unknown.

Material and Methods: Human lung cancer cell lines H1299 with overexpressing Aiolos (H1299-Aiolos) and A549 with overexpressing Aiolos (A549-Aiolos) were generated by stable transfection. Cell migration and invasiveness assay were done in these cells to demonstrate their invasion and migration ability. To further demonstrate whether Aiolos overexpression increased the metastatic ability *in vivo*, tail vein metastasis assay was performed. Sphere formation assay was used to determine their tumor-initiating capability. The expression levels of lung cancer CSC surface markers CD44 and CD133 were analyzed by flow cytometry and qRT-PCR. The effects of Aiolos overexpression on in vitro resistance to irradiation was determined by clonogenic cell survival assay.

Results: Aiolos overexpression induced EMT and increased migration/invasiveness in H1299 and A549 cells. Aiolos overexpression also increased metastatic ability *in vivo*. Aiolos overexpression upregulated the expression of Twist and its downstream target matrix metalloproteinase 16 (MMP16). EMT phenotypes, increased migration/invasiveness, and increase MMP16 expression of H1299-Aiolos and A549-Aiolos cells were reversed by siRNA-mediated repression of Twist expression or a phosphatidylinositol (PI) 3-kinase inhibitor. Overexpression of Aiolos upregulated the CSC-like properties in lung cancer cells, and were also reversed by a PI 3-kinase inhibitor.

Conclusions: Aiolos overexpression promotes EMT and CSC-like properties through upregulation of PI 3-kinase/Akt pathway in lung cancer cells. The information is helpful for developing therapeutic strategies targeting Aiolos expression for lung cancer treatment.

No conflict of interest

434 (PB-097) Poster

Inhibition of nuclear EGFR localization as a breast cancer therapeutic

C. Wiersma[1], J. Schroeder[2]. [1]*University of Arizona, Cancer Biology, Tucson, USA;* [2]*University of Arizona, Molecular and Cellular Biology, Tucson, USA*

Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, is frequently overexpressed in invasive carcinoma including glioblastoma, head and neck, and breast cancer. The primary role of EGFR is to transduce signals from the cell surface when a ligand (e.g., epidermal growth factor (EGF)) binds, resulting in increased proliferation, survival and migration. Membrane localized EGFR can also retrotranslocate to the nucleus where it can function as a transcriptional cofactor and alter gene expression directly. We have previously demonstrated that this activity is dependent on the oncogene MUC1, which colocalizes with EGFR at the cell surface and drives its retrotranslocation. Nuclear EGFR can act as a co-transcriptional activator to such oncogenes as Cyclin D1, iNOS, and COX2, and we hypothesize that it may be driving an epigenetic program to promote transformation and metastasis. To test this hypothesis, we have developed a peptide-based therapeutic against the nuclear localization signal (NLS) of EGFR, named ENLS4. We have found that treatment of breast cancer cell lines blocks the nuclear translocation of EGFR, resulting in a reduction in cell survival. We are now performing ChIP-seq to identify DNA binding sites targeted by EGFR, proteomic analysis to determine the interactions between chromatin remodeling proteins and nuclear EGFR determining the mechanism of action of ENLS4. Together, we expect that characterizing EGFR genomic targets and inhibition of EGFR nuclear localization holds the potential for improved therapeutics.

No conflict of interest

435 (PB-098) Poster

A phase I dose escalation multi-centre study of crizotinib (MET inhibitor) combined with binimetinib (MEK inhibitor) in patients with advanced solid tumours

R. Wilson[1], P. Hari Dass[2], S. Van Schaeybroeck[1], E. Elez Fernandez[3], R. Jones[4], A. Quinton[4], J. Houlden[5], L. Collins[5], C. Roberts[6], S. Love[6], M. Lawler[1], F. Di Nicolantonio[7], M. Grayson[8], A. Bardelli[9], P. Laurent-Puig[10], T. Maughan[11], J. Tabernero[3], M. Peeters[12], M. Middleton[13], C. Rolfo[14]. [1]*Queen's University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom;* [2]*Waikato Hospital, Department of Oncology, Hamilton, New Zealand;* [3]*Vall d'Hebron University Hospital, Medical Oncology Department, Barcelona, Spain;* [4]*Velindre Cancer Centre and University of Cardiff, Department of Oncology, Cardiff, United Kingdom;* [5]*University of Oxford, Oncology Clinical Trials Office OCTO, Oxford, United Kingdom;* [6]*University of Oxford, Department of Clinical Statistical Medicine, Oxford, United Kingdom;* [7]*University of Turin, Division of Molecular Genetics, Turin, Italy;* [8]*Northern Ireland Cancer Research, Consumer Forum, Belfast, United Kingdom;* [9]*University of Candiolo, Deaprtment of Oncology IRCCS, Candiolo, Italy;* [10]*Paris Descartes University, Department of Oncology, Paris, France;* [11]*University of Oxford, CRUK/MRC Oxford Institute for Radiation Oncology, Oxford, United Kingdom;* [12]*Antwerp University Hospital, Department of Oncology, Antwerp, Belgium;* [13]*Churchill Hospital, Cancer and Haematology Department, Oxford, United Kingdom;* [14]*University of Maryland School Of Medicine, Marlene and Stewart Greenebaum Comprehensive Cancer Centre, Baltimore, USA*

Background: The cMET-JAK-STAT3 survival axis is activated following MEK1/2 inhibition in *RAS* mutant (MT) colorectal cancer (CRC) models. Moreover, combination of MEK1/2 and c-MET inhibition results in increased cell death and anti-tumour activity in *RAS* MT CRC models, supporting the clinical investigation of combined MET/MEK inhibition in this patient group. We evaluated the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of oral crizotinib and binimetinib in combination to provide inhibition of both cMET and MEK.

Material and Methods: Patients with various advanced solid tumors received crizotinib (either 200 mg twice daily (BD) or 250 mg once daily (QD), continuously), combined with binimetinib (either 30 mg BD continuously on days 1–28 or on days 1–21 every 28 days). Evaluable patients for primary analysis were those who either completed cycle one or withdrew due to dose limiting toxicity (DLT). The rolling six design was used to establish the primary

Table 1 (abstract 435 PB-098): Baseline Characteristics of Dose Escalation of Crizotinib with Binimetinib

Binimetinib Crizotinib	Cohort A 30 mg BD days 1–28 200 mg BD (n = 8)	Cohort B 30 mg BD days 1–21 200 mg BD (n = 5)	Cohort C 30 mg BD days 1–21 250 mg OD (n = 7)
Age (years) median (range)	51 (33–72)	55 (40–65)	60 (46–70)
Gender n (%)			
Female	3 (37%)	3 (60%)	0
Male	5 (63%)	2 (40%)	7 (100%)
Number of prior lines of chemo			
0–1 n (%)	0	0	1 (14%)
2–3 n (%)	4 (50%)	3 (60%)	3 (43%)
4–5 n (%)	3 (38%)	1 (20%)	1 (14%)
≥6 n (%)	1 (13%)	1 (20%)	2 (29%)

outcome-recommended phase II dose and schedule (RP2D). PK and PD (skin +/−tumour biopsies) were analysed.

Results: Twenty patients (ECOG performance status 0–1) were recruited to the dose escalation part of this phase I trial (Table 1). Three patients withdrew early for reasons other than DLTs. Of the 8 Cohort A patients, 6 were evaluable and 2 patients experienced DLT (grade III transaminitis and creatinine kinase [CK] elevation). We reduced to interval dose scheduling of binimetinib where 2 of 5 Cohort B patients (grade III transaminitis and Grade IV CK elevation) had DLTs. In Cohort C with reduced crizotinib dosing, 1 of 7 patients (grade III fatigue) experienced DLT. The MTD was thus achieved at Cohort C. The most common treatment related adverse events were diarrhoea (11%), rash (9%), CK elevation (9%), fatigue (7%), and nausea (6%).

Conclusions: Crizotinib 250 mg OD continuously combined with binimetinib 30 mg BD at interval dosing (days 1–21 every 28 days) was the RP2D. The drug doses are pharmacologically active and the combination has a manageable safety profile. A dose expansion cohort of patients with *RAS* MT CRC has recently been recruited (n = 29) and efficacy results will be presented.

Conflict of interest: Ownership: 1. Robert Jones. 2. Mark Lawler. 3. Federica Di Nicolantonio. 4. Pierre Lauren-Puig. 5. Tim Maughan. 6. Josep Tabernero. 7. Marc Peeters. 8. Richard Wilson. 9. Mark Middleton. 10. Christian Rolfo. Advisory Board: 1. Orion Corporation. 3. Merck Bristol-Myers Squibb Boehringer Ingelheim. 5. Vertex. 6. Bayer Boehringer Lilly MSD Merck Serono Novartis Roche Sanofi Symphony Evolution Taiho Pharmaceuticals Genentech/Roche. 7. Amgen Bayer. 8. Merck Serono Sirtex Medical Amgen Servier Clovis Oncology Halozyme Bristol-Mayer Squibb. 9. Merck CytomX Therapeutics RigonTEC Bristol-Mayer Squibb Newlink Genetics Novartis. 10. Mylan MSD. Board of Directors: n/a for all co-authors. Corporate-sponsored Research: 1. Astra Zeneca. 3. Trovagene. 3. Roche Merck. 7. Roche Amgen Bayer. 8. Almac Group. 9. Immunocore Novartis Astra Zeneca Roche Amgen Milennium, Bristol-Mayer Squibb Vertix Merck Pfizer RigonTEC Replimune Array Biopharma TC Biopharm Regeneron. 10. OncoDNA Novartis Sanofi. Other Substantive Relationships: 1. Merck and Co Inc. 2. Pfizer – honoraria. 3. AmgenBohehringer Ingelheim Merck Serono Merck Roche Sanofi – all honoraria. 7. Roche Amgen Bayer Servier/Pfizer Sirtex Medical Merck. 8. Merck Amgen.

436 (PB-099) Poster

Association of oestrogen receptor β with inflammatory mediators in female patients with colorectal cancer

G. Topi[1], S.R. Satapathy[1], R. Ehrnström[2], M.L. Lydrup[3], A. Sjölander[1]. *[1]Lund University, Skane University Hospital, Department of Translational Medicine, Division of Cell Pathology, Malmö, Sweden; [2]Lund University, Skane University Hospital, Department of Translational Medicine, Division of Pathology, Malmö, Sweden; [3]Lund University, Skane University Hospital, Department of Clinical Sciences, Division of Surgery, Malmö, Sweden*

Background: The estrogen receptor β (ERβ) is the predominant ER in the colon mucosa. We have previously reported that high expression of ERβ is independently associated with a better prognosis in female patients with colorectal cancer (CRC). On the other hand, β-catenin plays a major role in CRC development. In addition, inflammatory mediators, such are cyclooxygenase-2 (COX-2), cysteinyl leukotriene receptor 1 and 2 (CysLT1R, CysLT2R) and prostaglandin D2 (PGD2) have been associated with CRC. In this study we aimed to investigate the correlation between ERβ and inflammatory mediators in patients with CRC and cell line experiments.

Material and Methods: A tissue microarray (TMA) of primary CRCs from 320 female patients was stained with the following antibodies: anti-ERβ (1:100), anti-COX-2 (1:200), anti-CysLT1R (1:100), anti-CysLT2R (1:10), anti-β-catenin (1:200) and anti-PGD2 (1:25). Immunohistochemistry technique was used to evaluate the staining intensity. Two colon cancer cells lines were stimulated with 60 μM of ERβ agonist (ERβ-041) for 48 h. The protein levels of interest inflammatory mediators were evaluated using the Western Blot method.

Results: Patients with high ERβ expression had significantly lower IRS for COX-2, CysLT1R and nuclear β-catenin expression and significantly higher IRS for CysLT2R, membrane β-catenin and PGD2 expression. ERβ was negatively correlated with COX-2 ($r = -0.32$; $p < 0.0001$), CysLT1R ($r = -0.12$; $p = 0.03$) and nuclear β-catenin ($r = -0.28$; $p < 0.0001$) expression and positively correlated with CysLT2R ($r = 0.60$; $p < 0.0001$), membrane β-catenin ($r = 0.50$; $p < 0.0001$) and PGD2 ($r = 0.43$; $p < 0.0001$) expression. Stimulation of colon cancer cell lines with ERβ agonist decreased the expression of CysLT1R and increased the expression of CysLT2R, which was stronger in the female colon cancer cell line HT-29.

Conclusion: High ERβ expression was significantly correlated with anti-proliferative and pro-apoptotic inflammatory mediators in female patients with CRC. These results strengthen our hypothesis for a beneficial role of ERβ in CRC patients. Further cell line experiments will be conducted to evaluate the cross talk between estrogen receptors and cysteinyl leukotriene receptors.

No conflict of interest

437 (PB-100) Poster

Identification of highly penetrant Rb-related synthetic lethal interactions in triple negative breast cancer

R. Brough[1], A. Gulati[1], S. Haider[1], R. Kumar[1], J. Campbell[1], E. Knudsen[2], S. Pettitt[1], C. Ryan[3], C. Lord[1]. *[1]Institute of Cancer Research, Breast Cancer, London, United Kingdom; [2]University of Arizona, Department of Medicine, Tucson, USA; [3]University College Dublin, Systems Biology Ireland, Dublin, Ireland*

Background: Although defects in the *RB1* tumour suppressor are one of the more common driver alterations found in triple negative breast cancer (TNBC), therapeutic approaches that exploit this have not been identified. The availability of several, large-scale, shRNA and siRNA screens, conducted in multiple tumour cell lines, some of which are derived from TNBCs, now make it possible to predict highly penetrant RB1-related synthetic lethal effects.

Materials and Methods: We integrated molecular profiling data with data from multiple genetic perturbation screens and identified candidate synthetic lethal (SL) interactions associated with *RB1* defects in TNBC. This was achieved using a refined data analysis pipeline that not only identified SL effects but also assessed their robustness or penetrance. We reasoned that highly penetrant synthetic lethal effects would be more robust in the face of molecular heterogeneity and would represent more promising therapeutic targets. Using protein-protein interaction and transcription factor binding data, we assessed the molecular connectivity of highly penetrant SL effects.

Results: A significant proportion of the highly penetrant *RB1* SL effects involved proteins closely associated with RB1 function, suggesting that this might be a defining characteristic. These included nuclear pore complex components associated with the MAD2 spindle checkpoint protein, the kinase and bromodomain containing transcription factor TAF1, multiple TAF1 transcriptional targets and multiple components of the SCF^{SKP} Cullin F box containing complex, including SKP2. Small molecule inhibition of SCF^{SKP} elicited an increase in $p27^{Kip}$ levels, providing a mechanistic rationale for RB1 SL. Transcript expression of SKP2, a SCF^{SKP} component, was elevated in *RB1* defective TNBCs, suggesting that in these tumours, SKP2 activity might buffer the effects of *RB1* dysfunction.

Conclusions: Here we describe a straightforward data analysis pipeline that allowed us to use *RB1* cell line annotation to interrogate both in-house and publically available large-scale, shRNA and siRNA screens to identify

highly penetrant candidate Rb-related synthetic lethal effects. We identified a series of pharmacologically tractable effects, one of which, SKP2, we validated using both genetic and pharmacological methods. We also noted that a significant proportion of the highly penetrant Rb SL effects in TNBC involved proteins closely associated with Rb function, suggesting that this might be a defining characteristic. Importantly, this straightforward analysis pipeline can be applied to any gene of interest.

No conflict of interest

438 (PB-101) Poster

A phase Ib study of the combination of MLN0128 (dual TORC1/2 inhibitor) and MLN8237 (Aurora A inhibitor, alisertib) in patients with advanced solid tumors, expansion cohort data

S.L. Davis[1], S. Leong[1], W. Messersmith[1], T. Purcell[1], E. Lam[1], B. Corr[1], A. Leal[1], C. O'bryant[2], A. Glode[2], N. Adler[1], N. Serkova[3], J. Diamond[1]. *[1]University of Colorado Cancer Center, Medical Oncology, Aurora, USA; [2]University of Colorado Anschutz Medical Campus, Pharmacy, Aurora, USA; [3]University of Colorado Anschutz Medical Campus, Radiology, Aurora, USA*

Background: MLN0128 is an oral inhibitor of mTOR kinase and mTORC1/2 signaling. Alisertib is an oral inhibitor of Aurora A kinase. Senescence and up-regulation of genes in the PI3K/AKT/mTor pathway were observed in patient-derived xenograft models (PDX) treated with alisertib to resistance, and tumor growth inhibition was demonstrated in these models treated with MLN0128 and alisertib in combination. An ongoing phase 1b clinical trial is evaluating the combination of MLN0128 and alisertib in patients with advanced solid tumors. Results of the dose escalation portion of this trial identifying the maximum tolerated dose (MTD) of the combination as MLN0128 2 mg daily on a continuous schedule and alisertib 30 mg BID days 1–7 of a 21-day cycle have been previously reported. Presented here are up-dated results from the dose expansion cohort of this trial.

Methods: Patients with advanced solid tumors, refractory to standard therapy, were treated with the combination of MLN0128 and alisertib at the MTD. In Cycle 1, patients were assigned to treatment with either MLN0128 or alisertib as a single-agent on days 1–7. For the remainder of the study, patients received combination treatment according to the MTD. Biopsies were performed in all patients prior to treatment initiation, Cycle 1 Day 7 (after single-agent lead-in), and Cycle 2 Day 7 (after combination treatment). Diffusion weighted MRI (DWI-MRI) was performed in a subset of patients with hepatic metastases at these time points, with assessment of apparent diffusion coefficients (ADC) as a potential biomarker of early treatment response. For a subset of patients who underwent baseline FDG-PET/CT for evaluation of disease, optional repeat assessment was performed Cycle 2 Day 7.

Results: Twenty patients with refractory cancers were treated in the dose expansion portion of this trial, with treatment ongoing in two patients. Represented tumor types include breast (9), colorectal (4), pancreatic (3), ovarian (2), renal cell (1), and uterine carcinoma (1). Median time on study was 2.5 cycles (range 1–15). No complete or partial responses were documented. Stable disease was observed in four patients (20%), including prolonged stable disease (15 cycles) in one patient with pancreatic cancer. The ADC of five patients who underwent DWI-MRI has been evaluated, with three patients with increased ADC values observed at Cycle 1 Day 7 noted to have decreased lesion size on Cycle 2 Day 7 imaging. Further assessment of this and additional correlatives is ongoing, with an up-date to be reported at the meeting.

Conclusions: In an expansion cohort of patients treated with the combination of MLN0128 and alisertib at the previously defined MTD, prolonged stable disease was observed in a patient with pancreatic cancer. Further expansion in a cohort of patients with refractory pancreatic adenocarcinoma is planned.

Conflict of interest: Other Substantive Relationships: Diamond: Grant funding for clinical trial, travel expenses for investigator meeting.

439 (PB-102) Poster

Targeting the PI3K pathway in HER2-positive gastric cancer

S. Toomey[1], A.M. Farrelly[1], M.J. Mezynski[1], A. Carr[1], P. Armstrong[1], J. Mcauley[1], J. Fay[2], E.W. Kay[2], M. Cremona[1], C. Holohan[3], N.M. Keegan[1], Y. Elamin[1], S. Rafee[3], B.T. Hennessy[1]. *[1]Royal College of Surgeons in Ireland, Molecular Medicine, Dublin, Ireland; [2]Royal College of Surgeons in Ireland, Pathology, Dublin, Ireland; [3]St. James's Hospital, Medical Oncology, Dublin, Ireland*

Background: HER2 is overexpressed in about 25% of gastric cancers and is correlated with more advanced tumours. PI3K pathway activating mutations have been reported in gastric cancer, however neither the association of HER2 overexpression with these mutations or the impact of aberrant PI3K pathway signalling on trastuzumab responsiveness been described. In HER2-positive breast cancer, targeting the PI3K pathway can overcome resistance to HER2-targeted therapies; however the role of PI3K inhibitors in sensitizing HER2-positive gastric cancers to trastuzumab or in overcoming trastuzumab resistance has yet to be addressed.

Materials and Methods: Gastric tumour samples were collected from 69 patients and stratified into HER2-positive and HER2-negative groups. Mass spectrometry-based genotyping (Agena Bioscience) was used for analysis of 105 hotspot, nonsynonymous somatic mutations in PIK3CA, EGFR, ERBB2, ERBB3 and ERBB4 in the tumours. A panel of HER2-positive gastric cell lines (N87, OE19, ESO26 and SNU16) was profiled for the same mutations and their anti-proliferative response to the PI3K inhibitor copanlisib alone and in combination with the HER2-targeted therapies trastuzumab and lapatinib was assessed.

Results: Patients with HER2-positive gastric cancer had significantly poorer overall survival compared to HER2-negative patients (15.9 months vs. 35.7 months). EGFR and ERBB family mutations occurred more frequently in HER2-negative than HER2-positive tumours. Mutations in PIK3CA were only identified in HER2-negative tumours. Mutations in PIK3CA or ERBB family genes did not have any effect on either progression free or overall survival. Mutations in PIK3CA (Q546H) and ERBB4 (M772L) were identified in ESO26 cells, while a mutation in ERBB2 (F254L) was identified in N87 cells. OE19 cells were resistant to copanlisib, while all other cell lines were sensitive, with IC50s ranging from 23.4 nm (N87) to 93.8 nm (SNU16). PIK3CA mutation status had no impact on copanlisib sensitivity. All cell lines except SNU16 were sensitive to lapatinib with IC50s ranging from 0.04 μM to 1.5 μM. OE19 and SNU16 were resistant to trastuzumab. The combination of lapatinib and copanlisib is synergistic in ESO-26 and OE-19 cells (ED_{50}: 0.83 ± 0.19 and 0.88 ± 0.13, respectively) and additive in NCI-N87 cells (ED_{50}: 1.01 ± 0.55). The combination of copanlisib and trastuzumab significantly improved growth inhibition compared to either therapy alone in N87, ESO26 and OE19 cells ($p < 0.05$).

Conclusions: Copanlisib is an effective monotherapy in some HER2-positive gastric cancer cell lines. Combinations of copanlisib and trastuzumab offer greater benefit than either drug alone, and may restore sensitivity to trastuzumab in cells with intrinsic resistance to trastuzumab. The addition of copanlisib to HER2 targeted therapy warrants further investigation in HER2-positive gastric cancer.

No conflict of interest

440 (PB-103) Poster

Mutational landscape assessed in tumor tissue and cell-free DNA during neratinib treatment of patients with HER2 mutated solid tumors

I. Spanggaard[1], O. Oestrup[2], B. Bertelsen[2], K. Staal Rohrberg[1], A.Y. de Schmidt[2], I. Viller Tuxen[1], L. Barlebo Ahlborn[2], J. Preuss Hasselby[3], E. Santoni-Rugiu[3], R. Lykke Marvig[2], U. Lassen[1], F. Cilius Nielsen[2], C. Westmose Yde[2], M. Mau-Soerensen[1]. *[1]Rigshospitalet, The Phase 1 Unit, Department of Oncology, Copenhagen, Denmark; [2]Rigshospitalet, Center for Genomic Medicine, Copenhagen, Denmark; [3]Rigshospitalet, Department of Pathology, Copenhagen, Denmark*

Background: The pan-HER kinase inhibitor neratinib has shown clinical activity in a range of solid tumors harboring activating HER2 mutations. However, anti-tumor activity varies between tumor types and different mutant variants. Here, we explore the dynamic changes in the mutational landscape of cell-free DNA (cfDNA) in serial blood samples and in pre- and on-treatment biopsies in patients with HER2 mutated cancers treated with neratinib.

Materials and Methods: Patients with refractory solid tumors were biopsied for whole exome sequencing as part of the Copenhagen Prospective Personalized Oncology (CoPPO) program to identify actionable driver mutations. Patients with activating HER2 mutations were offered treatment with neratinib as monotherapy or in combination with trastuzumab/fulvestrant either in a basket trial or in a patient named program. Repeated plasma sampling for cfDNA analysis was performed to assess the dynamics of the variant allele frequency (VAF) of activating HER2 mutations during therapy. When possible archival, on-treatment and/or post-progression biopsies were collected for comprehensive genomic analysis.

Results: From May 2013 to June 2018, fourteen of 752 patients included in CoPPO were identified as having HER2 mutations. Eight patients received neratinib either as monotherapy or in combination with trastuzumab (and fulvestrant in cases of estrogen receptor positive breast cancer). One patient received epirubicin+trastuzumab. Two patients received no HER2-targeted treatment. Three patients received no additional therapy due to rapid decline in performance status. Partial response was observed in 2/8 patients receiving neratinib, 3/8 had stable disease and 2 patients had progressive disease. One patient is awaiting first evaluation. In general, a decline in

HER2 VAF was observed upon initiation of neratinib treatment. In case of progression, an increase in VAF was observed prior to radiological detection of cancer metastases, supporting potential utility of cfDNA analysis for tracking the clinical response of neratinib treatment. Analysis of co-mutated genes and emergence of possible acquired resistance mechanisms to neratinib is on-going.

Conclusions: The results support the use of a neratinib-containing regimen in patients with somatic HER2 mutations. Analysis of HER2 mutation VAF in cfDNA from repeated plasma sampling during treatment shows promise for real-time monitoring of clinical response to HER2 targeting therapy.

Conflict of interest: Corporate-sponsored Research: Dr. Morten Mau-Soerensen has received a research grant from Puma Biotechnology. All other authors declare no potential conflicts of interest.

441 (PB-104) Poster

SEL120 – 34A, a potent and specific inhibitor of CDK8, as a potential treatment of acute myeloid and lymphoblastic leukemias

M. Mazan[1], E. Majewska[1], M. Mikula[2], K. Wiklik[3], M. Combik[3], A. Gołas[1], M. Masiejczyk[1], E. Fiedor[1], F. Malusa[3], A. Polak[4], A. Paziewska[5], M. Dąbrowska[2], A. Białas[6], K. Michalik[1], R. Windak[7], J. Ostrowski[2], P. Juszczyński[4], K. Brzózka[8], T. Rzymski[8]. *[1]Selvita S. A., R&D Biology, Krakow, Poland; [2]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Genetics, Warsaw, Poland; [3]Selvita S. A., Computational Drug Discovery, Krakow, Poland; [4]Institute of Hematology and Transfusion Medicine, Department of Experimental Hematology, Warsaw, Poland; [5]Medical Center for Postgraduate Education, Department of Gastroenterology, Hepatology and Clinical Oncology, Warsaw, Poland; [6]Selvita S. A., Discovery Chemistry, Krakow, Poland; [7]Selvita S. A., Clinical & Preclinical Operations, Krakow, Poland; [8]Selvita S. A., Research & Development, Krakow, Poland*

Background: Cyclin-dependent kinase 8 inhibitors (CDK8i) have anti-cancer activity in human acute myeloid leukemia (AML) cell lines that has been demonstrated both *in vitro* and *in vivo*. Inhibition of CDK8, Mediator-associated kinase, induces upregulation of Super-enhancer-associated genes in sensitive AML cell lines. Our preliminary results indicate also effectiveness of CDK8i in T-cell acute lymphoblastic leukemia (T-ALL) cell lines, however the exact mechanism of action here remains unknown. Due to poor outcome of current treatment in adult T-ALL, development of new therapies is urgently needed.

Material and Methods: Efficacy of the compound alone or in combination was tested in viability assays in a broad panel of cancer cell lines. Activity and mechanism of action of CDK8 inhibitor – SEL120-34A alone and in combination with other compounds was investigated by flow cytometry, western blotting and differential gene expression analysis. *In vivo* efficacy was tested in mice injected with leukemia cell lines. Sensitive and resistant cell lines panel was used for bioinformatic analysis of specific signatures.

Results: SEL120-34A showed differential activity in selected AML and T-ALL cells. We observed robust and dose-dependent inhibition of STAT5 phosphorylation on serine 726 (STAT5 pS726) in sensitive cell lines. Moreover, basal level of STAT5 pS726 in AML cell lines correlated with sensitivity to treatment with our compound. Differential gene expression analysis between sensitive and resistant leukemia cell lines demonstrated leukemia stem cell (LSC) – like signature present in responding cells that has been linked to resistance to standard therapies and relapsed disease. These findings were further corroborated by the presence of surface LSC markers in sensitive cells. Further comparison of transcriptomic changes upon treatment with SEL120-34A revealed changes in genes regulating differentiation and apoptosis. Prolonged treatment with SEL120-34A induced lineage commitment markers followed by reduced viability. We observed synergistic effects of SEL120-34A in several AML cell lines in combination with standard of care compounds like cytarabine and azacytidine and late phase experimental therapies effective in AML such as ABT-199.

Conclusions: SEL120–34A effectively inhibits phosphorylation of STAT proteins and induce CDK8-specific transcriptomic changes not only in previously demonstrated AML cell lines, but also in T-ALL models. Differential activity on LSC-like cells and synergistic activity with standard of care therapies warrants further clinical development, particularly in relapsed and refractory leukemia.

No conflict of interest

442 (PB-105) Poster

Miransertib and ARQ751 exhibit superior cell-death-inducing properties compared to other AKT inhibitors, and can overcome resistance to other allosteric AKT inhibitors

E. Kostaras[1], P. Cutillas[2], Y. Yu[3], R. Savage[4], B. Schwartz[3], I. Vivanco[1]. *[1]The Institute of Cancer Research, Cancer Therapeutics, London, United Kingdom; [2]Barts Cancer Institute, Centre for Haemato-Oncology, London, United Kingdom; [3]Arqule, Inc., Translational Research, Burlington, USA; [4]Arqule, Inc., Preclinical Development & Clinical Pharmacology, Burlington, USA*

Background: The serine/threonine kinase AKT is a critical effector of the PI3K signalling pathway, and is an attractive therapeutic target because of its frequent activation in human tumours and its role in the regulation of several hallmarks of cancer. As such, there has been significant effort in developing AKT inhibitors as cancer therapeutics. However, the clinical success of these compounds as single agents has so far been disappointing. Existing clinical AKT inhibitors can be classified, based on their mode of inhibition, into either ATP-competitive or allosteric. Recent data from our laboratory suggest that the latter may be able to inhibit both catalytic as well as non-catalytic activities, which we have also shown to regulate cancer cell survival. Miransertib (ARQ092) and ARQ751 are two novel allosteric and selective inhibitors of AKT1/2/3 that have shown promising anti-tumour activity in pre-clinical models.

Methods: We used miransertib, ARQ751, MK2206, GSK690693, and ipatasertib to treat breast cancer (MDA-MB-361, T47D, ZR75.1) and lung cancer cell lines (EBC1). Phosphoproteomic analysis was done using LC-MS.

Results: We have evaluated the cell-death inducing properties of these compounds in cell lines carrying activating PIK3CA mutations or MET gene amplification. Both compounds were highly active, and exhibited superior activity compared to MK2206, another allosteric AKT inhibitor, and two distinct ATP-competitive inhibitors. Interestingly, we have found that cell lines that have been made resistant to MK2206 through either chronic drug exposure or ectopic expression of an MK2206-binding-deficient AKT allele (W80A) remain sensitive to miransertib and ARQ751.

Given the disappointing clinical performance of AKT inhibitors as single agents, the therapeutic focus has now shifted towards the use of AKT inhibitors in combination regimens. To identify potential new co-targets for AKT, we used phosphoproteomic analysis using cell lines treated with a variety of AKT inhibitors. Allosteric and ATP-competitive inhibitors generated similar phosphopeptide signatures that were distinct from those generated by allosteric inhibitors. Through kinase substrate enrichment analysis (KSEA), we found that AKT inhibition was associated with activation of kinases in DNA repair pathways, and that combined inhibition of AKT and ATM was synergistic.

Conclusions: Our data suggest that Miransertib and ARQ751 have significant single agent activity AKT-dependent cell lines, and are superior to other AKT-targeting agents currently in clinical development. We also find that these compounds might be able to overcome at least some mechanisms of resistance to other AKT inhibitors. Finally, our data suggest that combined inhibition of AKT and ATM might be a rational therapeutic strategy to maximize therapeutic benefit.

Conflict of interest: Corporate-sponsored Research: Miransertib and ARQ751 were provided by Arqule, Inc. Funds for attendance to this conference were also provided by Arqule, Inc.

443 (PB-106) Poster

Harnessing the anticancer activity of the stapled peptide ALRN-6924, a dual inhibitor of MDMX and MDM2, using rational combination strategies for breast cancer and other malignancies

A. Annis[1], J.G. Ren[1], L.A. Carvajal[1], S. Santiago[1], N. Narasimhan[1], D. Sutton[1], S.S. Pairawan[2], V. Guerlavais[1], F. Meric-Bernstam[2], M. Aivado[1]. *[1]Aileron Therapeutics Inc., Research, Cambridge, USA; [2]MD Anderson, Investigational Cancer Therapeutics, Houston, USA*

Background: The purpose was to identify rational anticancer drug combinations with ALRN-6924.

Material and Methods: ALRN-6924 is a cell-penetrating stapled α-helical peptide that disrupts the interaction of the p53 tumor suppressor protein and its endogenous inhibitors, MDMX and MDM2. For TP53 wild-type (WT) tumors, this can restore p53-dependent cell cycle arrest and apoptosis leading to antitumor efficacy. ALRN-6924 was tested in combination with 29 drugs for synergistic *in vitro* anticancer activity. Select agents were further evaluated *in vivo*. Drugs listed in Table 1 were assayed in combination with ALRN-6924 using WST-1 and/or CyQUANT cell viability assays in

Table 1

Cancer	Cell line	Drug	CI*
Breast cancer	MCF-7	palbociclib	0.45 ± 0.17
		abemaciclib	0.57 ± 0.23
		ribociclib	0.39 ± 0.08
		paclitaxel	0.80 ± 0.05
		docetaxel	0.37 ± 0.02
		eribulin	0.66 ± 0.36
		everolimus	0.59 ± 0.28
		fulvestrant	0.41 ± 0.17[‡]
		carboplatin	0.49 ± 0.04
		gemcitabine	0.58 ± 0.05
		dexamethasone	No change
Colon cancer	HCT-116	5-FU	0.07 ± 0.03
Melanoma	A375	trametinib	0.41 ± 0.12
		dabrafenib	0.33 ± 0.15
		vemurafenib	0.48 ± 0.20
	MEL-JUSO	binimetinib	0.32 ± 0.10
		pimasertib	0.39 ± 0.08
		selumetinib	0.49 ± 0.08
T Cell Lymphoma	MOLT-3	romidepsin	0.38 ± 0.00
B Cell Lymphoma	DOHH-2	vincristine	0.09 ± 0.06
		cyclophosphamide	0.11 ± 0.13
		rituximab	0.40[†]
		doxorubicin	0.04[†]
	JVM2	ibrutinib	0.59 ± 0.27
Acute Leukemia	MV-4-11	cytarabine/Ara-C	0.68 ± 0.02
		azacitidine	0.49 ± 0.32
		decitabine	1.08 ± 0.07
		midostaurin	0.83 ± 0.04
		venetoclax	0.20 ± 0.03

*In vitro CI value at IC_{75} or ‡IC_{50}, average ± SD of ≥2 experiments. Values <0.9 are synergistic, 0.9–1.1 additive, >1.1 antagonistic. †Single experiment.

immortalized TP53-wildtype cancer cell lines. Synergy was quantified by the Chou-Talalay combination index (CI) method. *In vivo* ALRN-6924 combinations with palbociclib, abemaciclib, and nab-paclitaxel were tested in the MCF-7 breast cancer model in athymic nude mice.

Results: All the drugs evaluated except dexamethasone were additive or synergistic with ALRN-6924 *in vitro*; no antagonism was observed. In MCF-7 xenografts tumor growth inhibition was improved when ALRN-6924 was given in combination with palbociclib, abemaciclib, or nab-paclitaxel compared to single agent therapy. Pharmacodynamic biomarkers indicate on-mechanism activity. Body weights and mortality data suggest ALRN-6924 and combinations with nab-paclitaxel and palbociclib were tolerated at the doses tested. The combination with abemaciclib was tolerated with interruption and dose-reduction.

Conclusions: ALRN-6924 can be rationally combined with pathway-selective and chemotherapy agents. These results, plus promising ALRN-6924 safety and antitumor activity as a monotherapy (Meric-Bernstam et al., ASCO 2017) support the development of combination regimens for breast cancer and other malignancies.

Conflict of interest: Other Substantive Relationships: A. Annis, J-G Ren, L. A. Carvajal, S. Santiago, N. Narasimhan, D. Sutton, V. Guerlavais, and M. Aivado are employees of Aileron Therapeutics, Inc.

444 (PB-107) Poster

Gastro-intestinal carcinomas with RSPO3 gene fusion

Z. Gatalica[1], J. Xiu[2], J. Swensen[3], L. Faoro[4], A. Kapoun[4]. [1]*Caris Life Sciences, Molecular Diagnostics, Phoenix, USA;* [2]*Caris Life Sciences, Medical Affairs, Phoenix, USA;* [3]*Caris Life Sciences, Genetics, Phoenix, USA;* [4]*OncoMed Pharmaceuticals, Translational Medicine, Redwood City, USA*

Background: Gene fusions involving R-spondin family members RSPO2 and RSPO3 occur in approximately 10% of colon tumors. RSPO2/RSPO3 fusions and APC gene mutations are generally mutually exclusive (as both cause activation of the Wnt pathway). Therapies targeting this pathway (including anti-RSPO3) are being developed, and we sought to comprehensively characterize RSPO3 fusion driven cancers in order to gain insights to potential therapeutic biomarkers.

Material and Methods: Tumor DNA sequencing of 592 genes (NextSeq, Illumina, San Diego, CA) and RNA sequencing of 53 gene fusions (ArcherDx FusionPlex Assay, ArcherDX, Boulder, CO) were used along with immunohistochemistry for PD-L1 expression in the tumor cells (SP142 antibody, Ventana Medical Systems, Tucson, AZ).

Results: Forty-one RSPO3 fusion-positive cases were identified and included 36 cases arising in colon/rectum (CRC), 4 in small intestine and 1 in esophagus. Only PTPRK–RSPO3 fusions were detected. The most common additional somatic alteration was a pathogenic TP53 mutation (32/41 cases); all TP53 mutated cases had additional mutations in KRAS, NRAS or BRAF. Unexpectedly, two pathogenic APC gene mutations (p.S1465fs, p.E1097X) were detected in one case (CRC liver metastasis). Although the average mutational burden was low for the cohort (average = 8.7/Mb, range 4–16/Mb), 13 cases exhibited ≥10 mutations/Mb.

Chromosomal amplifications were detected in 8 cases: three of 12p (commonly FGF6/FGF23/CCND2) and one each of: 9p24 (PD-L1, PD-L2 and JAK2), 17q (HER2, MLLT6), 18q (ZNF521, SS18, SETBP1), 19q (AKT2, CDX2) or 22q (CRKL, CTCL1). No case exhibited mismatch repair deficiency or microsatellite instability (0/40). PD-L1 expression in tumor cells (TC+) was detected in 3/40 cases, including the case with 9p24 amplicon.

Targetable HER2 amplification was detected in the esophageal adenocarcinoma. Two patients were enrolled among the RSPO3 fusion patients in the OncoMed phase 1 trial (OMP-131R10, a first-in-class anti-RSPO3 antibody): one duodenal and one small bowel primary.

Conclusions: The majority of gastrointestinal adenocarcinomas driven by an RSPO3 fusion exhibit additional pathogenic mutations in genes that include TP53, KRAS, NRAS and BRAF. APC may also rarely be mutated. Immune checkpoint inhibition may be an option for rare cases with PD-L1 overexpression and high tumor mutational burden.

Conflict of interest: Ownership: Caris Life Sciences. OncoMed Pharmaceuticals.

445 (PB-108) Poster

Targeting tumour hypoxia with tarloxotinib improves the therapeutic efficacy of checkpoint blockade

R. Fu[1], K. Farrand[2], C. Guise[1], J. Smaill[1], I. Hermans[2], A. Patterson[1]. [1]*The University of Auckland, Faculty of Medical and Health Sciences, Auckland, New Zealand;* [2]*Malaghan Institute of Medical Research, Cancer Immunotherapy, Wellington, New Zealand*

Background: The magnitude of a T-cell response is regulated by various stimulatory and inhibitory receptors expressed on the cell surface. The interaction between inhibitory receptors and their ligands can be exploited by tumours to suppress the anti-tumour immune responses. Checkpoint blockade (CB) acts by inhibiting the interactions between these immuno-suppressive molecules, and thus relieves T-cells from such negative regulations. This therapeutic approach is capable of eliciting robust anti-tumour immune responses in cancer patients, but clinical studies have shown that only a minority of patients will experience durable responses, indicating underlying resistance mechanisms to CBs. Accumulating evidence suggests that tumour hypoxia can suppress the immune response via numerous pathways and T-cells must overcome such antagonism to kill cancer cells. We therefore hypothesised that the elimination of tumour hypoxia by administering tarloxotinib, a hypoxia-activated prodrug (HAP) of the potent irreversible EGFR/HER2 inhibitor tarlox-TKI, may improve the outcome of CBs in EGFR-dependent tumour models.

Material and Methods: Sensitivities of syngeneic murine cancer cells to tarloxotinib was evaluated using anti-proliferative assay in which cells were exposed under aerobic or hypoxic conditions for 4 hours. Total protein content was measured after 5-day incubation and is reflective of total cell proliferation. Cellular sensitivity was related to inhibition of the EGFR signalling network. Tarloxotinib metabolism was measured under anoxic conditions using mass spectrometry detection of metabolite (tarlox-TKI) formation in culture media. For *in vivo* and *ex vivo* studies, cells were inoculated subcutaneously to the flank of C57Bl/6 mice and the anti-tumour activities of tarloxotinib in combination with checkpoint blockade were evaluated. Tumour and lymphoid tissues were harvested to examine changes in hypoxic cell content, immune cell population, functionalities and proliferation of T-cells, and effect of HAPs on normal immunological functions.

Results: Tumour cell lines that are sensitive to tarloxotinib under hypoxia were identified and evaluated *in vivo*. *In vivo* studies showed that treatment with tarloxotinib reduced the hypoxic fractions within these tumours and delayed tumour growth when combined with CBs. Investigation of the immune cell populations in different tissues demonstrated favourable

changes in the percentage and number of different T-cell subsets and suppressor cells after the administration of tarloxotinib. Further studies showed increased antigen-release to the lymph nodes, increased T-cell proliferation in the spleen and changes in cytokine production profiles after tarloxotinib administration.

Conclusion: Tarloxotinib can be combined effectively with anti-PD1 or anti-CTLA4 to greatly improve their anti-tumour activity *in vivo*.

Conflict of interest: Ownership: Rain Therapeutics Inc holds worldwide development rights to tarloxotinib. Advisory Board: Dr Adam Patterson is a member of the Scientific Advisory Board of Rain Therapeutics Inc. Dr Jeff Smaill is a member of the Scientific Advisory Board of Rain Therapeutics Inc. Board of Directors: No conflicts. Corporate-sponsored Research: No conflicts. Other Substantive Relationships: No conflicts.

446 (PB-109) Poster

Inhibition of microRNA (miR) 141-3p induces autophagy through increased tuberous sclerosis 1 (TSC1) expression in esophageal cancer cells

P. Phatak[1], K. Asrani[2], I.E. Chesnick[3], A.W. Hamburger[4], J.M. Donahue[5]. [1]*Baltimore Veterans Affairs Medical Center – University of Maryland School of Medicine, Surgery, Baltimore, USA;* [2]*Johns Hopkins School of Medicine, Pathology, Baltimore, USA;* [3]*Baltimore Veterans Affairs Medical Center, Research, Baltimore, USA;* [4]*University of Maryland School of Medicine, Pathology, Baltimore, USA;* [5]*Baltimore Veterans Affairs Medical Center – University of Alabama at Birmingham School of Medicine, Surgery, Birmingham, USA*

Background: MiR-141-3p has been shown to be oncogenic in some malignancies, while functioning as a tumor suppressor in others. Its role in esophageal cancer is not well defined. Our prior studies show marked upregulation of miR-141-3p in the esophageal cancer lines TE7 and TE10 compared to esophageal epithelial cells. MiR-target sequence analysis predicts miR-141-3p to bind TSC1 mRNA with high affinity. TSC1 has been demonstrated to promote autophagy and function as a tumor suppressor. Study goals were to determine expression of TSC1 in esophageal cancer cells, explore the interaction between miR-141-3p and TSC1, and characterize the functional implications of this interaction.

Methods: Studies were performed in human esophageal cancer lines TE7, TE10, OE33, and FLO-1. MiR-141-3p and TSC1 mRNA expression levels were measured by real-time PCR. TSC1 protein expression levels were examined by Western blot. MiR-141-3p function was tested by its overexpression and silencing. Nascent protein synthesis was analyzed using the *Click-iT* detection assay. MiR-141-3p and TSC1 mRNA interaction was assessed using biotinylated RNA pull-down followed by digital droplet PCR and confirmed by luciferase assay. Autophagy was analyzed by CYTO-ID and immunoblots for LC3A/B and SQSTM1 (p62). Real-time cell migration and invasion were observed on an xCELLigenceRTCA.

Results: MiR-141-3p levels are markedly increased in TE7, TE10, and OE33 esophageal cancer cells compared to FLO-1 cells. TSC1 protein expression is reduced in these cells compared to FLO-1 cells. Silencing miR-141-3p in TE7 and OE33 cells lead to increased TSC1 protein levels, with no change in TSC1 mRNA levels. In reciprocal experiments, TSC1 protein expression levels decreased following miR-141-3p overexpression in FLO-1 cells, with no change in TSC1 mRNA levels. TSC1 mRNA translation decreased after ectopic expression of miR-141-3p in FLO1 cells. MiR-141-3p and TSC1 mRNA interaction was confirmed by enrichment of biotinylated TSC1 mRNA levels and with luciferase reporter constructs. Inhibition of miR-141-3p in TE7 cells led to increased autophagy, with a rise in LC3A/B expression and decreased SQSTM1(p62) expression, confirmed by live cell fluorescence assay. Finally, silencing miR-141-3p resulted decreased migration and invasiveness in TE7 cells.

Conclusions: Esophageal cancer cell line subsets exhibit differential expression of miR-141-3p. MiR-141-3p binds TSC1 mRNA, resulting in decreased TSC1 translation and protein expression. In cells exhibiting elevated miR-141-3p expression, silencing miR-141-3p results in increased TSC1 protein expression, leading to up-regulation of autophagy and reduction in migration and invasion.

No conflict of interest

Friday, 16 November 2018

POSTER SESSION

Paediatric Oncology

447 (PB-110) Poster

Anaplastic Lymphoma Kinase as an ADC target for the treatment of neuroblastoma

S. Leonard[1], R. Forfar[1], D. Moore[1], J. Large[2], C. Fernandes[3], R. Brown[3], C. Southern[1], D. Matthews[3], L. Friis[3], D. Taylor[1]. [1]*LifeArc, Biology, Stevenage, United Kingdom;* [2]*LifeArc, Chemistry, Stevenage, United Kingdom;* [3]*LifeArc, BioTherapeutics, Stevenage, United Kingdom*

Anaplastic Lymphoma Kinase (ALK) is a recognised neuroblastoma tumour antigen associated with the failure of developing neural crest tissue to completely differentiate. While ALK tyrosine kinase inhibitors have been successfully employed for the treatment of ALK-rearranged tumours, drug-resistance can limit use. Novel methods are therefore urgently needed to treat this rare paediatric cancer. As ALK is aberrantly expressed on neuroblastoma tumours and largely undetectable in normal tissue, an antibody drug conjugate (ADC) targeting this receptor may be a promising approach.

Through an immunisation campaign we identified a panel of monoclonal antibodies that can bind to immobilised antigen with high affinity. However, ALK proved to be a challenging target, particularly as it undergoes proteolytic cleavage at a major site in the extracellular domain where some candidates bind. Nevertheless, we have identified a lead antibody which effectively kills neuroblastoma cell lines when conjugated to a cytotoxic payload, progressing the development of an innovative therapeutic for this devastating disease.

No conflict of interest

448 (PB-111) Poster

Transcriptome analysis in childhood medulloblastoma identifies novel transcripts associated with survival

M. Mateos[1], D. Williamson[1], S. Bailey[1], S.C. Clifford[1]. [1]*Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom*

Background: Medulloblastoma is the commonest malignant brain tumour in childhood, with a progression-free survival (PFS) <45% for children with high-risk (HR) features. We sought to evaluate known (associated with curated genes) and novel transcripts in primary medulloblastoma samples, to identify prognostic biomarkers that outperformed current clinical risk-stratification schemes.

Materials and Methods: Our cohort consisted of 217 patients with clinical and RNA sequencing data (Illumina, 90 million paired-end reads). Functional and pathway analysis of transcripts significantly associated with overall survival (OS) and progression in univariate analysis was conducted. Survival analyses were performed for children aged 3–16 years, treated with curative intent. Multivariable survival analyses were adjusted for risk criteria (large cell anaplasia, metastases, *MYC*/*MYCN* amplification, resection, sex, molecular subgroup, *TP53* status, q13 loss) for (i) overall cohort (n = 145) (ii) Group3/Group4 (G3G4) (n = 95). Statistical analyses were performed in R, for categorical (<median, ≥median) and continuous expression data, and were corrected for multiple testing.

Results: The median age of the cohort was 7.5 years (range 0.24–43 years). From total >60,000 known transcripts, 9693 known transcripts that met criteria related to variability of expression were further assessed. Transcripts (n = 547) associated with OS in univariate cox regression were significantly enriched in cell junction, membrane and oxidoreductase functions. Transcripts associated with PFS in G3G4 showed enrichment in metabolic pathways (putrescine biosynthesis $P = 4.01E-03$, 4-hydroxyproline degradation $P = 6E-03$). Top pathways included the targetable *P38 MAPK – ERK1/2* pathway (featuring *NQO2*).

Three transcripts were independently significant for OS for the entire cohort (categorical data). *CXXC4* is a negative regulator of the canonical WNT/beta-catenin signalling pathway and alters DNA methylation. For G3G4, multivariable PFS analysis (adjusting for G3G4 HR status, *MYC*, q13 loss, sex), categorical and continuous expression data revealed four and 10 transcripts respectively that were independently prognostic. These included transcripts related to oxidoreductases (*NQO2, SELENOW*), neuronal toxicity (*STIL)* and an oncogenic kinase (*MELK*).

From 3150 novel transcripts, 217 were further assessed. Within G3G4, there were two novel transcripts that were independently significant in each of: OS (continuous), PFS (continuous) and PFS (categorical expression).

Conclusion: This study demonstrates that the transcriptome provides additional prognostic information in childhood medulloblastoma, beyond known clinico-pathological risk factors. The next step is to validate these markers in further cohorts and to exploit their use as biomarkers and/or therapeutic targets, particularly in G3G4 medulloblastoma.

No conflict of interest

449 (PB-112) Poster

Pharmacological monitoring of asparaginase (ASP) activity during Erwinia C. Asparaginase (ERW-ASP) treatment in pediatric patients with acute lymphoblastic leukemia (ALL), after an hypersensitivity reaction (HSR) to E. Coli PEG Asparaginase (PEG-ASP)

M. Zucchetti[1], T. Ceruti[1], C. Matteo[1], A. Colombini[2], D. Silvestri[3], V. Conter[2], F. Falcetta[1], L. Lo Nigro[4], C. Micalizzi[5], R.M. Mura[6], F. Petruzziello[7], M. Lebiu[2], E. Brivio[2], E. Barisone[8], L. Bettini[2], M. Rabusin[9], L. Vinti[10], M. Uggeri[3], M.G. Valsecchi[3], C. Rizzari[2]. *[1]IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Oncology, Milano, Italy; [2]Clinica Pediatrica Università di Milano-Bicocca, Ospedale S. Gerardo, Pediatria, Monza, Italy; [3]Università di Milano-Bicocca, Health Science, Monza, Italy; [4]Ospedale AOU Pliclinico Vittorio Emanuele, UOC Ematologia Oncologia Pediatrica/TMO, Catania, Italy; [5]IRCCS-Istituto G. Gaslini, Dipartimento Funzionale Ematoncologia, Genova, Italy; [6]Azienda Ospedaliera Brotzu, SC Oncoematologia Pediatrica e Patologia della Coagulazione, Cagliari, Italy; [7]AORN Santobono-Pausilipon, Oncoematologia, Napoli, Italy; [8]AOU Presidio Infantile Regina Margherita, Oncoematologia Pediatrica e CTO, Torino, Italy; [9]IRCCS Burlo Garofolo, SC Emato Oncologia Pediatrica, Trieste, Italy; [10]Ospedale Bambin Gesù, Ematologia Oncologia e medicina Trasfusionale, Roma, Italy*

Background: PEG-ASP is a cornerstone drug in the treatment of childhood ALL. However, 2–18% of the patients cannot successfully complete the PEG-ASP planned treatment due to the occurrence of a clinically evident HSR. ERW-ASP is an alternative formulation of ASP which is given to patients displaying HSR or SI in order to maintain the ASP plasma activity >100 IU/L (target value). A pharmacological monitoring of the ERW-ASP activity is performed in the patients enrolled in the AIEOP-BFM ALL protocols. The preliminary results of this study are here reported.

Patients and Methods: Forty-six patients received seven doses of ERW-ASP (20,000 IU/m^2 i.v. in 2 hours) every 48/72 hours to replace a single dose of PEG-ASP (2,500 IU/m^2) after an HSR. We used an enzymatic test (MAAT test, MEDAC) to evaluate the ERW-ASP serum activity in affected patients. Serum samples were classified in three different classes according to their ASP activity values: optimal (ASP activity ≥100 IU/L), border-line (ASP activity 50–100 IU/L) and inadequate (ASP activity <50 IU/L). We also compared the serum ASPase activity in samples taken at 48 hours or 72 hours post ERW-ASP dose.

Results: The ASP activity levels were analyzed in 331 samples of 46 patients was characterized by a marked inter-patient and intra-patient variability. It was optimal in the 53%, border-line in 21% and inadequate in 26% of samples. In particular, ASP activity measured 48-hours post-dose was ≥100 IU/L in 62% (123/199; mean 335 ± 283 IU/L), between 50 and 100 IU/L in 24% (79 ± 13 IU/L) and <50 IU/L in 14% (29 ± 18 IU/L) of the samples, respectively. Conversely the asparaginase activity detected in the samples withdrawn 72 hours after ERW-ASP administration were adequate in 16% only of samples, while it was inadequate in the vast majority of these.

Conclusions: ERW-ASP, administered instead of PEG-ASP in patients showing HSR, determines levels of adequate or border-line enzymatic activity in 86% of the samples when administered every 48 hours according to the timing suggested by the treatment protocol. Conversely, inadequate activity was detected 72-hours post-dose in 60% of the samples analyzed. These results highlight the need to follow more stringently the indication of administer ERW-ASP every 48 hours to ensure a better exposure of patients to the drug and therefore a continuous elimination of asparagine at systemic level.

No conflict of interest

450 (PB-113) Poster

RNA sequencing identifies differences in immune profiles of tumors from pediatric neuroblastoma patients with Opsoclonus myoclonus syndrome

M. Buchkovich[1], W. Jones[2], V. Weigman[1], M. Rosenberg[3], J. Maris[4]. *[1]Q2 Solutions – EA Genomics, Translational Genomics, Morrisville, USA; [2]Q2 Solutions – EA Genomics, Bioinformatics, Morrisville, USA; [3]Hebrew University of Jerusalem, Alexander Silberman Institute of Life Sciences, Jerusalem, Israel; [4]Children's Hospital of Philadelphia, Department of Pediatrics, Oncology Division, Philadelphia, USA*

Background: Pediatric Opsoclonus myoclonus syndrome (OMS) is a rare, paraneoplastic neurological disorder that presents in a small portion of pediatric neuroblastoma (NBL) cases. OMS causes ataxia, myoclonic jerks, opsoclonus, and disordered mood/behavior in a previously well child. Patients with OMS and NBL have improved tumor outcomes compared to children with NBL alone. Little is known about the molecular basis of OMS disease or the relationship between OMS disease and NBL restriction. Understanding this disorder may improve disease monitoring, offer prognostic indicators for OMS, and lead to improved immune therapy design for NBL. We used RNA sequencing (RNA-seq) data to identify HLA types and examine immune signatures in pediatric NBL tumors to better understand differences between patients with and without OMS.

Materials and Methods: We sequenced RNA from 38 patients with OMS from the Children's Oncology Group ANBL00P3 OMS clinical trial, as well as 5 high risk (HR) and 5 low risk (LR) NBL patients without OMS. We identified HLA types in these data as well as RNA-seq data from 164 NBL patients previously sequenced by the NCI TARGET initiative. Using fisher's exact tests, we tested for associations of HLA alleles with OMS.

We aligned sequence reads from the 38 OMS and 10 non-OMS samples, and compared gene counts to the Immune Landscape Signature (ILS) Portfolio. ILS contains 11 distinct but related groups of genes with continuous scores reflecting activity of immune system subcomponents in the tumor microenvironment. The signatures were derived initially from Newman et al (2015), refined using TCGA tumor expression profiles, and then validated for their predictive and prognostic value in multiple tumor cohorts and cancer types. We used t-tests to identify significant differences between ILS scores of OMS and non-OMS tumors.

Results: In the combined datasets, we identified 9 Class II HLA alleles associated with OMS (FDR q-value <0.05), of which 8 were enriched in OMS samples. Additionally, we detected significantly higher (FDR q-value <0.05) CD28 and CTLA4 expression, as well as increased cytotoxic lymphocyte, CD8, and B-cell ILS scores in the OMS samples.

Conclusions: Using only RNA sequencing, we identified differences in NBL tumor immune profiles of patients with and without OMS. These differences include enrichment of HLA Class II alleles in OMS samples, some of which have previously been associated with Type 1 diabetes, an autoimmune disease. We identified increased B-cell signature scores in OMS patients, consistent with observations of increased tumor infiltrating B-cell counts in these patients, as well as differences in other ILS scores. These analyses provide important insight into the immune profiles of NB tumors in patients presenting with OMS, and may help explain the improved NB outcomes in patients with comorbid OMS disease.

Conflict of interest: Advisory Board: V. Weigman is on the advisory board for Illumina.

451 (PB-114) Poster

The XPO1 inhibitor selinexor reverses aberrant NF-κB pathway activation in RELA-fusion positive ependymoma cells

M. Connelly[1], M. Mellado-Lagarde[1], A. Shelat[1]. *[1]St Jude Children's Research Hospital, Chemical Biology and Therapeutics, Memphis, TN, USA*

Background: Supratentorial RELA-fusion positive ependymoma (ST-EPN-RELA) is an aggressive pediatric brain tumor that is marked by the presence of a gene fusion involving *RELA*, the principal effector of canonical NF-κB signaling, and *C11orf95*, a less studied gene of unknown function. The translocation induces constitutive, aberrant NF-κB pathway activation, resulting in the increased expression of MDM2 and pro-survival members of the Bcl-2 family, and subsequent p53 inactivation and resistance to apoptosis. Exportin 1 (XPO1/CRM1) is the sole nuclear exporter of over 200 protein cargos bearing leucine-rich nuclear export sequences, including major tumor suppressor proteins. Recent reports suggest that the CNS

penetrant XPO1 inhibitor selinexor (SEL) can attenuate NF-κB transcriptional activity by inhibiting the nuclear export of IκB-α, an inhibitor of NF-κB. We hypothesized that SEL could reverse aberrant NF-κB pathway activation in RELA-fusion positive ependymoma.

Material and Methods: All studies were conducted in EP1NS cells grown under neurosphere conditions. Cell viability and the induction of apoptosis were assessed using CellTiter-Glo 3D (CTG-3D) and Caspase-Glo 3/7, respectively. CTG-3D was also used to study SEL in combination with compounds known to activate the p53 pathway. Gene expression was quantified using the Nanostring PanCancer panel and microarray analysis. Changes in expression were confirmed using qPCR and Western blot. The localization of IκB-α following SEL exposure was monitored using Western blot following isolation of the nuclear fraction, and immunofluorescence microscopy.

Results: SEL potently inhibited the viability of EP1NS cells (EC_{50} = 25 nM). Apoptosis peaked at more than 3.5-fold over DMSO control levels following 48 h continuous drug exposure. The combination of SEL with dactinomycin (DAC) was synergistic, and suggests significant potentiation of SEL by DAC concentrations <1 nM. SEL treatment had a profound impact on the levels of genes involved in DNA damage and repair, cell cycle and apoptosis, and chromatin modification. Quantitative PCR and Western blot confirmed upregulation of p53 responsive genes, including *MDM2* and *p21*, and a reduction in the expression of the previously reported RELA-fusion associated NF-κB target genes, *CCND1* and *L1CAM*. Studies of the role of IκB-α in mediating SEL drug response are in progress and will be reported when available.

Conclusions: SEL has the potential to be a targeted therapy for ST-EPN-RELA that suppresses aberrant NF-κB signaling while activating the p53 pathway. The combination of SEL with other inducers of the p53 pathway, such as DAC, MDM2 inhibitors, and AKT inhibitors, and DNA damaging agents, including ionizing radiation, warrants further investigation and may have therapeutic benefit.

No conflict of interest

452 (PB-115) Poster

Development of a physiologically relevant in vitro 3D model of neuroblastoma for reliable screening of disease biomarkers

J. Nolan[1], T. Frawley[1], R. Conlon[1], C. Curtin[2], F. O'brien[2], R. Stallings[1], O. Piskareva[1]. [1]*Royal College of Surgeons in Ireland, Molecular and Cellular Therapeutics, Dublin, Ireland;* [2]*Royal College of Surgeons in Ireland, Tissue Engineering Research Group, Dublin, Ireland*

Background: Neuroblastoma is a highly heterogeneous tumour accounting for 15% of pediatric cancer deaths. The main challenge in treating high risk neuroblastoma is the prevention of metastatic spread and the development of resistance to multiple chemotherapeutics. Interactions between tumour cells, the tissue microenvironment and neighboring cells act as powerful determinants of disease initiation, progression, treatment response and outcome. Exosomal miRNAs and proteins facilitate these interactions. The aim of this study was to investigate the growth of neuroblastoma cells in a physiologically relevant 3D collagen-nanohydroxyapatite (Coll-nHA) scaffold and to assess protein and miRNA expression in exosomes derived from cells grown on this *in vitro* model and *in vivo*.

Materials and Methods: In this study, Kelly (sensitive) and KellyCis83 (resistant) neuroblastoma cell viability and quantity in 3D scaffolds was determined by staining with Calcein AM for live and EthD-1 for dead cells and by DNA quantification using the Quant-iT™ Picogreen® dsDNA assay kit (Invitrogen). Cellular colonization and matrix deposition in scaffolds was assessed on deparaffinised sections using standard Hematoxylin & Eosin staining and cellular infiltration into scaffolds was assessed by nuclear fluorescent DAPI staining. Tumour growth *in vivo* was determined by bioluminescence imaging using IVIS Imaging system (Perkin Elmer) of female Hsd:Athymic Nude-Foxn1nu mice and by weight of primary tumour at time of sacrifice. Exosomes were purified from conditioned media and mouse plasma using Exoquick reagent (SBI). Total exosomal RNA was isolated using the miRNeasy Mini Kit (Qiagen) and profiled using TaqMan Array Human MicroRNA Cards and total protein was analysed by western blotting.

Results: Cell growth and viability in the 3D *in vitro* model closely replicated that seen *in vivo*. KellyCis83 exhibited higher growth than Kelly in the 3D scaffold, mirroring the higher tumour forming ability seen in vivo. Additionally both cell lines exhibited a much higher tolerance for cisplatin at a clinically relevant dose when grown in 3D models compared to 2D *in vitro*. Exosomes derived from KellyCis83 conferred increased colony forming ability to Kelly cells *in vitro*. Exosomes derived from KellyCis83 cells had significantly lower expression of miR-17, miR-92a, miR-106a and miR483-3p than exosomes from Kelly cells when grown in our 3D *in vitro* and *in vivo* models.

Conclusions: This study demonstrates the adaptation of a physiologically relevant 3D scaffold to enable the molecular dissection of the biological cues which underlie neuroblastoma disease progression. Through analysis of cell characteristics and profiling of cells grown in 3D in vitro and in vivo and the exosomes derived from these cells we were able to identify candidate miRNA biomarkers for further investigation in liquid biopsies.

No conflict of interest

453 (PB-116) Poster

Characterization of the genetic landscape of low mutation burden malignancies through multi-parallel genomic and immunologic analysis

T. Trippett[1], M. Berger[2], A. Narendran[3], D. Lyden[4], O. Kovalchuk[5], S. Bodour[6], M. Laquaglia[7], S. Ambati[1], N. Schultz[1], M. Roehrl[1]. [1]*Memorial Sloan Kettering Cancer Center, Pediatric Oncology, New York, USA;* [2]*Memorial Sloan Kettering Cancer Center, Pathology, New York, USA;* [3]*University of Calgary, Pediatric Oncology, Alberta, Canada;* [4]*Memorial Sloan Kettering Cancer Center/Weill Cornell Medical Center, Pediatric Oncology, New York, USA;* [5]*University of Lethbridge, Medical Oncology, Alberta, Canada;* [6]*Keck School of Medicine, Research, Los Angeles, USA;* [7]*Memorial Sloan Kettering Cancer Center, Pediatric Surgery, New York, USA*

Background: A comprehensive simultaneous approach encompassing genomic sequencing, whole exome, exosomal, methylation, transcriptome and proteomic analyses coupled with immunogenic profiling has the potential to unravel the genetic landscape in pediatric tumors and inform insightful therapeutic strategies for children.

Materials and Methods: Biospecimens obtained from 41 patients with low mutation pediatric solid tumor malignancies were analyzed using comprehensive, simultaneous multi-parallel molecular analysis of the genomic landscape. The types of pediatric malignancies included osteosarcoma, rhabdomyosarcoma, hepatocellular carcinoma, hepatoblastoma, and neuroblastoma. Molecular analysis was performed on tumor tissue, adjacent normal tissue and blood. Genomic sequencing on tissue and cell free DNA were conducted using MSK-IMPACT™ (Integrated Mutation Profiling of Actionable Cancer Targets), a custom hybridization-capture based assay developed at Memorial Sloan Kettering Cancer Center for targeted deep sequencing of all exons and selected introns as well as whole exome sequencing, where appropriate. Epigenomic analysis was conducted for methylation abnormalities using whole genome methyl bisulfite sequencing and assessment of transcriptome, small RNAome, signalome and interactome analyses using RNA-Seq. Proteomic analysis was conducted by mass spectroscopy. Tumor and plasma exosomes were isolated as potential biomarkers and analyzed for DNA, RNA, MiRNA and protein content to determine their influence on cellular response and trafficking of different cell types in the tumor microenvironment including immune cells and fibroblasts.

Results: Preliminary data has demonstrated the ability to detect certain alterations missed on targeted genomic sequencing alone. Prominent, distinct methylation abnormalities in solid tumors were detected using whole genome bisulfite sequencing and distinct pathway activation using RNA-Seq. Evidence has been demonstrated that genetic alterations in oncogenes or tumor suppressor genes may not be functionally relevant due to loss in translation, transcription or post translational modifications. Tumor DNA was detected in the tumor exosomes and the characteristic genetic alterations present in the tumors were identified in the plasma exosomes. Flow cytometry and immunofluorescence for immune profiling of tumors were performed in order to validate the immune cell signatures derived from the DNA sequencing of whole genome or exome.

Conclusion: Multi-parallel genomic and immunologic analysis is feasible and has the potential to characterize the genetic landscape of low mutation burden tumors and inform therapeutic strategies for this population of patients.

No conflict of interest