P001 The anti-angiogenic antibody BD0801 demonstrates better anti-tumor activity than bevacizumab and synergize with chemotherapies in multiple tumor models. Liting Xue, Jianxing Tang, Yuyin Ding, Lei Song, Shansen Xu, Yue Huang, Yan Wu, Wenjie Song, Renhong Tang, Wenqing Yang. Jiangsu Simcere Pharmaceutical Co. Ltd., Nanjing, China (Mainland).

Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) play a fundamental role in tumor growth and angiogenesis. An increasing number of evidences have suggested enhanced anti-tumor activity when combining anti-angiogenic agents with chemotherapies in the clinic. To support the clinical development of BD0801, a humanized rabbit anti-VEGF monoclonal antibody, we assessed anti-tumor activities of BD0801 using a series of preclinical tumor mouse models including human ovarian cancer OVCAR-8 and SK-OV-3, human non-small cell lung cancer (NSCLC) NCI-H460, and human renal cancer Caki-1 models, with bevacizumab being used as a benchmark. We also investigated the pharmacological kinetics (PK) of BD0801 in cynomolgus monkeys. BD0801 or bevacizumab were intravenously injected into tumor bearing mice at 0.8 mg/kg, 2.5 mg/kg or 7.5 mg/kg twice a week. Combinations of chemotherapy and BD0801 or bevacizumab were also investigated in some of the tumor models. BD0801 presented more potent anti-tumor activities than bevacizumab in ovarian cancer, NSCLC and renal cancer mouse models in a dose-dependent manner. The tumor growth inhibition of BD0801 was 2-20%, 34-66%, or 32-84% when dosed at 0.8 mg/kg, 2.5 mg/kg or 7.5 mg/kg, respectively. Besides, combination of 2.5 mg/kg BD0801 and 10 mg/kg paclitaxel presented a statistically stronger antitumor activity in human ovarian cancer models compared to both single treatments ($P<0.05$). Combination of 2.5 mg/kg BD0801, 10 mg/kg paclitaxel, and 80 mg/kg carboplatin also presented a stronger antitumor activity than BD0801 or chemotherapy alone in the human NSCLC model. In cynomolgus monkeys received a single intravenous injection of BD0801 at 1.5, 5, and 15 mg/kg, the half-life ($t_{1/2}$) of BD0801 was 39.4-142 h, the clearance (Cl) was 0.321-0.900 mL/h/kg, and the apparent volume of distribution (V_d) was 49.5-64.4 mL/kg. The peak concentrations (C_{max}) of BD0801 were proportional to the doses administered. No difference on the PK parameters was observed between female and male monkeys. Taken together, this study has demonstrated BD0801 as a more potent molecule than bevacizumab in some preclinical tumor models, supporting further clinical development plans for BD0801, especially in combination with chemotherapeutics.

P002 Metabolomic credentialling of murine chemical carcinogenesis model of urothelial cancer. Neveen A. Said. Wake Forest University Health Sciences, Winston Salem, NC.

Bladder cancer (BCa) is the most common malignancy of urinary system with increasing incidence and mortality and limited treatment options. Therefore, it is imperative to validate preclinical models that faithfully represent BCa cellular, molecular, and metabolic heterogeneity to develop new therapeutics. We performed metabolomic profiling of premalignant and non-muscle invasive bladder cancer (NMIBC) that ensued in chemical carcinogenesis N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) mouse model. We identified the enriched metabolic signatures that associate with premalignant and NMIBC. We found that enrichment of lipid metabolism is the forerunner of carcinogen-induced premalignant and NMIBC lesions. Cross-species analysis revealed the prognostic value of the enzymes associated with carcinogen-induced enriched metabolic in human disease. To date, this is the first study describing the global metabolomic profiles associated with early premalignant and NMIBC and provide evidence that these metabolomic signatures can be used for prognostication of human disease.

P003 *In vitro* and *in vivo* characterization of CCR8 humanized mouse model (HuGEMM™). Daniel He[1], Henry Li[2]. [1]Crown Bioscience Inc., Beijing, China (Mainland), [2]Crown Bioscience Inc., San Diego, CA.

Background: CCR8 is a C-C chemokine receptor with 7-transmembrane regions which belongs to the G-protein coupled receptor family. Recently, tumor-infiltrating $CCR8^+$ $CD4^+$ $Foxp3^+$ regulatory T (Treg) cells have been identified as a major immune-suppressive subset in the tumor microenvironment (TME), with CCR8 upregulated in tumor-infiltrating Tregs in patients with breast, lung and colorectal cancer. Among the tumor-infiltrating Treg cells, only 8%-10% of Tregs are CCR8 positive, which are regarded as the master driver populations for Treg-mediated immunosuppression. Thus, CCR8 is increasingly believed to be a potential therapeutic target with monoclonal antibodies specific to human CCR8 (hCCR8) inducing depletion of tumor-resident $CCR8^+$ Treg cells, reverse immunosuppressive signals to enhance the anti-tumor role of effector T cells, particularly in combination with other immune checkpoint inhibitors. Given the lack of cross-binding to mouse targets of human-specific antibodies, we developed a hCCR8 knock-in mouse model (CCR8 HuGEMM) to evaluate the *in vivo* therapeutic efficacy of hCCR8 antibodies. **Methods:** Since there is only one coding exon (exon 2) in both hCCR8 and mouse CCR8 genes, CCR8 HuGEMM was developed by CRISPR/Cas9 engineering to substitute the mouse CCR8 exon 2 for hCCR8 exon 2 in C57BL/6 mice. The hCCR8 expression in CCR8 HuGEMM was confirmed by flow cytometry analysis. The non-tumor-bearing CCR8 HuGEMM mice were then treated with anti-hCCR8 antibodies to investigate the immune cell phenotyping in blood and spleen. Finally, the CCR8 HuGEMM mice were inoculated with either MC38-OVA or Hepa1-6 syngeneic tumors in parallel to evaluate the anti-tumor responses of anti-hCCR8 antibodies *in vivo*. **Results:** The surface expression of hCCR8 on $CD4^+$ and $CD8^+$ T cells, derived from thymus as well as on $FoxP3^+$ Treg cells from CCR8 HuGEMM mice, was characterized by flow cytometry analysis. Monocytic myeloid-derived suppressor cells (MDSCs) ($CD3^-CD11b^+Ly6C^{hi}$) populations decreased significantly in non-tumor-bearing CCR8 HuGEMM mice upon treatment of anti-hCCR8 antibody compared to the control mice, which indicates that anti-hCCR8 may play a critical role in abrogating the immunosuppressive role of MDSC and further retard the MDSC-mediated Treg induction. Furthermore, MC38-OVA and Hepa1-6 syngeneic tumors were inoculated into the homozygous CCR8 HuGEMM mice and the mice were treated with 10mg/kg anti-hCCR8,4 days post tumor engraftment. The anti-hCCR8 alone led to ~30% tumor growth inhibition (TGI) in MC38-OVA tumor model, and ~50% tumor growth inhibition (TGI) in Hepa1-6 tumor model. Similarly, we found the tumor-resident $CCR8^+$ $CD4^+$ $FoxP3^+$ populations decreased significantly post-treatment which suggests that $CCR8^+$ Tregs potentially play an important immunosuppressive role in the TME. **Conclusions:** Our CCR8 HuGEMM model provides an important predictive preclinical model to evaluate the efficacy of hCCR8 antibodies alone or in combination regimens with other immune modulators.

P004 Orthotopic patient-derived xenografts are effective precision oncology models in predicting therapeutic response and acquired drug resistance. Jonathan Nakashima, Jantzen Sperry. Certis Oncology Solutions, San Diego, CA.

Patient-derived xenografts are a functional test system in a living organism, making them the leading assay for precision oncology and drug development. In vivo pharmacology studies are widely performed using subcutaneous implantation; however, due to significant changes in the tumor microenvironment, this methodology falls short of modeling the full complexities of human cancer. Recently evidence has shown different engraftment and growth rates and therapeutic responses if engrafted orthotopically (O-PDX). In this study we sought to compare the differences in pharmacological response between a subcutaneous PDX and O-PDX and apply O-PDX to predict therapeutic response and acquired resistance to therapy.

P005 Establishment and characterization of a panel of advanced prostate cancer patient-derived xenograft (PDX) models for cancer therapeutic evaluation. Jessie Jingjing Wang, Leilei Chen, Xueying Yang, Likun Zhang, Wubin Qian, Henry Qixiang Li. Crown Bioscience Inc., San Diego, CA.

Background: Prostate cancer is one of the most frequently diagnosed solid malignancies and is accountable for the second highest cancer-related deaths in males worldwide. Despite the success of targeting the androgen receptor signaling pathway with androgen deprivation therapy (ADT) and androgen receptor blockage (ARB), relapse and resistance to treatment leads to castration resistant prostate cancer (CRPC) or metastatic CRPC (mCRPC), resulting in poor clinical prognosis and therapeutic outcome. Prostate cancer is a highly heterogeneous disease, with different molecular pathways playing a role in the advanced disease, such as androgen receptor mutations, DNA repair gene deletions, P53 aberrations, RB1 function loss, as well as gene rearrangements, like TMPRSS2-ERG2. Preclinical models representing CRPC heterogeneity and clinically relevant responses to standard of care treatments are needed to better understand the biology and mechanisms of this malignancy. **METHODS:** CRPC PDX models derived from metastases of prostate cancer patients, which failed anti-androgen therapies, were established via subcutaneous engraftment in immunodeficient mice. Model classification and characterization were performed by histopathology and immunohistochemistry (IHC) with prostate-specific biomarkers. Genomic profiling of these models was performed by next generation sequencing (NGS). Chemotherapies such as docetaxel and target therapies, including anti-androgen, were evaluated in these prostate models. Tumor volume over time was used to determine growth rate and response to treatment in uncastrated and castrated mice. **RESULTS:** A series of CRPC PDX models derived from metastatic lesions in patients, including metastases from lymph nodes, bladder, ascites and rib/soft bone, were established and characterized. Histopathology by H&E staining presented distinct features of adenocarcinoma and the immunohistochemistry data indicated various expression levels of prostate-specific markers, such as prostate-specific antigen (PSA) and androgen receptor (AR). Most of the models showed loss of PTEN expression. Sequencing data also detected different gene aberrations such as TMPRSS2-ERG fusion, TP53 deletion and mutations, RB1 mutations, and AR alterations. *In vivo* modeling of these models presented tumor progression under castration. Docetaxel and enzalutamide, commonly used in mCRPC patients, presented varied responses. **CONCLUSIONS:** Prostate cancer presents high heterogenicity in clinical and molecular features, and treatment response. A panel of mCRPC PDX models have been established and characterized to provide clinically relevant preclinical models for prostate cancer therapeutic evaluation.

P006 Novel machine-learning tools improve cost-effective development of personalised immunotherapies: Lowering false positive rates in the search for actionable (personalised and largely shared) immunogenic neoantigens. Cedric Bogaert[1], Lena Pfitzer[1], Nil Adell Mill[2], Bruno Fant[1]. [1]myNEO, Ghent, Belgium, [2]University of Zurich, Zurich, Switzerland.

Personalised immunotherapy approaches rely on the ability of tumour-derived neoantigens to elicit a T-cell immune reaction able to recognise and kill the tumour cells expressing them. Clinical attempts to leverage the power of neoantigens have however yielded mixed results. This can mostly be attributed to the difficulty of finding truly immunogenic peptides from the set of novel peptides generated by mutations in a given cancer patient. In silico approaches can help alleviate this heavy cost by reducing the neoantigen search space, prioritising epitopes based on various parameters such as epitope expression or MHC binding likelihood. Here we present a suite of tools aimed at further assisting clinicians in selecting the most actionable peptides from a set of potential candidates. We developed neoMS, a neural network algorithm able to predict epitope presentation at the cell surface with unparalleled performance. The model achieves up to 0.61 precision at recall 0.4 on its test set, vastly outperforming the current industry standards. In addition, due to his sequence-based comparison method, neoMS exhibits extrapolation capabilities, achieving non-zero predictive power when evaluated on ground truth ligandome data derived from an HLA allele completely absent from the training set. In some cancer indications we showed, moreover, that the neoMS-predicted rate of neoantigen presentation can be used in combination with tumour mutational burden as a high-specificity predictor of response to immune checkpoint inhibitor treatment. Furthermore, the neoIM algorithm is able to discriminate, in an HLA-agnostic fashion, which of the presented peptides will elicit a T-cell immune reaction. This first-in-class algorithm is a random forest classifier specifically trained to classify short peptides of length 9-11 amino acids as immunogenic or non-immunogenic. neoIM vastly outperforms the currently available methods and can predict peptide immunogenicity with high accuracy (AUC=0.88). Interestingly, neoIM confirmed ELISPOT data obtained by Dillon et al. (2017) showing a response in 4 out of 11 breast cancer patients to a vaccine consisting of 9 MHC class-I restricted breast cancer-associated peptides. The 2 antigens that resulted in a CD8+ T-cell specific response were predicted by neoIM as the highest scoring showing its potential in finding the truly immunogenic neoantigens. Taken together, these tools decrease false positive rates significantly as they enable improved identification of immunogenic peptides and the predictions correlate with intensity of immune response and clinical benefits. As such, these tools represent a cost-efficient preliminary step in the search for actionable, immunogenic neoantigens.

P008 *myc* Gene and cancer variant analysis and network interaction: An in-silico analysis. Saeed Kabrah. Umm AlQura University, Makkah, Saudi Arabia.

The *myc* is a proto-oncogene that regulates the cellular process like cell growth, proliferation, metabolism, apoptosis and malignancy pathologies. *myc* deregulation occurs in several cancers and plays a significant role in disease development, metastatic potential, and therapeutic resistance. Drug targeting *myc* in cancer remains highly desirable with substantial challenges, delaying the direct *myc* targeted drugs's development. These drugs target the *myc* transcriptions deregulations in tumours cell by compound inhibiting the inducing of epigenetic silencing or regulation of G-quadruplex structures within the *myc* promoter. Proteins involved in *myc*'s post-translational modulation have been identified as significant surrogate targets for lowering *myc* activity downstream of aberrant cell stimulatory signals.

P009 A shared nearest neighbors approach for integrated, multi-platform networks and its application to the exploration of multiomics data from early-stage non-small cell lung cancers. Stephanie T. Schmidt, Neal Akhave, Alexandre Reuben, Tina Cascone, Jianhua Zhang, Jun Li, Junya Fujimoto, Lauren A. Byers, Beatriz Sanchez-Espiridion, Lixia Diao, Jing Wang, Lorenzo Federico, Marie-Andree Forget, Daniel J McGrail, Annikka Weissferdt, Shiaw-Yih Lin, Younghee Lee, Natalie Vokes, Carmen Behrens, Ignacio I. Wistuba, Andrew Futreal, Ara Vaporciyan, Boris Sepesi, John V. Heymach, Chantale Bernatchez, Cara Haymaker, Jianjun Zhang, Christopher A. Bristow, Timothy P. Heffernan, Marcelo V. Negrao, Don L. Gibbons. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The **I**mmunogenomi**C** pr**O**filing of **N**on-small cell lung cancer (NSCLC) Project (ICON) represents an ambitious undertaking to comprehensively characterize immuno-genomic diversity in NSCLC across diverse platforms. The depth and breadth of this cohort presented a unique opportunity to develop a specialized method for multi-platform data integration and exploration, which can be broadly applied to forthcoming large-scale patient profiling studies. Such a holistic approach can unlock insights for therapeutic targets, biomarkers, and treatment plans by providing a more complete view of phenomena driving disease pathogenesis and evolution. **Purpose**: We developed a novel shared nearest neighbors (SNN) approach to create an integrated network of ICON's multi-platform data and identified collections of closely related measurements within the resulting network tied to noteworthy patient characteristics, including recurrence and oncogenotype. **Methods**: The ICON dataset is derived from tumor and normal lung tissue samples collected from 150 patients at time of resection as well as blood samples collected then and at intervals during the year following. Tissue samples underwent RNA-sequencing (RNA-seq), whole exome sequencing, T-cell receptor sequencing, multiplex immunofluorescence for immune cells, and reverse phase protein array profiling; flow cytometry for immune cells was performed on tissue and blood samples. From these data, the ICON data network was built using an integrative approach based on the SNN algorithm in which genes were linked on the basis of their shared top correlates in orthogonal datasets. **Results**: The ICON data network currently includes over 20,000 genes linked by over 500,000 connections derived from correlations between RNA-seq and orthogonal platforms. We captured established associations between cancer-related genes and examined these along with new ones in the network. To do so, we used the InfoMap algorithm to extract more interpretable sub-networks, termed modules, from the ICON data network. Single sample gene set enrichment scores for each module were used in multivariate analysis to highlight modules linked to clinical characteristics of interest. As an example, we found modules significantly tied to disease recurrence. The most notable of these was strongly associated with metabolic pathways, and other modules associated with platelets and ion channels were also identified. The metabolic pathway module is being explored as a prognostic biomarker, underscoring the opportunites enabled by mining the network. **Conclusions**: Through the framework developed, we identified modules in the ICON data network significantly associated with important patient characteristics like recurrence and oncogenotype. We are validating the gene sets identified as potential biomarkers and are developing an interactive application to facilitate further mining of the network. Taken together, our SNN network-building approach enables the integration and exploration of patient data from diverse platforms.

P012 Genomic and clinical correlates of overall survival (OS) in men with newly diagnosed metastatic castration-sensitive prostate cancer (mCSPC) undergoing intensified androgen deprivation therapy (ADT). Nicolas Sayegh[1], Bennet Peterson[1], Roberto Nussenzveig[1], Adam Kessel[1], Taylor Ryan McFarland[1], Andrew Warren Hahn[2], Deepika Sirohi[1], Manish Kohli[1], Benjamin Louis Maughan[1], Umang Swami[1], Mark Yandell[1], Neeraj Agarwal[1]. [1]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, [2]MD Anderson Cancer Center, Houston, TX.

Background: Despite recent advancements in systemic therapy in men with mCSPC, disease remains fatal. Identification of novel genomic and clinical correlates of survival in this setting remain a significant unmet need. Methods: Newly diagnosed mCSPC undergoing intensified ADT i.e., ADT plus docetaxel or novel hormonal therapy and available tumor comprehensive genomic profiling (CGP) were included in the analysis. CGP was performed by a CLIA certified Next Generation Sequencing panel (Foundation Medicine) and involved the following genes: FAS, KDM6A, MYC1, PTEN, RB1, TMPRSS2, and TP53. All variants of unknown significance were removed, and mutated genes present in ≥5 % patients were included. Cox proportional hazards was used to assess relationships between OS and multiple variables (age at diagnosis, Gleason score, baseline PSA, de-novo disease, volume of disease, presence of visceral metastases and presence of genetic aberrations on CGP). Results: 127 patients were eligible and included. Higher baseline PSA (HR 1.45, 95% CI 1.09-1.9, P=0.0097), presence of visceral metastases (HR 4.41, 95% CI 1.26-15.39, P=0.0199) and genomic aberrations in MYC1 (HR 5.23, 95% CI 1.05-26.04, P=0.0433) and RB1 (HR 32.72, 95% CI 5.35-200.2, P=0.0002) were significantly associated with inferior OS. High volume disease trended to associate with poor OS, but was not statistically significant (HR 1.63, 95% CI 0.56-4.71, P=0.367). PTEN loss and other genomic aberrations were not associated with OS. See Table. Conclusions: In this real-world patient population of men with mCSPC undergoing intensified ADT we identify clinical and genomic markers associated with poor OS. This study has limitations as expected in a retrospective analysis. These data, upon external validation, may aid with development of a risk stratification model, counseling of patients, treatment decision making in the clinic, as well as further drug development.

P014 Clinical impact of evaluating serum IgG fractions in advanced non-small cell lung cancer treated with immune checkpoint inhibitors. Jun Oyanagi[1], Yasuhiro Koh[1], Yasuyuki Akiyama[2], Atsushi Morimoto[2], Koichi Sato[1], Shunsuke Teraoka[1], Daichi Fujimoto[1], Nahomi Tokudome[1], Atsushi Hayata[1], Yuichi Ozawa[1], Hiroaki Akamatsu[1], Masanori Nakanishi[1], Hiroki Ueda[1], Nobuyuki Yamamoto[1]. [1]Wakayama Medical University, Wakayama, Japan, [2]Tosoh Corporation, Kanagawa, Japan.

Background: Structure of N-linked glycan in immunoglobulin G (IgG) fragment crystallizable (Fc) region is associated with antigen-dependent cellular cytotoxicity (ADCC) activity. Although it has been known that N-glycan moiety is different between healthy individuals and cancer patients, the relationship between the N-glycan structure and treatment response in cancer patients is unknown. Here, we evaluated the putative ADCC activity by the N-glycan moiety of IgG-Fc using peripheral blood and analyzed its association with clinical benefits from immune checkpoint inhibitors (ICIs) in patients with advanced non-small cell lung cancer (NSCLC). **Patients and Methods:** Advanced NSCLC patients received nivolumab, pembrolizumab or atezolizumab treatment until disease progression or unacceptable toxicity. Serum samples were collected at baseline. Tumor responses were classified into complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) based on RECIST v1.1. Durable clinical benefit (DCB) was defined as patients whose response was lasting over 6 months. Three IgG fractions (area 1, 2 and 3) corresponding to three peaks identified by affinity chromatography using TSKgel FcR-IIIA-NPR column (Tosoh, Japan) were quantified and the putative ADCC activity was estimated based on the ratio of area 3, which associates with highest ADCC activity, to the whole area. All statistical analyses were carried out using JMP Pro software (ver. 14.0). Mann-Whitney U-test was conducted. Time to event analysis was conducted using Kaplan-Meier methods and the log-rank test. **Results:** Ninety-seven patients were registered in the study between Jan 2016 and Nov 2018 at Wakayama Medical University. Characteristics of the patients were as follows: median age, 70 (range, 49-91); male. 78%; smoker, 79%; previous treatment ≥1, 77%; performance status 0-1, 82%; stage III/IV, 31/69%; squamous/non-squamous/unknown, 29/69/2%; nivolumab/pembrolizumab/atezolizumab, 46/42/12%. The objective response rate was 25.0% (24/96) and the disease control rate was 55.2% (53/96). The DCB rate was 45.4% (44/96). The median progression free survival (PFS) was 72 days (95%CI, 49 to 127) and median overall survival (OS) was 310 days (95% CI, 229 to 475). When time-to-event analysis was conducted by dividing into two groups at the median of the ratio of area 3 to the whole area, OS was significantly longer in patient with higher ratio than those with lower one in both entire cohort (median OS, 952 vs 482; 95% CI, 482 days-not reached and 233-744 days; log rank p=0.0292) and 2nd line cohort (median OS, 952 vs 292; 95% CI, 453 days-not reached and 120-692 days; log rank p=0.0010). Evaluation of IgG fraction was not associated with tumor response or PFS in this study cohort. **Conclusion:** Our results suggest that evaluation of the N-glycan moiety of IgG-Fc in peripheral blood has a potential as a prognostic biomarker in advanced NSCLC patients treated with ICIs.

P015 Biomarker results supporting selection of RP2D from a phase 1b study of ORIC-101, a glucocorticoid receptor antagonist, in combination with enzalutamide in patients with metastatic prostate cancer progressing on enzalutamide. Anneleen Daemen, Shravani Barkund, Ann Johnson, Aleksandr Pankov, Amber W. Wang, Haiying Zhou, Pratik S. Multani, Edna Chow Maneval, Lori S. Friedman, Rupal Patel. ORIC Pharmaceuticals, South San Francisco, CA.

Background: Preclinical studies have shown that activation of the glucocorticoid receptor (GR) leads to resistance to antiandrogens, which is reversed by GR inhibition. ORIC-101 is a potent, selective, orally bioavailable small molecule GR antagonist undergoing clinical development in combination with enzalutamide in patients with metastatic prostate cancer (NCT04033328). **Methods:** Ten patients were enrolled in the dose escalation portion of the Phase 1b study during which three dose levels of ORIC-101 given once daily were evaluated in combination with 160 mg enzalutamide. Translational markers were measured both pre-treatment and on study, including PD biomarkers, blood cortisol, GR and AR protein levels, and genomic alterations. PD biomarkers FKBP5, GILZ and PER1 were assessed by RT-qPCR in peripheral blood mononuclear cells (PBMCs), collected on multiple days and times in the first two cycles before and after dosing. PD modulation and target coverage were evaluable in 9 out of 10 patients. In tumor biopsies with >50 tumor cells, GR, AR and PTEN protein status were evaluated by immunohistochemistry (IHC). Liquid biopsies were collected before treatment, at the end of Cycle 2, and/or the end of treatment: nuclear GR and ARv7 protein levels were measured on circulating tumor cells (CTCs) by immunofluorescence (Epic Sciences, San Diego, CA), mutations and copy number alterations in cancer genes were captured in plasma with the 500-gene GuardantOMNI® ctDNA assay (Redwood City, CA). **Results:** PD suppression after one dose of ORIC-101 was observed in PBMCs of all patients. ORIC-101 reached steady-state target engagement within the first cycle in 6 out of 9 evaluable patients across dose levels, including at the RP2D. Nuclear GR protein was detected by IHC in the 6 pre-treatment biopsies, with H-scores ranging from 11 to 298 (median 95, IQR 55-148). Reduction of GR protein was observed in 3 out of 4 matched on study biopsies (pre-treatment H-scores 110-298), indicating potential elimination of GR positive tumor cells, while the fourth patient had low baseline GR levels (H-score 11). Pre-treatment AR protein levels were consistently high (IHC H-scores 230-300). AR alterations observed in ctDNA or CTCs were L702H (n=1 patient), H875Y (n=1), amplification (n=2), and ARv7 (n=2). PTEN protein aberrations were identified in two patients. The amount of tumor shedding in this cohort ranged from 0.24 to 82% (median 2.2%, IQR 0.54-12.1%), comparable to a clinical cohort of ~10,000 prostate tumors profiled with Guardant360® (Guardant Health). Prevalent genomic alterations at time of enrollment included TP53, RB1, BRCA2, ATM and SPOP, with minimal new genomic changes occurring on treatment. **Conclusions:** Biomarker data from patients enrolled in the Phase 1b study provide evidence of PD modulation, steady-state target coverage, and on-target tumor cell eradication at all dose levels. Tumor characterization in association with clinical outcome provides an opportunity to refine the patient population during the ongoing dose expansion portion of the study.

P018 Integrative proteomics of PARP1 protein complexes and post-translational modifications implicates DDR and AKT-mTOR signaling in mediating response or primary resistance of ovarian carcinoma cells to PARP1 inhibitors. Ou Deng, Sweta Dash, Thales Nepomuceno, Bin Fang, Douglas Marchion, John Koomen, Alvaro N. Monteiro, Uwe Rix. Moffitt Cancer Center, Tampa, FL.

Background: About 40-50% of epithelial ovarian cancers (EOC) show defects in DNA repair by homologous recombination (HR), which are mostly associated with *BRCA1/2* loss-of-function mutations. The PARP inhibitors (PARPis) olaparib, niraparib and rucaparib were recently approved for treatment of ovarian cancer patients with platinum sensitivity and recurrent ovarian cancer who carry inactivating *BRCA1/2* mutations. These targeted drugs produce significant response rates ranging from 40-60% in patients with *BRCA*-linked advanced EOCs, but resistance is a continuing challenge. Whereas several studies have reported various mechanisms of acquired resistance to PARPis, the mechanisms of primary resistance are still poorly understood. Our goal is to develop predictors of PARPi response and to identify new targets for combination therapy to overcome primary resistance. We apply a novel integrated proteomics approach to develop mechanism-based biomarkers of response or primary resistance and to identify new therapeutic targets for rational combination approaches that can overcome resistance to single agent PARPi therapy. **METHODS:** The isogenic EOC cell line pair UWB1.289 with *BRCA1* deletion (parental; UWB) and reconstituted with ectopic *BRCA1* (UWB+B) was used. The effects of FDA approved PARPis on *BRCA1*-null and *BRCA1*-reconstituted UWB1.289 cells regarding short- and long-term cell viability were determined by CellTiterGlo and crystal violet assays. Chemical proteomics, global phosphoproteomics and ADP-ribosylation proteomics were used to identify the components of PARP1-based multiprotein complexes as well as protein post-translational modifications in the DNA damage signaling network in *BRCA1/2*-linked EOC cells. Specific PARP1-engaged protein complexes were further determined by immunoblotting. Frozen *BRCA1*-proficient and deficient ovarian cancer patient tumor samples collected at the time of debulking were also characterized by chemical proteomics. **RESULTS:** Cell viability assays confirmed the expected correlation between PARPi response and *BRCA1/2* status. Chemical proteomics followed by validation with co-immunoprecipitation revealed differential composition of the PARP1/2-Ku70/Ku80 protein complexes in PARPi-sensitive UWB compared to UWB+B cells. Global phosphoproteomics and ADP-ribosylation proteomics further indicated that rucaparib induced the cell cycle and c-NHEJ pathways in UWB cells, but down-regulated the MAPK pathway in UWB+B cells. In addition, our results showed that inhibition of AKT PARylation and AKT-mTOR signaling may help to preserve cell viability in UWB+B cells after rucaparib treatment. Consistently, synergy with DNAPKi and AKTi was more pronounced in UWB+B cells. **CONCLUSION:** Ovarian cancers that do not respond to PARPi displayed significant changes in PARPi-engaged protein complexes as well as post-translational protein modifications. The combination of chemical, phospho- and ADP-ribosylation proteomics can generate a systems view of PARP1 complexes and diverse drug compensatory signaling in EOC.

P020 Schlafen 11 (SLFN11) as a predictive biomarker of the response to TAS1553, a novel small molecule ribonucleotide reductase subunit interaction inhibitor. Hiroto Fukushima, Hiroyuki Ueno, Takuya Hoshino, Wakako Yano, Hiraku Itadani, Miki Terasaka, Sayaka Tsukioka, Takamasa Suzuki, Shoki Hara, Yoshio Ogino, Khoon Tee Chong, Tatsuya Suzuki, Yoshihiro Otsu, Satoshi Ito, Nozomu Tanaka, Seiji Miyahara. Taiho Pharmaceutical Co., Ltd., Tsukuba, Japan.

Background: Ribonucleotide reductase (RNR) plays a crucial role in dNTP biosynthesis, which is required for DNA synthesis and repair, and is thought to be an attractive cancer therapeutic target. However, the precise significance of RNR inhibition remains to be elucidated, since reported RNR inhibitors exhibit limited pharmacological potency and off-target effects. We have developed a highly potent and novel small molecule RNR inhibitor, TAS1553, and reported that TAS1553 disrupted the protein-protein interaction between RNR subunits and exhibited the broad antiproliferative activity against human cancer cells in both *in vitro* and *in vivo* via oral administration. Phase-I study is currently ongoing and the identification of a predictive biomarker is essential to maximize clinical benefit of TAS1553. Here, we report a predictive biomarker of the response to TAS1553, and a clinical development strategy with the biomarker use. **Material and methods:** TAS1553 was synthesized at Taiho Pharmaceutical Co., Ltd. DNA replication stress, apoptosis, caspase activity and cellular growth inhibition induced by TAS1553 were assessed by western blotting, immunofluorescence staining, Caspase-Glo 3/7 assay and CellTiter-Glo® 2.0 assay, respectively. Cells were transfected with 2 nM siRNAs against SLFN11 (siSLFN11) and used for caspase-3/7 activation assay and cell proliferation assay. **Results:** TAS1553 induced intracellular pChk1, pRPA2 and γH2AX, followed by cleavage of PARP and caspase-3, suggesting that TAS1553 causes massive DNA replication stress. Then, we explored factors, involved in DNA replication stress, to predict response to TAS1553. Cytotoxicity profiling revealed broad antiproliferative activity of TAS1553 against both human hematological and solid cancer cell lines in a dose-dependent manner (GI_{50} = 228-4150 nmol/L), and global gene expression profiling revealed that cells with high SLFN11 mRNA expression showed a higher sensitivity to TAS1553. TAS1553 exerted growth inhibitory activity without any cell killing effect against cells with low SLFN11 expression even at a concentration of 10 μmol/L, but cell killing activity against cells with high SLFN11 expression. Depletion of SLFN11 by siSLFN11 treatment in A673 cells which have high SLFN11 expression suppressed cytotoxic effect but not the growth inhibitory effect of TAS1553. Furthermore, we observed suppression of caspase-3/7 activation induced by TAS1553 in A673 cells transfected with siSLFN11. SLFN11 appeared to sensitize cancer cells to TAS1553 via promoting apoptosis. **Conclusions:** TAS1553, a novel orally available RNR inhibitor, showed potent antitumor activity in preclinical models of both hematological and solid tumors. Thus, TAS1553 could be a promising therapeutic agent for cancer, and SLFN11 could be a predictive biomarker in order to maximize clinical response to TAS1553.

P021 Genomic biomarkers for response to 9-ING-41, a small molecule selective glycogen synthase kinase-3 (GSK-3) inhibitor, in pancreas cancer: Preliminary results.

Brittany A. Borden[1], Andre De Souza[1], Devalingam Mahalingam[2], Steven Powell[3], Pamela N. Munster[4], Kelsey Huntington[1], Andrew Mazar[5], Ludmila Calvacante[6], Francis J. Giles[6], Wafik S. El-Deiry[1], Howard P. Safran[1], Benedito A. Carneiro[1]. [1]Brown University, Providence, RI, [2]Northwestern University, Chicago, IL, [3]Sanford Health, Sioux Falls, SD, [4]UCSF, San Francisco, CA, [5]Monopar Therapeutics, Wilmette, IL, [6]Actuate Therapeutics, Fort Worth, TX.

Background: 9-ING-41, a small molecule specific GSK-3 inhibitor, demonstrated favorable efficacy and safety in a Phase I clinical trial evaluating 9-ING-41 monotherapy and 8 chemotherapy combinations in 236 patients (pts) that has since been expanded to a Phase II multicenter study (NCT03678883). Interim results documented encouraging clinical activity, especially in patients with advanced pancreatic ductal adenocarcinoma (PDAC) and durable responses in melanoma and ATLL. Potential genomic biomarkers for 9-ING-41 response have not yet been evaluated. **Methods:** Next-generation sequencing (NGS) of tumor samples was obtained, whenever possible, for those receiving 9-ING-41 alone or in combination with chemotherapy across all sites. Genomic sequencing reports were reviewed for somatic alterations deemed potentially actionable or biologically relevant. For those with PDAC, best response was determined using RECIST 1.1 for those who completed at least two cycles of treatment. Chi-square frequency statistics were used to show the observed versus expected rate of pathogenic variants between patients with disease control (complete response [CR], partial response [PR] or stable disease [SD]) and progressive disease (PD). **Results:** NGS results were available for 135 pts across 11 sites. Most common histologies were PDAC (n=32), colorectal (n=17), and melanoma (n=9). Among pts with PDAC, NGS results were available for 32 pts (23 tumor samples, 9 ctDNA). Four patients received 9-ING-41 monotherapy; 28 received 9-ING-41 combined with gemcitabine/nab-paclitaxel (n=18), gemcitabine (n=7), or irinotecan (n=3). The most frequently mutated genes among PDAC included *KRAS* (n=22 pts), *TP53* (n=21), *CDKN2A* (n=12), *SMAD4* (n=4), *CDKN2B* (n=4), *MTAP* (n=3), *ATM* (n=3), *AKT2* (n=2), and *ARID1A* (n=2). Of these 32 pts, 25 pts were evaluable for response: 2 pts had CR, 2 PR, 9 SD, and 12 PD. Among the pts with CR, one had tumor without pathogenic variants, the other displayed *TP53* and *KRAS* mutations. Pts with PR: one tumor had no pathogenic mutations, the other tumor harbored mutations in *ARID1A, TP53, FGF14,* and *ROS1*. Of the nine patients with SD, 8 had *KRAS* mutations, 5 had *CDKN2A* loss of function, and 4 inactivating mutations in *SMAD4*. Eight out of 12 pts with PD had *KRAS* mutations. There were no significant differences between pts who had disease control and those with PD in the frequency of *KRAS* ($\chi 2$= 0.0189, *P*=0.89), *TP53* (χ 2= 0.0712, *P*=0.78), or *SMAD4* (χ 2= 1.9631, *P*= 0.1611). **Conclusions**: 9-ING-41 has shown clinical benefit in patients with PDAC independent of tumor somatic mutational profile. Preliminary analysis does not reveal pathogenic mutations that are associated with clinical benefit. Additional biomarker studies are ongoing.

P022 Clinical and genomic characteristics of tropomyosin receptor kinase (TRK) fusion cancer in community oncology practice. Andrew Klink[1], Abhishek Kavati[2], Ruth Antoine[2], Awa Gassama[2], Tom Kozlek[2], Ajeet Gajra[1]. [1]Cardinal Health, Dublin, OH, [2]Bayer Pharmaceuticals LLC, Whippany, NJ.

Introduction: *NTRK* gene fusions have been observed across age groups in a variety of tumor types and are implicated in approximately 1% of all solid tumor cancers. The FDA approved larotrectinib (2018) and entrectinib (2019) for the treatment of TRK fusion cancer in adult and pediatric populations (larotrectinib: all ages; entrectinib: ≥12 years old). Given the rarity of *NTRK* gene fusions, both approvals were based on trials recruiting small numbers of patients in single arm trials. Characteristics of patients harboring *NTRK* gene fusions in the real-world setting are limited. This study reports the real-world demographic, clinical, and genomic characteristics in patients harboring an *NTRK* gene fusion. **Methods**: A retrospective, observational cohort study of adult patients with a known *NTRK* gene fusion and diagnosed with any advanced or metastatic solid tumor between January 1, 2016 and December 31, 2019 was conducted. Characteristics and testing patterns were abstracted from patient medical records by 19 medical oncologists (89.5% community-based; 10.5% academic/teaching hospital) across all geographic areas in the US (21.1% Northeast, 10.5% Midwest, 36.8% South, 31.6% West). Descriptive statistics were used to summarize patient characteristics and testing patterns. **Results**: Among the 110 patients included in the study, the median (range) patient age at advanced/metastatic diagnosis was 62 (39-77) years. The majority of patients (58.2%) were male and white (79.1%). Among the 15 solid tumor types observed, lung (24.5%), cholangiocarcinoma (13.6%), pancreatic (10.9%), and colorectal (10.0%) were reported in at least 10% of the study cohort. Half (50.0%) of patients had an *NTRK1* gene fusion, and 27.3% and 20.0% had *NTRK3* and *NTRK2* gene fusion, respectively. The five most commonly reported *NTRK* gene fusion partners were *ETV6-NTRK3* (13.6%), *TPM3-NTRK1* (7.3%), *PPL-NTRK1* (4.5%), *SQSTM1-NTRK1* (4.5%), and *TPM3-NTRK2* (4.5%). Median time from initial cancer diagnosis to *NTRK* gene fusion testing was 11.5 (interquartile range, 1-149) days. Next generation sequencing was used to identify *NTRK* gene fusions in 69.1% of patients (8.2% performed as confirmation to immunohistochemistry testing), followed by fluorescent in situ hybridization studies (FISH) in 14.5% of patients and unknown in 16.4% of patients. **Conclusion**: TRK fusion cancer was most commonly observed in lung, cholangiocarcinoma, pancreatic, and colon cancers. Patients harboring *NTRK* gene fusions are being identified soon after initial cancer diagnosis across a variety of tumor types and age groups in routine clinical practice. Early detection of *NTRK* gene fusions can inform the clinician regarding use of TRK inhibitors earlier in the course of disease when indicated.

P023 Achieving synergism in combination chemotherapy for gastric cancer treatment.
Ozen Leylek, Gulnihal Ozcan. Koç University, Istanbul, Turkey.

Gastric cancer (GC) is the fifth most common cancer and the fourth leading cause of deaths from cancer. Although the combination of conventional chemotherapeutics with molecular targeted agents targeting tumor-specific biomarkers has increased the treatment efficacy in certain types of cancer such as breast cancer, similar success could not be achieved in GC treatment. Therefore, there is an urgent need for combination protocols that can increase the therapeutic success in GC therapy. Synergism is the most important requirement for the success of drug combinations in cancer therapy. Besides the choice of anti-cancer agents included in combination protocols, the order of application and the time interval between drug applications, are important determinants of synergism. In this study, we aim to test whether sequential application of conventional chemotherapeutic-molecular targeted agent pairs can transform antagonistic response into synergistic response in GC. To achieve the goals of this study, we have screened dual combinations of five different chemotherapeutics from anthracyclins, platinium derivatives, taxanes, fluoropyrimidines and topoisomerase inhibitors, and three different molecular-targeted agents targeting EGFR, mTOR or c-Met in four different GC cell models to identify synergistic and antagonistic drug pairs. The most antagonistic drug pairs are selected to test whether sequential application schedules can transform antagonism into synergism for these drug pairs. Drug pairs that display strongest synergism in concomitant applications are also further analyzed in detail to investigate their potential in GC treatment. The degree of synergism is measured with Chou-Talalay Method, and apoptosis, autophagy and cell cycle responses were investigated to identify possible molecular mechanisms of synergism. Our data shows that sequential application schedules can transform antagonism into synergism. We believe that the treatment schedules that will be defined in this study can increase the success of chemotherapeutic-molecular targeted agent combinations in GC.

P024 Antitumoral effect of telotristat ethyl as a single agent in neuroendocrine tumor cell lines and potential synergies. Arantzazu Sierra Ramirez[1], Marinela Méndez[2], Adrián Plaza[1], Andrés Pastor[1], Javier Molina Cerrillo[3], Pablo José Fernández Marcos[1], Teresa Alonso Gordoa[4], Enrique Grande Pulido[5]. [1]IMDEA Food, Madrid, Spain, [2]CNIO, Madrid, Spain, [3]Hospital Ramon y Cajal, Madrid, Spain, [4]Hospital Ramón y Cajal, Madrid, Spain, [5]MD Anderson Medical Center, Madrid, Spain.

Neuroendocrine tumors (NET) comprise a rare and heterogeneous family of tumors arising from cells throughout the diffuse endocrine system. Carcinoid syndrome, a common gastrointestinal and lung NET complication, is mainly caused by serotonin release into the bloodstream, promoting diarrhea, flushing and carcinoid heat disease. By inhibiting the tryptophan hydroxylase, telotristat ethyl diminishes the synthesis of serotonin and improves carcinoid-related bowel movements. However, to our knowledge, there is no data showing the potential activity of telotristat ethyl as anti-tumoral agent and not only as a symptom-reliever . The purpose of this study is to analyze possible anti-tumoral effects of telotristat ethyl in NETs. For this, we used the pancreatic NET cell lines BON-1 and QGP1, and the gastrointestinal cell line HROC57. We treated them with telotristat ethyl as a single agent or in combination with common NET therapies, such as somatostatin analogues, mTOR inhibitors, standard chemotherapy and tyrosine kinase inhibitors. We analyzed the effects of these treatments on cellular viability, apoptosis markers, cell cycle and signaling pathways. Telotristal ethyl as single agent induced apoptosis measured by MTT in all NET cell lines after 72 hours of treatment, with IC50 in the µM range. Telotristat ethyl maintained this apoptotic effect even after serotonin addition. Surprisingly, neither the somatostatin analog octreotide nor the standard NET chemotherapy capecitabin/temozolomide induced apoptosis in BON-1, QGP-1 nor HROC57, suggesting that these cell lines are somatostatin analog- and chemotherapy-resistant. mTOR inhibition by everolimus treatment in NET cell lines induced apoptosis as monotherapy with IC50 in the µM range. Telotristat ethyl combination with everolimus produced a strong synergetic effect, dramatically decreasing cell viability in all NET cell lines. Mechanistically, combination of telotristat ethyl with everolimus promoted increasing levels of the apoptosis marker Anexin V with no major effects on cell cycle. mTOR inhibition by everolimus, alone and in combination with telotristat ethyl, decreased phosphorylated levels of the mTOR pathway readout protein S6 and elevated phosphorylated p42/44 MAPK levels, with no clear effects of telotristat ethyl. Our results show that telotristat ethyl has the capacity to inhibit pancreatic and GI NET cells tumor growth as a single agent and seems synergistic when combined with mTOR inhibitors but not with antiangiogenics or chemotherapy.

P025 NUC-3373 is a more potent inhibitor of thymidylate synthase than 5-FU and reduces generation of toxic metabolites. Jennifer Bré, Alison L. Dickson, Oliver J. Read, Ying Zhang, Peter Mullen, Clarissa M. Czekster, David J. Harrison. University of St Andrews, St Andrews, United Kingdom.

Background NUC-3373 is a novel anti-cancer agent designed to replace 5-FU, one of the most widely used therapies across a broad range of tumors, including colorectal cancer (CRC). 5-FU exerts its main anti-cancer activity via the active metabolite, fluorodeoxyuridine-monophosphate (FUDR-MP), which binds and inhibits thymidylate synthase (TS), preventing the conversion of dUMP into dTMP and disrupting DNA synthesis and repair. NUC-3373, a phosphoramidate transformation of FUDR-MP, is designed to bypass 5-FU resistance mechanisms associated with transport, activation and breakdown, and reduce generation of toxic metabolites including FUTP, which is incorporated into RNA causing myelosuppression and gastrointestinal toxicity, and FBAL which causes hand-foot syndrome. Thymidine supplementation rescues cells from NUC-3373 induced cytotoxicity, supporting a DNA targeted mode of action. The aim of this study was to further evaluate the differences between NUC-3373 and 5-FU. **Methods** CRC cell lines HCT116 and SW480 were exposed to sub-IC_{50} doses of NUC-3373 or 5-FU (0.1-25 μM) for 6h. At specific time-points, cells were harvested and analyzed as follows: TS inhibition by western blot, metabolite levels by mass spectrometry (LC-MS & LC-MS/MS) and cell cycle by flow cytometry. In rescue experiments, NUC-3373 and 5-FU were supplemented with thymidine (8 μg/mL) for 24h and cell survival assessed at 96h post-treatment. **Results** In both cell lines, NUC-3373 was a more potent inhibitor of TS than 5-FU with a higher proportion of TS protein bound to FUDR-MP at low concentrations. At 24h, 10 μM 5-FU was required to achieve the same level of TS binding as 0.1 μM NUC-3373 in HTC116 cells and as 0.5 μM NUC-3373 in SW480 cells. TS inhibition by NUC-3373 was almost maximal by 6 hours and was sustained for at least 48 hours. NUC-3373 generated significantly higher levels (50x) of free FUDR-MP compared to 5-FU, resulting in a more pronounced increase in dUMP levels (5x compared to 5-FU). At 48h, NUC-3373 treated cells remained arrested in S phase, while 5-FU treated cells had reverted to a normal cell cycle. FUTP was present in cells exposed to low doses of 5-FU (0.5 μM) but was not detectable in NUC-3373 treated cells. Finally, thymidine supplementation did not alter cell sensitivity to 5-FU but rescued cells treated with NUC-3373. **Conclusion** NUC-3373 generates higher intracellular levels of FUDR-MP and is a more potent inhibitor of TS than 5-FU, leading to more pronounced effects on cell cycle arrest and perturbation of the nucleotide pool that can result in misincorporation of uracil into DNA. Furthermore, NUC-3373 did not generate FUTP, consistent with the observation that patients treated with NUC-3373 in clinical studies have experienced much lower rates of FUTP-related toxicities. NUC-3373 is a potent TS inhibitor with a favorable safety profile.

P026 NUC-7738 alters oxidative phosphorylation and causes terminal differentiation in acute myeloid leukemia cells. Akbar M. Shahid, In Hwa Um, Oliver J. Read, David J. Harrison. University of St Andrews, St Andrews, United Kingdom.

Background NUC-7738, a phosphoramidate transformation of 3'deoxyadenosine (3'dA), is specifically designed to generate the active anti-cancer metabolite 3'-deoxyadenosine monophosphate (3'-dAMP) directly in cells, bypassing key cancer resistance mechanisms of transport, activation and breakdown. NUC-7738 is currently in a Phase I clinical study to assess safety and determine the recommended dose in patients with advanced solid tumors and lymphoma. Acute myeloid leukemia (AML) cells exhibit impaired differentiation, uncontrolled proliferation and are highly dependent on mitochondrial metabolic processes for survival and chemoresistance. The aim of this study was to determine the effect of NUC-7738 on oxidative phosphorylation (OXPHOS) proteins and resulting changes in mitochondria morphology and activation of the intrinsic apoptotic pathway. **Methods** AML cell lines OCI-AML3 and HL-60 were treated with NUC-7738 for 48 hours. Cell differentiation was determined by morphologic modification with Giemsa stain and assessing the expression of surface markers CD11b and CD163 by flow cytometry. Immunoblotting was performed for Mitochondrial Electron Transport Chain Complex I subunit NDUFB8, Complex II subunit SDHB, Complex III subunit UQCRC2, Complex IV subunit COX II and ATP synthase subunit ATP5A. Mitochondria morphology was assessed by confocal microscopy with MitoTracker-Deep Red CMXRos dye. Annexin-V, DAPI, Bcl-2, cytochrome c, and cleaved-caspase 7 were assessed by flow cytometry to determine intrinsic apoptosis. **Results** NUC-7738 increased the proportion of CD11b+ and CD163+ cells in a dose-dependent manner from 6.1% to 28.7% and 9.6% to 26.9%, respectively. CD11b positivity was also increased in HL-60 cells from 14.5% to 31.1%. NUC-7738 induced morphological differentiation as indicated by decreased nucleo-cytoplasmic ratio, appearance of multi-lobed nuclei and vacuolization of the cytoplasm in OCI-AML3 cells. Decreased nucleo-cytoplasmic ratio was also evident in HL-60 cells. NUC-7738 decreased the protein expression of all 5 complexes of oxidative phosphorylation in OCI-AML3 cells at 48 hours and this metabolic disruption was also evident from distinct changes in mitochondria morphology. NUC-7738 activated the intrinsic apoptotic pathway, causing sequestration of BCL-2 and release of cytochrome c, resulting in a decrease in the percentage of live OCI-AML3 cells from 76% to 37% at 48 hours. **Conclusion** Through the ability of NUC-7738 to inhibit OXPHOS complexes with consequent terminal differentiation and activation of mitochondrial-mediated apoptosis, it exploits the mitochondrial characteristics of certain cancer cells, including their greater dependence on OXPHOS for survival than normal cells. NUC-7738 offers a potential treatment option for patients with a variety of malignancies.

P027 MUC1-C integrates chronic activation of the type I and II interferon pathways in treatment resistance of triple-negative breast cancer. Nami Yamashita[1], Atsushi Fushimi[1], Yoshihiro Morimoto[1], Atrayee Bhattacharya[1], Mark Long[2], Song Liu[2], Donald Kufe[1]. [1]Dana-Farber Cancer Institute, Boston, MA, [2]Roswell Park Comprehensive Cancer Center, Buffalo, NY.

Chronic inflammation is intimately linked to cancer progression and resistance to treatment. Triple-negative breast cancer (TNBC) is a recalcitrant malignancy largely unresponsive to cytotoxic, targeted and immunotherapeutic agents. The MUC1-C transmembrane protein, which is aberrantly expressed in TNBCs, evolved in mammals to provide protection of epithelia from the external environment. MUC1-C is activated in epithelial cell responses to inflammation and damage, and induces inflammatory, proliferative and remodeling signaling pathways that are associated with wound healing. However, prolonged MUC1-C activation in settings of chronic inflammation promotes cancer progression and treatment resistance. Recent studies in TNBCs have demonstrated that MUC1-C drives intrinsic activation of type II interferon (IFN) pathway that is linked to chronic inflammation and immune evasion (Yamashita N, et al. JITC 2021). In extending this work, we have now found that MUC1-C activates the type I IFN pathway, which contributes to genomic instability and DNA damage tolerance in TNBC cells. Importantly, our results further demonstrate that MUC1-C interacts with interferon regulatory factor 1 (IRF1) as a key regulatory node in integrating activation of the type I and II IFN pathways. We show that stimulation of TNBC cells with IFN-γ induces nuclear complexes of MUC1-C and IRF1 that drive expression of effectors, such as IDO1 and WARS, which promote immunosuppression in the tumor microenvironment (TME). Of translational relevance, analysis of human TNBC bulk and single cell RNA-seq datasets demonstrated that MUC1 associates with (i) IDO1 and WARS expression, and (ii) depletion and dysfunction of CD8+ T cells in the TNBC TME. Our results further demonstrate that MUC1-C and IRF1 complexes are necessary for induction of RIG-I, MDA5 and STING expression and for IFN-β production in TNBC cells. Consistent with these findings, we show that MUC1-C is necessary for activation of the downstream type I IFN pathway and IFN-related DNA damage resistance gene signature, which includes ISG15, a ubiquitin-like protein that links chronic inflammation and DNA damage resistance. Of translational relevance, studies in BRCA wild type and mutant TNBC cells treated with carboplatin or the PARP inhibitor olaparib demonstrate that MUC1-C is necessary for expression of RIG-I, MDA5, cGAS, STING and ISG15 and that targeting MUC1-C abrogates this response. As a result, MUC1-C is a target for inhibiting DNA damage tolerance and synergistically sensitizing TNBC cells to platinum-based agents and olaparib. In summary, these findings in TNBC uncover an essential role for MUC1-C and IRF1 in chronic activation of the type I and II IFN pathways that promote immune evasion and resistance to replicative stress. Our findings also support MUC1-C as a target for TNBC treatment with chimeric antigen receptor T cells, antibody-drug conjugates and a functional inhibitor that are under preclinical and clinical development.

P028 Proteomic approaches define rocaglates as translation remodelers with multiple protein targets. Tyler A. Cunningham, J. J. David Ho, Paola Manara, Stephen Lee, Jonathan H. Schatz. Sylvester Comprehensive Cancer Center at the University of Miami Miller School of Medicine, Miami, FL.

Intro: Deregulated protein synthesis is a common trait across solid and hematologic malignancies and an attractive target for cancer therapy. Rocaglates compounds that inhibit eukaryotic initiation factor 4A1 (eIF4A1), the essential DEAD-box RNA helicase that resolves mRNA 5'UTR secondary structures during cap-dependent translation initiation. Rocaglates' unique mechanism of action causes sequence-selective mRNA binding by eIF4A1, clamping the inactive helicase onto the transcript. This suppresses translation globally and affects many oncogenic and pro-survival transcripts in particular. Zotatifin, the first-in class synthetic rocaglate, is currently in Phase I clinical trials for the treatment of solid tumors and as an antiviral against SARS-CoV2. Currently, eIF4A1 and DDX3 are the only reported targets of rocaglate-mediated RNA clamping. Employing unbiased proteomic approaches, we have discovered that rocaglates, thought to act as pure eIF4A/translation inhibitors, extensively remodel the translation machinery and translatome. Additionally, mass-spec interrogation for proteins interacting with specific RNA sequences reveals novel targets of rocaglate-mediated, sequence-specific RNA clamping. Methods: We conducted original mass-spectrometry analyses of translational reprogramming by rocaglates. TMT-pSILAC assessed acute changes in protein production, while MATRIX, which captures high-resolution profiles of the translation machinery, revealed translation factors that drive reprogramming in response to rocaglate exposure. We validated results biochemically, in cellulo, and in vivo using patient-derived xenograft (PDX) mouse models. To probe existing and novel rocaglate RNA-clamping targets, we developed unbiased "clampome" assays – in cellulo protein-RNA-pull downs followed by mass-spec analysis of proteins with increased binding to RNA in the presence of rocaglates. Results: We find rocaglates, including zotatifin, have effects far more complex than simple "translational inhibition" as currently defined. Indeed, translatome analysis by TMT-pSILAC revealed myriad up-regulated proteins that drive hitherto unrecognized cytotoxic mechanisms. The GEF-H1 guanine exchange factor, for example, drives anti-survival RHOA/JNK activation, suggesting novel candidate biomarkers of rocaglate clinical outcomes. Translation-machinery analysis by MATRIX identifed rocaglate-induced dependence on specific translation factors including eEF1ε1 that drive remodeling. Novel rocaglate RNA-binding targets revealed by clampome studies remain under detailed evaluation as mediators of drug activities. Discussion: Our original proteome-level interrogation revealed that the complete cellular response to these historical "translation inhibitors" is mediated by comprehensive translational landscape remodeling. Effects on a broader suite of RNA binding proteins than eIF4A1 alone we suggest mediate the potent antitumor activities of these unique compounds, elucidation of which permits development of novel precision approaches to targeted translational deregulation in cancer.

P029 CDC7 inhibitor-induced replication stress generates inflamed aneuploid cells to sensitize immune checkpoint inhibitors. Tomoko Y. Morita[1], Jie Yu[2], Yukie Kashima[3], Kosuke Tanaka[1], Yumi Hakozaki[1], Shun-ichiro Kageyama[4], Akito Nakamura[2], Eric Lightcap[5], Huifeng Niu[5], Karuppiah Kannan[2], Akihiro Ohashi[1]. [1]Exploratory Oncology Research & Clinical Trial Center, National Cancer Center, Kashiwa, Japan, [2]Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA, [3]Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan, [4]National Cancer Center Hospital East, Kashiwa, Japan, [5]Millennium Pharmaceuticals, Inc., Cambridge, MA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA.

Background: Cell division cycle 7 (CDC7), a serine/threonine kinase, plays important roles in the initiation of DNA replication. We developed a highly specific CDC7 inhibitor, TAK-931, as a next-generation of replication stress (RS) inducer. In this study, we preclinically investigated novel aspects of TAK-931 on antitumor efficacy and immunity to evaluate the therapeutic potential of TAK-931 with immune checkpoint inhibitors (ICIs).**Methods:** TAK-931-treated HeLa cells were subjected to cell growth assay, senescence-associated galactosidase (SA-b-gal) activity assay, transcriptome analysis (RNA-seq), and single-cell RNA-seq (scRNA-seq). The flowcytometry (FCM)-based immune profiling panel studies in J558 allograft syngeneic mouse models were performed at Shanghai Medicilon Inc. In vivo efficacy studies in J558 allograft models in combination with anti-mPD-1, anti-mPD-L1, and anti-mCTLA-4 antibodies were performed at Shanghai Medicilon Inc. **Results:** TAK-931 intensively induced RS to consequently generates senescence-associated secretory phenotype (SASP) aneuploid cells, which highly expressed inflammatory cytokines and chemokines. In transcriptome analyses, inflammatory cytokine and chemokine hallmarks were also significantly and intensively enriched in the TAK-931-induced aneuploid cells; 5 out of top-6 enriched hallmarks were inflammatory related. The scRNA-seq analyses revealed that advanced aneuploidy was closely associated with activations in the inflammatory-related and SASP-associated pathways. The FCM-based immune profiling panel studies demonstrated that the tumor infiltrating immune cells (TIICs), such as CD8+ T cells, CD4+ T cells, PD-1+CD8+ T cells, and PD-1+CD4+ T cells, were significantly accumulated in TAK-931-treated J558 mouse allografts, while immune suppressive CD45+ myeloid derived suppressor cells (MDSCs) were significantly decreased. Single-agent treatment with TAK-931 exhibited significant antitumor efficacy and immunity in J558 syngeneic allografts, which confirmed by tumor re-challenging studies. We finally demonstrated that combination treatment with TAK-931 and ICIs (anti-mPD-1, anti-mPD-L1, and andti-mCTLA-4 antibodies) enhances antiproliferative activities in preclinical syngeneic mouse models. **Conclusions:** These preclinical findings suggest the therapeutic potential of TAK-931 in antitumor efficacy and immunity, which may improve clinical benefit of the currently-used immunotherapy by combination treatment.

P030 Chemoprevention by the mutant p53 reactivator SLMP53-2 on ultraviolet radiation-induced skin cancer. Joana B. Loureiro[1], Rita Ribeiro[1], Nair Nazareth[1], Tiago Ferreira[2], Adelina Quaresma[2], Valentina Barcherini[3], Laura Marabini[4], Paula A. Oliveira[2], Maria M. M. Santos[3], Lucília Saraiva[1]. [1]LAQV/REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal, [2]CITAB, Universidade de Trás os Montes e Alto Douro, Vila Real, Portugal, [3]Research Institute for Medicine (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal, [4]Università degli Studi di Milano, Milano, Italy.

The growing incidence of skin cancer (SC) is a global public health concern. Preventive approaches mostly embrace the sunscreen use against ultraviolet radiation (UVR), the main SC etiologic factor. Yet, the increasing SC incident have suggested that additional preventive agents could complement the sunscreen, helping to reverse this trend. Mutant p53 (mutp53) represents a promising target in SC prevention, since its occurrence is a key early event in UVR-induced SC. Thus, the inhibition of mutp53 formation by mutp53 reactivators, which are able to restore the wild-type-like function to p53, would be a valuable strategy for SC prevention. Recently, our group identified the tryptophanol-derived oxazoloisoindolinone SLMP53-2 as a new mutp53 reactivator. Herein, we aimed to study the potential of SLMP53-2 as a chemopreventive drug against UVR-induced SC. For that, assays with normal human keratinocyte HaCaT cells were performed. Cells were pre-treated with SLMP53-2 for 24h, followed by exposure to distinct UVB doses and further analysis. For *in vivo* studies, FVB/N mice were topically pre-treated for 1h prior to UVB radiation (180 mJ/cm^2) and the skin was collected 24h after for immunohistochemistry/immunofluorescence analysis. Pre-treatment of HaCaT cells with SLMP53-2 enhanced cell survival in response to UVB with promotion of cell cycle arrest in G0/G1 phase, upregulation of p21 protein levels, reduction of UVB-induced apoptosis, and inhibition of JNK activity. SLMP53-2 also protected cells from UVB-induced ROS generation and from its consequent lipid peroxidation and protein carbonylation. It enhanced DNA repair through upregulation of the protein and mRNA levels of XPC (xeroderma pigmentosum complementation group C) and DDB-2 (DNA damaged binding protein 2) from the NER (nucleotide excision repair) pathway. In fact, SLMP53-2 depleted UVB-induced DNA damage, as evidenced by a reduction of DNA in comet tails, γH2AX staining and cyclobutane pyrimidine dimers (CPDs) levels. SLMP53-2 also showed promising activity in suppressing UVB-induced inflammation, decreasing COX-2, IL-6 and TNF-α protein levels and reducing NF-kB nuclear translocation and DNA binding ability. Moreover, SLMP53-2 enhanced the expression of key players in keratinocytes differentiation, namely NOTCH1 and keratin 1. Consistently, pre-treatment with SLMP53-2 reduced mutp53 protein levels by potentially restoring its wild-type p53 form, which may underlie the increased resistance of HaCaT cells to UVB harmful effects. Importantly, *in vivo* studies supported that SLMP53-2 pre-treatment of UVB-irradiated mice promoted the survival of epidermal cells, reducing the DNA damage by enhancement of DNA repair and downregulating mutp53 expression levels. Further, it reduced the expression of inflammatory-related proteins and promoted cell differentiation. Collectively, these results support a promising chemopreventive activity for SLMP53-2 against UVB-induced skin damage.
We thank FCT/MCTES for the projects UIDB/50006/2020, UIDP/04423/2020 and fellowship SFRH/BD/128673/2017 (J.B. Loureiro).

P031 MANTRA: A randomized, multicenter, phase 3 study of the MDM2 inhibitor milademetan (RAIN-32) versus trabectedin in patients with de-differentiated liposarcoma.

Mrinal Gounder[1], Gary Schwartz[2], Robin Jones[3], Shreyaskumar Patel[4], Silvia Stacchiotti[5], Andrew Wagner[6], Vijaya Tirunagaru[7], Naisargee Shah[7], Richard Bryce[7], Robert Doebele[7].
[1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Columbia University School of Medicine, New York, NY, [3]Royal Marsden Hospital/Institute of Cancer Research, London, United Kingdom, [4]The University of Texas MD Anderson Cancer Center, Houston, TX, [5]Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy, [6]Dana Farber Cancer Institute, Boston, MA, [7]Rain Therapeutics Inc., Newark, CA.

Background: p53 plays a central role in tumor suppression and maintenance of genome integrity. Murine double minute 2 (MDM2) is a ubiquitin ligase that inhibits p53 transcriptional activity and induces p53 degradation through ubiquitination. *MDM2* amplification occurs in many cancers but is universal in well-differentiated (WD) or de-differentiated (DD) liposarcomas (100% of cases) [Cancer Genome Atlas Research Network. Cell 2017]. Current therapies for WD/DD liposarcomas include anthracycline-based chemotherapy, eribulin, and trabectedin. Inhibition of the MDM2-p53 interaction is a promising therapeutic approach to restore p53 tumor suppressor activity in liposarcomas. Milademetan (RAIN-32) is a small-molecule MDM2 inhibitor that inhibits the MDM2-p53 interaction and restores p53 function at nanomolar concentrations. In a phase 1 study, milademetan showed promising efficacy in patients with WD/DD liposarcoma when administered on an intermittent schedule (260 mg on Days 1–3 and 15–17 every 28 days), with a median progression-free survival (PFS) of 7.4 months [Gounder et al. AACR-NCI-EORTC 2020]. MANTRA (RAIN-3201) is a randomized, multicenter, open-label, phase 3 registration study designed to evaluate the efficacy and safety of milademetan versus trabectedin in patients with unresectable or metastatic DD liposarcoma with disease progression on ≥1 prior systemic therapies, including ≥1 anthracycline-based regimen (EudraCT: 2021-001394-23). **Methods:** Eligible patients are ≥18 years of age with histologically confirmed unresectable and/or metastatic DD liposarcoma, with or without a WD component, who have received ≥1 prior systemic therapies, including ≥1 anthracycline-based regimen, with radiographic evidence of progression within 6 months before study entry. Prior treatment with trabectedin or an MDM2 inhibitor is not permitted. Patients will be randomly assigned (1:1) to receive milademetan (260 mg once daily orally Days 1–3 and 15–17 on a 28-day cycle) or trabectedin (1.5 mg/m^2 as a 24-hour intravenous infusion every 3 weeks). Randomization is stratified by Eastern Cooperative Oncology Group performance status (0 or 1) and number of prior treatments for liposarcoma (≤2 or >2). Tumor response will be evaluated by RECIST v1.1 at Weeks 8, 16, 24, and 32, and then every 12 weeks. Primary endpoint: PFS by blinded independent central review. Secondary endpoints: overall survival; disease control rate; objective response rate; duration of response; PFS by investigator assessment; safety; health-related quality of life. Exploratory endpoints: molecular markers in peripheral blood and/or tumor tissue; milademetan pharmacokinetics. To demonstrate a 3-month increase in PFS (from 3 to 6 months) corresponding to a hazard ratio of 0.5, approximately 160 patients will be required to observe 105 events with 93.9% power and 2-sided significance level of 5%. MANTRA is currently open to enrollment.

P032 Berzosertib plus irinotecan in patients with TP53 mutant gastric/gastroesophageal junction adenocarcinoma: A phase II study. Satya Das[1], G. Dan Ayers[1], Jennifer Whisenant[1], Anwaar Saeed[2], Edward Kim[3], Vaia Florou[4], George Yacoub[5], Percy Ivy[6], Charles Kunos[6], Jordan Berlin[1]. [1]Vanderbilt University Medical Center, Nashville, TN, [2]Kansas University Medical Center, Kansas City, KS, [3]University of California Davis Comprehensive Cancer Center, Davis, CA, [4]Huntsman Cancer Institute, Salt Lake City, UT, [5]Wake Forest University University Health Sciences, Winston-Salem, NC, [6]National Cancer Institute, Bethesda, MD.

Background: Nearly 50% of patients with advanced gastric/gastroesophageal junction (GEJ) adenocarcinoma possess somatic TP53 mutations, with most mutations falling within exons 2 or 4-11. Though the presence of TP53 mutations has historically been considered a poor prognostic factor in advanced gastric/GEJ adenocarcinoma, cancer cells with mutations in TP53 tend to depend on ataxia telangiectasia and Rad3-related protein kinase (ATR) as a primary mediator of DNA damage repair (DDR). In preclinical studies, topoisomerase 1 inhibitors and ATR inhibitors demonstrate synergy in TP53 mutant gastrointestinal cancer cell lines. Based on the dearth of active later-line treatments in patients with advanced gastric/GEJ adenocarcinoma, we initiated a combinatorial study of the highly selective ATR inhibitor berzosertib (formerly VX-970/M6620) with irinotecan in this disease population. **Methods:** The study is a single arm phase II trial of berzosertib plus irinotecan in patients with advanced (progressive on at least one prior line of therapy) TP53 mutant (with mutations in exons 2 or 4-11) gastric/GEJ adenocarcinoma and is sponsored by the National Cancer Institute [NCT03641313]. The primary endpoint of the study is objective response rate (ORR) with key secondary endpoints of overall survival (OS), progression-free survival (PFS), duration of response (DOR) and measuring pharmacodynamic biomarkers of DNA damage induction (γ-H2AX, KAP1 p-Ser 824 and p-ATR) from on-treatment biopsies in select patients. Exploratory aims of the study are to assess ORR, OS, PFS and DOR in patients based upon the presence of other tumor DDR mutations (e.g. BRCA1, BRCA2, MRE11, RAD50, RAD51, NBN, ATM). The study utilizes a Simon's Optimal two-stage design to assess ORR. If 2 or more responses are not observed in the first 9 response-eligible patients on the study, it will close for futility. If the interim efficacy threshold is achieved, 14 more response-eligible patients will be enrolled for a total of 23. A safety lead-in is being utilized for the initial 6 patients as the RP2D of the experimental combination had not yet been fully defined from its preceding phase I study. **Results:** The initial 6 patients have been treated with irinotecan (180 mg/m2 D1,D15 every 28 days) and berzosertib (270 mg/m2 D1,D15 every 28 days). No dose limiting toxicities were observed during cycle 1 in these patients and thus this dose was established as a safe dose for the remainder of the study patients. Patients have experienced grade 3/4 adverse events in 7 instances (most have been hematologic with 1 case of grade 4 neutropenia and 1 case each of grade 3 anemia, neutropenia and thrombocytopenia). Of the initial 6 patients, 1 has demonstrated a partial response while 3 others have demonstrated stable disease on initial response assessment (by RECIST 1.1). **Conclusion:** Berzosertib plus irinotecan appears to be safe in patients with TP53 mutated gastric/GEJ adenocarcinoma. The interim efficacy analysis, along with pharmacodynamic assessments of DNA damage induction, remain pending.

P033 CC-95775, a reversible, oral bromodomain and extra-terminal (BET) inhibitor in patients with advanced solid tumors (STs): Results of a phase 1 study. Thierry Lesimple[1], María José de Miguel[2], Cristophe Le Tourneau[3], Mariano Ponz-Sarvise[4], Marie Paule Sablin[5], Diego Salas Benito[4], Bishoy Hanna[6], Henry Chang[7], Xin Wei[8], Marta Ocejo Garcia[9], Pilar Lardelli[9], Tania Sánchez[9], Josep Lluís Parra Palau[9], Zariana Nikolova[10], Emiliano Calvo[2]. [1]Centre Eugène Marquis, Rennes, France, [2]START Sanchinarro, Madrid, Spain, [3]Institut Curie, Paris, France, [4]Clínica Universidad Navarra, Navarra, Spain, [5]Institute Curie, Paris, France, [6]BMS, Summit, NJ, [7]BMS, San Francisco, CA, [8]BMS, Berkeley Heights, NJ, [9]BMS, Seville, Spain, [10]BMS, Boudry, Switzerland.

Background: BET proteins are epigenetic readers and activators of oncogenic pathways in cancer. CC-95775 is a novel oral small molecule bromodomain inhibitor. It is a non-specific inhibitor with potent activity against all 4 BET family members (BRD2, BRD3, BRD4, BRDT), and shows additional activity towards several non-BET bromodomain proteins. **Methods:** CC-95775-ST-001 is a phase 1 dose-escalation study of CC-95775 in patients with advanced STs. Primary objectives were to determine safety and recommended phase 2 dose (RP2D). Secondary and exploratory objectives were pharmacokinetics (PK), pharmacodynamics (PD) and antitumor activity. Four dose levels (DLs), from 400 to 1200 mg, administered on 4 consecutive days (Day 1 to Day 4) followed by 24 days off, every four weeks (Q4W), were evaluated. **Results**: As of 16 Apr 2021, 24 evaluable patients were enrolled and treated. The RP2D was 1200 mg (300 mg on days 1-4 and 24 days off, Q4W). One patient treated at 800 mg and two at 1200 mg had dose-limiting toxicities: QT prolongation, left ventricular ejection fraction (LVEF) decreased and abnormal T wave. The most common treatment-related adverse event (TRAE) was thrombocytopenia in 11 patients (45.8%), 2 of them grade 3 (8.3%) and 1 grade 4 (4.2%). Safety profile consisted mainly of gastrointestinal and general disorders. Five patients (20.8%) had transient serious TRAEs: nausea, QT prolongation, abnormal T wave, posterior reversible encephalopathy syndrome and acute kidney injury. Eleven patients 11 (45.8) had disease stabilization, 9 of them with a duration of ≥ 16 weeks and 4 of them ≥ 24 weeks: melanoma, chondrosarcoma, adenoid cystic carcinoma and chordoma. Plasma exposures increased in a dose-proportional manner across DLs. Across all dose groups, median Tmax was between ~ 2-4 h post-dose, indicating rapid absorption. The terminal half-life was approximately 30 h and repeated dosing leads to drug accumulation, as expected: ~2 - 3 fold for AUC and Cmax. CC-95775 induced ≥50% decrease of the PD biomarker CCR1 at the 4-hour timepoint in the 1200 mg cohort. **Conclusions:** CC-95775 was well tolerated and showed preliminary antitumor activity in heavily pretreated patients with advanced malignancies. The RP2D was 1200 mg Q4W. The favorable PD profile improved tolerability and enabled less frequent dosing. Further evaluation of CC-95775 alone or in combination in STs is warranted.

P035 A phase 1 pharmacokinetic trial of single agent trametinib a MEK inhibitor in advanced cancer patients with hepatic dysfunction: An NCI Organ Dysfunction Working Group (ODWG) study (NCI 9591). Pei Jye Voon[1], Eric X. Chen[1], Helen X. Chen[2], Albert C. Lockhart[3], Solmaz Sahebjam[4], Karen Kelly[5], Ulka N. Vaishampayan[6], Vivek Subbiah[7], Albiruni R. Razak[1], Daniel J. Renouf[8], Sebastien J. Hotte[9], Arti Singh[1], Philippe L. Bedard[1], Aaron R. Hansen[1], Ivy S. Percy[2], Lisa Wang[1], Lee-Anne Stayner[1], Lillian L. Siu[1], Anna Spreafico[1]. [1]Princess Margaret Cancer Centre, Toronto, ON, Canada, [2]Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, [3]Washington University in St. Louis, St. Louis, MO, [4]Mofitt Cancer Center, Tampa, FL, [5]UC Davis Comprehensive Cancer Center, Sacramento, CA, [6]Karmanos Cancer Institute, Detroit, MI, [7]MD Anderson Cancer Center, Houston, TX, [8]British Columbia Cancer Agency, Vancouver, BC, Canada, [9]Juravinski Cancer Centre, Hamilton, ON, Canada.

Background Trametinib (Mekinist ®) is an oral bioavailable MEK 1/2 inhibitor that is FDA approved in combination with BRAF inhibitor Dabrafenib for *BRAFV600* mutant solid tumors. The single agent recommended phase 2 dose (RP2D) is 2 mg daily (QD). No clinical data is available on recommendation of trametinib dosing in various degrees of hepatic dysfunction (HD). This study was designed to evaluate RP2D, maximum tolerated dose (MTD), and pharmacokinetic (PK) profile of trametinib as primary endpoints in patients (pts) with genomically unselected advanced solid tumors with various degrees of HD. **Methods** Advanced cancer pts with ECOG ≤2, adequate renal and bone marrow functions, were stratified (NCI ODWG Criteria) into 4 HD groups: normal (NO), mild (ML), moderate (MD), severe (SV). NO group was enrolled as control subjects and was not evaluable for dose limiting toxicity (DLT). Trametinib was given QD on a 28-days cycle, with dose escalation based on a "3+3" design within each HD group (starting dose: NO, ML: 2mg; MD: 1.5 mg; SV: 1mg). Due to the long half-life of trametinib, PK samples were collected at days 15-16 in cycle 1. Differences in PK parameters among liver function groups were evaluated using analysis of variance (ANOVA). **Results** Out of 46 pts enrolled (2 pts ineligible), 44 (NO=17, ML=7, MD (1.5mg)=4, MD (2mg)=5, SV (1mg)=9, SV (1.5mg)=2) were evaluable for safety and 22 for PK analysis. The most common cancer type was GI-non CRC cancer (n=16, 36%). The most common all-grade treatment related adverse events (TRAEs) were acneiform rash (NO=53%, HD=48% of pts), nausea (NO=65%, HD=22%), diarrhea (NO=53%, HD=26%) and fatigue (NO=59%, HD=15%). Grade 3/4 TRAEs occurred in 27% (n=12) of pts (NO=8, 47%; HD=4, 15%). No treatment related deaths occurred. DLT was evaluable in 15 pts (ML=6, MD (1.5mg)=3, MD (2mg)=2, SV (1mg)=3 and SV (1.5mg)=1). One DLT (grade 3 acneiform rash) was observed in an SV pt (1.5mg). Dose interruptions or reductions due to TRAEs occurred in 15 pts (34%) [NO=9, 53%; ML=2, 29%; MD (1.5mg)=1, 33%; MD (2mg)=2, 33%; SV (1mg)=1, 11%; SV (1.5mg)=1; 50%]. Best response was stable disease in all HD groups (33 to 75%) and 54% in NO group. There were no significant differences for PK parameters of C_{max} (p=0.18), C_{min} (p=0.16), C_{avg} (p=0.62), or AUC_{0-24} (p=0.11) (NO vs ML, NO vs MD, NO vs SV, ML vs MD, ML vs SV, MD vs SV), when trametinib was normalized to 2 mg dose. However, only limited PK data were available for the MD (n=3) and SV (n=3) groups compared to NO (n=10) and ML (n=6) groups. Trametinib is heavily protein bound, with no correlation between serum albumin level and unbound trametinib fraction (p=0.26). **Conclusion** RP2D for trametinib in ML pts is 2 mg QD. There are insufficient number of evaluable pts to declare RP2D for MD and SV HD groups. No DLTs were noted in the highest dose cohorts that reached 3 evaluable pts: 1.5 mg QD in MD

group, and 1 mg QD in SV group. It may be appropriate for pts with MD and SV HD to start trametinib at 1.5 mg QD and 1 mg QD respectively, and monitored closely for toxicity.

P036 Trial in progress: Combination of the dual RAF/MEK inhibitor VS-6766 with the mTOR inhibitor everolimus with expansion in patients with KRAS mutant NSCLC.

Thubeena Manickavasagar[1], Chara Stavraka[2], Kaiser Anam[2], Silvia Coma[3], Sanjib Chowdhury[3], Jonathan A. Pachter[3], Tom Parker[1], Mona Parmar[1], Laura Finneran[1], Emma Hall[1], James Spicer[2], Anna Minchom[1], Udai Banerji[1]. [1]The Institute of Cancer Research and The Royal Marsden Hospital NHS Trust, London, United Kingdom, [2]King's College London, Guy's Hospital, London, United Kingdom, [3]Verastem Oncology, Needham, MA.

The RAS/RAF/MEK/ERK (MAPK) pathway is a frequently deregulated oncogenic pathway in cancer driven by RAS and RAF mutations as well as being a key signaling pathway downstream of deregulated receptor tyrosine kinases. Although RAF and MEK have been validated as anticancer targets and several BRAF and MEK inhibitors are FDA approved, acquired resistance develops in most patients. Preclinically, inhibition of RAF or MEK has been found to activate AKT/mTOR signaling as a potential resistance mechanism, and combination of MEK inhibition with an inhibitor of the AKT/mTOR pathway yields improved anti-tumor activity. However, combined inhibition of MEK with an AKT or mTOR inhibitor has typically shown poor clinical tolerability. VS-6766 is a unique dual RAF/MEK inhibitor which blocks MEK activity without the compensatory MEK activation that limits the efficacy of other MEK inhibitors. VS-6766 has shown clinical responses as a single agent in gynecological cancers and KRAS mutant non-small cell lung cancer (NSCLC) (Guo Lancet Oncology 2020), and has shown clinical responses in combination with the focal adhesion kinase (FAK) inhibitor defactinib in patients with low-grade serous ovarian cancer and KRAS mutated NSCLC. To explore the combination of VS-6766 with blockade of the AKT/mTOR pathway, we tested the combination of VS-6766 with the mTOR inhibitor everolimus for potential synergy across a variety of RAS pathway mutations and tumor types in preclinical experiments. In 3D proliferation assays in vitro, VS-6766 was synergistic with everolimus in reducing viability of cell lines representing multiple MAPK pathway alterations, including KRAS (G12C, G12D, G12V and G13D), BRAF V600E, NRAS and NF1 mutations. Synergy of VS-6766 with everolimus was observed in 8/9 NSCLC, 16/20 colorectal cancer, 7/10 melanoma and 5/7 pancreatic cancer cell lines tested. These preclinical data support testing the combination of VS-6766 with everolimus in patients with KRAS mutant NSCLC as well as in numerous other cancer indications with various MAPK pathway alterations. A phase I study is ongoing to assess the combination of VS-6766 with everolimus with an intermittent dosing schedule. The clinical trial uses a rule-based design of 3+3 dose escalation cohorts, initially with once weekly dosing of VS-6766 in combination with everolimus. If this was found to be tolerable, the combination of twice weekly dosing of both compounds was to be explored. Following establishment of the recommended phase 2 dose (RP2D) for the combination, an expansion cohort (n = 10) in patients with KRAS mutant NSCLC was planned. The trial is currently recruiting into an expansion cohort of patients with *KRAS* mutated NSCLC ;NCT02407509, (Trial in Progress).

P037 A phase 1/2 dose escalation and expansion study of OP-1250 in adults with advanced and/or metastatic hormone receptor-positive (HR+), HER2-negative (HER2-) breast cancer. Carlos Alemany[1], Manish Patel[2], Zahi Mitri[3], Joseph Sparano[4], Virginia Borges[5], Della Makower[4], Pam Klein[6], Julia Lawrence[6], Trinh Le[6], Jo Anne Zujewski[6], Erika Harmilton[7]. [1]Advent Health Cancer Institute of Florida, Orlando, FL, [2]Florida Cancer Specialists, Sarasota, FL, [3]Knight Cancer Institute at OHSU, Portland, OR, [4]Montefiore Medical Center, Bronx, NY, [5]Anschutz Cancer Pavilion, Aurora, CO, [6]Olema Oncology, San Francisco, CA, [7]Sarah Cannon Research Institute, LLC, Nashville, TN.

Background: Endocrine therapy administered sequentially as monotherapy or in combination with targeted therapy is the primary treatment for HR+, and HER2- metastatic breast cancer (MBC). Most patients with HR+, HER2- MBC will develop resistance to available therapies. More effective therapies are needed for HR+, HER2- MBC and for the treatment of endocrine therapy-resistant disease. OP-1250 is a small molecule Complete Estrogen Receptor ANtagonist (CERAN) that completely inactivates ER, blocking the activity of both the AF1 and AF2 transcriptional activation functions, inhibits ER-driven breast cancer cell growth, and induces ER degradation. OP-1250 demonstrates anti-cancer activity in vitro and in vivo, including activity against mouse models of metastases in the brain and tumors with activating mutations in ESR1. OP-1250 is orally bioavailable with a favorable pharmacokinetic (PK) profile enabling once-daily dosing. OP-1250's complete ER antagonism is hypothesized to result in superior efficacy compared to agents that only partially antagonize and/or degrade but do not completely antagonize ER. Trial design: Goals of this phase 1/2 are to determine the Dose Limiting Toxicity (DLT), Maximum Tolerated Dose (MTD) and/or Recommended Phase 2 Dose (RP2D), to characterize the safety and PK profile, and to determine the preliminary activity of OP-1250 in subjects with HR+, HER2- MBC. Ph 1 (Dose Escalation) will evaluate escalating doses of orally administered OP-1250 to determine the safety, pharmacology, MTD (if any) and/or the RP2D. Cohorts of 3 to 6 subjects will be sequentially enrolled and monitored for DLTs during cycle 1. Eligibility criteria include males, and both pre- and post-menopausal females, age 18 or older, with ER+, HER2- advanced or MBC (pre-menopausal women must be on an LHRH antagonist); prior CDK4/6 and SERD and fulvestrant are permitted; ECOG of 0 or 1. The objectives of the phase 1 are: identification of the DLT, MTD and/or RP2D and assessment of the safety and tolerability and PK of OP-1250. Objectives of the phase 2 are to assess the objective response rate (ORR) of OP-1250 in 1) subjects with HR+, HER2- MBC who have progressed on endocrine therapy and have no evidence of central nervous system (CNS) metastases, 2) in patients with non-measurable disease, and 3) in patients with CNS disease. Correlative analyses include ER, PR, Ki67 in tumor biopsies and ctDNA pre- and post-therapy for activating mutations in ESR1. Summary: OP-1250, a complete estrogen receptor antagonist (CERAN), is currently being evaluated in a phase 1/2 study in ER+, HER2- MBC. For more information, please contact clinical@olema.com (NCT04505826)

P038 Early phase II clinical trial results for 4-demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) in adolescents and young adults (AYA) with brain cancers. Lee Roy Morgan[1], Roy S. Weiner[2], Tallat Mahmood[3], Marcus L. Ware[4], Andrew H. Rodgers[5], Manish Bhandari[6], Philip Friendlander[7]. [1]DEKK-TEC, Inc, New Orleans, LA, [2]Tulane University School of Medicine, New Orleans, LA, [3]Detroit Clinical Research Center, Lansing, MI, [4]Ochsner Medical Center, New Orleans, LA, [5]DEKK-TEC, Inc, New Orleans, LA, [6]The Christ Hospital, Cincinnati, OH, [7]Tisch Cancer Institute, New York, NY.

Background: 4-Demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) is a poly-chlorinated pyridine cholesteryl carbonate with a MOA *via bis*-alkylation of DNA @ N^7-guanine and N^4-cytosine that is currently in Phase II trial in AYA subjects with cancer that have CNS involvement. The aims for the trials are to assess clinical responses, monitor toxicities, safety and confirm the MTDs for IV administered DM-CHOC-PEN (IND 68,876) to AYA subjects with cancer involving the CNS. We report here responses and toxicities observed to date in Phases I & II DM-CHOC-PEN clinical trials with AYA subjects that had cancer involving the CNS. **Subjects & Methods**: DM-CHOC-PEN was administered as a 3-hr IV infusion once every 21 days to AYA subjects. The dosing schedule was 2-tiered: subjects with liver involvement received 85.8 mg/m^2 and subjects with normal liver function received 98.7 mg/m^2. **Results:** nineteen (19) AYA subjects with CNS involvement have been treated to date. The common tumor types treated were oligoastrocytoma, astrocytoma, GBM; leukemia (ALL), lymphoma (NHL), melanoma, breast and lung cancers (NSCL). Three (3) AYA patients are currently being followed with diagnoses of breast cancer, astrocytoma, and lung cancer and have had good qualities of life at 12, 59 and 72+ mos. All patients have been followed with lab tests, scans (RECIST 1.1) and virtual exams. The drug was well tolerated. The most common adverse effect was fatigue (17%). No neuro/cognitive, liver dysfunction, hematological, cardiac, renal or GI toxicities were observed. PK modeling revealed that AUCs were parallel for all dose levels. The C_{max} for DM-CHOC-PEN and DM-PEN (4-demethylpenclomedine, a metabolite) were 3 and 24 hours, respectively for the AYA subjects; similar to what was seen for older adults. The drug and metabolite were still detectable in plasma and rbcs for 21 to 50 days in the AYA (15 – 39 y/o) group *vs*. 3 to < 21 days (previously reported for adult subjects (>60 y/o) (AACR #1185, 2013). Differences in PK profiles between AYA and older adult subjects will also be reviewed in depth. **Conclusion**: DM-CHOC-PEN is safe for usage and has produced objective responses with manageable toxicities in AYA subjects with cancer involving the CNS. Complete data on subject responses and observed toxicities will be presented. We propose a 3-stage mechanism for drug entry into the CNS and into cancer cells *via* reversible binding with RBCs and then association with L-glutamine transport into cells. **Supported by NCI/SBIR grants – R43/44CA132257 and R43CA203351 and NIH NIGMS 1 U54 GM104940.**

P039 A phase 1 dose escalation study of protein arginine methyltransferase 5 (PRMT5) inhibitor PRT543 in patients with advanced solid tumors and lymphoma. Meredith McKean[1], Manish R. Patel[2], Robert Wesolowski[3], Renata Ferrarotto[4], Eytan M. Stein[5], Alexander N. Shoushtari[5], David Mauro[6], John Viscusi[6], Peggy Scherle[6], Neha Bhagwat[6], William Sun[7], Rachel Chiaverelli[6], Eric Mintah[6], Shekeab Jauhari[8], Laura Finn[9], Neil D. Palmisiano[10], Robert A. Baiocchi[3]. [1]Sarah Cannon Research Institute, Tennessee Oncology, Nashville, TN, [2]Sarah Cannon Research Institute, Florida Cancer Specialists & Research Institute, Sarasota, FL, [3]Ohio State University Comprehensive Cancer Center, Columbus, OH, [4]The University of Texas MD Anderson Cancer Center, Houston, TX, [5]Memorial Sloan Kettering Cancer Center, New York, NY, [6]Prelude Therapeutics, Research & Development, Wilmington, DE, [7]Prelude Therapeutics, Research & Development, Wilmington, DE, [8]Florida Cancer Specialists and Research Institute, Lake Mary, FL, [9]Ochsner Health System's Hematology and Stem Cell Transplant Program, New Orleans, LA, [10]Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA.

Background: PRMT5 catalyzes symmetric arginine dimethylation of protein substrates with important roles in cancer cell growth/survival. PRT543 is a potent, selective, oral PRMT5 inhibitor with robust preclinical efficacy (Bhagwat *AACR* 2020). An open-label Phase I study of PRT543 in unselected patients (pts) with advanced solid tumors and hematologic malignancies (NCT03886831) is ongoing. Dose escalation results from pts with solid tumors and lymphoma are presented herein. **Materials and Methods:** This study assesses safety, pharmacokinetics, pharmacodynamics, and preliminary tumor response (RECIST v1.1) of PRT543 administered at varying schedules beginning with twice weekly (BIW) and increasing to once daily (QD) with doses ranging from 5-50 mg in 28-day cycles. Dose escalation followed a 3+3 design. Serum symmetric dimethylarginine (sDMA) and intron retention, a marker of PRMT5-mediated mRNA splicing fidelity, were assessed as measures of PRMT5 target engagement and function, respectively. **Results:** As of 18 June 2021, 49 unselected pts with measurable disease refractory to established therapies had enrolled (17 pts BIW, 11 pts 5x/week [wk], 21 pts QD). Median number of prior lines of systemic therapies was 3. Six of 21 pts dosed at 25-50 mg QD experienced dose limiting toxicity (DLT) of thrombocytopenia, and 1 pt of 6 dosed at 45 mg 5x/wk had DLT of fatigue. Most frequent treatment-related adverse events (TRAEs), any grade, in all regimens were fatigue (n=20, 41%), nausea (n=14, 29%), thrombocytopenia (n=13, 27%), and anemia (n=12, 24%). Grade ≥3 TRAEs in all regimens occurring in ≥5 pts included thrombocytopenia (n=10, 20%) and anemia (n=6, 12%). Cytopenias were reversible and managed with dose modifications. 44 pts discontinued treatment, mainly due to disease progression, with 2 due to AEs (Grade 3 thrombocytopenia and Grade 4 cholangitis). The 45 mg 5x/wk regimen was selected as the expansion dose. PRT543 demonstrated dose-dependent increases of C_{max} (nM) and AUC (nM.hr). At the expansion dose, $T_{½}$ was 15 hrs, and plasma drug exposures (C_{max}, 2142 nM; AUC, 26,293 nM.hr) exceeded those for preclinical efficacy models. Serum sDMA decreased in a dose-dependent manner reaching 77% reduction with 50 mg QD and 69% reduction with the expansion dose. Increased intron retention was seen in peripheral blood mononuclear cells. A complete response (CR) has been maintained for >1 yr in a pt with homologous recombination deficiency-positive (HRD+) ovarian cancer who remains on study therapy. An additional 5 pts exhibited stable disease ≥6 mo. **Conclusions:** PRT543 was well tolerated with a favorable safety profile. Dose-dependent inhibition of target engagement and functional activity of PRMT5 were observed. PRT543 demonstrated encouraging clinical

activity with a CR in HRD+ ovarian cancer and prolonged stable disease in multiple pts. An expansion phase in biomarker-selected solid tumor cohorts is ongoing.

P041 Initial results from a phase 1b study of ORIC-101, a glucocorticoid receptor antagonist, in combination with enzalutamide in patients with metastatic prostate cancer.

Wassim Abida[1], Neeraj Agarwal[2], Andrew W. Hahn[3], Neal Shore[4], Paul Sieber[5], Tanya Dorff[6], Mathew Rettig[7], Mathew Smith[8], Paul Monk[9], Rongda Xu[10], Ann Johnson[10], Anneleen Daemen[10], Edna Chow Maneval[10], Pratik S. Multani[10], Rupal Patel[10], Michael J. Morris[1].
[1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, [3]MD Anderson Cancer Center, Houston, TX, [4]Carolina Urologic Research Center, Myrtle Beach, NC, [5]Keystone Urology, Lancaster, PA, [6]City of Hope, Duarte, CA, [7]VA Greater Los Angeles, Los Angeles, CA, [8]Massachusetts General Hospital, Boston, MA, [9]Ohio State Comprehensive Cancer Center, Columbus, OH, [10]ORIC, South San Francisco, CA.

Background: Upregulation of the glucocorticoid receptor (GR) is a potential mechanism of resistance to enzalutamide and other androgen receptor (AR) modulators in prostate cancer. Preclinical studies have demonstrated that GR activation can bypass enzalutamide-mediated AR inhibition and support prostate cancer cell growth. Overexpression of GR is associated with poor outcomes in castration-resistant prostate cancer patients (CRPC) treated with enzalutamide. ORIC-101 is a potent and selective orally bioavailable, small molecule antagonist of GR. Mechanistically, ORIC-101 inhibits GR transcriptional activity and blocks the pro-survival signals mediated by the activated nuclear hormone receptor. **Methods:** A modified interval 3+3 (i3+3) design was used to assess safety, pharmacokinetics (PK), and pharmacodynamics (PD) to select the Recommended Phase 2 Dose (RP2D) of ORIC-101 in combination with enzalutamide in patients with metastatic CRPC progressing on enzalutamide 160 mg, dosed once daily (NCT04033328). ORIC-101, at doses ranging from 80 to 240 mg once daily, given in a continuous dosing regimen, was added to enzalutamide at the time of disease progression. Plasma PK and PD biomarkers were assessed on multiple days and times before and after dosing. PD modulation in blood-derived peripheral blood mononuclear cells (PBMCs) was assessed by RT-qPCR for GR target genes. Antitumor activity was assessed by Prostate Cancer Clinical Trials Working Group 3 (PCWG3) and RECIST 1.1. **Results:** 10 patients were treated in 3 cohorts in the dose escalation portion of the study. ORIC-101 exposure increased with dose and no drug-drug interaction (DDI) was observed that necessitated reduction from the standard enzalutamide dose of 160 mg. No dose limiting toxicities were observed at any dose level. Based upon plasma exposure and PD modulation, the RP2D was established as 240 mg ORIC-101 plus 160 mg enzalutamide, both dosed once daily continuously in 28-day cycles. All adverse events (AEs) were Grade 1 or 2 with the most common (>15%), treatment-related AEs being fatigue (40%), nausea (30%), constipation (20%), decreased appetite (20%), high aspartate aminotransferase (20%), high alkaline phosphatase (20%), and headache (20%). There were no Grade ≥3 treatment-related AEs. Biomarker data demonstrated ORIC-101 induced reduction in GR target gene expression in PBMCs, indicating PD modulation across dose levels of ORIC-101. Data will be updated at the time of the presentation. **Conclusions:** Preliminary evidence suggests that ORIC-101 effectively modulates GR and has an acceptable tolerability profile when combined with enzalutamide. Dose expansion is ongoing at the RP2D.

P042 Phase I/II first-in-human study of TT-10 (A2AB inhibitor) as a single agent in subjects with advanced selected solid tumors. Sushant Kumar, Kasim Mookhtiar, Desa Rae Pastore, Brian Schwartz, Vijay Reddy. Tarus Therapeutics, North Bergen, NJ.

Immunotherapy has emerged as a potent tool for cancer treatment by activating the immune system to recognize and attack cancer cells. Immune checkpoint blockage has shown notable clinical success in several malignancies such as advanced non-small cell lung cancer, melanoma, advanced kidney cancer and several other indications, as demonstrated with the approval of CTLA-4 and PD-1/PD-L1 checkpoint inhibitors. However, the efficacy of checkpoint inhibitors to date is limited to only a subset of patients, even within the same cancer type. Thus, the continued focus in the cancer immunotherapy field is to identify new immunosuppressive pathways that allow cancer cells to evade immune surveillance. One emerging pathway of interest is the purinergic pathway, in which adenosine is recognized as a key metabolic checkpoint, and high levels of extracellular adenosine have been evidenced to mediate profound tumor resistance. In this first-in-human Phase I/II study, we explore selective inhibition of the adenosine A_{2A} receptor ($A_{2A}R$) with TT-10, an oral immune-oncology agent, as a treatment option for subjects with select solid tumors. TT-10 is being evaluated as a single agent, in an open-label, non-randomized, multicenter, dose-escalation (phase 1) and dose-expansion (phase 2) study in subjects with renal cell cancer (RCC), castrate resistant prostate cancer (CRPC) and non-small cell lung cancer (NSCLC) (NCT# NCT04969315). Phase 1 (Cohort A) will explore ascending doses of TT-10, taken orally (10, 20, 50, 100, 200 mg, BID), utilizing a 3+3 design in 28-day cycles to identify the maximum tolerated dose (MTD) and/or recommended Phase 2 dose (R2PD). Enrollment will be open to all three indications. Phase 2 will begin once RP2D is determined and will enroll up to 60 subjects, with approximately 15 subjects per cohort (B.) RCC, (C.) CRPC, (D.) NSCLC (E.) Exploratory Tumor Biopsy). The primary objectives of this study are to evaluate the safety, tolerability, and MTD or the RP2D of TT-10 monotherapy. Secondary objectives are to obtain a preliminary estimate of efficacy, evaluate PK and anti-tumor activity of TT-10 monotherapy, with exploratory objectives inclusive of identification of candidate biomarkers and biomarkers that might predict response, as well as, exploring if adenosine signature and/or receptor expression in tumor tissue at baseline might predict response. Subjects must have locally advanced, recurrent or metastatic neoplastic disease that is not curable by currently available, failure to respond to standard therapy or for whom no appropriate therapies are available, and have a life expectancy of $\geq$ 3 months to be eligible for the trail. Select subjects that would like to participate in Cohort E.) Exploratory Tumor Biopsy must have an accessible tumor for pre and post dose biopsies. Enrollment of phase 1 is expected to start October 2021.

P043 A double-blind randomized, placebo-controlled trial of oral administration with human papillomavirus (HPV) type 16 E7-expressing *Lactobacillus*-based vaccine, BLS-ILB-E710c, for the treatment of cervical intraepithelial neoplasia (CIN2/3). Jae Kwan Lee[1], Seung Hun Song[2], Young Tae Kim[3], Chi-Heum Cho[4], Chan Joo Kim[5], Young-Chul Park[6]. [1]Korea University Guro Hospital, Seoul, Korea, Republic of, [2]Comprehensive Gynecologic Cancer Center, CHA Bundang Medical Center, CHA University, Seongnam-si, Korea, Republic of, [3]Department of Obstetrics and Gynecology, Institute of Women's Medical Life Science, Yonsei University College of Medicine, Severance Hospital, Seoul, Korea, Republic of, [4]Department of Obstetrics and Gynecology, School of Medicine, Keimyung University, Daegu, Korea, Republic of, [5]Department of Obstetrics and Gynecology, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of, [6]BioLeaders Corporation, Yongin, Korea, Republic of.

Background - Despite preventive HPV vaccines is implemented worldwide, current treatments for high grade cervical intraepithelial neoplasia are ablative, and no pharmacological treatments are available. Here we evaluated safety of BLS-ILB-E710c, HPV 16 E7-expressing *Lactobacillus*-based vaccine, and explored its efficacy for histopathological regression in women with CIN2/3. **Methods** - Safety and efficacy of BLS-ILB-E710c were assessed in CIN2/3 associated with HPV16 and its related HPV types in a randomized, double-blind, placebo-controlled phase 2b study. Total of 116 patients was recruited from fourteen hospitals in South Korea, and were randomized (2:1) to receive 1000 mg BLS-ILB-E710c or placebo for 5 days at 1, 2, 4, and 8 weeks. The primary endpoint was histopathological regression to CIN1 or normal pathology at 16 weeks after the first dose. Full analysis set (FAS) and Per-protocol set (PPS) analyses were based on patients receiving at least one oral vaccination, and on patients receiving four rounds of oral vaccination without protocol violations, respectively. The safety population included all patients who enrolled. The trial is registered at clinicaltrials.gov (number NCT03274206). **Findings** - Total of 116 patients were randomized, and 113 received either BLS-ILB-E710c (n=75) or placebo (n=38). The oral vaccination was well tolerated and no serious vaccine-related AEs occurred. No differences were showed between the BLS-ILB-E710c and placebo groups for patient background and adverse events. In the full analysis set (FAS) no statistically significant difference was noted between the two groups of histopathological regression at 16 weeks. However, the distribution by Bethesda system (for cervical cytology) in CIN2 patients showed significant differences between two groups (P=0.0304), which can affect histopathological regression. Sub-group analysis in FAS is performed to reduce the bias; histopathological classification (CIN2, CIN3) and cytological classification (≤ASCUS, LSIL, HISL). Therefore, in CIN3 sub-group, BLS-ILB-E710c recipients showed 13.33% of higher histopathological regression at 32 weeks than placebo recipients, and interestingly represented the statistically significant difference when histopathological regression at 32 weeks compared with 16 weeks (percentage point difference 34.87 [95% CI 7.78–61.96]; P=0.0216). In BLS-ILB-E710c recipients of CIN3 sub-group with histopathological regression, E7-specific CD8+ T lymphocyte immune responses were induced at 32 weeks (P= 0.0323). In addition, in HSIL sub-group, BLS-ILB-E710c recipients showed higher histopathological regression at 32 weeks than placebo recipients in similar with CIN3 sub-group analysis. **Interpretation** – Based on the finding this study, BLS-ILB-E710c could induce the local immune response by recruiting HPV16 E7-specific CD8+ T cells to cervical lesions, leading to histologic regression. To assess

the efficacy of BLS-ILB-E710c as a novel *Lactobacillus*-based oral vaccine, it is necessary to define the population and time frame for phase 3 trial.

P044 A phase 1 dose escalation study of protein arginine methyltransferase 5 (PRMT5) brain penetrant inhibitor PRT811 in patients with advanced solid tumors, including recurrent high-grade gliomas. Gerald S. Falchook[1], Jon Glass[2], Varun Monga[3], Pierre Giglio[4], David Mauro[5], John Viscusi[6], Peggy Scherle[6], Neha Bhagwat[6], William Sun[6], Rachel Chiaverelli[6], Eric Mintah[6], Lydia Clements[7], Tanner M. Johanns[8], Meredith McKean[9]. [1]Sarah Cannon Research Institute at HealthONE, Denver, CO, [2]Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, [3]University of Iowa Hospitals and Clinics, Iowa City, IA, [4]The Ohio State University Wexner Medical Center, Columbus, OH, [5]Prelude Therapeutics, Research & Development, Wilmington, DE, [6]Prelude Therapeutics, Research & Development, Wilmington, DE, [7]Christiana Care Health Services, Christiana, DE, [8]Washington University School of Medicine, St. Louis, MO, [9]Sarah Cannon Research Institute, Tennessee Oncology, Nashville, TN.

Background: PRMT5 catalyzes symmetric arginine dimethylation of protein substrates with important roles in cancer cell growth/survival, including glioblastoma (GBM) cells. PRT811 is a brain penetrant, potent, selective, oral PRMT5 inhibitor with preclinical efficacy in a GBM orthotopic model (Zhang *AACR* 2020). An open-label Phase I study of PRT811 in patients (pts) with advanced solid tumors and recurrent high-grade glioma (NCT04089449) is ongoing. Dose escalation results are presented herein. **Materials and Methods:** This study assesses the safety, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary anti-tumor activity (RECIST v1.1, Lugano, & RANO) of PRT811 administered at varying doses/schedules (15-600 mg daily [QD]; 300 mg twice daily [BID]). Serum symmetric dimethylarginine (sDMA) and intron retention, a marker of PRMT5-mediated mRNA splicing fidelity, were assessed as measures of PRMT5 target engagement and function, respectively. **Results:** As of 18 June 2021, 38 unselected pts with recurrent or refractory disease had enrolled (22 solid tumor, 16 GBM). Median number of prior lines of systemic therapies was 2. No dose-limiting toxicities have been identified. Most frequent treatment-related adverse events (TRAEs), any grade, were nausea (n=10, 26%), vomiting (n=7, 18%), diarrhea (n=4, 11%), fatigue (n=4, 11%), constipation (n=3, 8%), and pruritis (n=3, 8%). Decreased lymphocyte count (n=1, 3%) and vomiting (n=1, 3%) were the only Grade ≥3 TRAEs. 28 pts discontinued treatment, mainly due to disease progression. No pts discontinued due to treatment-emergent AEs (TEAEs). PRT811 exhibited linear PK characteristics. At the highest evaluated dose (600 mg QD), $C_{max,}$of 3777 nM, AUC of 12,487 nM.hr, and $T_{½}$ of 5.8 hrs were observed. Comparison of PK parameters of 300 mg administered either QD or BID demonstrated similar PK with no accumulation with the BID schedule. Serum sDMA levels decreased by 80% with 600 mg QD. PRMT5-mediated mRNA splicing fidelity in peripheral blood mononuclear cells showed increased intron retention in specific transcripts at the higher PRT811 doses, indicating PRMT5 functional activity was decreased. A partial response was observed in 1 pt with GBM (>9 months duration) who remains on study. In addition, a pt with uveal melanoma (spliceosome SF3B1 mutation) achieved a 25% decrease in tumor burden per RECIST v1.1. An additional 3 pts with solid tumors had stable disease ≥6 mo. **Conclusions:** PRT811 was well tolerated with a favorable safety profile when administered at either QD or BID schedules. Target engagement and inhibition of PRMT5 functional activity were observed across multiple dose levels. Preliminary evidence of antitumor activity was observed in GBM and uveal melanoma. The expansion phase of the study will be initiated in select tumor types upon establishing the recommended expansion dose.

P045 LUMINOS-103: A basket trial evaluating the safety and efficacy of PVSRIPO and PVSRIPO in combination with anti-PD-1/L1 checkpoint inhibitors in patients with advanced solid tumors. Brant A. Inman[1], Matthew I. Milowsky[2], Raj S. Pruthi[3], Marshall Posner[4], Melissa J. Polasek[5], Shannon R. Morris[5], Lori Mixson[5], Kristin Orr[5], Elizabeth M. H. Woodson[5], Andrea T. Kelly[5], W. Garrett Nichols[5], Arjun V. Balar[6]. [1]Duke University Medical Center, Durham, NC, [2]University of North Carolina Lineberger Cancer Center, Chapel Hill, NC, [3]University of California San Francisco Medical Center, San Francisco, CA, [4]Icahn School of Medicine at Mount Sinai, New York, NY, [5]Istari Oncology, Inc., Morrisville, NC, [6]New York University Langone Health, New York, NY.

Background: PVSRIPO, a novel intratumoral viral immunotherapy, infects cells via CD155, which is widely expressed on solid tumors and antigen-presenting cells (APC). Infection is lethal in malignant cells, but a unique, activating, nonlethal infection of local APCs yields type-I/III interferon (IFN)-dominant inflammation with subsequent anti-tumor T-cell priming and activation. In preclinical models, PVSRIPO-dependent inflammation upregulated the PD-1/L1 pathway, and greater anti-tumor response was observed with PVSRIPO + anti-PD-1/L1 (αPD-1/L1). Promising clinical activity with PVSRIPO monotherapy was observed in patients (pts) with recurrent glioblastoma and advanced αPD-1–refractory melanoma (Desjardins 2018 *NEJM*, Beasley 2021 *JITC*). Collectively, these results warrant further clinical investigation of PVSRIPO ± αPD-1/L1. **Trial design, objectives, and eligibility criteria:** LUMINOS-103 (NCT04690699) is a phase (Ph) 1/2, open-label, multi-center, single-arm basket trial evaluating repeat administration of PVSRIPO ± αPD-1/L1 in adults with solid tumors. Trial objectives are to assess the safety and tolerability of PVSRIPO monotherapy in each cohort in Ph 1 and the safety, tolerability, and antitumor efficacy of PVSRIPO + αPD-1/L1 in each cohort in Ph 2. The first two study cohorts include pts with muscle-invasive bladder cancer being treated in the neoadjuvant setting (A) and pts with metastatic bladder cancer being treated in the $1^{st}/2^{nd}$ line setting (B); these cohorts have been described previously (Inman 2021 *Virtual AACR Annual Meeting*). Cohort C includes pts with resectable, locally advanced head and neck squamous cell carcinoma (HNSCC) being treated in the neoadjuvant setting; Cohort D includes pts with recurrent/metastatic HNSCC with a PD-L1 Combined Positive Score ≥1 being treated in the 1st line setting. Eligibility: HNSCC pts must have histologically or cytologically proven SCC of the oral cavity, oropharynx, hypopharynx, or larynx. All pts must have prior and boosted PV immunization and tumors amenable to injection and biopsy. Key exclusion criteria: Requirement for oxygen supplementation, systemic or intratumoral therapy ≤6 months prior to the first dose of study drug, CNS metastases requiring immediate treatment, systemic immunosuppressive medications ≤4 weeks prior to the first dose of study drug, and severe active comorbidities. Pts who are HIV+, HBV+ or HCV+ are eligible provided they meet certain criteria. **Endpoints**: Primary endpoints include safety (all), tolerability (all), surgical complication rate (A, C), pathologic treatment effect/response (A, C), and objective response rate (B, D). Secondary endpoints include overall survival (all), pathologic downstaging and relapse-free survival (A, C), duration of response and progression-free survival (B, D), and assessment of tumor/blood biomarkers (all).

P046 ENGOT-ov60/GOG3052/RAMP 201: A phase 2 study of VS-6766 (dual RAF/MEK inhibitor) alone and in combination with defactinib (FAK inhibitor) in recurrent low-grade serous ovarian cancer (LGSOC). Susana N. Banerjee[1], Bradley J. Monk[2], Els Van Nieuwenhuysen[3], Kathleen N. Moore[4], Ana Oaknin[5], Michel Fabbro[6], Nicoletta Columbo[7], David M. O'Malley[8], Robert L. Coleman[9], Jonathan Pachter[10], Andrew Koustenis[10], Gloria Patrick[10], Lorna Leonard[11], Rachel Grisham[12]. [1]The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, United Kingdom, [2]Arizona Oncology (US Oncology Network), University of Arizona College of Medicine, Phoenix, AZ, [3]University Hospital Leuven, Leuven, Belgium, [4]Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, [5]Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain, [6]ICM Val d'Aurelle, Montpellier, France, [7]European Institute of Oncology, IRCCS, Milan, Italy, [8]The Ohio State University College of Medicine, Columbus, OH, [9]US Oncology Research, The Woodlands, TX, [10]Verastem, Needham, MA, [11]Institute of Cancer Research, Royal Cancer Hospital, London, United Kingdom, [12]Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY.

Background: VS-6766 is a unique small molecule inhibitor that blocks MEK kinase activity and RAF phosphorylation of MEK. This mechanism of blockade has been shown to limit compensatory MEK activation, thereby potentially enhancing efficacy of MEK inhibition. Defactinib, (VS-6063), an orally active small molecule, is a potent adenosine 5'- triphosphate (ATP) competitive, reversible inhibitor of focal adhesion kinase (FAK). Defactinib has shown synergistic activity with BRAF and MEK inhibitors in both in vitro and in vivo solid tumor models. Prior molecularly unselected studies with single agent MEK inhibitors have shown response rates up to 26% in recurrent LGSOC. A third of patients with recurrent LGSOC harbor somatic KRAS mutations. FAK inhibition has been shown to induce tumor regression when combined with RAF, MEK or RAF/MEK inhibitors in in vivo models of KRAS mutant ovarian cancer. The combination of VS-6766 and defactinib is currently being evaluated in the ongoing Investigator Sponsored FRAME study (NCT03875820). In this proof of concept study, durable objective responses have been reported in recurrent LGSOC patients, particularly those with KRAS mutations including patients who have had a prior MEK inhibitor (Banerji et al AACR 2020). Based on preclinical studies demonstrating efficacy of both VS-6766 and the VS-6766/defactinib combination and preliminary results of the FRAME study, the phase II ENGOT-ov60/GOG3052 has been developed in recurrent LGSOC. **Methods:** This is a Phase II, adaptive, two-part, multicenter, parallel cohort, randomized, open label study designed to evaluate the efficacy and safety of VS-6766 versus VS-6766 in combination with defactinib (NCT04625270). The study will be conducted in two parts. Part A will determine the optimal regimen based on confirmed overall response rate (independent radiology review) in KRAS-mutated LGSOC. Part B will determine the efficacy of the optimal regimen identified in Part A in KRAS-mutated and KRAS wild-type LGSOC. The minimum expected enrollment is 52 subjects with KRAS-mutated tumors (32 subjects in Part A and 20 in Part B) and 36 with KRAS wild-type tumors in Part B. Patients will be randomized to receive VS-6766 (4.0 mg PO, twice weekly 3 weeks on, 1 week off) or VS6766 with defactinib (VS-6766 3.2 mg PO, twice weekly + defactinib 200 mg PO BID 3 weeks on, 1 week off) till progression. Key inclusion criteria include histologically confirmed LGSOC, presence of KRAS mutation (Part A), prior systemic therapy for metastatic disease and up to 1 prior line of MEK/RAF inhibitor therapy permitted. This international study is open to enrollment.

P047 A phase 1/2 trial of CBX-12, an alphalex™ peptide drug conjugate, in patients with advanced or metastatic refractory solid tumors. Anthony Tolcher[1], Joseph Paul Eder[2], David Sommerhalder[1], Sophia Gayle[3], Paul Pearson[3], Deb Chapman[3], Arthur P. DeCillis[3], Anish Thomas[4], Funda Meric-Bernstam[5]. [1]Next Oncology, San Antonio, TX, [2]Yale University, New Haven, CT, [3]Cybrexa Therapeutics, New Haven, CT, [4]National Cancer Institute, Bethesda, MD, [5]MD Anderson Cancer Center, Houston, TX.

Background Tumor-targeted drug delivery technologies are urgently needed to overcome the lack of tumor selectivity, a major drawback of conventional chemotherapy. In addition, the acidic intercellular microenvironment in solid tumors traps weak acid/base chemotherapy agents, preventing necessary intracellular concentrations in tumour cells. An alphalex conjugate, which contains a low-pH insertion peptide, a linker, and a payload, is designed to overcome these limitations. Unlike antibody drug conjugates, these peptide drug conjugates target tumors in an antigen-agnostic manner. At pH ≥7.0, the peptide is unstructured. In the low-pH tumor microenvironment, the peptide forms an alpha helix, inserts directionally in the cell membrane delivering the linker and payload intracellularly where the linker is cleaved. CBX-12 consists of the pH-sensitive peptide, a self-immolating linker, and the topoisomerase 1 (TOP1) inhibitor exatecan. Methods CBX-12-101 is a first-in-human, open-label, dose-escalation, safety, pharmacokinetics (PK), and biomarker study of CBX-12 in patients with advanced or metastatic refractory solid tumors. CBX-12 is administered as a 1-hour intravenous infusion. Two dosing schedules, daily x 5 every 3 weeks (Part A) and daily x 3 every 3 weeks (Part B), are being evaluated. The starting dose in Part A is 0.25 mg/kg. Single-patient cohorts will be enrolled initially, with dose-escalations up to 100% of the prior dose, until a patient has a ≥ Grade 2 adverse event (AE) considered possibly related to CBX-12 during Cycle 1 (the DLT period), at which time 2 additional patients will be enrolled in that cohort, and a 3 + 3 design will subsequently be utilized. Further dose escalations may be no more than 50% of the prior dose. After at least 2 cohorts have been evaluated in Part A, Part B will open for accrual. Maximum tolerated doses and recommended phase 2 doses will be determined for each dosing schedule. The PK of the CBX-12 conjugate and free exatecan will be determined. The stability of the conjugate in circulation will be evaluated by the ratio of CBX-12 to free exatecan. Pre- and on-treatment tumor biopsies are required. Biomarker and pharmacodynamic evaluations including measuring intratumor exatecan levels, TOP1, gamma H2AX and schlafen-11 are planned. Antitumor activity will be assessed per RECIST v 1.1. Phase 2 expansion cohorts are planned in patients with platinum-resistant ovarian and small cell lung cancer. As of July 2021, patients are enrolling in Part A Cohort 2 at a dose of 0.50 mg/kg.

P048 A phase 2 study of VS-6766 (dual RAF/MEK inhibitor) RAMP 202, as a single agent and in combination with defactinib (FAK inhibitor) in recurrent KRAS-mutant (KRAS-MT) non-small cell lung cancer (NSCLC). D. Ross Camidge[1], Jonathan Pachter[2], Andrew Koustenis[2], Gloria Patrick[2], David R. Spigel[3]. [1]University of Colorado Denver Department of Medical Oncology, Aurora, CO, [2]Verastem, Needham, MA, [3]The Sarah Cannon Cancer Center, Nashville, TN.

Background: VS-6766 is a unique small molecule inhibitor of both RAF and MEK. In contrast to several other MEK inhibitors available, VS-6766 blocks both MEK kinase activity and RAF phosphorylation of MEK. This sequential blockade mechanism enables VS-6766 to block MEK signaling more consistently without the compensatory and paradoxical activation of MEK that reduces the efficacy of other small molecule inhibitors of MEK and RAF. Defactinib (VS-6063), an orally active small molecule, is a potent adenosine 5'- triphosphate (ATP) competitive, reversible inhibitor of focal adhesion kinase (FAK). Defactinib has shown synergistic activity with BRAF and MEK inhibitors in both in vitro and in vivo preclinical solid tumor models. Specifically, in several human tumor cell lines with mutations in RAS or BRAF or wildtype RAS and BRAF, defactinib has shown synergy with MEK inhibitors or VS-6766. In mouse models of KRAS mutant ovarian cancer, BRAF-mutant melanoma or uveal melanoma, FAK inhibition has been shown to induce tumor regression when combined with RAF, MEK or RAF/MEK inhibitors, while the single agents have only induced tumor stasis. The combination of VS-6766 and defactinib is currently being evaluated in the Investigator Sponsored FRAME study. Preliminary efficacy results are available for a small number of subjects with KRAS mutated NSCLC treated with a combination of VS-6766 and defactinib. Of the 10 subjects with NSCLC, 1 subject with KRAS-G12V- mutated cancer achieved PR while 3/10 subjects received treatment for ≥24 weeks. In an updated analysis of response in 15 subjects with KRAS-mutant NSCLC, there were 2 PRs and 7 SDs (ORR: 13%). The two subjects with KRAS G12V- mutated NSCLC both achieved PR (ORR: 100%). **Methods:** This is an adaptive, two-part, Phase 2, multicenter, parallel cohort, randomized, open label study designed to evaluate the efficacy and safety of VS-6766 versus VS-6766 in combination with defactinib in subjects with recurrent NSCLC(NCT04620330). The study will be conducted in two parts. Part A will determine the optimal regimen, either VS-6766 monotherapy or VS-6766 in combination with defactinib based on confirmed overall response rate as assessed by independent radiology review. Part A will consist of three arms in KRAS mutated NSCLC Arm 1 monotherapy of VS-6766, Arm 2 the combination of VS-6766 and defactinib in KRAS G12V mutated and Arm 3 the combination in other KRAS mutated. Part B will determine the efficacy of the optimal regimen identified in Part A in KRAS mutated NSCLC. Subjects enrolled must have histologic or cytologic evidence of NSCLC, measurable disease according to RECIST V1.1 and known KRAS mutation. The study will enroll up to 377 subjects globally with 57 subjects (32 KRAS G12V & 25 KRAS other) in Part A and an additional 144 KRAS G12V and 176 KRAS other in Part B. This study is open for enrollment.

P049 Phase II trial of TRC102 (methoxyamine HCl) in combination with temozolomide (TMZ) in patients with advanced non-small cell lung cancer. Mohamad A. Salkeni[1], Geraldine O'Sullivan Coyne[1], Jennifer Zlott[1], Naoko Takebe[1], Ning Ma[2], Larry Rubinstein[1], Richard Piekarz[1], Jared Foster[1], Brandon Miller[1], Ralph Parchment[2], Abdul R. Naqash[1], Andrea Voth[2], James Doroshow[1], Alice Chen[1]. [1]National Institutes of Health, Bethesda, MD, [2]Frederick National Laboratory for Cancer Research, Frederick, MD.

Background: TRC102 binds to abasic sites in DNA, inhibiting the base excision repair (BER) pathway, which is implicated in resistance to alkylating agents. Preclinical xenograft studies in mice demonstrated a synergistic anti-tumor effect when TRC102 was combined with alkylating agents. TRC102 given in combination with temozolomide in a phase 1 trial demonstrated acceptable toxicity and antitumor activity in patients (pts) with colorectal cancer (CRC), non-small cell lung cancer (NSCLC, squamous cell carcinoma) and granulosa cell ovarian cancer (Oncotarget 2020). Pharmacodynamic (PD) analysis revealed treatment-induced induction of nuclear Rad51 signal, indicating DNA damage repair (DDR) response and tumor biopsy data indicated MGMT methylation correlated with tumor response in CRC (Cancer Cell, 2020). Here we report data from a phase II expansion cohort of this combination in pts with NSCLC. Methods: We conducted a single arm open label trial in a pt cohort with advanced NSCLS, who had received at least one line of therapy in the metastatic setting. Pts who received immunotherapy (ICI) and/or molecularly targeted therapy were eligible. Pts received 125 mg TRC102 (100 mg for BSA < 1.6) and 150 mg/m2 TMZ given orally daily for days 1-5 of 28-day cycle (C). Restaging CT scans were carried out after every 2 C and evaluated using RECIST 1.1. Blood from all pts was analyzed for circulating tumor cells (CTCs) at baseline and the beginning of each C. A two-stage design was employed, with at least 2 objective responses needed among the first 16 pts treated on study in order to proceed to the second stage. Results: Sixteen pts with NSCLC (12 adenocarcinoma, 4 squamous) were enrolled between 11/2016 and 6/2021; 12 men and 4 women, median age of 69 yrs (range 33-80), 1 Hispanic, 3 Asian, 2 Black/African American, and 10 Caucasian. Pts received a median of 3 prior lines of therapy (range 1-7), including ICI in all but 1 pt. Twelve pts were evaluable for response. Three pts experienced clinical progression before completing C 2, and 1 pt withdrew consent before completing C 2. Median number of Cs received by pts was 3 (range 1-14); 9 pts experience stable disease (SD) as best response lasting median of 12 wks (range 8-56 wks), and 4 pts experienced prolonged SD lasting greater than 6 Cs (24 wks). Treatment-related grade 3 adverse events reported include lymphopenia (4) and anemia (1). No grade 4 or 5 events were noted. Conclusion: TRC102 in combination with temozolomide was well tolerated with notable efficacy. Although no objective tumor responses were observed in this heavily pretreated population, 9 of 15 pts experienced SD, lasting ≥ 24 wks in 4 pts. Given the limited options for NSCLC treatment following ICI and TKI treatment, this regimen merits further evaluation. Analysis of CTCs is ongoing, as well as additional biomarker evaluation in other treatment cohorts. Funded by NCI Contract No. HHSN261200800001E

P050 Trial in progress: A phase 2 multicenter study of autologous tumor-infiltrating lymphocyte (TIL, LN-145) cell therapy in patients with metastatic non-small cell lung cancer (mNSCLC). Erminia Massarelli[1], Selda Samakoglu[2], Viktoria Gontcharova[2], Guang Chen[2], Madan Jagasia[2], Friedrich Graf Finckenstein[2], Ammar Sukari[3]. [1]City of Hope Comprehensive Cancer Center, Duarte, CA, [2]Iovance Biotherapeutics, Inc., San Carlos, CA, [3]Karmanos Cancer Institute, Detroit, MI.

Patients with mNSCLC without actionable driver mutation(s) who have progressed after cytotoxic chemotherapy plus immune checkpoint inhibitors (ICI) ± bevacizumab have limited treatment options and represent an unmet need. The safety and efficacy of TIL cell therapy for patients with mNSCLC who failed to respond or progressed on nivolumab has been evaluated in a Phase 1 clinical trial (Creelan B. AACR 2020), demonstrating an objective response rate (ORR) of 25% including 17% durable CR. This provides a clear rationale for initiation of IOV-LUN-202 study, evaluating TIL cell therapy with LN-145 in patients with mNSCLC without actionable driver mutation(s), who have progressed on or following a single line of approved systemic therapy consisting of combined ICI + chemotherapy ± bevacizumab. **Trial Design** IOV-LUN-202 (NCT04614103) is a prospective, open-label, multi-cohort, non-randomized, multicenter Phase 2 study. Cohorts 1 and 2 (each, n=40) are enrolling patients with mNSCLC based on tumor proportion score (TPS; Cohort 1, TPS <1%; Cohort 2, TPS ≥1%) at metastatic diagnosis prior to ICI use. Cohort 3 (TPS <1%; n=15) uses core biopsies for tumor acquisition in patients unable to undergo a surgical harvest, and Cohort 4 is designated for patients requiring retreatment. LN-145 is a cryopreserved infusion product generated at centralized GMP facilities in a 22 day manufacturing process. All patients receive TIL therapy consisting of nonmyeloablative lymphodepletion with cyclophosphamide (60 mg/kg × 2) + fludarabine (25 mg/m^2 × 5), followed by a single infusion of autologous LN-145 (Day 0) and up to 6 doses of IL-2 (600,000 IU/kg). Key eligibility criteria include age ≥ 18 y, 1 prior line of therapy, ≥1 lesion(s) available for TIL generation and a remaining RECIST-measurable lesion, and ECOG PS 0–1. For each cohort the primary endpoint is ORR per RECIST 1.1. Secondary endpoints are safety, complete response rate, duration of response, disease control rate, progression-free survival, overall survival, and efficiency of generating LN-145 from tumor core biopsies (Cohort 3).

P051 Identification of an orally bioavailable dual Cyclin K glue degrader - CDK12/13 inhibitor. Edward K. Ainscow[1], Adrian Campbell[2], Michael Cripps[3], Robert Workman[2], Stuart Thomson[2], Kam Chohan[2], Damien Crepin[2], Mahiro Sunose[2], Jamie Patient[2], Jane Kendrew[2], Ash Bahl[1]. [1]Carrick Therapeutics, Dublin, Ireland, [2]Sygnature Discovery, Nottingham, United Kingdom, [3]Histologix, Nottingham, United Kingdom.

CDK12 and CDK13 regulate gene expression through translation and splicing of mRNA. Impaired activity of CDK12 has been shown to reduce the expression of a range of genes involved in DNA homologous repair leading to the induction of a 'BRCAness' phenotype in cells. CDK12 inhibitors have the potential in many different indications, including overcoming emergent resistance to PARP inhibition, enhancing the efficacy of PARP inhibition in patients with BRCA1/2 wt status [1] and extending the efficacy of immune checkpoint inhibition through immunogenic cell death [2]. Recently, certain classes of CDK12 inhibitors have also demonstrated 'glue degrader' properties [1]: causing proteosomal degradation of the obligate co-factor for CDK12 and 13, Cyclin K. Such glue-degraders have the capability of enhancing cellular potency and specificity over classical kinase inhibitors. Taking as a starting point the selective CDK7 inhibitor samuraciclib, we have taken a rational design approach to develop inhibitors that preferentially inhibit CDK12. By utilising differences in the active site between CDK7 and CDK12 we were able to increase potency against CDK12 in biochemical assays by > 100 fold, with no significant inhibition of kinases outside the CDK class. Cellular potency of these molecules was evaluated in the Ewing's sarcoma cell line A673, which is known to be sensitive to CDK12 inhibition [4]. Remarkably the potency of the molecules to induce cell death was greater than their biochemical potency against CDK12 by > 10 fold. Analysis of Cyclin K levels in cells demonstrated that the molecules lead to Cyclin K degradation. Furthermore, cellular potency was reduced by the presence of the neddylation inhibitor MLN4924 confirming that the compounds enhanced potency was mediated by glue degradation of Cyclin K. The molecules were found to cause potent inhibition of DNA homologous repair genes including BRCA1, FANCF and ERCC4 and lead to the accumulation of DNA strand breaks as assessed by the marker γ-H2AX. In the BRCA wt ovarian cancer cell line, OV-90, strong synergy was observed in the ability to induce cell death with a range of PARP inhibitors. In A673 tumour bearing mice, dosing with the compounds was found to cause significant reduction in the levels of cyclin K in tumours, which was sustained for 24 hr. Additionally, assessment of CDK12 protein in these tumours also showed a reduction, suggesting greater turnover of CDK12 protein in the absence of Cyclin K. Repeat dose studies at doses that lead to Cyclin K degradation in tumours demonstrated that the compounds were well tolerated, with no weight loss in mice. Two molecules, CT7439 and CT7510, were found to have oral bioavailability in mice that indicate that they would be suitable for once-daily oral administration in humans and are being progressed through regulatory toxicity studies. [1] Johnson, *et al*, (2016) Cell Rep. 17, 2367 [2] Li, *et al*, (2020) Cancer Lett. 495, 12 [3] Słabicki, *et al*, (2020) Nature 585, 293 [4] Iniguez, *et al*, (2018) Cancer Cell 33, 202

P053 VAL-083 (dianhydrogalactitol) synergizes with PARP inhibitors in BRCA-proficient and BRCA-deficient ovarian cancer models. Anne Steino[1], Guangan He[2], Beibei Zhai[3], Jeffrey Bacha[4], Dennis M. Brown[5], Mads Daugaard[3], Zahid H. Siddik[2]. [1]Kintara Therapeutics, Inc., Vancouver, BC, Canada, [2]MD Anderson Cancer Center, Houston, TX, [3]Vancouver Prostate Centre, Vancouver, BC, Canada, [4]Formerly Kintara Therapeutics, Inc., Vancouver, BC, Canada, [5]Kintara Therapeutics, Inc., Menlo Park, CA.

Platinum (Pt)-based chemotherapy plays a key role in ovarian cancer treatment, but patients frequently develop Pt-resistance. Dysfunctional p53 is implicated in Pt-resistance, comprising a therapeutic challenge in high grade serous ovarian carcinoma (HGSOC), where p53 is universally mutated. Attempts to overcome Pt-resistance in HGSOC include agents blocking the DNA repair pathways, most notably PARP inhibitors (PARPi), leading to accumulation of DNA double strand breaks (DSBs) and cancer cell death. Sensitivity to PARPi is correlated with deficiencies in the homologous recombination (HR) DNA repair system, which accurately repairs DSBs. BRCA1 and BRCA2 are key proteins in HR, and mutated BRCA1/2 are well-established biomarkers for PARPi sensitivity. PARPi treatment of Pt-sensitive ovarian cancers have improved progression free survival; however, improvements in overall survival have not been achieved and a 5-year survival rate of 40% remains in ovarian cancer. Additionally, resistance to PARPi has emerged as a significant clinical challenge. VAL-083 is a first-in-class bifunctional DNA damaging agent with demonstrated clinical activity against a range of cancers, including ovarian. VAL-083 rapidly induces interstrand cross-links at guanine-N^7 leading to DSBs, activation of the HR repair pathway and cancer cell death. VAL-083 is able to overcome cisplatin-resistance in a panel of ovarian cancer *in vitro* models. We have also shown that VAL-083 maintains activity independent of prominent DNA repair mechanisms implicated in resistance to numerous chemotherapeutics including cisplatin and PARPi such as MGMT, non-homologous end-joining and mismatch repair. Cancer cells thus rely heavily on a functional HR pathway for repair of VAL-083-induced DSBs. This rationalizes VAL-083 combination therapy with agents inducing DSBs or blocking their repair, including PARPi. Taken together, these data highlight VAL-083's potential for targeting Pt-resistant HGSOC in combination with PARPi. METHODS: The cytotoxicity of VAL-083 in combination with PARPi (olaparib, niraparib, rucaparib, veliparib or talazoparib) against HR-impaired (BRCA siRNA knockdown) and HR-proficient (control siRNA) ovarian cancer cells was studied. RESULTS: We report increased VAL-083 cytotoxicity against HR-impaired A2780 cancer cells. In addition, synergy between VAL-083 and olaparib, talazoparib and niraparib in both HR-proficient and deficient settings. VAL-083 combination with rucaparib produced synergistic cytotoxicity in the HR-deficient setting, while VAL-083 combination with veliparib was additive. These data demonstrate that VAL-083 can synergize with some PARPi in both HR-proficient and HR-deficient settings. CONCLUSION: Our results demonstrate a distinct DNA-damaging mechanism for VAL-083, resulting in the ability to overcome cisplatin-resistance in HR-impaired tumors. In addition, VAL-083 synergized with several PARPi, particularly olaparib, rucaparib and talazoparib, in both HR-proficient and deficient ovarian tumor cells.

P054 RP-3500: A novel, potent and selective ATR inhibitor that is effective in pre-clinical models as a monotherapy and in combination with PARP inhibitors. Anne Roulston[1], Michal Zimmerman[1], Robert Papp[2], Alex Skeldon[3], Charles Pellerin[3], Émilie Dumas-Bérube[3], Valerie Dumais[4], Stephane Dorich[3], Sara Fournier[1], Li Li[1], Marie-Ève Leclaire[1], Shou Yun Yin[1], Amandine Chefson[3], Hunain Alam[1], William Yang[1], Chloe Fugère-Desjardins[1], Sabrina Hammond[5], Kathryn Skorey[6], Amina Mulani[6], Victoria Rimkunas[1], Artur Veloso[1], Martine Hamel[1], Rino Stocco[1], Yael Mamane[1], Zuomei Li[5], Jordan Young[1], Mike Zinda[1], Cameron Black[1]. [1]Repare Therapeutics Inc., Saint-Laurent, Canada, [2]Repare Therapeutics Inc., Saint-Laurent, Canada, [3]Ventus Therapeutics Inc., Saint-Laurent, Canada, [4]Ventus Therapeutics Inc, Saint-Laurent, Canada, [5]Amplia Pharmatek Inc., Saint-Laurent, Canada, [6]NuChem Sciences Inc., Saint-Laurent, Canada.

Background: Ataxia telangiectasia and Rad3-related (ATR) protein kinase is a key mediator of cellular DNA damage repair (DDR) and is activated in response to DNA replication stress. ATR is attractive as a drug target in tumors with loss-of-function alterations in complimentary DDR pathways, including ataxia-telangiectasia mutated (ATM) and BRCA. **RESULTS:** RP-3500 is a novel, orally bioavailable clinical-stage ATR kinase inhibitor. RP-3500 is highly potent with ATR kinase IC_{50} values of 1.0 and 0.33 nM in biochemical and cell-based assays, respectively. It is highly selective with >30-fold selectivity over mTOR and >2000-fold selectivity over ATM, DNA-PK and PI3Kα kinases. Preclinical tumor xenograft models harboring synthetic lethal (SNIPRx) gene mutations whose loss of function sensitizes to RP-3500 were selected for *in vivo* studies. RP-3500 treatment resulted in potent single-agent *in vivo* efficacy and/or tumor regression in multiple models at minimum effective doses (MED) of 5–7 mg/kg once daily. Pharmacodynamic assessments validated target engagement, with a proportional relationship between tumor pCHK1(Ser345) inhibition and circulating RP-3500 plasma levels (IC_{80} = 18.6 nM). Circulating free plasma levels of RP-3500 at the MED indicate that exposure above the *in vivo* tumor pCHK1(Ser345) IC_{80} for 10–12 hours is sufficient for efficacy and dose proportional phosphorylation of DNA damage markers γ-H2AX, pDNA-PKcs and pKAP1. *In vitro*, tumor cells with ATM loss exhibited increased susceptibility to RP-3500 and a 3-day compound exposure was sufficient to generate a sustained DNA damage response compared to cells with functional ATM expression. In ATM-deficient mouse models, short-duration intermittent (weekly 3 days on/4 days off or 5 days on/2 days off) dosing schedules maximized tumor growth inhibition while minimizing the impact on hematology parameters, including red blood cell depletion. These results emphasize the reversible nature of erythroid toxicity with RP-3500 and the advantage of intermittent dosing schedules to alleviate anemia. The 3 days on/4 days off intermittent treatment schedule also substantially improved the efficacy and tolerability of RP-3500 and PARP inhibitor combinations compared to continuous treatment schedules. Intermittent treatments of RP-3500 given concomitantly with reduced doses of olaparib or niraparib demonstrated synergistic efficacy in Granta-519 (ATM mutn) and SUM149PT (BRCA1mutn) models with minimal hematological adverse effects and superior efficacy to sequential treatment. **CONCLUSIONS:** These results provide a strong preclinical rationale to support clinical investigation of the novel ATR inhibitor RP-3500 on an intermittent schedule as a monotherapy and in combination with PARP inhibitors as a means of maximizing clinical benefit. RP-3500 is currently evaluated in the ongoing phase 1 TRESR (Treatment Enabled by SNIPRx) study (NCT04497116).

P055 Targeting Krebs-cycle-deficient renal cell carcinoma with PARP inhibitor and low-dose alkylating chemotherapy. Daiki Ueno[1], Amrita Sule[2], Jiayu Liang[3], Jinny van Doorn[2], Ranjini Sundaram[2], Randy Caliliw[4], Huihui Ye[4], Rong Rong Huang[4], Jing Li[5], Karla Boyd[6], Ranjit S. Bindra[2], Juan C. Vasquez[6], Brian M. Shuch[1]. [1]Department of Urology, David Geffen School of Medicine at UCLA, Los Angeles, CA, [2]Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, [3]Department of Urology, West China Hospital/ School of Medicine, Chengdu City, China (Mainland), [4]Department of Pathology & Laboratory Medicine, UCLA, Los Angeles, CA, [5]Karmanos Cancer Institute, Wayne State University, Detroit, MI, [6]Department of Pediatrics, Yale University School of Medicine, New Haven, CT.

Loss-of-function mutations in genes encoding the Krebs cycle enzymes fumarate hydratase (FH) and succinate dehydrogenase (SDH) lead to excess accumulation of fumarate and succinate, respectively. Germline mutations in FH lead to a genetic predisposition to hereditary leiomyomatosis and renal cell cancer (HLRCC)-associated RCC. Similarly, loss-of-function alterations of SDH, most commonly SDHB, are associated with SDH-deficient neoplasms, including RCC. FH and SDHB deficient RCC tends to be aggressive and metastasize early with very limited treatment options. The oncometablites produced by these mutations, fumarate and succinate, competitively inhibit αKG-dependent dioxygenases, resulting in dysregulated DNA methylation and histone modification. We have previously demonstrated that elevated levels of fumarate and succinate both suppress the homologous recombination (HR) DNA-repair pathway through inhibition of the lysine demethylase KDM4B, resulting in aberrant hypermethylation of histone 3 lysine 9 (H3K9) at loci surrounding DNA breaks and masking a local H3K9 trimethylation signal that is essential for the proper execution of HR. In this study, we sought to identify novel treatment approaches that exploit genomic instability in FH- and SDHB-deficient RCC. Temozolomide (TMZ), an alkylating agent, mediates its cytotoxic effect by attaching methyl groups to DNA (O6-guanine, N7-guanine and N3-adenine). [16] N3-MetA and N7-MetG repair are mediated by the base excision repair (BER) in a process involving PARP. While the combination of PARP inhibitors with TMZ has been shown to increase TMZ-induced cytotoxicity, clinical trials investigating combination therapy have been hampered by significant toxicity. Using CRISPR/Cas9, we developed new syngeneic FH- and SDHB-deficient murine models of RCC. We demonstrate that FH- and SDHB-deficient cells have accumulation of fumarate and succinate leading to an increase in unresolved DNA double-strand breaks (DSBs). Treatment with PARP inhibition and temozolomide results in marked *in vitro* cytotoxicity in FH- and SDHB-deficient cells, even at low concentrations of TMZ (50 μM). *In vivo*, the combination of standard dose BGB-290 and low-dose TMZ (3mg/kg/dose) results in significantly delayed tumor progression in an SDHB deficient RENCA model without any significant increase in toxicity. Notably, the TMZ dose of 3mg/kg/dose is significantly lower than the 50mg/kg/dose that is commonly used for *in vivo* studies. Taken together, these findings provide the basis for a novel therapeutic strategy exploiting HR deficiency in FH and SDHB-deficient RCC with combined PARP inhibition and low-dose alkylating chemotherapy. Furthermore, the development of a new syngeneic mouse model provides a tool for the future study of immunotherapy in Krebs-cycle-deficient RCC.

P056 Polθ inhibitors elicit *BRCA*-gene synthetic lethality and target PARP inhibitor resistance. Diana A. Zatreanu[1], Helen M. R. Robinson[2], Omar Alkhatib[2], Marie Boursier[2], Harry Finch[2], Lerin Geo[2], Diego Grande[2], Vera Grinkevich[2], Robert Heald[2], Sophie Langdon[2], Jayesh Majithiya[2], Claire McWhirter[2], Niall M. B. Martin[2], Shaun Moore[2], Joana Neves[2], Eeson Rajendra[2], Marco Ranzani[2], Theresia Schaedler[2], Martin Stockley[2], Kimberley Wiggins[2], Rachel Brough[1], Sandhya Sridhar[1], Aditi Gulati[1], Nan Shao[1], Luned M Badder[3], Daniela Novo[1], Eleanor G. Knight[1], Rebecca Marlow[1], Syed Haider[1], Elsa Callen[4], Graeme Hewitt[5], Joost Schimmel[6], Remko Prevo[7], Christina Alli[8], Amanda Ferdinand[8], Cameron Bell[8], Peter Blencowe[8], Mathew Calder[8], Mark Charles[8], Jayne Curry[8], Tennyson Ekwuru[8], Katherine Ewings[8], Andre Nussenzweig[9], Marcel Tijsterman[6], Andrew Tutt[1], Simon J. Boulton[5], Geoff S. Higgins[7], Steve Pettitt[1], Graeme C. M. Smith[2], Christopher J. Lord[1]. [1]Institute of Cancer Research, London, United Kingdom, [2]Artios Pharma, Cambridge, United Kingdom, [3]The Institute of Cancer Research, London, United Kingdom, [4]NCI NIH, Bethesda, MD, [5]The Francis Crick Institute, London, United Kingdom, [6]LUMC, Leiden, Netherlands, [7]MRC-University of Oxford, Oxford, United Kingdom, [8]CRUK-Therapeutic Discovery Laboratories, Cambridge, United Kingdom, [9]NCI NIH, Bethesda, MD.

To target DNA repair vulnerabilities in cancer, we discovered nanomolar potent, selective, low molecular weight, allosteric inhibitors of the polymerase function of DNA polymerase Theta (Polθ), including ART558. ART558 inhibits the major Polθ-mediated DNA repair process, Theta-Mediated End Joining (TMEJ), without targeting Non-Homologous End Joining. Moreover, we show that exposure to ART558 can elicit DNA damage and synthetic lethality in *BRCA1*- or *BRCA2*-mutant tumour cells and enhances the effects of a PARP inhibitor. Genetic perturbation screening revealed that defects in the 53BP1/Shieldin complex, which are a cause of PARP inhibitor resistance, result in *in vitro* and *in vivo* sensitivity to Polθ polymerase inhibitors. Mechanistically, ART558 increases biomarkers of single-stranded DNA and synthetic lethality in 53BP1-defective cells. The inhibition of DNA nucleases that promote end-resection reversed these effects, suggesting that resection via *Exo1* or *Blm-Dna2* being a cause, at least in part, of the ART558 sensitivity phenotype. Taken together, these observations describe a drug class that elicits *BRCA*-gene synthetic lethality and PARP inhibitor synergy, as well as targeting a biomarker-defined mechanism of PARPi-resistance.

P057 The ubiquitin-dependent ATPase p97 removes cytotoxic trapped PARP1 from chromatin. Dragomir Krastev[1], Shudong Li[2], Yilun Sun[3], Yves Pommier[3], Kristijan Ramadan[4], Christopher J. Lord[5]. [1]The Institute of Cancer Research, London, United Kingdom, [2]University of Oxford, Oxford, United Kingdom, [3]National Cancer Institute, NIH, Bethesda, MD [4]University of Oxford, Oxford, United Kingdom, [5]The Institute of Cancer Research, London, United Kingdom.

Poly-(ADP-ribose) polymerase inhibitors (PARPi) elicit anti-tumour activity in homologous recombination defective cancers by promoting cytotoxic, chromatin-bound, "trapped" PARP1. How cells process trapped PARP1 remains unclear. By exploiting wild-type or trapping-resistant PARP1 transgenes combined with either a rapid immunoprecipitation mass-spectrometry of endogenous proteins (RIME)-based approach or PARP1 Apex2-proximity labelling linked to mass-spectrometry, we generated proteomic profiles of trapped and non-trapped PARP1 complexes. This combined approach identified an interaction between trapped PARP1 and the ubiquitin-regulated p97 ATPase (aka VCP). Subsequent experiments demonstrated that upon trapping, PARP1 is SUMOylated by the SUMO-ligase PIAS4 and subsequently ubiquitinated by the SUMO-targeted E3-ubiquitin ligase, RNF4, events that promote p97 recruitment and p97 ATPase-mediated removal of trapped-PARP1 from chromatin. Consistent with this, small molecule p97 complex inhibitors, including a metabolite of the clinically-used drug disulfiram, CuET that acts as a p97 sequestration agent, prolong PARP1 trapping and thus enhance PARPi-induced cytotoxicity in homologous recombination defective tumour cells and patient-derived tumour organoids. Taken together, these results suggest that p97 ATPase plays a key role in the processing of trapped PARP1 from chromatin and the response of homologous recombination defective tumour cells to PARPi.

P058 Anti-tumor activity of ATR inhibitor BAY 1895344 in patient-derived xenograft (PDX) models with DNA damage response (DDR) pathway alterations. Christian X. Cruz Pico[1], Dali Li[1], Christopher D. Lanier[1], Kurt Evans[1], Maria G. Raso[1], Timothy DiPeri[1], Yasmeen Rizvi[1], Min Jin Ha[1], Huiqin Chen[1], Ming Zhao[1], Argun Akcakanat[1], Xiaofeng Zheng[1], Gokce Toruner[1], Erkan Yuca[1], Stephen Scott[1], Antje M. Wengner[2], Timothy A. Yap[1], Funda Meric-Bernstam[1]. [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Bayer, Berlin, Germany.

Introduction: The ATR (ataxia-telangiectasia and Rad3 related protein) kinase inhibitor BAY 1895344 is currently in clinical development for the treatment of advanced solid tumors and has demonstrated promising antitumor activity in heavily pretreated patients with various advanced solid tumors, particularly those with *ATM* deleterious mutations and/or loss of ATM protein. There is further need to identify robust predictive biomarkers to optimize patient selection for ATR inhibitors. For that purpose, we tested the antitumor activity of BAY 1895344 in patient-derived xenograft (PDX) models that are characterized by a variety of DDR alterations. Methods: PDX models were characterized by genomic sequencing and ATM loss by immunohistochemistry (IHC). PDX models with deleterious *ATM*, *BRCA1* or *BRCA2* mutations or loss derived from a variety of histologies were tested. BAY1895344 treatment was tested with two monotherapy regimens 20 mg/kg and 40 mg/kg PO BID both 3 days on/4 days off. For *in vivo* studies, the percent tumor volume change per time point was calculated as a relative level of tumor growth change from baseline: , where is the tumor volume at time and is the tumor volume at baseline. T/C ratio was defined as the ratio of tumor volume change in treated vs control group. An event in each animal was defined as a doubling of tumor volume from initial tumor volume. Event free survival was analyzed by Kaplan-Meier survival analysis. Results: Seventeen PDX models from sixteen patients were treated with BAY 1895344. The PDX models spanned multiple tumor types: breast, colon, pancreas, and cholangiocarcinoma. BAY 1895344 has shown potent and dose-dependent antitumor activity. Strongest activity was observed with BAY 1895344 at 40 mg/kg PO BID applied for 3 days on and 4 days off treatment, achieving a regression or T/C ratio <0.4 in 6 of 17 models. Eleven models showed statistically significant prolongation of event-free survival. BAY 1895344 had anti-tumor activity in PDX models with ATM loss as well as *BRCA* alterations. Notably BAY 1895344 had antitumor activity in an ATM-deleted PDX model with acquired PARP inhibitor resistance generated in the lab as well as a PDX model generated from a *BRCA*-mutant patient with clinically acquired PARP resistance. Conclusion: ATR inhibition via treatment with BAY 1895344 shows potent antitumor activity as monotherapy in selected models that are characterized by certain DDR alterations and even those that have developed resistance to PARP inhibition. Further analyses are ongoing to define predictors of sensitivity to BAY 1895344 as well as pharmacodynamic markers of efficacy/response as a single agent or in rational combinations

P059 Exploiting mutant PPM1D-induced metabolic defects with nanoparticle-encapsulated NAMPT inhibitors. Matthew A. Murray, Yazhe Wang, Ranjini Sundaram, Jason Beckta, Mark Saltzman, Ranjit Bindra. Yale University, New Haven, CT.

Diffuse Intrinsic Pontine Glioma (DIPG) is a leading cause of death in pediatric cancer, with an abysmal <1% 5-year survival rate due to lack of effective treatment options. A significant effort is needed to understand the genetic landscape of this disease to develop novel therapeutic strategies and modalities. Recently, the Bindra laboratory found that a truncation mutation in the gene *PPM1D,* found in ~30% of DIPG cases, causes global epigenetic alterations that lead to therapeutic vulnerabilities. They found that mutant PPM1D activity results in loss of the NAD+ biogenesis protein NAPRT that can be therapeutically targeted by inhibitors of another protein in the NAD+ biogenesis pathway NAMPT (NAMPTi), providing a viable therapeutic strategy for these cancers. Some NAMPTis have been FDA-approved for clinical usage, but present with systemic and retinal toxicity. Moreover, while there are few NAMPTis that can pass the blood brain barrier through systemic delivery, studies show diminished concentrations at the target site. Together, current studies show that the use of NAMPTis for precision targeting of CNS cancers such as DIPG with mutant PPM1D status is a promising therapeutic strategy, but impractical given the limitations of these drugs. However, recent developments in drug delivery systems offer a chance to overcome these issues. Tools such as nanoparticle (NP) drug delivery and unique injection set-ups such as convection-enhanced delivery (CED) allow for drugs such as NAMPTis to be reconsidered for clinical usage. To circumvent the challenges presented by these drugs, **we have developed and characterized nanoparticles that encapsulate NAMPTis (NAMPTi-NP) and use CED for sustained intratumoral delivery.** Thus far, we have fabricated and optimized PLA-PEG copolymeric nanoparticles capable of encapsulating the NAMPTi, GMX-1778. We characterized these nanoparticles based on (1) hydrodynamic diameter, (2) zeta potential, and (3) stability within an artificial cerebrospinal fluid solution. We have performed both *in vitro* and *in vivo* assays to determine the functionality of the NAMPTi-NPs through (1) cellular uptake studies via immunofluorescence, (2) functional analysis via NAD^+ quantification, (3) short- and long-term cell viability assays to determine sensitivity to the NAMPTi-NP, and (4) *in vivo* biodistribution studies to assess sustained retention of NAMPTi-NP with CED intracranial injections. We find that NAMPTi-NPs have immediate and sustained cellular uptake, loss of NAD^+ post-treatment indicating effective targeting, and enhanced sensitivity in long-term viability studies in mutant PPM1D models. Lastly, these NAMPTi-NP display prolonged retention in brain tissue compared to free drug injection over time. With further *in vivo* validation, this NP-based strategy will be a powerful tool for targeting mutant PPM1D DIPG and other cancers.

P061 *De novo* design of importin-*a*-specific NLS sequences for nuclear-targeted therapeutics. Olga Bednova, Alexis Rioux-Chevalier, Dipika Patel, Mathieu Boudreau, Jeffrey Victor Leyton. Université de Sherbrooke, Sherbrooke, QC, Canada.

Background: Research focused on the application of nuclear localization sequence (NLS)-based therapeutics has been a topic of intense interest to medicine since the core principles governing nuclear transport were awarded the Nobel Prize in 1999. Despite these efforts, efficient nuclear localization has been difficult. A major obstacle is that NLSs have cationic-net charges (abundance of lysines/arginines) required for appropriate interactions with the nuclear transporter importin-a (Impa). Once in the blood stream, cationic charges can cause strong non-specific cellular uptake and are rapidly cleared from the plasma compartment, which prevents sufficient tumor cell uptake and, hence, nuclear localization. Here, describe a *de novo* computational approach for generating 43 novel NLSs (based on 3 different NLS classes) that contained amino acid substitutions to achieve net-neutral charge states. We also introduce a novel nuclear isolation-quantitative flow cytometric method for determining nuclear localization efficiency. **Material and methods** An algorithm was created based on complete interaction binding affinity strengths for 20 x 20 pairs of octapeptides consisting of the 20 common amino acids. 9 PDB files were selected as templates and were comprised of viral, RNA processing, and transcription initiation protein NLSs bound to Impa. *In silico* alanine scanning on all NLS templates generated rankings on amino acid sensitivities for each position in the sequences. Non-sensitive residues were then subjected to residue scanning for generating net-neutral charged NLSs. Computational docking studies generated predictive binding scores relative to wild type NLSs. Favorable NLS candidates were genetically fused to GFP. In contrast to utilizing fluorescence microscopy, which cannot determine nuclear localization efficiency, we created a method to isolate nuclei from transiently transfected CHOK1 cells and quantify nuclear localization by flow cytometry. **Results** 2-8 net-neutral NLSs with good binding for Imga could be generated for each PDB file. The net-neutral mutants often contained an abundance of glutamic and/or aspartic acid substitutions, and were able to bind to Impa residues to compensate for the replaced amino acids. Transfected GFP-NLS constructs displayed variable fluorescence expression kinetics. Therefore, we created an in-house plasma membrane lysis protocol to isolate intact nuclei. Quantitative evaluations are currently underway by evaluating nuclei fluorescence by flow cytometry at various time points. Thus far, the tested GFP-NLSs are able to localize to the nucleus. **Conclusions** An important objective of computational protein design is the generation of high affinity peptides as a precursor to the development of therapeutics, and as a tool to aid researchers in understanding governing interaction principles of specific complexes. We have achieved both the development of potential NLS peptides for overcoming the cationic-sequestration barrier, and for understanding novel NLS principles to further advance the NLS-therapeutics field.

P062 The CL2A-SN38 linker-payload system conjugated to trastuzumab results in improved cellular cytotoxicity over time relative to T-DM1. Hardeep Singh, Victor Jeffrey Leyton. Université de Sherbrooke, Sherbrooke, QC, Canada.

Background The approved antibody-drug conjugate (ADC) sacituzumab-govitecan (SG) that exploits the topoisomerase I inhibitor SN38, represents a substantial advance in the ADC field. SN38 is the first payload with low nanomolar cytotoxicity. This deviates from current design principles, which utilize ultracytotoxic payloads. The CL2A linker has a short non-cleavable seven polyethylene glycol segment that enables a higher number of hydrophobic SN38 molecules to be attached to reduced cysteines. CL2A is also designed to permit the release of SN38 in an acidic environment via a pH-sensitive benzyl carbonate bond to SN38's lactone ring. There is no cathepsin B cleavage site. Key to the performance of SG is the SN38 release half-life of 1-2 days. This also deviates from very stable linker systems that require full ADC internalization and lysosomal processing for payload release. As a result of these novel ADC properties, SG is given in higher and repeated doses, which results in slow release at the tumor site and enhanced uptake by target cancer cells and reduced toxicity on healthy cells due to low potency SN38 alone. Our goal is to further evaluate the CL2A-SN38 system when conjugated to trastuzumab. **Material and methods** Trastuzumab was conjugated to CL2A-SN38 to reduced cysteines (T-SN38). Monitoring the changes in DAR over time was performed to determine the stability of T-SN38 in PBS at pH values of 7.4, 5.72, and 4.58, to mimic circulating blood, early endosomes, and lysosomes, respectively. Cytotoxicity assays for 72 h on HER2-positive ovarian cancer SKOV3.ip and HER2-negative CHO cells were performed and compared to the approved anti-HER2 ADC trastuzumab-emtansine (T-DM1). Cytotoxicity assays were also performed between T-SN38 and T-DM1 at their respective IC_{50} values and the percent of cell survival determined daily from 24-96 h. **Results**T-SN38 was generated with a DAR of 6.97. Surprisingly, the half-life of SN38 release in acidic pH was extremely slow ($t_{1/2}$=~120 h) and at the final evaluated time point (120 h) 69% of SN38 remained conjugated to trastuzumab. As anticipated, as a function of concentration T-DM1 was significantly more potent than T-SN38 (IC_{50}=0.0013 vs 0.0832 mg/mL). However, when cytotoxicity was evaluated as a function of time T-SN38 switched to the more potent ADC at the late time points. T-SN38 killed SKOV3.ip cells 36% more effectively than T-DM1 at 96 h. **Conclusions** Our data from SN38 release experiments in acidic buffer suggest that pH-induced cleavage of the CL2A linker is not a contributable release mechanism of SN38 when bound to trastuzumab and is counterintuitive for what is currently described for SG. However, traceless release of hydrophobic SN38 capable of traversing cell membranes is only possible by hydrolysis of the benzyl carbonate bond. Our cytotoxicity studies over time suggest that pH-dependent release does occur. Further investigations are ongoing to more precisely determine the contribution of the carbonate bond for SN38 release and cytotoxic potency.

P063 Novel prodrugs coupled with albumin nanoparticle encapsulation improves antitumor effects of the nucleoside analog gemcitabine. Neil Raheja[1], Jason Kahana[2], Robin Jackman[2], Curtis Monnig[2]. [1]Consultant, San Diego, CA, [2]January Therapeutics, San Diego, CA.

Nucleoside analogs such as gemcitabine have been widely used to treat many solid tumors. Despite their near universal use and well understood mechanisms of action, this class of chemotherapeutic agents has notable shortcomings including rapid metabolism, limited systemic exposure, acquired resistance, and dose-limiting toxicities. Albumin-based nanoparticles offer several advantages for drug delivery including direct tumor targeting, low risk of immunogenicity, and ease of synthesis and manufacturing. We have modified numerous nucleoside drugs of clinical significance to form new prodrugs and use nanoparticle encapsulation in an attempt to improve the therapeutic index of the parent drugs. Different prodrugs of gemcitabine were designed to evade drug resistance mechanisms, and methods were developed to form stable lyophilized nanoparticle formulations. Nanoparticles were characterized by particle size (~40-65 nm) and polydispersity, and dose-dependent effects on the proliferation of human tumor cell lines were compared to the response to the parent drug gemcitabine. To evaluate effects in vivo, nanoparticle formulations were dosed intravenously (IV) to immunocompromised mice bearing patient-derived xenografts (PDx). The prodrug JTX-836 (IC_{50}=0.46 nM) was ~10x more potent against BxPC-3 pancreatic adenocarcinoma cells than the parent gemcitabine (IC_{50} = 5.2 nM) in vitro. IV dosing of JTX-836 albumin nanoparticle formulation improved tumor growth inhibition in PDx models of both pancreatic ductal adenocarcinoma (CTG-0282 TumorGraft, Champions Oncology) and ovarian cancer (CTG-1180, Champions Oncology) when compared to IV gemcitabine dosing. These results demonstrate the potential for nanoparticle encapsulation of prodrugs to improve the antitumor activity of existing nucleoside analog therapeutics and support continued development of albumin-based delivery of gemcitabine for the treatment of human cancer.

P064 Novel engineering of therapeutic *Fusobacterium nucleatum* phage for colorectal cancer treatment. Lihi Ninio-Many[1], Yael Zigelman[1], Nufar Buchshtab[1], Yifat Elharar[1], Gal Eylon[1], Eliya Gidron[1], Dikla Berko-Ashur[1], Julian Nicenboim[1], Lior Zelcbuch[1], Einav Safyon Gartman[1], Sharon Kredo-Russo[1], Noga Kowalsman[1], Ilya Vainberg Slutskin[1], Iddo Weiner[1], Inbar Gahali-Sass[1], Naomi Zak[1], Sailaja Puttagunta[2], Merav Bassan[1]. [1]BiomX, Ness Ziona, Israel, [2]BiomX, Branford, CT.

Fusobacterium nucleatum (*FN*) is enriched in human colorectal tumors [1] and its presence is correlated with poor prognosis [2]. Bacteriophages ("phages") are naturally occurring, self-amplifying viruses that are highly specific for particular bacterial species and strains and are recognized to have a high intrinsic safety. They offer a promising treatment strategy to both target *FN* associated with colorectal cancer (CRC) and, by phage engineering, to deliver an anti-tumoral immune-stimulating payload that may enhance the effectivity of other anti-cancer therapies such as checkpoint inhibitors in unresponsive colorectal cancer patients. The major challenge in engineering phages that target *FN* is the optimization of the eukaryotic payloads for high expression in this bacterial host. Since no data is available about codon usage and non-coding genetic sequences in the genome of *FN* bacteria, two computational approaches were used to optimize the codon usage for elevated payload expression of the first selected payload, murine IL-15. These approaches involved identifying ribosome binding sites and elongation speeds to optimize codon usage. Following design of an IL-15 expression vector, expression of this eukaryotic payload in *FN* bacteria was tested *in vitro*. For phage engineering, a plasmid containing the designed payload sequence was used to introduce the selected IL-15 encoding sequences into the phage genome. *FN* phage was successfully engineered to deliver a payload encoding the sequence of murine cytokine IL-15. Targeting of *FN* with engineered phage resulted in IL-15 protein expression in phage-infected bacteria *in vitro*. Given the specificity of *FN* for CRC tumors and the ability to locally express a eukaryotic protein by using *FN* targeting phage to deliver a payload, phage therapy may offer novel treatment approaches for patients with CRC.

P065 Proteogenomic characterization of CDK4/6 inhibitor-resistant ER+ breast cancer.

Christopher T. Chen[1], Ignaty Leshchiner[2], Liz Martin[2], Harry Kane[2], Kahn Rhrissorrakrai[3], Filippo Utro[3], Chaya Levovitz[3], Michael Gillette[2], Shankha Satpathy[2], Christopher Pinto[4], Daniel McLoughlin[4], Read Allen[4], Brian P. Danysh[2], Kara Slowik[2], Raquel A. Jacobs[2], Steven Carr[2], Laxmi Parida[3], Gad Getz[2], Dejan Juric[4]. [1]Stanford University, Palo Alto, CA, [2]Broad Institute of MIT and Harvard, Cambridge, MA, [3]IBM Watson Research, Yorktown Heights, NY, [4]Massachusetts General Hospital, Boston, MA.

Studies of therapeutic resistance in cancer have conventionally focused on identification of acquired exome mutations in tissue or circulating DNA at progression. However, this strategy has generated limited insights into resistance to CDK4/6 inhibitor and endocrine therapy combinations, which are the key first-line treatment modality in ER+ breast cancer. We utilized an alternative approach of integrated proteogenomic analysis of 8 pairs of pre- and post-treatment biopsies, 67 rapid autopsy samples, and 17 plasma samples from 12 ER+ breast cancer patients treated with CDK4/6 inhibitor combinations at a large academic center. In addition to whole exome sequencing, RNA sequencing, and immunohistochemistry (IHC) profiling on all tissue samples, we performed deep-scale mass spectrometry-based proteomics and phospho-proteomics on 35 rapid autopsy samples from 5 patients that had sufficient protein yields for analysis. We describe a patient with acquired Rb IHC loss after treatment progression without a *Rb1* genetic alteration detected in exome sequencing or Rb transcript loss at her post-progression biopsy. Integrated proteogenomic analysis of ten autopsy lesions from this patient revealed convergent Rb protein loss across all tumor lesions, including lesions with and without Rb1 exome alterations. *ESR1* mutations were frequently acquired at post-treatment biopsies at high cancer cell fractions, but a pre-existing *ESR1*-mutant subclone was nevertheless lost in a patient who acquired a concurrent *Rb1*-mutant subclone in the same tumor lesion. This suggests *ESR1* mutations confer a relative fitness advantage that can nevertheless be mitigated in the presence of more potent synchronous resistance mechanisms. To investigate whether circulating tumor DNA reflects DNA shed specifically by progressing tumor lesions, we modeled the DNA shedding of tumor lesions into the plasma for 3 patients with both rapid autopsy and serial plasma samples available. In some instances, new lesions began shedding significant amounts of DNA months prior to their initial radiographic appearance. Together, these results illustrate the value of integrated multi-omics interrogation of different sample types to investigate therapeutic resistance and advance precision oncology.

P066 Gain and loss of function genome-wide CRISPR screens identify Hippo signalling as an important driver of resistance in EGFR mutant lung cancer. Matthias Pfeifer[1], Jonathan Brammeld[2], Stacey Price[2], Matthew Martin[1], Hannah Thorpe[1], Aurelie Bornot[3], Ercia Banks[3], Nin Guan[4], Shanade Dunn[1], Maria Lisa Guerriero[3], Daniel O'Neill[3], James Pilling[3], Davide Gianni[3], James Brownell[4], Paul Smith[1], Ultan McDermott[1]. [1]Oncology R&D, AstraZeneca, Cambridge, United Kingdom, [2]Wellcome Sanger Institute, Cambridge, United Kingdom, [3]Discovery Sciences, BioPharmaceuticals R&D, Cambridge, United Kingdom, [4]Oncology R&D, AstraZeneca, Waltham, MA.

Drug resistance is ultimately the cause of death for most cancer patients – even initially strong responses to treatment are usually followed by the emergence of resistance over time. This suggests the existence of residual or persistent cancer cells, creating a reservoir that ultimately gives rise to stable resistance. These drug tolerant persisters ('the deadly survivors') have been described for over a decade in numerous studies; they are often present as a minor fraction of the total tumour population and may exploit non-genetic (transcriptional) programs to allow the cells to survive drug treatment. 10-20% of lung adenocarcinoma patients harbour activating mutations in *EGFR*. Although treatment with the EGFR kinase inhibitor osimertinib has improved overall survival in such patients, almost all patients ultimately develop drug resistance. We carried out parallel genome-wide CRISPR gain and loss of function screens in *EGFR* mutant lung cancer cell lines treated with EGFR inhibitors, to identify the genes and pathways that may be important in enabling the survival of persister cells. We observed recurrent resistant genes in previously identified resistance pathways including PI3K (PTEN, TSC1, TSC2), MAPK (KRAS, NF1, MET), cell death (BCL2L11, BAX), the mediator complex (MED24, MED19) and ubiquitination (KCTD5, KEAP1). A secondary screen of 63 resistance genes that combined high content microscopy with CRISPR gene knockout demonstrated that 21% (13/63) of genes were associated with increased nuclear localisation of YAP1/WWTR1, key activators of the Hippo pathway. A closer review of the CRISPR screen data confirmed that many resistance hits are members of this pathway - upstream regulators (NF2, AMOTL2), core signalling genes (LATS1, LATS2), main effectors (WWTR1, YAP1), transcriptional co-effectors (TEAD3, FOSL1, VGLL4) and the SWI/SNF complex (ARID2, SMARCA4, SMARCB1, PBRM1). Hippo signalling is mediated through YAP1 and WWTR1 which bind to TEAD transcription factors and activate transcriptional programs affecting cell proliferation and apoptosis. We confirmed using CRISPR that knockout (NF2) or overexpression (YAP1, WWTR1) of key Hippo genes in the *EGFR* mutant lung cancer cell lines PC-9, HCC827 and HCC4006 resulted in up to 60-fold increased resistance to osimertinib and increased expression of canonical Hippo transcriptional targets. We therefore reasoned that the Hippo pathway might be involved in maintaining the survival of drug tolerant persister cells in this setting. Acute treatment of *EGFR* mutant cell lines with osimertinib was associated with increased nuclear localisation of YAP1 and WWTR1 and increased expression of canonical Hippo transcriptional targets. Furthermore, the combination of osimertinib and a TEAD inhibitor (K-975) almost completely abolished the survival of drug tolerant persister cells following treatment, indicating that this pathway is an important survival mechanism following drug treatment. Consequently, we propose Hippo signalling as an important target mechanism for the prevention of resistance to osimertinib.

P067 VRN10s, a series of HER2 inhibitors to overcome NRG-mediated drug resistance and acquired-drug resistant HER2 mutations. Chan Mi Park[1], Jihye Yoo[1], Mingyeong Son[2], Hong-ryul Jung[1], Jin-Hee Park[3], Jeongbum Son[2], Eunhwa Ko[3], Sunghwan Kim[1]. [1]Voronoi, Incheon, Korea, Republic of, [2]VoronoiBio, Incheon, Korea, Republic of, [3]B2SBio, Incheon, Korea, Republic of.

The overexpression of Human epidermal growth factor receptor 3 (HER3) and increased Neuregulin (NRG) production are well known resistance mechanisms to Human epidermal growth factor receptor 2 (HER2) kinase inhibitors. FDA-approved HER2 kinase inhibitors show significantly decreased activities in the presence of NRG, which induces HER2-HER3 heterodimer and activates HER3. VRN10s, HER2 kinase inhibitors, maintained potency to inhibit proliferation of HER2-positive breast cancer cell lines even in the presence NRG1. Western blot confirmed that VRN10s inhibited phosphorylation of HER2 and HER3 as well as downstream signaling, such as AKT and ERK pathways. Emergence of acquired resistance mutations against tyrosine kinase inhibitors (TKI) are also nearly inevitable, but less is known about the acquired drug resistance mutations of the HER2 kinase domain. To predict the drug resistance mutations against two representative HER2 TKIs, Tucatinib (non-covalent) and Neratinib (covalent), we performed an ENU mutagenesis assay and identified HER2 mutant sequences from clonal cells survived from Tucatinib and Neratinib treatment in Ba/F3 HER2 WT cells. There were 9 types of Tucatinib-resistant mutations. The most common Tucatinib-resistant mutation type was T862A/S, which was found in 5 out of total 15 clones. All types of mutations showed 6~70 folds resistance for Tucatinib compared to WT. These mutant types were also resistant against another non-covalent HER2 TKI, Lapatinib. In case of Neratinib, HER2 S963P was derived from two different clones, showing 4~8 folds resistance against Neratinib compared to WT. However, a series of VRN10 was relatively sensitive toward all Tucatinib or Neratinib resistant mutants. Based on our *in vitro* assay and ENU mutagenesis studies, VRN10s can be a new option to overcome HER2 TKI drug resistance derived by HER3 activation or HER2 mutagenesis.

P068 Different treatment schemes cause distinct PARP inhibitor resistance mechanisms in *BRCA2*-mutant ovarian cancer cells. Tzu-Ting Huang[1], Jayakumar R. Nair[1], Nitasha Gupta[1], Tomomi M. Yamamoto[2], Benjamin G. Bitler[2], Jung-Min Lee[1]. [1]National Cancer Institute, National Institutes of Health, Bethesda, MD, [2]University of Colorado, Aurora, CO.

Poly (ADP-ribose) polymerase inhibitors (PARPis) represent a major advance in ovarian cancer management, now as treatment for recurrent ovarian cancer and as a maintenance therapy following platinum-based chemotherapy in the upfront and platinum-sensitive recurrent settings. Yet, a growing number of patients progress through PARPis, creating a need for further understanding on resistance mechanisms. We hypothesized that different treatment dosing/timing schemes would cause distinct mechanisms of acquired resistance to PARPis. To test this hypothesis, we exposed *BRCA2*-mutant PEO1 ovarian cancer cells to 0.5 μM olaparib, doubling its concentration up to 40 μM over 3–4 months (PEO1/OlaJR). The second PARPi-resistant PEO1 cell line (PEO1/OlaR) was developed using a relatively low initial dose of 5 nM olaparib, and gradually increasing its concentration up to 20 μM over 6 months. To confirm PARPi resistance, cell proliferation and viability were measured by MTT and colony formation assay, respectively. Both cell lines were resistant to two different PARPis beyond clinically achievable concentrations (olaparib IC_{50} 141.7–372.8 μM and rucaparib IC_{50} 37.1–51.2 μM) and demonstrated restored homologous recombination (HR) repair as evidenced by increased HR-reporter activity (1.32 to 1.45-fold compared to PEO1, $p < 0.05$) and RAD51 foci formation (5.2 to 6.1-fold compared to PEO1, $p < 0.001$). For PEO1/OlaJR, distinct resistance mechanisms to PARPi were noted: 1) promoting drug efflux confirmed by increased MDR efflux activity (1.92-fold compared to PEO1, $p < 0.001$) and increased protein levels of an ABCB1 transporter (7.2-fold compared to PEO1); and 2) enhancing HR repair restoration, likely via reduced REV7 protein expression (0.43-fold compared to PEO1). On the other hand, PEO1/OlaR exhibited a mesenchymal cell phenotype with greater invasion ability as measured by transwell invasion assay (2.25-fold compared to PEO1, $p < 0.001$) and restored HR repair with upregulation of TRIP13 protein (1.85-fold compared to PEO1). PEO1/OlaR also showed increased cell survival, possibly via activation of PI3K/AKT signaling, as evidenced by increased p-AKT (2.12-fold compared to PEO1). No loss of 53BP1 was observed in either PEO1/OlaJR or PEO1/OlaR based on whole exome sequencing and immunoblotting of 53BP1. *BRCA2* reversion mutation status is currently under investigation. Altogether, our findings suggest that mechanisms of resistance to PARPis arise differently based on the duration and concentrations of drug treatment, highlighting the importance of appreciating interplay between specific treatment schemes and the downstream consequences. Thus, developing tailored therapeutic strategies should consider various mechanisms of PARPi resistance.

P069 GDF15 contributes to the maintenance of the drug-tolerant persister state in cells responding to eribulin. Chiara Bellio[1], Marta Emperador[1], Esther Zamora[2], Violeta Serra[1], Cristina Saura[2], Bruce A. Littlefield[3], Josep Villanueva[1]. [1]Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain, [2]Vall d'Hebron Institute of Oncology (VHIO); Breast Cancer Program, Vall d´Hebron Univ. Hospital, Barcelona, Spain, [3]Eisai Inc., Cambridge, MA.

A major obstacle in the fight against cancer is the development of drug resistance in response to therapy. Acquired resistance can be mediated by a small population of drug-tolerant persister cancer cells (DTPs), which are characterized by an increased capacity of adaptation to various stresses. DTPs are a small slow-cycling population of cells that tolerate cancer drugs, and most likely are responsible for generating a stable resistance. Growth differentiation factor 15 (GDF15) is a well-established marker of cellular stress. It is a member of the TGF-β superfamily, and while its physiological expression is barely detectable in most human somatic tissues, it is prominently induced under stress conditions to maintain cell and tissue homeostasis. Evidence suggests that GDF15 can be secreted in different cancer types. Nevertheless, GDF15 can have opposite effects depending on cellular context and disease stage, and further studies are needed to confirm its biological role in cancer. The working hypothesis of this study is that GDF15 plays a functional role in mediating the eribulin tolerance in DTP cells. MDA-MB-231 and MCF7 cell lines were treated for a month with an IC80 of eribulin. Initially, cells were sensitive to eribulin and approx. 85 % of the cells died after 7 days of treatment, then for about three weeks the surviving cells remained in a quiescent-DTP state. After approx. 3 weeks of treatment with a high dose of eribulin, cells started to proliferate in the presence of the drug, and we assume that at this point a stable mechanism of resistance is established. Interestingly, the analysis of the GDF15 levels during the chronic treatment showed that GDF15 expression/secretion was only detectable in the DTP state, suggesting a role of GDF15 in the maintenance of DTPs. To demonstrate the GDF15 protecting role against eribulin, we conducted loss of GDF15 function experiments, targeting both GDF15 and its receptor GFRAL. siGDF15 cells showed higher sensitivity to eribulin treatment than the siCTRL cells. We confirmed the same results targeting the GFRAL receptor, confirming an autocrine role of the secreted GDF15 in the cells that survive eribulin treatment. These results open the possibility for combining eribulin and a GDF15 antibody to specifically target DTP cells, and overcome eribulin resistance. Moreover, we envision that the circulating levels of GDF15 in metastatic breast cancer patients could be used as a pharmacodynamic biomarker during eribulin treatment. Furthermore, we are confident that GDF15 could help to better understand the onset of acquired resistance to eribulin in metastatic breast cancer patients.

P070 Targeting SOX10 deficient cells to reduce resistance to targeted therapy in melanoma. Claudia Capparelli, Timothy Purwin, Andrew Aplin. Thomas Jefferson University, Philadelphia, PA.

Cellular plasticity contributes to intra-tumoral heterogeneity and phenotype switching, traits that enable tumor cell adaptation to metastatic microenvironments and resistance to targeted therapies. Despite these wide-ranging effects, the key mechanisms that underlie tumor cell plasticity remain poorly understood. We studied the role of SOX10, a neural crest lineage transcription factor, and its role in cutaneous melanoma plasticity. SOX10 was heterogeneously expressed in melanoma patient samples. Loss of SOX10 was sufficient to induce a slow cell cycling phenotype *in vitro* and *in vivo*, which was associated with invasive properties including expression of mesenchymal genes and extracellular matrix, as well as tolerance to BRAF and/or MEK inhibitors. Long-term exposure of co-mixtures of SOX10-proficient and SOX10-deficient cells to targeted therapy selected for SOX10 knockout cells. Furthermore, cell lines generated from BRAF-MEK inhibitor resistant xenografts showed dramatic reductions in SOX10 expression. To identify synthetic lethal interactions with SOX10 loss, we screened a drug compound library and identified the class of cellular inhibitor of apoptosis protein-1/2 (cIAP1/2) inhibitors as selectively inducing cell death in SOX10-deficient cells. Combining cIAP1/2 inhibitor with BRAF/MEK inhibitors delayed the onset of acquired resistance in melanomas *in vivo*. Together, these data suggest that SOX10 mediates phenotypic switching in cutaneous melanoma to produce a targeted inhibitor tolerant state that is likely a prelude to the acquisition of resistance. Furthermore, we provide a novel strategy by selectively eliminating SOX10-deficient cells.

P071 Phosphoproteomics identifies Mig6 as a key mediator of adaptive resistance to ALK/ROS1 oncogene inhibition. Nan Chen[1], Anh T. Le[1], Eric A. Welsh[2], Bin Fang[2], Eric B. Haura[2], Robert C. Doebele[1]. [1]University of Colorado Anschutz Medical Campus, Aurora, CO, [2]Moffitt Cancer Center, Tampa, FL.

Tyrosine kinase inhibitors (TKIs) targeting ALK and ROS1 fusions have significantly improved outcomes for patients with non-small cell lung cancer harboring these oncogenes. However, complete responses are rarely achieved, and therapeutic resistance eventually emerges from residual tumor. To control or eradicate residual disease pre-emptively, it is important to understand the molecular mechanism contributing to the adaptive resistance under initial exposure to oncogene inhibition. Our lab previously demonstrated that EGFR mediates responses to TKIs in oncogene fusion positive cancers, but the detailed mechanism underlying adaptive EGFR signaling is still not clear. We performed global and tyrosine-specific phosphoproteomic analyses to investigate the signaling reprogramming following 2-hr treatment of crizotinib, a dual ALK/ROS1 inhibitor, in an EML4-ALK cell line (H3122) and a TPM3-ROS1 patient-derived cell line (CUTO28). Pathway analysis revealed "ErbB signaling pathway" and "regulation of ErbB signaling pathway" were enriched in the proteins with differentially expressed phosphosites under crizotinib treatment. Mig6, a protein that negatively regulates EGFR, showed a significant decrease of phosphorylation of Y394/Y395 following crizotinib treatment. Phosphorylation of these 2 residues has been shown to be essential for Mig6 binding and subsequent inhibition of EGFR, suggesting that EGFR activity may be released from Mig6 inhibition rapidly following ALK or ROS1 inhibition. Mig6 transcripts and protein levels were also reduced by following ALK/ROS1 inhibition. The MEK inhibitor trametinib decreased Mig6 transcripts and protein with a similar magnitude as ALK/ROS1 inhibition, suggesting that the regulation of Mig6 expression by ALK/ROS1 inhibition is mediated by the MAPK pathway. Mig6 knock-down was able to rescue cell survival and p-Erk suppression from ALK/ROS1 inhibition, but failed to rescue when co-treated with afatinib, a pan-HER inhibitor. Neither Mig6 knockdown nor overexpression altered the phosphorylation of EGFR itself. Instead, phosphorylation of Y239 and Y240 on Shc1, a signaling adapter shared by ALK, ROS1, and EGFR, was rescued by Mig6 knockdown under ALK/ROS1 inhibition. This is consistent with the previously reported structures showing Mig6 and Shc1 compete for the same substrate binding cleft on EGFR. Combination of afatinib and ALK/ROS1 TKIs eliminated residual colony formation in 2 weeks. Crizotinib-resistant H3122 demonstrated loss of Mig6 and resistance could be reversed using either afatinib or Mig6 overexpression. In summary, this study identified Mig6 as a novel regulator for ErbB signaling-mediated adaptive and acquired resistance to ALK/ROS1 TKIs. This resistance mechanism may have broader implications for other oncogene-driven cancers given the observed Mig6 regulation by the MAPK pathway. These data provide additional support for co-inhibition of EGFR in oncogene-driven cancers, such as ALK or ROS1, to reduce the burden of residual disease by eliminating Mig6-mediated EGFR survival pathways.

P072 Combining ATR inhibitors with carboplatin in chemoresistant TNBC conditionally reprogrammed cells and patient-derived xenografts. Juliet Guay. McGill University, Montreal, Canada.

Background: Breast cancer is the second leading cause of cancer related death worldwide in women and triple negative breast cancer subtype is associated with a poor prognosis. TNBC patients are treated mostly with chemotherapy. In addition to the standard of care, the DNA crosslinking agent Carboplatin is often used in the treatment of both early and metastatic TNBC. A fraction of patients presents a good response, but the majority are resistant or develop resistance to chemotherapy. Overcoming chemotherapy resistance in TNBC is a major clinical unmet need and the identification of novel treatments against chemoresistant TNBC is necessary. Method: We used conditional reprogramming to establish 5 patient-derived TNBC cell lines (CRCs) from patient-derived xenografts (PDXs) generated from chemotherapy resistant tumors. All cell lines were resistant to carboplatin, and we performed shRNA high throughput screens to identify genetic vulnerabilities that could resensitize these cells to Carboplatin. We identified ataxia telangiectasia and Rad3-related protein (ATR) as a target in one of the cell lines screened. Validation of this target was performed by targeted shRNA ATR knockdown and pharmacologically with commercially available ATR inhibitors both in vitro and in vivo. Results: We confirmed that ATR inhibition resensitizes TNBC CRC to Carboplatin with shRNA ATR. We used Ceralasertib and Elimusertib, 2 ATR inhibitors, to pharmacologically validate this hit. We tested the effect of the combination of each ATR inhibitors with carboplatin (IC25) using alamar blue assays and calculated the combination index with the Chou Talalay method in all 5 cell lines. We found the combination with Elimusertib to have a more potent synergy (Average CI=0.43) than the combination with Ceralasertib (Average CI=0.68). To further explore the mechanism of ATR inhibition in this combination we measured the key targets and observed a loss of ATR protein by western blot when the cells were exposed to the combination with Elimusertib-Carboplatin but not with Ceralasertib-Carboplatin. Pharmacological inhibition of ATR main effector Chk1 with Rabusertib or by knockdown did not recapitulate the synergy observed with Elimusertib-Carboplatin, suggesting the synergy observed is independent of Chk1 activity. Surprisingly, ATR inhibition or knockdown still modulated major cell cycle players. Finally, we performed *in vivo* validation using the PDX from which the cell line had been derived. Our results demonstrated that addition of low doses of Elimusertib to Carboplatin significantly delayed tumor growth compared to carboplatin treatment alone and prolonged survival in animal models. Conclusion: We highlighted the efficacy of ATR inhibition to resensitize drug resistant TNBC to Carboplatin as supported by our in vitro and in vivo results. Our data also sheds light on a potentially novel mechanism of action of the Elimusertib drug involving ATR loss of expression. Our findings imply that all ATR inhibitors are not equal, and they might be associated with different mechanisms of action.

P073 Pharmacological inhibition of IRAK4 with CA-4948 is beneficial in marginal zone lymphoma models with secondary resistance to PI3K and BTK inhibitors. Francesca Guidetti[1], Alberto J. Arribas[1], Filippo Spriano[1], Laura Barnabei[1], Reinhard von Roemeling[2], Elizabeth Martinez[2], Emanuele Zucca[3], Francesco Bertoni[1]. [1]Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, Switzerland, [2]Curis, Inc., Lexington, MA, [3]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland.

Background. Marginal zone lymphoma (MZL) is a heterogeneous B-cell malignancy for which no standard treatment exists. B-cell receptor (BCR) signaling dysregulation is a hallmark of MZL and can mimic antigen dependent BCR activation. However, single treatment with BCR signaling inhibitors shows limited complete response rate and relapsing patients. IRAK4 is a protein kinase downstream the Toll-like receptor signaling. CA-4948 is the first-in-class inhibitor of IRAK4 and it is in phase 1/2 studies for patients with relapsed/refractory clinical studies with favorable activity in lymphomas and myeloid neoplasms. Here, we assessed IRAK4 inhibition with CA-4948 against a panel of MZL cell lines in which we have developed secondary resistance to inhibitors of PI3K (idelalisib, copanlisib) or BTK (ibrutinib). **Methods**. Models with secondary resistance were developed by continuous exposing cell lines derived from splenic MZL (Karpas 1718 and VL51) to high doses of drugs for long period of times. Resistant and parental lines were exposed to increasing doses of CA-4948, alone or in combination with compounds they are resistant to. Cell viability was tested by MTT assay (72h treatment). Synergy of combinations was evaluated according to the Chou-Talalay combination index (CI), as well as potency and efficacy, estimated according to the MuSyC algorithm. **Results.** The parental Karpas1718, bearing a *MYD88* L265P mutation, had an IC50 of 3.29 µM for CA-4948 as single agent, while the parental VL51 and all resistant models presented IC50 values in the range of 19-35 µM. IRAK4 inhibition with CA-4948 had strong benefit when combined to downstream BCR inhibitors. In resistant cells, CA-4948 was strongly synergistic with idelalisib, ibrutinib and, at lesser extent, with copanlisib. CA-4948 in combination with ibrutinib was strongly synergistic especially in the VL51 ibrutinib resistant model compared to the parental one. CA-4948 (from 1 to 5 µM) recovered sensitivity to ibrutinib at IC50 values close to the parental condition. Similarly, CA-4948 in combination with idelalisib was also synergistic and increased sensitivity to idelalisib in idelalisib resistant VL51. Less benefit was seen adding CA-4948 to copanlisib: the combination was synergistic in VL51 parental cell, but with only weak efficacy. In the Karpas1718, CA-4948 was synergistic either in idelalisib resistant or in parental, with improved benefit in the latter, in which synergistic effect was observed when combined with both idelalisib and ibrutinib. The synergistic activity of CA-4948 was mainly due to improved efficacy rather than potency of the combinatorial partners. **Conclusions.** Our results in MZL cell lines show that the IRAK4 inhibitor CA-4948 is synergistic with small molecules targeting BCR signaling, especially with idelalisib and ibrutinib. Data also suggest that concentrations of CA-4948 comparable to those achieved in patients might overcome or reduce secondary resistance to the tested tyrosine kinase inhibitors.

P074 STAT3 pathway in palbociclib resistance in breast cancer cell lines and the role of inhibitors such as tocilizumab and silymarin. Kevin Doello[1], Cristina Mesas[2], Víctor Amecua[1], Marco Fuel[2], Laura Cabeza[2], Raúl Ortiz[2]. [1]Virgen de las Nieves Hospital, Granada, Spain, [2]Centro de Investigación Biomédica, Universidad de Granada, Granada, Spain.

Introduction Resistance to cyclin inhibitors such as palbociclib is a challenge in the treatment of hormone-dependent metastatic breast cancer. Likewise, triple negative tumors are resistant to these inhibitors. The role of the present research is to identify the role that the transcription factor STAT3 (antiapoptotic transcription factor) and the inhibitors of the STAT3 pathway, such as tocilizumab (interleukin 6 receptor inhibitor) and silymarin (extract of milk thistle containing silibinin, a STAT3 inhibitor), could have in this situation. Methods Breast cancer cell lines MCF-7 (positive for estrogen receptors) and MDA-MB-231 (triple negative) were cultured in DMEM + 10% FBS for 72 hours with different concentrations of palbociclib, tocilizumab and silymarin in order to obtain cell growth inhibition values (IC50) in each compound and combinations of palbociclib with tocilizumab or silymarin. In these cases, the combination indexes (CI < 1 (synergy) and CI > 1 (antagonism)) were calculated using CompuSyn software. Likewise, an RNA extraction was carried out from the untreated cell lines to determine the basal expression levels of STAT3 by RT-qPCR. Results The IC50 of palbociclib in MCF-7 was 3.14 µM and in MDA-MB-231, 29.69 µM. The results of RT-qPCR for the basal expression of STAT3 revealed its absence of expression in MCF-7 and the presence of expression in MDA-MB-231, which could be related to the greater resistance to palbociclib of this last cell line. Tocilizumab did not present cytotoxicity *per se* and silymarin did not reach IC50 value in the cell lines studied (both compounds in doses up to 100 µM). Regarding the combinations of silymarin (50 µM and 75 µM) and palbociclib (0.1 µM and 1 µM), a synergistic effect (CI < 1) was detected in all the combinations studied in MCF-7 and in most of the same in MDA-MB-231 (except 75 µM silymarin and 0.1 µM palbociclib). However, in the case of the combination of tocilizumab (10 µM and 50 µM) and palbociclib (0.1 µM, 1 µM and 5 µM in MCF-7, and 5 µM, 10 µM and 25 µM in MDA-MB-231) synergy was only obtained in half of the combinations studied in both cell lines. Conclusion STAT3 factor could be related to resistance to cyclins in hormone-dependent breast cancer and to the lower sensitivity to these drugs observed in triple-negative breast cancer. The synergistic combination of palbociclib with silymarin, a STAT3 inhibitor, in both the hormone-sensitive and triple negative cell lines indicates that silymarin could help to enhance the effect of palbociclib in breast cancer and, even, help to reverse the resistance to this drug both in the hormone dependent and triple negative, although further studies would be needed to confirm this fact.

P076 Novel mechanisms of acquired TKI resistance in ROS1+ NSCLC. Logan C. Tyler[1], Anh T. Le[1], Hala Nijmeh[2], Liming Bao[2], Kristen Turner[3], Jason Christianson[3], Robert C. Doebele[1]. [1]University of Colorado - Anschutz Medical Campus, Aurora, CO, [2]Colorado Genetics Laboratory - Anschutz Medical Campus, Aurora, CO, [3]Boundless Bio, Inc, San Diego, CA.

ROS1 tyrosine kinase inhibitors (TKIs) entrectinib and crizotinib have significantly improved outcomes for patients with ROS1 gene fusion positive (ROS1+) lung adenocarcinoma. However, drug resistance inevitably develops, leading to disease progression. Approximately one-third of patients resistant to ROS1 TKIs demonstrate on-target ROS1 kinase domain (KD) mutations (Dziadziuszko et al. ESMO 2019), but the mechanism of resistance in the majority of patients remain unknown or poorly characterized. The purpose of this study was to model and characterize mechanisms of acquired ROS1 TKI resistance. To investigate resistance to ROS1 TKIs, we used patient-derived cell lines CUTO28 (TPM3-ROS1) and CUTO37 (CD74-ROS1) to generate drug-resistant derivatives to entrectinib (CUTO28-ER, CUTO37-ER) or crizotinib (CUTO28-CR,CUTO37-CR) in vitro. We utilized several techniques to probe the mechanisms driving resistance, including DNA and RNA sequencing, fluorescence in-situ hybridization (FISH), cell proliferation assays, and western blotting. DNA sequencing of the ROS1 KD in all resistant cell lines did not reveal any mutations. CUTO28-ER cells displayed sensitivity to MET-selective TKIs in cell proliferation assays. Consistent with MET dependency, MAPK and Akt pathways were inhibited by treatment with a MET-selective, but not a ROS1-selective, TKI. Western blot demonstrated MET overexpression and interphase FISH confirmed MET gene amplification (MET:CEP7 ratio 4.2) compared to parental cells (MET:CEP7 ratio 1.0). Notably, metaphase FISH revealed extrachromosomal DNA (ecDNA) amplification of MET. CUTO28-CR cells displayed sensitivity to Src family kinase (SFK) TKIs, and addition of pan-HER TKI afatinib resulted in synergistic inhibition of proliferation and downstream MAPK signaling. CUTO37-ER cells displayed sensitivity via proliferation and signaling to pan-HER inhibitor afatinib, and phosphorylation of EGFR Y845 consistent with Src-mediated activation of EGFR. Parental CUTO28 and CUTO37 cells were not sensitive to SFK or HER inhibition. Interphase FISH revealed modest EGFR gene amplification in CUTO28-CR cells—(EGFR:CEP7 ratio 2.1) compared to parental (EGFR:CEP7 ratio 1.0)—but not in CUTO37-ER or CUTO37-CR cells. HER2 gene amplification was not identified in any of the cell lines. Stimulation with epidermal growth factor (EGF) markedly enhanced MAPK signaling in CUTO28-CR, CUTO37-ER, and CUTO37-CR cells to a greater extent than in corresponding parental cells, consistent with EGFR or HER2 dependence. In conclusion, we demonstrated novel mechanisms of resistance to ROS1 TKIs entrectinib and crizotinib. These include ecDNA amplification of MET as an alternate oncogene driver and Src/EGFR axis as a driver of bypass signaling. Combination strategies with existing TKIs could be explored in patients with ROS1 TKI resistance. The discovery of ecDNA MET amplification is particularly intriguing; ecDNA is associated with more aggressive cancers and next generation sequencing analyses do not typically involve amplicon reconstruction to detect ecDNA.

P077 SCO-101 mediates re-sensitization of irinotecan (SN38) resistant colorectal cancer cells. Jan Stenvang, Nicklas Lindland Roest, Peter Michael Vestlev, Bo Rode Hansen. Scandion Oncology, Copenhagen, Denmark.

Introduction: Annually, more than 500.000 die from Colorectal Cancer (CRC). Three types of chemotherapy are currently being used worldwide to treat CRC patients: 5FU, oxaliplatin and irinotecan. Unfortunately, chemotherapy resistance is present or develops in the majority of metastatic CRC patients. Resistance to anti-cancer drugs represents the main cause of cancer-related deaths. Thus, re-sensitization of chemotherapy resistant cancer cells constitutes a highly unmet medical need. The small molecule drug, SCO-101, is an oral drug and has passed 4 Phase I clinical trials where it demonstrated excellent PK and favourable safety profile. **Materials and Methods:** We used pairs of parental (sensitive) and SN38-resistant (SN38 is the active metabolite of irinotecan) human colon cancer cell lines (HT29 and LoVo). Inhibition of ABCG2 was known re-sensitize the SN38 resistant cells and SCO-101 was investigated for effects on ABCG2 by dye-flux assays, bi-directinal fluz assays and vesicular uptake assays. The impact on the levels of ABCG2 was investigated in the presence or absence of SCO-101. Furthermore, a kinase screen revealed that the kinase SRPK1 was inhibited by SCO-101 and the importance of SRPK1 activity in re-sensitization of SN38 resistant colon cancer cells was investigated by two different synthetic SRPK1 inhbitors. The effect of SCO-101 was investigated by cell viability assays (MTT/PresoBlue) *in vitro* during 72h exposure to SCO-101 and/or SN38. **Results:** Updated results from the various flux assays clearly demonstrated that SCO-101 inhibited the activity of ABCG2 (BCRP). Protein analysis further demonstrated that SCO-101 causes degradation of ABCG2/BCRP and in silco docking predicted SCO-101 to bind in the part of ABCG2 whwere also SN38 binds. Cell viability assays with two different SRPK1 inhibitors demonstrated that this kinase is involved in re-sensitization to SN38. Exposing SN38-resistant cells to the combination of SCO-101 and SN38, had a significant inhibitory effect on cell viability compared to either drug alone, suggesting an additive or synergistic effect between these two drugs with SCO-101 re-sensitizing the resistant cells to SN38. **Conclusion and future perspectives:** These preclinical studies demonstrate that SCO-101 sensitizes SN38-resistant colon cancer cells to SN38 through inhibition of the SRPK1 kinase and inhibition/degradation of the ABCG2/BCRP drug efflux pump. In conclusion, SCO-101 represents a unique drug with an innovative dual mechanism of action. Scandion Oncology has reported the outcome of the first part of the ongoing Phase II clinical CORIST trial with metastatic CRC patients, who have developed acquired resistance to irinotecan containing treatment. These patients were be re-exposed to the combination of irinotecan containing treatment and SCO-101 (www.clinicaltrials.gov/ct2/show/NCT04247256?term=scandion&draw=2&rank=2).

P078 Aurora A kinase inhibition with VIC-1911 overcomes intrinsic and acquired resistance to KRAS^{G12C} inhibition in *KRAS(G12C)*-mutated lung cancer. Jong Woo Lee, Sundong Kim, Cindy Yang, Barbara Burtness. Yale Cancer Center, Yale University School of Medicine, New Haven, CT.

Oncogenic *KRAS* mutation is common in non-small cell lung cancer (NSCLC) and portends poor outcome. Direct KRAS^{G12C} inhibitors have clinical activity; however, intrinsic and acquired resistance to these drugs limits their utility. Aurora A Kinase (AURKA), a mitotic cell cycle regulator, has been considered as a key druggable KRAS effector and mediates adaptive resistance to direct KRAS^{G12C} inhibitors. We found that AURKA expression correlated with poor outcome of lung cancer patients in caBIG, GEO and TCGA, and its expression was greater in *KRAS*-mutated NSCLC cells resistant to KRAS^{G12C} inhibitor sotorasib (AMG510) compared to normal human tracheobronchial epithelial (NHTBE), lung fibroblast, and *KRAS* wild-type cells. We have previously demonstrated synergistic antitumor effects for combination AURKA and WEE1 inhibition. Here, we explored a novel combination of AURKA and KRAS^{G12C} inhibition in *KRAS(G12C)*-mutated NSCLC cells intrinsically resistant to the KRAS^{G12C} inhibitor, along with a combination of AURKA and WEE1 inhibition in mutant KRAS(G12C) lung cancer cells with acquired resistance to the inhibitor. To overcome resistance to the KRAS^{G12C} inhibitor sotorasib, we tested combination treatment with VIC-1911, a newly synthesized selective AURKA inhibitor, with sotorasib in sotorasib-resistant human lung cancer cells. Cooperative screening, Loewe plotting, and clonogenic survival assays were performed to determine synergistic antitumor effects. In addition, we established *KRAS(G12C)*-mutated human lung cancer cell lines with acquired resistance to sotorasib through the means of escalated incremental dosing or sorting non-quiescent cells after drug exposure. Synergy was further confirmed with cell cycle distribution, phenotypic mitotic catastrophe on confocal microscopy, and induction of apoptosis. Interestingly, cells intrinsically resistant to sotorasib showed profound synergistic suppressive effect on cancer cell survival with addition of VIC-1911 compared to sotorasib-sensitive cells (Loewe synergy scores: NCI-H1792=16.03; HCC44=14.37; NCI-H358=1.48). Further, the combination of AURKA and WEE1 inhibition synergistically induced greater cell death in NCI-H358 lung carcinoma cells harboring acquired resistance to sotorasib, with dramatic induction of Bim. Moreover, the combination of AURKA and WEE1 inhibition resulted in a synergistic tumor control in *KRAS/TP53*-mutated lung cancer xenograft models. Our findings suggest AURKA activation leads to both intrinsic and acquired resistance to sotorasib in *KRAS(G12C)*-mutated NSCLC; therefore, addition of AURKA inhibition to sotorasib may be a promising therapeutic approach in NSCLC with intrinsic resistance to direct KRAS^{G12C} inhibitors, while the combination of AURKA and WEE1 inhibition merits exploration in acquired resistance to these agents.

P079 The impact of BCL2 expression on sensitivity to the novel Aurora kinase B inhibitor AZD2811 in small cell lung cancer. Azusa Tanimoto[1], Carminia M. Della Corte[1], Kavya Ramkumar[1], Robert J. Cardnell[2], Allison C. Stewart[2], Carl M. Gay[2], Lauren Averett Byers[2], Qi Wang[3], Li Shen[3], Jing Wang[3], Jon Travers[4]. [1]The University of Texas MD Anderson Cancer Center Department of Thoracic/Head & Neck Medical Oncology, Houston, TX, [2]The University of Texas MD Anderson Cancer Center Department of Thoracic/Head & Neck Medical Oncology, Houston, TX, [3]The University of Texas MD Anderson Cancer Center of Bioinformatics & Computational Biology, Houston, TX, [4]Astrazeneca, Early Oncology R&D, Cambridge, United Kingdom.

Purpose: Small cell lung cancer (SCLC) is a highly lethal malignancy, with rapidly-acquired therapeutic resistance. In contrast to non-SCLC, treatment strategies based on molecular subtypes have not been well established. Aurora kinase family proteins (AURKA and AURKB) are essential for cell division, regulating chromosomal segregation during mitosis, and are upregulated in cancer. Our group has demonstrated that cMYC-driven SCLC tumors were susceptible to an AURKA inhibitor, alisertib, making AURK proteins an attractive targeted therapeutic approach. A novel AURKB inhibitor, AZD2811NP (nanoparticle), is now being investigated in relapsed SCLC patients (NCT02579226), but molecular mechanisms of resistance have not yet been identified. Here, we hypothesize that targeting predictive markers related to the effect of AZD2811 may reinforce susceptibility to AURKB inhibition. **Experimental Design:** We evaluated susceptibility to AZD2811 in 63 human-derived SCLC cell lines using 96-hour proliferation assays. To identify translatable biomarkers of response, we correlated AZD2811 IC50 values with genomic (whole exome sequencing, WES), transcriptomic (RNASeq), and proteomic profiling (Reverse Phase Protein Array, RPPA) data. We validated changes in apoptosis and DNA damage markers induced by AZD2811 in SCLC cells infected with the lentivirus vectors expressing BCL-2 and shRNA against BCL-2 using western blot. We used SCLC patient-derived xenograft (PDX) models with distinct BCL-2 profiles to evaluate in vivo antitumor effect. **Results:** In the SCLC cells treated with AZD2811, 15/63 (24%) and 10/63 (16%) cell lines showed high sensitivity (IC50<30 nM, Cmax) and intermediate sensitivity (IC50=30-100nM). Comparing protein expression, we found that cMYC (Fold change, FC:2.5; $P = 0.015$) were a positive biomarker of sensitivity, while high BCL-2 (FC:1.86; $P = 0.032$) associated with resistance. cMYC-high SCLC cell lines that were susceptible to AZD2811 became resistant when BCL-2 was overexpressed. Conversely, BCL-2 knock-down enhanced response to AZD2811, inducing apoptosis and DNA damage, in BCL-2-high cells that were originally resistant to single-agent AZD2811. Similar to Bcl-2 knock-down, treatment with venetoclax (a BCL-2 inhibitor routinely used in other cancers) enhanced apoptosis and DNA damage induction in combination with AZD2811 in cells overexpressing BCL-2. In PDX models with high BCL-2, combination of AZD2811 and venetoclax prominently induced tumor regression and apoptosis compared with each monotherapy, while there was no significant difference in PDX models with low BCL-2 between the combination and the monotherapy. **Conclusions:** Our preclinical results indicate that high BCL-2 expression reduced the efficacy of AZD2811 in SCLC models, but that Bcl2-driven resistance could be overcome with the combined use of BCL-2 and AURKB inhibitors. Findings support a promising rational combination therapy for BCL-2-high SCLC to enhance response to aurora kinase B inhibition.

P080 Analysis of clinical *BRCA1/2* reversions identifies hotspot mutations and predicted neoantigens associated with therapy resistance. Stephen J. Pettitt, Jessica Frankum, Marco Punta, Stefano Lise, John Alexander, Yi Chen, Syed Haider, Andre N. J. Tutt, Christopher J. Lord. Institute of Cancer Research, London, United Kingdom.

Although PARP inhibitors and platinum salts form part of the standard-of-care for cancers with homologous recombination (HR) defects, drug resistance does emerge and is often caused by reversion mutations in *BRCA1* or *BRCA2*. Reversion mutations are secondary mutations that restore functional protein or compensate for frameshift mutations. To better understand the nature and aetiology of these mutations, we collated, codified and analysed over 200 reversion mutations from 24 published studies. Most reversions have been reported as case reports or small clinical series – we have collected all published reports into a single freely-accessible database to which further cases can be submitted. The majority of reported reversion mutations are from *BRCA1/2* mutant serous ovarian cancer, reflecting the longer use of platinum and PARP inhibitors in this disease. Our analysis identified reversion "hotspots" and "deserts" in the N- and C-terminal coding regions (respectively) of *BRCA2*, suggesting that pathogenic mutations in these domains may be at higher or lower chance of reversion, an effect not seen for *BRCA1*. Missense and splice-site pathogenic mutations in *BRCA1/2* also appeared less likely to revert than frameshift-causing mutations. Unexpectedly, whilst most reversions were <100 bp "second site" deletions, microhomology use was not universal, especially in *BRCA1*-mutant cancers, suggesting that multiple DNA repair processes cause reversion and these vary in *BRCA1 vs. BRCA2* mutant tumours. Reversions in *BRCA1* mutant tumours were less likely to be mediated by deletions. Many reversions contain novel protein sequence not found in the wild type protein, for example where a stretch of out-of-frame protein sequence is retained between the pathogenic and reversion mutations. We modelled MHC binding affinities for these sequences and found that many reversions were predicted to encode potentially immunogenic neopeptides, suggesting a route to the treatment of reverted disease. These observations have implications for how drug resistance might be managed in *BRCA*-mutant cancers.

P081 ***In vitro*** **activity and efficacy of novel dual PARP-HDAC inhibitors.** Sarah Truong[1], Fariba Ghaidi[1], Louise Ramos[1], Jay Joshi[1], Dennis Brown[2], Neil Sankar[2], John Langlands[2], Jeffrey Bacha[2], Wang Shen[2], Mads Daugaard[1]. [1]Rakovina Therapeutics & Vancouver Prostate Centre, Vancouver, BC, Canada, [2]Rakovina Therapeutics, Vancouver, BC, Canada.

Introduction: Inhibition of poly adenosine diphosphate-ribose polymerase (PARP) is an effective treatment strategy against tumors with homologous recombination (HR) DNA repair deficiencies. Combination treatments utilizing PARP inhibitors (PARPi) in concert with inhibitors of key DNA repair or PARPi-resistance pathways, may expand the utility of PARPi beyond cancers harboring germ-line HR deficiencies. Acetylation and deacetylation of histones is an important regulatory event in the DNA damage response. Combining histone deacetylation inhibitors (HDACi) and PARPi have sensitized PARPi-resistant cells to treatment; however, combination regimens often require sequential administration to manage overlapping toxicities and accommodate diverse pharmacokinetics, constituting a significant limitation of these strategies in a clinical setting. Here, we test the activity of kt-3000 series, a novel bi-functional class of small molecules with dual PARP and HDAC inhibitor activity in HR-proficient cancer cells where PARPi have historically lacked single-agent activity. **Methods:** PARP enzyme activity was measured using the Trevigen Universal Colorimetric PARP Assay Kit. HDAC activity was measured using nuclear extracts of treated cells and the HDAC Fluorometric Activity Assay Kit from Cayman Chemical, as per the manufacturer's protocol. PARP activity was measured by fluorescence in C41 cells following compound treatment for 2 hours and PARP-activation with 1M H_2O_2 by staining cells with an anti-PARP Ab followed by a FITC-coupled secondary antibodies. Cell survival EC50 values were obtained by treating cells with a range of inhibitor concentrations, then quantifying cell confluency after 72-hours of treatment based on images taken with an Incucyte S3 system. **Results:** kt-3000 series compounds are potent inhibitors of PARP and HDAC with IC50 values for PARP enzyme activity in the low nM range, comparable to those of FDA-approved PARP inhibitors (olaparib, rucaparib, niraparib, and talazoparib) and IC50 values for the inhibition of HDAC activity comparable to FDA-approved HDAC inhibitors (panabinostat, belinostat, and vorinostat). Fluorescent assays of PARP activity in C41 cells also resulted in IC50 values in the nM range and cell survival EC50 values of the dual inhibitors are comparable to FDA-approved PARP inhibitors alone and in similar range with FDA-approved HDAC inhibitors alone. **Conclusion:** Our novel dual PARP-HDAC inhibitors show potent inhibition of PARP activity *in vitro* comparable to FDA-approved PARP inhibitors and also show potent inhibition of HDAC activity. The potency and activity of kt-3000 series compounds exhibit potential superiority to FDA-approved PARP inhibitors and HDAC inhibitors, in formulation as a single molecule. Development of these multi-target inhibitors will target unmet medical needs in the treatment of HR-proficient cancer types with dysregulation of histone deacetylation.

P082 Distinct and significant anti-cancer efficacy of plinabulin in patient derived small cell lung cancer (SCLC) 3D soft agar clonogenic assays. George K. Lloyd[1], James Tonra[2], Claudia Goettlich[3], Tobias Deigner[4], Ramon Mohanlal[2], Lan Huang[5]. [1]BeyondSpring Pharmaceuticals Inc., Redwood City, CA, [2]BeyondSpring Pharmaceuticals, New York, NY, [3]Charles River Discovery Research Services Germany GmbH, Freiburg, Germany, [4]Charles River Discovery Research Services Germany GmbH, Freiburg, Germany, [5]BeyondSpring Pharmaceuticals Inc., New York, NY.

Plinabulin (Plin) is a new chemical entity and a selective immunomodulating microtubule-targeting agent (SIMBA), which exerts immune-enhancing effects through increasing dendritic cell (DC) maturation and DC-dependent antigen presentation to CD4+ and CD8+ T-Cells. Here we evaluated whether Plin also exerts direct anti-cancer effects against approximately 80 patient derived (PDX) models. Plin was characterized for its ability to inhibit anchorage independent growth and *ex vivo* colony formation of PDX tumor cells in semi-solid medium. The compound was investigated in PDX models of various cancer types, using a 3D clonogenic assay in a 96-well format. Briefly, tumor cell suspensions were prepared from subcutaneous xenografts in NMRI nu/nu mice and seeded into 96 well ultra-low attachment plates within a layer of semi-solid medium. Plin was added to the assay plate at either day 2 or day 7 and tested at 9 concentrations in half-log increments up to a concentration of 3 µM. Supernatant was exchanged 24 h after dosing and cells were further incubated at 37°C and 7.5 % CO_2 up to a total assay time of 8 or 13 days. Colony counts based on image analysis were utilized to evaluate efficacy. Plin inhibited tumor colony formation in a concentration-dependent manner. With Plin treatment beginning 2 days after PDX cell seeding, the most sensitive cancer types were small cell lung cancer (mean absolute IC_{70} = 0.035 µM; n = 7), bladder cancer (mean absolute IC_{70} = 0.038 µM; n = 9), and soft tissue sarcoma (mean absolute IC_{70} = 0.057 µM; n = 10). Melanoma models were observed to be the least sensitive to Plin monotherapy, with a mean absolute IC_{70} of 1.105 µM (n = 19). With Plin treatment beginning 7 days after PDX cell seeding, based on absolute IC_{70} values, 9 out of 68 tumor models were sensitive towards plinabulin. The most sensitive histotypes were gastric cancer (Asian, mean abs. IC_{70} = 0.319 µM, n = 3), small cell lung cancer (mean abs. IC_{70} = 0.385 µM, n = 7; 3 below 50 nM), osteosarcoma (mean abs. IC_{70} = 0.624 µM, n = 3), and central nervous system cancer (mean abs. IC_{70} = 1.521 µM, n = 6). Overall, the most responsive models based on IC_{70} were the small cell lung cancer models 2156 (abs. IC_{70} = 0.015 µM), 1129 (abs. IC_{70} = 0.032 µM) and 650 (abs. IC_{70} = 0.032 µM), and the osteosarcoma model 1186 (abs. IC_{70} = 0.027 µM). Soft tissue sarcoma models (n = 8), Her2-enriched breast cancer models (n = 6), melanoma models (n = 9), bladder cancer models (n = 6), and gastric cancer models (Caucasian, n = 3) were observed to be the least sensitive cancer types with a mean IC_{70} value > 3 µM. In conclusion, using patient-derived tumor models in a 3D clonogenic assay, SCLC tumor types were the most sensitive to Plin. These results provide mechanistic support for the preliminary positive results recently reported with Plin in combination with Nivolumab and Ipilimumab in SCLC patients (Malhotra ASCO 2021).

P083 Metastatic NSCLC -Re-challenging with first generation TKI after a drug free holiday after resistance to 3rd generation TKI. Suryakanta Acharya. Swami Vivekananda Cancer Hospital, Darbhanga, India.

BACKGROUND Lung cancer is the most common cancer worldwide in males & a good number of patients present with metastatic disease. Though EGFR mutated patients respond to EGFR TKIs, resistance develops ultimately. Overcoming 3rd generation EGFR TKI resistance is a big challenge. METHOD 20 patients of metastatic adenocarcinoma, confirmed with core biopsy & IHC (EGFR positive) were offered Erlotinib initially. Osimertinib was offered to those who developed EGFR TKI resistance due to T790M mutation (10 cases). All 10 patients eventually developed resistance to Osimertinib. All patients were given 3 months drug free holiday & then re-challenged with Erlotinib. RESULT 4 out of 10 (40%) patients responded to the therapy. 6 patients had progression & were managed by systemic chemotherapy & radiation. CONCLUSION Literature shows up to 50% metastatic adenocarcinoma, EGFR positive patients who had developed EGFR TKI resistance to 1st & 3rd generation TKIs, responds to re-challenge with first generation TKI after a drug free holiday. In this series 40% patients responded. This approach should be tried & non-responders can be offered systemic chemotherapy & radiation.

P084 DCC-3116, a first-in-class selective inhibitor of ULK1/2 kinases and autophagy, synergizes with EGFR inhibitors osimertinib and afatinib in NSCLC preclinical models.

Madhumita Bogdan, Mary J. Timson, Hikmat Al-Hashimi, Yu Zhan, Bryan D. Smith, Daniel L. Flynn. Deciphera Pharmaceuticals, Lawrence, KS.

Background: Activating mutations in EGFR have been reported in ~30% of patients with non-small cell lung cancer (NSCLC). Three generations of small molecule EGFR kinase inhibitors have been approved by the FDA to treat these patients, however, multiple mechanisms of resistance cause cancer progression. In addition to drug-resistant mutations that arise and re-activate EGFR, other signaling pathways can be activated to cause resistance. Although EGFR inhibitors such as osimertinib and afatinib (also a pan-ErbB inhibitor) have demonstrated clear clinical benefits, patients inevitably develop resistance. Herein we demonstrate mutant EGFR NSCLCs activate autophagy upon treatment with EGFR inhibitors as a drug resistance mechanism. Therefore, a combination of an EGFR inhibitor with an autophagy inhibitor has the potential to deepen and prolong responses and improve patient outcomes. **Materials and Methods:** Human cancer cells with *EGFR* mutations were cultured using recommended complete medium. Inhibition of ULK1/2 was measured through standard biochemical assays and cellular readouts including NanoBRET and ELISA-based ATG13 phosphorylation assays. Autophagosome formation was measured using the CytoID assay (Enzo Life Sciences). For *in vivo* studies, NCI-H1975 cells that harbor an EGFR T790M resistance mutation were inoculated into BALB/c nude mice. Statistical analyses for the *in vivo* studies were performed using the Student's t-test. **Results:** Erlotinib, gefitinib, osimertinib, and afatinib activated autophagy 3–4-fold over basal levels in the HCC827 cell line (EGFR exon 19 deletion) as measured by increases in phosphorylated ATG13, a cellular substrate of the autophagy-initiating kinases ULK1/2. DCC-3116, an investigational potent and selective dual inhibitor of ULK1 (IC_{50} 6 nM) and ULK2 (9 nM) in cellular assays, inhibited both EGFR-induced and basal phosphorylation of ATG13 with IC_{50} values of 61–66 nM. Treatment of the NCI-H1975 EGFR mutated (L858R/T790M) NSCLC cell line with osimertinib or afatinib induced autophagy 3-fold over basal levels. DCC-3116 potently inhibited osimertinib and afatinib induced phosphorylation of ATG13 with IC_{50} values of 91 nM and 71 nM, respectively, and inhibited the increase in autophagosomes induced by these agents. Importantly, these *in vitro* effects translated to *in vivo* efficacy. The combination of DCC-3116 with osimertinib or afatinib resulted in significantly greater tumor responses than single agent treatments in the NCI-H1975 NSCLC xenograft model (combination vs. osimertinib $p = 0.0005$; combination vs. afatinib $p = 0.0001$; osimertinib combination vs vehicle $p < 0.0001$; afatinib combination vs. vehicle $p < 0.0001$). These data provide a strong rationale to study the combination of the ULK inhibitor DCC-3116 with EGFR inhibitors such as osimertinib and afatinib in cancer patients. DCC-3116 is currently in a Phase 1 clinical trial in patients with advanced solid tumors with a documented RAS or RAF mutation (NCT04892017).

P086 Identification of pharmacodynamic and sensitivity biomarkers for TACH101, a pan-inhibitor of KDM4 histone lysine demethylase. Frank Perabo[1], Sanghee Yoo[1], Chandtip Chandhasin[1], Joselyn Del Rosario[2], Young K. Chen[2], Ellen Filvaroff[3], Jeffrey A. Stafford[4], Stephen Quake[5], Michael F. Clarke[5]. [1]Tachyon Therapeutics, Inc., Houston, TX, [2]Bristol Myers Squibb, San Diego, CA, [3]Bristol Myers Squibb, San Francisco, CA, [4]858 Therapeutics, San Diego, CA, [5]Stanford University, Stanford, CA.

Background: TACH101 is a novel, selective and potent inhibitor of KDM4 in development for advanced cancers. KDM4 plays a key role in epigenetic regulation by removing methyl marks on histone H3K9 and H3K36. Overexpression of KDM4 leads to dysregulation of transcription, cell cycle, and DNA replication/repair processes and has been associated with many cancer types. **Methods**: Candidate pharmacodynamic (PD) and sensitivity biomarkers for TACH101 were evaluated in vitro and in vivo using cancer cell lines, mouse xenograft models, gene microarrays, ChIP-seq, and ChIP-qPCR. **Results:** Evaluation of differential gene expression from tumor tissue identified several candidates as potential PD markers for TACH101 activity. In particular, direct binding of KDM4 to Protein Phosphatase 1 Regulatory subunit 10 (PPP1R10 or PNUTS) was confirmed by ChIP-seq in cell lines from esophageal (KYSE-150), breast (MDA-MB-231) and lymphoma (OCI-Ly19) cancers. TACH101 treatment caused 86% repression of PNUTS mRNA, as well as a 51% increase in H3K9me3, a mark of repressed transcription. A 78% decrease in H3K36me3 at the PNUTS gene was also observed. PNUTS as a target of KDM4 was validated via ChIP-qPCR in xenograft tumors of KYSE-150, OCI-Ly19 and SU-60 (colorectal). In vivo, maximum inhibition of PNUTS was reached at 8 hours post TACH101 administration, when TACH101 concentration was 100-200 nM in tumors. In addition, extensive bioinformatics analysis using >300 cancer cell lines showed that cell lines with MSI-high (MSI-H) status tended to be more sensitive to TACH101 in vitro. This association was found with other markers of MSI-H status such as MMR gene mutations, MLH1 methylation status, and overall mutation frequency. To further test this association, TACH101 was evaluated in a panel of patient-derived xenograft (PDX) and organoid models. The results showed a strong correlation of TACH101 sensitivity with MSI-H status (IC50 ranges 1-150 nM). **Conclusions:** Candidate PD biomarkers of TACH101 activity for clinical evaluation have been identified and further studies are ongoing to explore current and additional markers. In addition, MSI-H status was associated with increased sensitivity to TACH101, which may be useful for enriching for select patient populations in clinical studies.

P087 Targeting LSD1 protein scaffolding function in FET-rearranged sarcomas with SP-2577. Galen Rask[1], Emily R. Theisen[1], Aundrietta D. Duncan[2], Daniela Y. Santiesteban[2]. [1]Nationwide Children's Hospital, Columbus, OH, [2]Salarius Pharmaceuticals, Inc., Houston, TX.

The RNA-binding proteins, FUS, EWSR1, and TAF15 (FET protein family) are frequently fused to transcription factors, and the resulting oncogenic fusion proteins characterize various sarcomas. These fusion proteins act as aberrant transcription factors and promote oncogenic gene expression profiles. FET family rearranged sarcomas include Ewing Sarcoma, desmoplastic round cell tumors, myxoid liposarcoma, and clear cell sarcoma, amongst others. A growing body of work suggests that, independently of Lysine Specific Demethylase 1 (LSD1) demethylase activity, LSD1's association with *FET* family oncogenic transcription factors is critical for FET-rearranged sarcoma development and progression. Extensive ongoing work aims to characterize the functional relationship of LSD1 and EWS/FLI, as well as the anti-tumor effect of disrupting that interaction. SP-2577 is an oral, first-in-class, small molecule with reversible, noncompetitive inhibition of LSD1 demethylase activity (IC_{50}: 25–50 nM) and, importantly, also disrupts LSD1 protein-protein interactions. *In vitro* and *in vivo* data demonstrate SP-2577, or analogs, reverses EWS/ETS-mediated transcriptional regulation at both up- and downregulation target genes, leading to significant tumor growth inhibition in Ewing Sarcoma mouse xenograft studies. In this study, we set out to expand our understanding of SP-2577's efficacy in diverse FET-rearranged malignancies including desmoplastic small round cell tumor (DSCRT: EWSR1/WT1), myxoid liposarcoma (ML: FUS/DDIT3), and clear cell sarcoma (CCS: EWS/ATF1). To examine whether these additional FET-rearranged sarcomas are sensitive to LSD1 scaffolding inhibition, we measured viability of Ewing sarcoma cells A673, TC32, TTC-446, and SK-N-MC, clear cell sarcoma cell lines SU-CCS-1, DTC-1, myxoid liposarcoma cell lines DL-221, 402-91, and 1765-92, and DSCRT patient derived xenograft organoids from SJDSRCT046151, X0069, SJDSRCT046155 after 96 hours of treatment with the clinical compound SP-2577, a first generation compound SP-2509, an inactive control, SP2513, and, a TCP-scaffold based, irreversible inhibitor of LSD1, OG-L002. While SP-2513 and OG-L002 had no activity against these cells SP-2509 and SP-2577 demonstrated potent activity across cell lines in vitro. Preliminary in vivo assessment of SP-2577 efficacy in DSCRT PDX SJDSRCT046151 resulted in a significant delay in time to event ($p<0.02$). Together, these data demonstrate that the unique scaffolding inhibition function of SP-2577 is essential for reducing cell viability through LSD1 targeting. Currently, we are elucidating changes to the transcriptome and proteome following SP-2577 treatment, in vitro. These data, in combination with results from the ongoing dose expansion phase 1 trial of SP-2577 in select sarcoma patients, may offer further mechanistic insight into the dependency of FET-rearranged sarcomas on LSD1 scaffolding functions and potentially lead to identifying biomarkers of sensitivity and response.

P088 Combined inhibition of histone deacetylases and EZH2 for the treatment of Wilms tumors. Hongbing Liu, Nhi Ngo, Chao-Hui Chen, Samir S. El-Dahr. Tulane, New Orleans, LA.

Wilms tumor (WT) is the most common childhood kidney cancer. It is believed to arise from immature kidney cells--nephron progenitor cells (NPCs)—which have failed to differentiate properly. Altered epigenetics is central to oncogenesis in many pediatric cancers. The critical contribution of epigenetic dysregulation to pediatric tumors provides a compelling rationale for the therapeutic potential of epigenetic drugs. Histone deacetylases (HDACs, especially HDAC1/2) and Enhancer of Zeste Homolog 2 (EZH2), a histone H3K27 methyltransferase, have been demonstrated to play a critical role in self-renewal and differentiation of mouse nephron progenitor cells (NPCs). In addition, altered expression and mutations of HDACs and EZH2 have been linked to many human cancers, including WT. As such, they are among the most promising therapeutic targets for cancer treatment. For sinstance, the treatment of Panobinostat, an FDA-approved pan-HDAC inhibitor, leaded to apoptosis and growth suppression of WT cells. EZH2 is highly expressed in WT and has been demonstrated to be associated with WT progression. We reasoned that WT results from unrestrained proliferation of progenitor cells due to overactive HDAC1, HDAC2, and EZH2. To prove this, we collected 5 human WT specimens. Immunostaining demonstrated dramatically higher level of HDAC1, HDAC2, SIX1, and SIX2 in the tumor tissues compared with adjacent normal tissues, strongly suggesting a critical role for the overactive HDAC1/2 in the SIX1/2 activation to promote cell proliferation in WT. Gain-of-function of SIX1 or SIX2 has been demonstrated to be closely correlated with high proliferation and aberrant differentiation of WT. Importantly, SIX2 marks the cancer stem cell population in WT. In addition, knockdown of HDAC1/2 by siRNAs or CRISPR gene editing resulted in downregulation of SIX1/2 in human embryonic kidney 293 cells and G401 cells derived from a Wilms tumor. Published studies also showed that treatment of Trichostain (a potent pan HDAC inhibitor) significantly reduced the Six1 expression in vertebrate neural crest and Hodgkin lymphoma cells. All these support the regulation of SIX1/2 expression by HDAC1/2. Concomitant inhibition of HDACs and EZH2 has proven to be highly synergistic and very potent for the treatment of many types of human cancers. Promisingly, combined treatment benzamide-based HDAC1/2-selective inhibitor Merck60 and Tazemetostat (an FDA-approved EZH2 inhibitor) synergistically suppressed cell growth in a wide range of dose combinations in 293T and G401 cells. In summary, our studies demonstrated elevated expression and activity of HDAC1/2 in WT, positive regulation of SIX1/SIX2 by HDAc1//2 during tumorigenesis, and synergic effect of HDAC1/2 and EZH2 inhibition in suppressing tumor cell proliferation. Thus, targeting HDAC1/2 and EZH2 may provide a promising therapeutic approach to treat WT and other pediatric kidney cancer with low toxicity and low side effects. Furthermore, availability of epigenetic drugs will facilitate the translation of this research into effective therapies.

P089 Discovery of novel small-molecule inhibitors for an epigenetic modulator WDR5.

Cindy Huang, Shirley Guo, Ping Cao. BridGene Biosciences, Inc., San Jose, CA.

WDR5, the histone H3 lysine 4 (H3K4) presenter WD repeat-containing protein 5, is a widely expressed protein that forms complexes with H3K4 methyltransferases MLL1-MLL4 and partner proteins including RBBP5, ASH2L, DPY30, and MYC. It plays an important role in epigenetic machinery assembly, transcriptional regulation, and chromatin regulation. As a result, WDR5 has become an increasingly attractive target for therapeutic intervention against cancers, including mixed-lineage leukemia, neuroblastoma, breast cancer, bladder cancer, pancreatic cancer, and colorectal cancer. Using its proprietary chemoproteomic platform IMTAC^{TM} (Isobaric Mass Tagged Affinity Characterization), BridGene has screened its unique covalent library against live-cell proteomes and discovered small-molecule ligands for hundreds of hard-to-drug targets, including transcription factors, splicing factors, epigenetic modulators, and E3 ligases. WDR5 is among these targets that BridGene has discovered a novel ligand for. Preliminary characterization of the "hit" compound, BGS1989, showed that it interacts with WDR5 in a dose-dependent manner, and it can inhibit MLL1 methyltransferase activity at low μM potency. After one round of chemical optimization, we identified an improved inhibitor BGS2597 that has ~200 nM potency in the WDR5-MLL interaction and H3K4 methylation assays. Additional optimizations are being conducted to further improve BGS2597's activity and selectivity for WDR5. Comprehensive characterizations are being performed to delineate which of WDR5's functions are modulated by BGS2597. The WDR5 inhibitor discovered using IMTAC^{TM} platform provides an excellent starting point for the development of new drugs targeting WDR5-dependent cancers.

P092 A novel approach to target drug-resistance in thyroid cancer by regulating Annexin 7 (ANXA7)/p21 axis. Surya Radhakrishnan, Alakesh Bera, Narayanan Puthillathu, Nahbuma Gana, Madhan Subramanian, Eric Russ, Anubhuti Paria, Swarnarup Paria, Stephen Rothwell, Harvey B. Pollard, Meera Srivastava. USUHS, Bethesda, MD.

Thyroid cancer is the most common endocrine malignancy in the United States. The survival rate of thyroid cancer patients is high. However, there is a group of patients with poor prognosis due to the development of drug resistance. Differential expression and mutation of the BRAF, Met, and p53 genes have been correlated with the progression and aggressiveness of this cancer, and over 60% of thyroid cancer patients were found to have a V600E mutation in BRAF. ANXA7 is a multifunctional protein that has been found to act as a tumor suppressor in many cancers. In this study, we focused on the expression of ANXA7 in relation to the BRAF mutation and its functional role in regulating thyroid cancer progression, aggressiveness, and drug-sensitivity. High-throughput RNA-seq and protein array studies indicated a lower ANXA7 protein expression linked with thyroid cancer. Additionally, ANXA7 was found to be lower in the thyroid cancer cell lines with the BRAF mutation. To test ANXA7's role in regulating drug-sensitivity, thyroid cancer cells were treated with different BRAF and MEK inhibitors. Both groups of inhibitor treatment on the thyroid cancer cells resulted in an increase in ANXA7 expression, a decrease in Ph-ERK, and an increase in apoptotic markers. We also found that the cyclin-dependent kinase inhibitor p21 is a novel regulator of BRAF mediated chemoresistance, and elevation of both p21 and ANXA7 through the combination of drugs resulted in a synergistic effect in the apoptotic pathway. Together, these results provide new insights into thyroid malignancy and its drug resistance mechanism involving the ANXA7/p21/BRAF/MAPK pathway. Future translational-based approaches with high-throughput functional screenings are necessary to develop a novel and effective ANXA7-based therapeutic strategy for thyroid cancer.

P093 Proteomic analysis reveals a mechanism of resistance to radiation mediated by microvesicles in glioma. Elena Panizza, Brandon D. Regalado, Fangyu Wang, Robert J. Munroe, Nathaniel M. Vacanti, Marc A. Antonyak, Richard A. Cerione. Cornell University, Ithaca, NY.

Glioma accounts for 80% of malignant brain tumors. Standard treatment for glioma involves surgical resection followed by radiation therapy and chemotherapy, however, tumors recur leading to death in 12 to 18 months on average. Gliomas contain tumor-initiating cells (glioma stem cells, GSCs) that contribute causally to resistance to radiation therapy. GSCs shed large amounts of extracellular vesicles (EVs), which can potentiate growth, therapy resistance and invasiveness of less-aggressive cells present within the tumor microenvironment. However, how EVs mediate these effects is still not well-understood. To gain insights into mechanisms of resistance to radiation, we profiled the proteome of patient-derived GSCs that were either left untreated or treated with a standard therapeutic dose of ionizing radiation (IR). We identify a subset of patient-derived GSC lines that are resistant to radiation. Particularly, we find the cell line GSC-267 displaying a pronounced vesicular transport function. Microvesicles (MVs) but not exosomes shed by GSC-267 can strongly potentiate the proliferation of recipient cells. Subsequent proteomic analysis of EVs highlights elevated levels of nicotinamide phosphoribosyltransferase (NAMPT) within the MVs shed by GSC-267 as well as in the cells of origin. NAMPT is a central enzyme in NAD^+ metabolism, is overexpressed in a subset of glioma patients, and correlates with poor patient survival. We find that the NAMPT protein is transferred to recipient cells via MVs derived from GSC-267. Furthermore, presence of NAMPT within MVs, and not solely overxpression within cells, is required for MVs to be able to rescue the proliferation of fibroblasts treated with a radio-mimetic compound. NAMPT inhibition ablates the proliferative gain induced by MVs derived from GSC-267. Supplementation with nicotinamide mononucleotide, the enzymatic product of NAMPT, does not enhance the effect of MVs derived from GSC-267, indicating a NAD^+-dependent mechanism. MVs isolated from NAMPT knock-down GSC-267, which no longer carry the NAMPT protein, are not able to rescue the proliferation of recipient cells that were treated with IR. Finally, to more closely model the tumor microenvironment, we employ radiation-sensitive GSC-1079 as recipient cells. Transfer of MVs derived from GSC-267 is able to rescue the viability of GSC-1079 treated with a therapeutic dose of IR. In summary, MVs derived from the radiation-resistant GSC-267 are able to spread aggressive traits in the tumor microenvironment determining an overall shift towards a resistant phenotype. We identify the transfer of the enzyme NAMPT via MVs to be a causative mediator of resistance to radiation. Specific therapeutics targeting NAMPT have been tested in the context of other types of cancer but have shown a poor safety profile. Further analyses may indicate molecular mediators of the ability of NAMPT to confer resistance to radiation, which may lead to identifying novel targets for the treatment of glioma.

P094 Targeted sequencing revealed clonal genetic changes in spatially different foci in urothelial carcinoma of bladder. M. Talha Ugurlu[1], Rachel Goldberg[2], Pritam Sadhukhan[2], Mohammad O. Hoque[3]. [1]Department of Otolaryngology-Head and Neck Surgery, The Johns Hopkins University, School of Medicine, Baltimore, MD, [2]Department of Otolaryngology-Head and Neck Surgery, The Johns Hopkins University, School of Medicine, Baltimore, MD, [3]Department of Otolaryngology-Head and Neck Surgery, Department of Urology, Department of Oncology, The Johns Hopkins University, School of Medicine,, Baltimore, MD.

Urothelial Carcinoma of Bladder (UCB) is a disease of multiple origins of the same bladder with varying in morphology and genetic changes. The origin of spatially different tumors in the same bladder has been proposed to occur through clonal changes with intraluminal migration or field effect with synchronous transformation of multiple cells by carcinogens. A clear molecular understanding of the origin of multifocal UCB will have important implications for the proper tailoring of personalized targeted treatment and diagnostics. Despite efforts to identify intertumoral heterogeneity, the clonal origin of UCB is still debated. To further understand the genetic mechanism behind the multifocality of UCB, we performed targeted next-generation sequencing (tNGS) on 39 UCB lesions obtained from 15 cystectomized bladders. Germline distant muscle DNA was used as a control. We technically validated selected mutational events by using ultra-sensitive droplet digital PCR (ddPCR). Our data showed that multifocal UCB is of clonal origin, with lesions from individual patients displaying nearly identical mutational landscapes, with few heterogeneous events that may have been acquired at a later point. tNGS demonstrated similar genetic drivers within the same patient and reveal that clonal expansion is an early event of tumorigenesis. In addition, we found distinct gene mutations in different UCB patients suggesting prominent interpatient heterogeneity of clonal events in the UCB. Among others, our result showed that TP53, FGFR3, and NOTCH4 are the most frequently mutated genes in UCB samples. ddPCR assay of mutational events on TP53, FGFR3, AKT, PIK3CA genes confirmed TNGS data and solidified that mutation in TP53, FGFR3, AKT, PIK3CA genes are associated with tumorigenesis of uroepithelium. In summary, this study conveys insight into the clonal origin of multifocal UCB and demonstrates that TNGS is a sensitive and reliable method for interrogating the molecular landscape of UCB. These findings also may inform clinical practice as sequencing of a single focus from a patient presenting with the multifocal disease may be sufficient to understand the mutational profile and guide personalized treatment and risk stratification.

P095 Isogenic CRISPR anchor screens identified actionable nodes to CHK1/2 inhibitor prexasertib in TP53 mutant cancer. Teng Teng, Stephen Paik, Shan-chuan Zhao, Ashley Choi, Steven A. Lombardo, Shangtao Liu, Samuel R. Meier, Yi Yu, Jannik N. Anderson, Alan Huang, Fang Li, Xuewen Pan. Tango Therapeutics Inc., Cambridge, MA.

TP53 is the most mutated tumor suppressor gene in human cancers and is a master regulator of a wide array of cellular processes including but not limited to cell cycle progression and DNA damage response. Due to its diverse functions, therapies specifically exploit the vulnerabilities created by TP53 loss remained elusive. We established a panel of TP53 isogenic pair cell line models by knocking out TP53 from WT cell lines and observed that CHK1/2 inhibitor prexasertib showed preferential selectivity toward TP53 KO cell lines. To thoroughly examine additional nodes which could further exploit this selective sensitivity, we carried out genome-wide prexasertib anchor screen in A549 isogenic lines employing CRISPRn, CRISPRi and CRISPRa platforms. From these functional genomics screens, we identified that targeting the anti-folate pathway genes (TYMS, DHFR) showed selective synergy with prexasertib specifically in TP53 KO cells. Secondly, a panel of iron metabolism genes was identified across different CRISPR platforms as candidate TP53 context specific synthetic lethal hits synergize with prexasertib. And lastly, additional sub-context was identified which may inform patient selection strategy for prexasertib as single agent.

P096 Using CRISPR-Cas9 screens to identify microRNA involved in aggressive prostate cancer phenotypes. Jonathan Tak-Sum Chow, Daniel K. C. Lee, Martino Marco Gabra, Norman Fu, Keyue Chen, Leonardo Salmena. University of Toronto, Toronto, ON, Canada.

Background: Prostate cancer (PCa) is the most common cancer diagnosis in Canadian men. For many, the tumour can remain "dormant" negating a need for treatment, but if the tumour progresses and becomes metastatic, prognosis is poor. Despite the introduction of new treatments, metastatic PCa remains a lethal and incurable disease. This is invariably due to the onset of castration-resistance in advanced cases producing a more aggressive tumour with increased metastatic potential. We hypothesize that microRNA can promote these aggressive PCa phenotypes due to their ability to regulate diverse gene networks simultaneously. Methods: We have generated miRKOv2, the second version of our microRNA CRISPR Knockout library using the latest on- and off-target scoring algorithms and other microRNA-specific features for CRISPR-Cas9 guide RNA (gRNA) design. miRKOv2 will be used in three separate screens to identify microRNA that are essential for PCa growth, microRNA that are involved in PCa metastasis, and microRNA that can confer castration-resistance, respectively. Results: Next generation sequencing of the plasmid miRKOv2 library showed a median gRNA coverage of ~1100X. In silico comparisons to other microRNA libraries demonstrate that miRKOv2 is a superior sgRNA library. DU145 cells stably expressing Cas9 are highly active and proof of principle growth assays demonstrate that essential gene knockout and subsequent decrease in cell viability is detectable. Generation of a Cas9+ PCa cell line panel and screens are ongoing. Conclusion: miRKOv2 is an improved microRNA-focused CRISPR library with high gRNA coverage. Proof of principle assays demonstrate that knockout of essential genes results in a reduction in cell viability, indicating that a CRISPR dropout screen is appropriate for our in vitro model. This project aims to identify microRNA dependencies in metastatic and castration-resistant PCa that can be leveraged into new biomarkers and therapies.

P097 Comparative single cell transcriptome profiling of primary tumors, CTCs and metastatic sites from a bladder cancer PDX model. Tomas Vilimas[1], Brandie Fullmer[1], Alyssa Chapman[1], Li Chen[1], Ting-Chia Chang[1], Rini Pauly[1], Biswajit Das[1], Chris Karlovich[1], Yvonne A. Evrard[1], Howard Stotler[1], Michelle M. Gottholm-Ahalt[2], Tara Grinnage-Pulley[1], Melinda G. Hollingshead[2], James H. Doroshow[3], P. Mickey Williams[1]. [1]Frederick National Laboratory for Cancer Research, Frederick, MD, [2]National Cancer Institute, Frederick, MD, [3]National Cancer Institute, Bethesda, MD.

Background: A PDX bladder cancer model, BL0293-F563, grows large subcutaneous tumors, spontaneously metastasizes to the liver and bone, and sheds high numbers of circulating tumor cells (CTCs). This PDX model provides a unique opportunity to explore the relationships between primary tumors, CTCs and metastatic cell subpopulations. **Methods:** BL0293-F563 tumors (available from the NCI Patient-Derived Models Repository [https://pdmr.cancer.gov/] and originally developed by Jackson Laboratories) were implanted into NSG mice and and primary tumors, metastatic nodules in the liver, and blood were collected at maximal allowable tumor burden. Tumor tissue was dissociated using Miltenyi Tumor Dissociation Kit with OctoDissociator, and Human CTCs were enriched from whole mouse blood through negative selection with anti-mouse CD45 and anti-mouse MHC-1 magnetic beads. Single cell sequencing was done using 10X Genomics 3' gene expression assay v3.1. Sequencing libraries were prepared using 10X Genomics Chromium and 3' gene expression kit v3.1. Data processing and analysis was done using 10X Genomics' Cell Ranger pipeline, Seurat, and consensus non-negative matrix factorization. **Results:** Using Seurat FindNeighbors, cells in the aggregated dataset were classified into 17 distinct clusters. All clusters were comprised of cells from multiple sites (primary tumor, CTCs, metastases), but three clusters were enriched in CTCs and one cluster was composed of mostly primary tumor cells. All clusters exhibited an epithelial-like gene expression signature score, suggesting that CTC shedding was occurring without prominent epithelial-mesenchymal transition. Consistent with expected differences in oxygenation states, CTC-enriched clusters exhibited a lower hypoxia gene expression score than primary tumor and metastasis-enriched clusters. CTC-enriched clusters also showed higher expression of oxidative phosphorylation genes, suggesting metabolic differences between CTCs and cells from primary tumors and metastases. Based on Human Primary Cell Atlas phenotype prediction, several clusters were associated with stem cell like phenotypes. Additionally, two of three CTC-enriched clusters had elevated expression of mitosis-associated genes, suggesting that at least some populations of CTCs are not quiescent but actively cycling. **Conclusions:** Utilizing single cell gene expression profiling, we have linked the gene expression profile of CTCs to specific cell subpopulations in primary tumors and metastases. We show that CTC-enriched cell clusters appear to maintain an epithelial phenotype. Subpopulations of CTC cells exhibited enrichment of stemness-associated transcripts and features of active cell cycling.

P098 A chemoproteomic platform for identifying small-molecule modulators of protein-protein interactions, discovering new cancer targets, and revealing previously unknown targets for well-known drugs. Cindy Huang, Vivian Zhang, Ning Deng, Irene Yuan, Linda Pullan, C. Glenn Begley, Ping Cao. BridGene Biosciences, Inc., San Jose, CA.

BridGene Biosciences has developed a novel chemoproteomic platform IMTAC^{TM} (Isobaric Mass-Tagged Affinity Characterization) for discovering small-molecule inhibitors of protein-protein interactions, disclosing new cancer targets, and identifying previously unknown targets for known drugs. The key components of the IMTAC^{TM} platform include a unique library of drug-like covalent small molecules containing an alkyne tag, live-cell screening, and quantitative mass spec analysis. The IMTAC^{TM} analysis begins with treating live cells with probes from the covalent library, proceeds with enrichment of the probe-modified proteins, and then employs Mass Spec to identify the probe-modified proteins and determine the relative binding affinity. This powerful chemoproteomic platform enables systematic mapping of the direct interactions of small-molecules across the proteome. A prominent application of BridGene's IMTAC^{TM} platform is to identify hits for "hard to drug" targets from live-cell screening. IMTAC ^{TM} screening is well suited for identifying drug-like ligands for such hard-to-drug targets, including those with shallow binding pockets or temporary pockets formed under certain cellular settings. Lead optimization can be immediately initiated after confirmation of the ligand's binding to relevant targets. Using the IMTAC^{TM} platform, BridGene has discovered small-molecule ligands for a number of "hard-to-drug" targets as well as oncogenic mutants, including GTPases (e.g. RhoA), transcription factors (e.g. TEAD), splicing factors (e.g. SRSF1), epigenetic modulators (e.g. WDR5), E3 ligases, etc. The potency of the hits ranges from low nanomolar to micromolar. Because of the IMTAC^{TM} platform's proteome-wide profiling capability, BridGene has also, for the first time, revealed new targets for some well-known drugs. Combining IMTAC ^{TM} screening with phenotypic screening, BridGene is also discovering new/unknown targets that drive certain disease phenotypic changes. IMTAC^{TM} is a novel platform to discover small molecule drugs for hard-to-drug targets. It allows the mapping of targets for small molecules on a proteome-wide scale, which can provide comprehensive selectivity information to facilitate lead optimization and lower off-target toxicity. IMTAC^{TM} has the potential to redefine precision medicine, discover new drugs and new targets, and identify new indications of known drugs.

P099 Radionuclide imaging of low-density-lipoprotein receptor (LDLR)-overexpressing glioblastoma: A preclinical study of Gallium-68 RMX-VH. Izabela Tworowska[1], Leo Garcia Flores II[1], Xuewei Qu[1], Cédric Malicet[2], Nilesh Wagh[1], David Ranganathan[1], Jonathan Nowak[2], Pascaline Lecorche[2], Jamal Temsamani[2], Ebrahim S. Delpassand[1]. [1]RadioMedix, Houston, TX, [2]Vect-Horus, Marseille, France.

Introduction: One of the factors that limit the efficacy of the drugs, especially in primary brain tumors, is the permeability of the blood-brain barrier (BBB). The low-density lipoprotein receptor (LDL-R) expressed at the BBB mediates the transport of endogenous ligands through the BBB. We developed a new radiolabeled peptide targeting both the human and murine LDLR and able i) to cross the BBB and ii) to target tumors such as glioblastoma that express high levels of the LDLR. The objective of this study was to determine the LDLR targeting properties, the pharmacokinetics of ^{68}Ga radiolabeled RMX-VH in a glioblastoma model that expresses the human LDLR (hLDLR). **Methods:** The nonclinical studies of 68Ga-RMX-VH were completed in U87MG, A172, U373 glioblastoma cancer cell lines and xenografts mice models. The studies determined *in vivo* time-dependent accumulation of this agent, *in vitro* dose-depended cellular uptake, and cellular competition studies. We compared the tumor-specific accumulation of 68Ga-RMX-VH and normal organ distribution in female and male athymic nude mice. Radiotracer, RMX-VH (10-30ug) was labeled with isotope-Ga68 (10-25mCi, ITM GmBH). U87MG and A172- derived xenografts were generated in athymic nude mice (10 weeks) and PET/CT images were acquired using G4 PET/Xray camera (Sofie Biosciences) at 1h, 2h, 3h, and 4h post-injection. The followed up biodistribution studies were done at 30 min, 1h, 2h, and 3 hrs. post-injection. The organs and tumor were collected, weighed, and the tissue radioactivity was measured with Wizard2 Gamma Counter (Perkin-Elmer, Waltham, MA). The percentage of injected dose per gram of tissue (%ID/g) was calculated and decay-corrected. **Results:** Our studies confirmed the in vitro and in vivo selectivity and specificity of 68Ga-RMX-VH toward LDLR-positive tumors. 68Ga-RMX-VH is a small peptide (MW: 1432.7 g/mol) and renal excretion was expected as a route of agent elimination. The tumor-specific uptake of radiotracer in U87MG xenografts was 1.8%ID/g at 1h and remained unchanged at 3h post-injection. The kidney retention of the agent reached 12.2%ID/g and decreased to 10.2%ID/g. The accumulation of radiotracer in the liver, ovaries, and intestine correlated well with the known normal expression of LDLR. The tumor-to-muscle ratio was 5.97 at 1h; increased to 26.1 and 22.39 at 2h and 3h. The elimination t1/2 of radiotracer was only 18.6 min, with a clearance CL of 502 ml/min. Adsorption from the site of administration is rapid as the Cmax was 5 min. The tumor-specific uptake and normal organ distribution of 68Ga-RMX-VH were lower in male mice than in females. This correlated with differences in lipid and lipoproteins metabolism in males and females. **Conclusions:** RMX-VH showed favorable hLDLR targeting properties in *vitro* and *in vivo* in glioblastoma mice models. Our results suggest that hLDLR may serve as a target for imaging for glioblastoma. The first-in-human exploratory IND study of Ga68-RMX-VH will be initiated in Q2 of 2021.

P100 Use of a novel checkpoint inhibitor peptide ligand in a first-in-human phase 1 trial for adults with recurrent glioblastoma. Michael R. Olin[1], G. Elisabeth Pluhar[2], Ingunn Stromnes[1], Anne Eaton[1], Shannon Lynn[1], Emily Greengard[1], Christopher L. Moertel[1]. [1]University of Minnesota, Minneapolis, MN, [2]University of Minnesota, St. Paul, MN.

Cancer immunotherapy has revolutionized clinical management for many systemic malignancies by generating durable control and rendering a significant improvement on an otherwise dismal prognosis. Unfortunately, these therapies often elicit adverse events including autoimmunity and death. In addition, these therapies are not successful in all solid tumors and show no effect on cancers of the central nervous system. Our research is on the CD200 checkpoint that modulates the immune system through the inhibitory receptor (CD200R1) and activation receptors (CD200ARs). We demonstrated that targeting CD200ARs with our checkpoint inhibitor peptide ligand (CD200AR-L) activates the immune system and renders it impervious to the inhibitory effects of the tumor-associated protein, CD200. In a preclinical trial in pet dogs with spontaneous high-grade glioma (n=31), CD200AR-L added to tumor lysate vaccines increased the median OS and PFS compared to TL alone. We suggest these results were due to the ability of CD200AR-L to modulate multiple immune checkpoints. During the characterization of CD200AR-L, we discovered signaling molecules shared by the CD200 and PD-1/PD-L1 pathways, suggesting that these immune checkpoints are interconnected. We initiated a first-in-human, single-center dose-escalation phase 1 clinical trial (NCT04642937) utilizing a 3+3 design. Induction therapy consisted of injection of CD200AR-L (3.75 ug/kg BW) with an allogeneic-brain allogeneic brain tumor vaccine (GBM6-AD) weekly for 4 weeks while monitoring for dose-limiting toxicities. Six patients enrolled in this first cohort; 5 patients were at first recurrence and 1 at second, 5 had MGMT-promoter unmethylated cancers. Although all patients completed induction, one experienced a dose-limiting toxicity of grade III encephalopathy. Non-dose limiting toxicities included lymphopenia (n=1) and immunotherapy-related cerebral edema (n=2) that was mitigated with a bevacizumab rescue protocol. There were no local injection site reactions or other grade I-II toxicities. Three patients were taken off study for radiographic disease progression that was confirmed by histopathology (n=1) or by progressive neurological decline (n=2). The patient with 2 prior recurrences remains stable with no progression on protocol-directed maintenance therapy. . Immune stimulation, completed in 3 of 6 patients, correlated with increases in CD4/CD8 T cells, NK and NKT cells between weeks 2 and 4 post vaccination. A reduction in immunosuppression was documented by decreases in the expression of CD200R1, PD-1, PD-L1 and CTLA4 on CD4/8 T-cells, CD14, CD11c and MDSC populations compared to basline (n=5). The ability of PBMCs to overcome immunosuppression was shown by increases in IL-2, IL-6 and TNF alpha between weeks 2 and 4 after restimulation with PHA. Immune memory was suggested in 2 patients by increases in IFNg and TNFa. **CONCLUSION:** Therapy with CD200AR-L in combination with GBM6-AD was well - tolerated with early evidence of an immunological response. Dose escalation enrollment in the Phase 1 Trial continues.

P101 Combined metronomic chemo-immunotherapy in head and neck cancers: An experience from the developing and resource poor country. Irappa Madabhavi[1], Malay Sarkar[2], Pham Nguyen Quy[3], Apurva Patel[4]. [1]Department of Medical and Pediatric Oncology, Kerudi Cancer Hospital, Bagalkot, J N Medical College, Belagavi, and Nanjappa Hospital, Shimoga, Karnataka, India, Bagalkot, India, [2]Department of Pulmonary Medicine, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India, Shimla, India, [3]Department of Medical Oncology, Kyoto Miniren Central Hospital, 2-1 Uzumasa Tsuchimotocho, Ukyoku, Kyoto, Japan 616-8147, Kyoto, Japan, [4]GCRI, Ahmedabad, Ahmedabad, India.

Background: Head and Neck squamous cell carcinomas (HNSCC) have proven to be inherently resistant to systemic treatments; with limited treatment option after progression on systemic chemotherapy in HNSCCs Immunotherapy has a role to play with improved results. **Methods:** All 7 patients with advanced, metastatic-HNSCCs, received Inj. Nivolumab 240mg flat dose or 3mg per kg, intravenously every 2 weekly along with low dose capecitabine 500mg twice a day, were prospectively assessed. Patient's clinical, hematological and staging characteristics were described and Clinical Benefit Rate (CBR) was calculated. **Results:** Total 7 patients received the combined metronomic chemo-immunotherapy (CMCI). Majority of patients were belonging to ECOG-PS 1(66%), with all patients being in stage IV disease. Three, three &one patient received Immunotherapy as 5th, 3rd and 4th line of therapy respectively. Nivolumab and low dose Capecitabine was used in all 7 patients. CBR was seen in 71.42% (5/7) patients, one patient died due hepatitis and hepatic encephalopathy, and one patient was having progressive disease, and one patient with stable disease discontinued of treatment because of financial constraints and kept on capecitabine alone. Majority tolerated therapy well with no grade 3/4 Immune related adverse events (IRAEs). One patient required supportive therapy with packed red cell transfusion and Albumin infusions. **Conclusions:** To conclude nivolumab along with metronomic chemotherapy with low dose capecitabine was very well tolerated and exhibited antitumor activity with CBR of 71% in extensively pretreated patients with HNSCCs. Additional studies of Nivolumab and metronomic chemotherapy and immuno-immuno combination therapy in these diseases are ongoing.

P102 NTX-1088, A potent first-in-class, anti-PVR mAb, restores expression and function of DNAM1 for optimal DNAM1-mediated antitumor immunity. Pini Tsukerman[1], Anas Atieh[1], Akram Obeidat[1], Keren Paz[2], Guy Cinamon[3], Tihana Lenac Roviš[4], Lea Hirsl[4], Paola Kucan Brilc[4], Stipan Jonjic[4], Ofer Mandelboim[5]. [1]Nectin Therapeutics, Jerusalem, Israel, [2]Nectin Therapeutics, New York, NY, [3]Nectin Therapeutics, Tel Aviv, Israel, [4]MEDRI, Rijeka, Croatia, [5]HUJI, Jerusalem, Israel.

The poliovirus receptor (PVR, CD155) represents a resistance mechanism to approved immune checkpoint inhibitors (ICIs). It is a key regulator of immune activation, that modifies immune function through multiple mechanisms. Increased levels of PVR expression on tumor cells have been associated with resistance to anti-PD-(L)1 therapy in clinical settings, while loss of PVR led to reduced tumor growth in multiple pre-clinical models. Targeting PVR using blocking mAbs offers an attractive therapeutic approach for patients with advanced cancer. NTX-1088 is a first-in-class, potent, anti-PVR mAb being developed for the treatment of solid tumors. The antibody binds to PVR with high affinity, blocks its interactions with TIGIT and CD96, and thus interrupt their immunosuppressive signaling. However, NTX-1088 forte is manifested through its ability to block the critical interaction between PVR and the costimulatory receptor DNAM1 (CD226). This blockade prevents internalization of DNAM1, restores its expression on the surface of immune cells and results in a robust antitumor activation. NTX-1088 was tested using several tumor and immune cell co-culture systems. Various cancer cell lines were co-incubated with relevant immune effector cells from healthy human donors, in the presence of NTX-1088, as a single agent and in combination with anti-PD-1 mAb (pembrolizumab). NTX-1088 significantly increased immune cell activation, as measured by IFNg release from activated polyclonal CD8+ T cells, induction of CD137 and killing of tumor cells. When tested in combination with pembrolizumab, NTX-1088 further increased all measured activation parameters, suggesting a potential synergistic effect. When compared to anti-TIGIT mAb (tiragolumab), NTX-1088 demonstrated clear superiority in its ability to activate T and NK cells. Furthermore, NTX-1088 in combination with pembrolizumab was significantly superior to the combination of pembrolizumab with anti-TIGIT mAb. Interestingly, NTX-1088 as a single agent showed a comparable effect to that of the combined blockade of TIGIT and CD112R, and further synergized with anti-CD112R for maximal activity. NTX-1088 was the only intervention that significantly restored DNAM1 levels, whereas blockade of DNAM1 reduced the activity of NTX-1088 to levels comparable to that of anti-TIGIT mAb. Humanized murine models confirmed the above observations; NTX-1088 exhibited strong efficacy, inducing a robust tumor growth inhibition, accompanied by significantly higher prevalence of CD137+, DNAM1+, CD8+ tumor infiltrating cells, compared to control treated mice. This is the first report of drug-induced DNAM1 restoration and immune activation. NTX-1088 shows, for the first time, exclusive triple mechanism of action, whereby simultaneous and effective blockade of TIGIT and CD96 is complemented by the efficient restoration of DNAM1. This is a step change in antitumor immune activation, which will soon be tested in the clinic.

P103 The introduction of a single strain of *Bacillus* into a germ-free environment did not impact the anti-PD-1 efficacy in a MC38 syngeneic model. Tao Yang[1], Bonnie Xiaobo Chen[1], Rongfei Lu[1], Xiaoyu An[1], Mingfa Zang[1], Jingjun Li[1], Sheng Guo[1], Wubin Qian[1], Jian Fei[2], Tongyang Hao[3], Edward Xu[4], Henry Li[1]. [1]Crown Biosciences, San Diego, CA, [2]Shanghai Model Organisms Center, Inc., Shanghai, China (Mainland), [3]GemPharmatech Co., Ltd, Nanjing, China (Mainland), [4]Cyagen Biosciences Co., Ltd, Suzhou, China (Mainland).

Background. Although immunotherapy has led to exceptional and durable clinical response, the majority of patients respond poorly to the current immunotherapies. Growing evidence has linked some of the poor responsiveness to the gut microbiota, and the modulation of gut microbiome composition is becoming a promising new strategy to enhance immune checkpoint inhibitor (ICI) treatment outcome. Mouse tumor modelling under germ-free (GF) conditions combined with introduction of defined bacterial strains could be a useful approach to investigate the impact of microbiota on ICI efficacy, as well as understanding the underlying mechanisms. We previously demonstrated that GF mice exhibited a significantly poor response to anti-PD-1 therapy when compared to the specific pathogen free (SPF) mice in a subcutaneous MC38 colorectal cancer model, which is consistent with other reports. **Methods**. Introduction of a single strain of *Bacillus* in the GF-environment was assessed for its impact on the anti-mouse PD-1 monoclonal antibody (mAb) therapy in GF-mice and SPF mice, both for efficacy and pharmacodynamics tumor infiltrating lymphocytes (TILs) profiling. C57BL/6 mice were inoculated subcutaneously with MC38 tumor cells and when the tumors were reached 80-120mm^3, the mice were randomized for isotype or anti-PD-1 mAb treatment. Fecal sample 16S rRNA analysis (NGS) was used to confirm the gut bacteria status. **Results.** The MC38 tumor in GF mice has significantly fast baseline growth kinetics, even with the introduction of *Bacillus* under GF conditions compared to SPF mice, suggesting tumor immunity was not enhanced by *Bacillus*. Despite the introduction of *Bacillus*, GF mice also showed reduced response to anti-PD-1 treatment when compared to the SPF mice as previously reported, further confirming that introduction of *Bacillus* had minimal effects on the efficacy of anti-PD-1 therapy. Moreover, the SPF mice and GF mice with *Bacillus* exhibited distinct profiles of TILs, consistent with distinct efficacies as observed. GF free mice showed a lower frequency of CD45+ TILs in comparison to SPF mice. In addition, GF mice exhibited lower frequency of CD8+ TILs and TIL- NKT when compared to the SPF mice, both of which are consistent with the stronger efficacy seen in SPF mice. Meanwhile, GF mice also exhibited higher granulocytic myeloid derived suppressor cells (gMDSC) and lower M_1/M_2 ratio, both of which imply a more suppressive tumor microenvironment in GF mice. Fecal sample analysis using 16S rRNA analysis confirmed a single strain of *Bacillus* was indeed introduced into the guts of all the GF mice. **Conclusions**. The GF conditions provide a useful environment for the investigation of specific microbiota strains on the impact on ICI treatment outcome. In summary, the introduction of *Bacillus* in GF mice did not impact the efficacy of anti-PD-1, thus suggesting that other strain(s) of gut microbiota in SPF mice may impact this and need to be investigated.

P104 Therapeutic targeting of TREM1 with PY159 promotes myeloid cell reprogramming and unleashes anti-tumor immunity. Erin Mayes, Vladi Juric, Mikhail Binnewies, Pamela Canaday, Tian Lee, Subhadra Dash, Joshua L. Pollack, Joshua Rudolph, Vicky Huang, Xiaoyan Du, Nadine Jahchan, Asa J. Ramoth, Shilpa Mankikar, Manith Norng, Carlos Santamaria, Kevin P. Baker, Linda Liang. Pionyr Immunotherapeutics Inc., South San Francisco, CA.

Myeloid cells present in the tumor microenvironment can exist in immunosuppressive states that impede productive anti-tumor immunity. One strategy for targeting these immunosuppressive mechanisms is reprogramming of myeloid cells from immunosuppressive to immunostimulatory, resulting in the removal of the immune inhibition and unleashing of anti-tumor immunity. Triggering receptor expressed on myeloid cells-1 (TREM1) is an immunoglobulin superfamily cell surface receptor expressed primarily on neutrophils and subsets of monocytes and tissue macrophages. TREM1 signals through the association with DAP12 adaptor protein and mediates proinflammatory signaling, amplifies the host immune response to microbial pathogens, and has been implicated in the development of acute and chronic inflammatory diseases. TREM1 is also enriched in tumors, specifically on tumor-associated myeloid cells. To investigate the potential of TREM1 modulation as an anti-cancer therapeutic strategy, we developed PY159, an afucosylated humanized anti-TREM1 monoclonal antibody. We found that PY159 does not deplete TREM1-expressing cells, but rather acts as a TREM1 agonist. *In vitro* human blood assays showed that PY159 treatment upregulated activation markers on monocytes and stimulated neutrophil chemotaxis, as assayed by flow cytometry, transcriptional analysis, and *in vitro* migration assays. Furthermore, PY159 induced a selective set of proinflammatory cytokines and chemokines, which was dependent on PY159 afucosylation. We validated TREM1 expression in human tumors by single-cell RNAseq, immunohistochemistry, and flow cytometry, and found that it is expressed on myeloid populations, including tumor-associated neutrophils (TAN), tumor-associated macrophages (TAM), and monocytic myeloid-derived suppressive cells (mMDSC). We showed that PY159 can also induce proinflammatory cytokines and chemokines in dissociated human tumors *in vitro*, demonstrating that PY159 can reprogram tumor-associated myeloid cells. Finally, *in vivo* treatment of mice with a surrogate anti-mouse TREM1 antibody, PY159m, promoted anti-tumor efficacy in several syngeneic mouse tumor models, both as single-agent and in combination with checkpoint inhibitors, such as anti-PD-1 antibody. Together, these results demonstrate that therapeutic targeting of TREM1 with a TREM1 agonist antibody, PY159, promotes myeloid cell reprogramming and anti-tumor immunity. PY159 safety and tolerability have been demonstrated in non-human primates, and safety and efficacy of PY159 are currently being evaluated in first-in-human clinical trial (NCT04682431) including solid tumors that are resistant and refractory to standard of care therapies.

P105 Targeting VSIG4, a novel macrophage checkpoint, repolarizes suppressive macrophages which induces an inflammatory response in primary cell *in vitro* assays and fresh human tumor cultures. Steve Sazinsky, Ani Nguyen, Mohammad Zafari, Ryan Phennicie, Joe Wahle, Veronica Komoroski, Kathryn Rooney, Craig Mizzoni, Boris Klebanov, Jessica Ritter, Denise Manfra, Igor Feldman, Tatiana Novobrantseva. Verseau Therapeutics, Bedford, MA.

VSIG4 (V-set immunoglobulin-domain-containing 4) is a B7 family related protein with known roles as a complement receptor involved in pathogen clearance, via interactions with C3 fragments, as well as a negative regulator of T cell activation by an undetermined mechanism. VSIG4 is expressed in tumor associated macrophages with exquisite specificity. In cancer, increased expression of VSIG4 has been associated with worse survival in multiple indications, including non-small cell lung cancer, multiple myeloma, ovarian cancer, stomach cancer and glioma, suggesting an important role in tumor immune evasion. Based upon computational analysis of transcript data across thousands of primary cancer and normal tissue samples, we hypothesized that VSIG4 has an important regulatory role in promoting M2-like immune suppressive macrophages in the tumor microenvironment, and that targeting VSIG4 via a monoclonal antibody could relieve VSIG4-mediated macrophage suppression by repolarizing tumor associated macrophages (TAMs) to an inflammatory phenotype capable of coordinating an anti-tumor immune response. Through a series of *in vitro* and *ex vivo* assays we demonstrate that anti-VSIG4 antibodies repolarize M2 macrophages and induce an immune response culminating in T cell activation. Anti-VSIG4 antibodies upregulate pro-inflammatory cytokines in M-CSF plus IL-10 driven monocyte-derived M2c macrophages, as well as in TAM-like macrophages *in vitro* derived from monocytes cultured in the presence of ascites fluid from ovarian cancer patients. To determine whether anti-VSIG4-induced macrophage repolarization can activate T cells, monocyte-derived M2c macrophages were co-cultured with autologous T cells in the presence of staphylococcal enterotoxin B (SEB) activation and anti-VSIG4 antibodies. Here, anti-VSIG4 antibodies upregulate both pro-inflammatory myeloid-derived cytokines (GM-CSF) and T cell-derived cytokines (IFNγ). To extend these observations to a relevant translational model, we treated fresh, patient-derived tumor samples with anti-VSIG4 antibodies and relevant controls *ex vivo*. Across numerous patient-derived samples, which included multiple tumor types, anti-VSIG4 treatment resulted in a significant upregulation of cytokines involved in TAM repolarization and T cell activation, and chemokines involved in immune cell recruitment, at levels greater than observed by treatment with anti-PD-1 or a clinical macrophage repolarizing agent (anti-ILT-4). Taken together, these data suggest that VSIG4 represents a promising new target capable of stimulating an anti-cancer response via multiple key immune mechanisms.

P106 Reprogramming regulatory T cells (Treg) using a MALT1 inhibitor for cancer therapy. Peter Keller[1], Irina Mazo[1], Yun Gao[1], Vijayapal Reddy[1], Francisco Caballero[1], Sam Kazer[1], Dannah Miller[2], Roberto Gianani[2], James E. Marvin[3], Bret Stephens[4], Gregory L. Beatty[5], Ulrich H. von Andrian[6], Thorsten R. Mempel[7]. [1]Monopteros Therapeutics, Boston, MA, [2]Flagship Biosciences, Broomfield, CO, [3]University of Utah, Salt Lake City, UT, [4]Rincon Biosciences, Salt Lake City, UT, [5]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [6]Harvard Medical School, Boston, MA, [7]Center for Immunology & Inflammatory Diseases, Massachusetts General Hospital, Boston, MA.

Background Despite transforming effects of immune checkpoint blockade (ICB) therapy, objective response rates are low for most solid tumors. In the tumor microenvironment (TME), regulatory T cells are functionally unstable, likely due to changes in Treg metabolism seen in the tumor milieu. Destabilized Treg are susceptible to reprogramming wherein they can be induced to lose their immunosuppressive function and to secrete interferon-gamma (IFN-g). Thus, Treg reprogramming offers a novel strategy to sensitize unresponsive tumors to ICB. Notably, blockade of MALT1 protease induces Treg reprogramming in the TME but without affecting Treg in healthy tissue. MPT-0118, an orally dosed MALT1 inhibitor, was developed to reprogram Treg in the TME and is currently being assessed in patients with advanced tumors. *Approach* Treatments included MPT-0118 and anti-PD-1. *In vivo* studies in mice assessed anti-tumor effects using D4M.3A, B16F10, and MC38 syngeneic tumors. Human and mouse tumor tissues were evaluated for Treg reprogramming by *in situ* hybridization or flow cytometry. Studies in rats and dogs assessed pharmacokinetics and safety. *Results* MPT-0118 demonstrated dose-dependent *in vivo* anti-tumor activity. Consistent with the hypothesis that Treg reprogramming supports anti-tumor immunity by initiating IFN-g-driven tumor inflammation, the effect was strongest in combination with anti-PD-1 and in models that are not responsive to ICB alone. MPT-0118-treated tumors showed an increase in IFN-g-secreting Treg, associated with decelerated tumor growth. *Ex vivo*, MPT-0118 induced Treg reprogramming in tumors resected from patients with colorectal and endometrial cancers. While MPT-0118 caused Treg to produce IFN-g, no changes in the frequencies of Treg circulating in blood were detected in rats. Modeling of the human effective dose and toxicology studies demonstrate a >2x therapeutic window in patients. *Conclusions* MPT-0118 Treg reprogramming represents a novel strategy with the potential to improve responses to ICB therapy in solid tumors. A Phase 1/1b dose-escalation and cohort-expansion clinical trial evaluating MPT-0018 is underway.

P107 PSGL-1 blocking antibodies repolarize tumor associated macrophages, reduce suppressive myeloid populations and induce inflammation in the tumor microenvironment, leading to suppression of tumor growth. Ani Nguyen[1], Jessica Ritter[1], Mohammad Zafari[1], Denise Manfra[1], Veronica Komoroski[1], Brian O'Nuallain[1], Ryan Phennicie[1], Kevin Kauffman[1], Dominika Nowakowska[1], Joe Wahle[1], Steve Sazinsky[1], Michael Brehm[2], Igor Feldman[1], Tatiana Novobrantseva[1]. [1]Verseau Therapeutics, Bedford, MA, [2]University of Massachusetts Medical School, Worcester, MA.

Suppressive myeloid populations in the tumor microenvironment are associated with worse survival of cancer patients and low effectiveness of T cell checkpoint inhibitors. Recently, several early clinical studies have produced positive data for therapies aimed at repolarizing suppressive myeloid populations in the tumor microenvironment. One new macrophage repolarizing target, PSGL-1, is expressed at high levels on immuno-suppressive TAMs and differentiated M2 macrophages. PSGL-1 has been shown to have an immune-modulatory activity, which includes its role in maintaining a suppressive functional macrophage state. To assess the ability of PSGL-1 antibodies to convert macrophages and the tumor microenvironment from an immuno-suppressive toward a pro-inflammatory state, we employed *in vitro* primary macrophage and multi-cellular assays, *ex vivo* patient-derived tumor cultures, and a humanized mouse PDX model. We have determined that our lead anti-PSGL-1 antibody repolarized M2-like macrophages to a more M1-like state both phenotypically and functionally as assessed in primary *in vitro* macrophage assays. Transcriptomics profiling of M2c macrophages showed that the anti-PSGL-1 antibody upregulated TNF-a/NF-kB and chemokine-mediated signaling, while downregulating oxidative phosphorylation, fatty acid metabolism and Myc signaling pathways, consistent with a broad M2-to-M1 shift of the macrophage state. Furthermore, these repolarized M1-like macrophages enhanced the inflammatory response in complex multi-cellular assays. The PSGL-1 antibody also showed efficacy in a humanized mouse PDX model of melanoma. The antibody suppressed tumor growth to a significantly greater degree compared to anti-PD-1. At the cellular and molecular levels, the anti-PSGL-1 treatment led to a more enhanced inflammatory microenvironment, including a reduced M2:M1 macrophage ratio, and an increase in systemic pro-inflammatory mediators. Compared to anti-PD-1 monotherapy, anti-PSGL-1 alone and in combination with anti-PD-1 increased the fraction of effector $CD8^+$ T cells among the infiltrating T cells. Significant combination effects of anti-PSGL-1 plus anti-PD-1 were seen at the cellular and molecular levels within the tumor tissue, the spleen, and peripheral blood. Lastly, pre-clinical efficacy of our lead anti-PSGL-1 antibody was demonstrated using *ex vivo* cultures of fresh patient-derived tumors that preserve the cellular heterogeneity of the TME. Anti-PSGL-1 increased production of inflammatory cytokines and chemokines involved in immune activation of the TME and T cell recruitment. The data presented here provide biological and mechanistic support for clinical testing of antibodies targeting PSGL-1 for the treatment of cancer.

P108 In vivo CRISPR screens identify E3 ligase COP1 as a modulator of macrophage infiltration and cancer immunotherapy target. Xiaoqing Wang[1], Collin Tokheim[1], Shengqing S. Gu[1], Binbin Wang[2], Qin Tang[3], Yihao Li[1], Nicole Traugh[4], Zexian Zeng[1], Yi Zhang[1], Boning Zhang[1], Jingxin Fu[1], Tengfei Xiao[1], Wei Li[5], Clifford Meyer[1], Peng Jiang[2], Paloma Cejas[1], Klothilda Lim[1], Henry Long[1], Myles Brown[1], X. Shirley Liu[1]. [1]Dana-Farber Cancer Institute, Boston, MA, [2]National Institutes of Health, Bethesda, MD, [3]Salk Institute, San Diego, CA, [4]Tufts University, Boston, MA, [5]George Washington University, Washington, DC.

Despite remarkable clinical efficacies of immune checkpoint blockade (ICB) in cancer treatment, ICB benefits in triple-negative breast cancer (TNBC) remain limited. Through pooled *in vivo* CRISPR knockout (KO) screens in syngeneic TNBC mouse models, we found that inhibition of the E3 ubiquitin ligase COP1 in cancer cells decreases the secretion of macrophage-associated chemokines, reduces tumor macrophage infiltration, enhances tumor immunity and ICB response. Transcriptomics, epigenomics, and proteomics analyses revealed COP1 functions through proteasomal degradation of the C/ebpδ protein. COP1 substrate TRIB2 functions as a scaffold linking COP1 and C/ebpδ, which leads to polyubiquitination of C/ebpδ. COP1 inhibition stabilizes C/ebpδ to suppress the expression of macrophage chemoattractant genes. Our integrated approach implicates COP1 as a target for improving cancer immunotherapy efficacy by regulating chemokine secretion and macrophage infiltration in the TNBC tumor microenvironment.

P109 Targeting BRD4 in T cells with self-delivering RNAi PH-894 for immunotherapy.

Melissa Maxwell[1], Daisuke Ujiie[2], Benjamin Cuiffo[1], Brianna Rivest[1], Dingxue Yan[1], James Cardia[1], Jeroen Melief[2], Rolf Kiessling[2], Simon P. Fricker[1]. [1]Phio Pharmaceuticals, Marlborough, MA, [2]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden.

BRD4 is a member of the family of BET proteins that act as epigenetic readers and regulators of gene transcription. BRD4 is a known driver of tumor growth via enhancement of transcription of oncogenes such as Myc. More recent evidence also suggests a role of BRD4 in T cells. T cell differentiation and T cell exhaustion is epigenetically regulated and BRD4 has been associated with T cell differentiation. Available small molecule inhibitors of BET proteins do not target BRD4 specifically, but also inhibit other members of the BET protein family. We therefore investigated the effects of BRD4 silencing on T cells with the INTASYL™ compound PH-894, a self-delivering RNAi specifically targeting BRD4. INTASYL self-delivering RNAi molecules consist of an asymmetric duplex structure, a small duplex region (≤ 15 base pairs), a single-stranded phosphorothioate tail, and chemical modifications to confer stability, hydrophobicity, and efficient cellular uptake. Primary human T cells from healthy donors, either unstimulated or activated by OKT3 or anti-CD3/CD28 beads, were treated with PH-894. Additional studies were performed with $CD8^+$ T cells magnetically sorted from primary human T cells. Silencing of BRD4 mRNA was assessed by RT-qPCR, and protein by flow cytometry. Phenotypic markers of T cell activation, differentiation and function were assessed by flow cytometry and cytokine secretion by cytometric bead array. Expanded and PH-894-treated $CD8^+$ T cells were studied for IFNγ production upon coculture with allogeneic melanoma cell lines. Silencing of BRD4 mRNA and protein by PH-894 was concentration dependent and durable for up to 10 days in activated and naïve T cells. Silencing was specific for BRD4 with no effect on BRD2 or BRD3 expression. In activated T cells there was a suppression of IL-10 secretion and no effect on inflammatory cytokine levels. Markers of T cell activation were elevated on naïve PH-894-treated T cells with a shift to an increased $CD8^+/CD4^+$ ratio. In addition, BRD4 silencing in activated $CD8^+$ T cells coincided with a decreased expression of transcription factors typically induced immediately upon TCR activation and are known drivers of T cell differentiation towards an effector phenotype. Moreover, $CD8^+$ T cells expanded in the presence of PH-894 produced increased levels of IFNγ when cocultured with two allogeneic melanoma cell lines. These data translated *in vivo* with PH-894 treatment eliciting a dose associated inhibition of tumor growth in a CT26 murine colon carcinoma model. *Ex vivo* analysis of tumors found PH-894 efficacy was associated with decreased expression of BRD4 in tumor infiltrating CD3 T cells, and a reduction of $Foxp3^{hi}$ $CD25^{hi}$ $CD4^+$ $CD3^+$ Tregs, compared with control tumors. Specific silencing of BRD4 with PH-894 resulted in phenotypic changes on T cells associated with T cell activation and reduced immunosuppression. These data suggest that BRD4 not only plays a role on tumor cells but can also regulate T cell function and that PH-894 can reprogram T cells to provide enhanced immunotherapeutic activity.

P110 Neandertal introgressions contribute to upper aero-digestive tract tumor patient survival and identify patients who may benefit from STING agonist treatment. Antonio Gualberto[1], Tenghui Chen[1], Catherine Scholz[1], Jason Luke[2]. [1]H3 Biomedicine, Cambridge, MA, [2]University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Admixture of archaic (Neandertal and Denisova) and ancestral genes enhanced natural immunity during out of Africa migrations and modulates modern susceptibility to autoimmunity and cancer. We investigated a series of functional interactions between archaic and ancestral STING1 and TLR genes to identify vulnerabilities that could be addressed by STING agonist therapy. **Methods:** Gene variants from 10,389 cancer patients (pts) were obtained from TCGA. Archaic sequences were accessed using UCSD genome browser 410. Linkage disequilibrium was investigated using LDlink v5.0. Patient 1 was treated within NCT04144140. **Results:** STING1 variants were overrepresented in gastroesophageal cancer pts. V48V (rs7447927-C>G) with GWAS of lower rate of Asian esophageal cancer was in linkage disequilibrium with the reference alleles of the partially active HAQ and REF variants, and with rs13153461, present in Neandertal sequences. STING1 rs7447927-G was also associated with HLA A*24:02 ($p<0.001$), A*02:06 ($p=0.01$), and A*31:01 ($p=0.02$), of Neandertal origin. We then investigated a potential epistasis between STING1 rs7447927-G and TLR variants associated with decreased H. pylori prevalence: Neandertal-like TLR10 I775V (rs4129009) and ancestral TLR6 S249P (rs5743810). No independent prognosis was identified in 32 TCGA legacy studies; however, in upper aerodigestive (oral cavity, oropharynx, esophageal, gastric, biliary tract) tumor pts who carried reference/heterozygous TLR6 S249P and/or reference TLR10 I775, Neandertal-associated STING1 rs7447927-G zygosity was a strong predictor of survival. Hazards ratio for rs7447927-GG vs GC (4.8 vs 2.7 yrs. median survival), and GG vs CC (4.8 vs 1.8 yrs.) were respectively 0.71 and 0.53, N=713, $p=0.0003$. Patient 1 was a 75 yr.-old male esophageal cancer pt with GERD, Barrett's esophagus and TLR6 S249/STING1 rs7447927-GC, who was progressing from anti-PD1 and chemotherapy and received 75 microg intra-tumoral injections of the STING agonist E7766. Non treatment-related grade 2 events of anemia and hyponatremia, serum IFNbeta and IP10 levels induction, 20% tumor size reduction including abscopal effects, and 6.3 months PFS benefit were observed. **Conclusion:** Genotyping of STING1, TLR6-10 variants could contribute to identify pts who may benefit from E7766 treatment.

P111 Therapeutic KRAS^{G12C} inhibition alleviates KRAS-driven immunosuppression.

Edurne Mugarza, Febe Van Maldegem, Jesse Boumelha, Chris Moore, Sareena Rana, Miriam Llorian Sopena, Phil East, Miriam Molina Arcas, Julian Downward. The Francis Crick Institute, London, United Kingdom.

Therapeutic KRAS^{G12C} inhibitors may substantially improve KRAS-mutant cancer patient care, considering the encouraging clinical data emerging from ongoing clinical trials. Since oncogene-targeting therapies often lead to resistance, the development of combinatorial therapies will be of utmost importance to achieve long-lasting tumour control. Given the known immunosuppressive role of KRAS, clinical trials investigating the combination of KRAS^{G12C} inhibitors with immune checkpoint blockade are currently underway for NSCLC patients. Here, we show that KRAS^{G12C} inhibition using MRTX1257 alleviates KRAS-driven immune suppression, through a myriad of mechanisms including the augmentation of tumour cell-intrinsic interferon responses. Despite harbouring a large number of somatic mutations, orthotopic 3LL (KRAS^{G12C}DNRAS) tumours are able to evade anti-tumour immune responses. Combining multicolour flow cytometry, RNA sequencing and imaging mass cytometry, we observed significantly increased T cell activation and infiltration after MRTX1257 treatment in addition to enhanced antigen presentation cells and gene sets, and reduced myeloid cells, creating an immune permissive microenvironment in these lung tumours. Despite these profound changes, combination of KRAS^{G12C} inhibitors and anti-PD-(L)1 antibodies fail to show synergism in our model, in contrast with Amgen's published data using an immunogenic model of colorectal cancer. This data suggests that a pre-existing anti-tumour immunity may be crucial for clinical responses to KRAS inhibitors and immune checkpoint blockade combinations. Henceforth, ongoing clinical trials investigating this approach will likely only benefit a subset of patients and further research will be needed to examine novel therapeutic approaches and combinations for refractory patients.

P112 Treatment with the dual-mechanism ERK inhibitor, ASTX029, alters myeloid cell differentiation. Christopher Hindley[1], Andrea Biondo[1], Kim-Hien Dao[2], Lynsey Fazal[1], Alpesh Shah[1], Martin Sims[1], Nicola Wilsher[1], Harold Keer[2], John Lyons[1]. [1]Astex Pharmaceuticals, Cambridge, United Kingdom, [2]Astex Pharmaceuticals, Inc., Pleasanton, CA.

The inhibition of aberrant MAPK pathway activity is a clinically validated approach which has resulted in the approval of agents targeting tumors driven by activating mutations in BRAF and KRAS. Although the overall response rate to MAPK-targeting agents is high, duration of response is often limited by the emergence of acquired resistance. In contrast, immune checkpoint inhibitors (ICI) such as the anti-PD1 therapy, pembrolizumab, have a lower response rate but induce more durable responses. It has been demonstrated that inhibition of aberrant MAPK pathway activity enhances immune activation. For example, preclinical studies show that treatment with the $BRAF^{V600E}$ inhibitor dabrafenib or the $KRAS^{G12C}$ inhibitor sotorasib induces a pro-inflammatory tumor microenvironment (TME), which is associated with increased anti-tumor immunity. Further, studies using syngeneic, MAPK-activated in vivo models have demonstrated that the combination of MAPK-targeting agents and ICI results in synergistic inhibition of tumor growth. ASTX029 is a dual-mechanism ERK1/2 (ERK) inhibitor, inhibiting both the catalytic activity and phosphorylation of ERK, which is currently undergoing clinical development as part of a Phase 1/2 trial in advanced solid tumors (NCT03520075). ASTX029 has good oral bioavailability and shows potent inhibition of tumor growth in preclinical models bearing activating mutations in the MAPK pathway. We have previously investigated the immunomodulatory effects of ASTX029 using an in vivo syngeneic tumor model and observed that treatment with ASTX029 resulted in a pro-inflammatory TME, with increased interferon signaling consistent with published data describing the effects of treatment with dual-mechanism ERK inhibitors (Kidger *et al*., Mol Cancer Ther, 2020). We also observed an increased expression of antigen presentation genes. Using digital spatial profiling, we evaluated the expression of 31 proteins in immune infiltrates and observed a significant decrease in CD14 and a significant increase in MHC class II in ASTX029-treated tumors compared to untreated tumors. We therefore investigated the immunomodulatory effects of ASTX029 using primary human monocytes under conditions that induce macrophage differentiation and polarisation. Treatment with ASTX029 induced a decrease in CD14 and an increase in MHC class II cell surface expression, consistent with our previous in vivo mouse model data. In addition, we observed changes in cell surface expression of phenotypic markers, such as CD206, following treatment with ASTX029. These data support our previous observations and demonstrate that ERK inhibition by ASTX029 leads to phenotypic changes during monocyte to macrophage differentiation. Our data provide a strong rationale for the combination of ASTX029 with agents which aim to modulate the myeloid compartment or response to myeloid signaling.

P113 Image-based quantification of immunotherapeutic effect on the tumor-immune interactions in 3D co-cultures. Lidia Daszkiewicz, Gera Goverse, Nataliia Beztsinna, Marjan van de Merbel, Benjamin Visser, Tomas Veenendaal, Emma Spanjaard, Kuan Yan, Leo Price. Crown Bioscience Netherlands B.V., Leiden, Netherlands.

INTRODUCTION Despite the increasing number of immunotherapies available in preclinical cancer research and drug discovery pipelines, the number of patients benefiting from these immunotherapies remains extremely low. Moreover, the failure of immunotherapies in clinical trials remains very high to date. These high failure rates could be attributed to the lack of deep understanding the mechanism of action of drug candidates and to the absence of clinically relevant preclinical models that are suitable for drug screening purposes. To address these issues, we have developed an *in vitro* drug screening platform suitable for high-throughput testing in physiologically relevant 3D environment. Our preclinical drug testing platform is based on the co-culture of tumor cells with immune cells in 3D. Image analysis is applied to read out effects of drug candidates on immune cell migration, immune cell infiltration and tumoroid killing. **MATERIALS AND METHODS** 3D tumor cultures from human cancer cell lines (including breast, lung and colon cancer) or patient-derived colorectal organoids (from HUB Organoid Technology) were generated by embedding tumor cells in 3D ECM-like matrix constituted from protein hydrogel. Subsequently, different immune subsets, including PBMCs from healthy donors, T cells, NK cells or macrophages, were stained with cell tracker and added to the cultures. The cultures were treated with different immune-modulators (e.g. superantigens, activating antibodies, T cell engagers, CSFR1 inhibitor or STING agonist). The effect of immune-modulators on immune cell infiltration and their killing was assessed by high-content imaging and quantified after morphometric analysis with the proprietary Ominer® software. Immune cell-mediated killing was confirmed by measurement of IFNγ secretion in the culture supernatants. **RESULTS** Image-based analysis allowed for the dissection of complex tumor-immune cell interactions in the 3D cultures. Moreover, morphometric analysis revealed different levels of immune cell infiltration and tumoroid killing upon treatment with different immune-modulators. These effects could be reduced by inhibitory signals from the tumor microenvironment (TME) and were confirmed by the levels of IFNγ secreted by the immune cells. **CONCLUSION** Our *in vitro* platform allows quantitative image-based analysis of 3D tumor-immune cell co-cultures in a high-throughput manner, based on spatially resolved information in a more physiologically-relevant setting compared to traditional 2D cultures. This image-based analysis could be employed to dissect the effect of immunotherapy on different cell populations with the TME representing a promising tool to improve our understanding of the mechanism of action of novel treatments, treatment relapse and combination strategies, to eventually lead to a better clinical performance.

P114 Engineered hydrogel elucidates contributions of matrix mechanics to esophageal adenocarcinoma and identify matrix-activated therapeutic targets. Ricardo Cruz-Acuña[1], Claudia Loebel[2], Jason A. Burdick[2], Anil K. Rustgi[1]. [1]Columbia University Irving Medical Center, New York, NY, [2]University of Pennsylvania, Philadelphia, PA.

Changes in the tumor microenvironment arbitrated by a stiffened ECM are associated with tumor aggression and enable increased propensity towards metastasis. For instance, *in vitro* (2D) studies have implicated ECM properties in EAC progression, suggesting their exploitation for esophageal therapy following surgical resection. However, these studies are limited by the lack of 3D intercellular interactions, underscoring the need for physiologically relevant 3D culture models (e.g., organoids) that better recapitulate human cancer and its microenvironment to elucidate underlying mechanisms. Engineered hydrogels are an evolving and important component of 3D organoid culture systems, especially to introduce tunable physicochemical matrix signals that have been investigated in tumor progression and metastasis. Furthermore, patient-derived tumor organoids have become an attractive pre-clinical *in vitro* model to study cancer biology and evaluate response to therapeutics. Therefore, we have engineered a visible light-mediated hydrogel platform that supports the development of patient-derived Barrett's esophagus (BE) organoids, a precursor to esophageal adenocarcinoma (EAC), as well as EAC organoids. This synthetic biomaterial platform allows control over hydrogel stiffness to better recapitulate the mechanically dynamic esophageal cancer microenvironment, and may help identify therapeutic targets in EAC organoids. Our preliminary data have demonstrated that BE and EAC organoid density, size and proliferation can be controlled by synthetic ECM biomechanical properties, demonstrating the significance of understanding the independent contributions of ECM properties to EAC development, which is inherently limited in biologically-derived materials (e.g. Matrigel^{TM}). Furthermore, our data show that increased hydrogel stiffness promotes increased expression of mutant TP53, and increased SOX9 expression as a result of YAP1 activation, suggesting that matrix mechanics have a significant role in conferring stem-like properties to EAC organoids via activation of canonical oncogenic signaling pathways. Ongoing studies involve identifying matrix-activated therapeutic targets via small-molecule inhibition of TP53 and YAP1, as a function of matrix stiffening, and identifying differentially expressed genes using high-throughput transcriptome sequencing between stiffened hydrogel-encapsulated EAC organoid conditions. Our work is significant because it establishes a biomaterial platform that overcomes the limitations of current 3D organoid culture methods to elucidate the role of the tumor microenvironment in EAC tumorigenesis and to identify disease-relevant therapeutic targets. Successful completion of this work will also provide an opportunity to further establish the engineered biomaterial as a platform to potentially elucidate the mechanisms of, and therapy targets for, other human adenocarcinomas in the context of changes in the mechanics of tumor microenvironment.

P115 Novel patient avatar platform for oncology drug testing using 3D *ex vivo* models derived from fresh patient tumor tissues. Nataliia Beztsinna[1], Fanny Grillet[2], Niels Meesters[1], Donny van der Meer[2], Lidia Daszkiewicz[1], Kuan Yan[1], Emma Spanjaard[1], Willemijn Vader[2], Leo Price[1]. [1]Crown Bioscience Netherlands B.V., Leiden, Netherlands, [2]VitroScan B.V., Leiden, Netherlands.

Introduction The staggeringly high failure rate of clinical trials for oncology drugs can be attributed to many factors, including suboptimal *in vitro* and *in vivo* models that fail to recapitulate the complexity of the human tumor microenvironment (TME) or predict patient response. Translational human 3D cell culture models, such as patient-derived tumor organoids, have begun to bridge the gap between tissue culture systems and patients in the clinic. However, even in these advanced models, the endogenous cells of the TME, such as tumor infiltrating lymphocytes (TILs), fibroblasts, macrophages and other immune cells, are absent. These TME components have been shown to express important drug targets and play a critical role in both tumor progression and modulation of the response to drugs. Here we present a novel patient avatar platform that combines a short-term 3D *ex vivo* tumor culture system with high content image (HCI)-based analysis. Patient tumor tissues from pleural fluid, ascites, surgical resections or biopsy were tested *ex vivo* to preserve tumor heterogeneity and resident immune cells, removing the need for artificial co-culture systems. This study entailed a detailed quantification of tumor sensitivity to targeted therapies, standard of care, and novel (immune) drugs and drug combinations, tested on different cancer types. **Methods** Patient tumor tissues were obtained from ongoing clinical trials in the Netherlands as well as from commercial tissue providers, and processed within 24 hours to preserve the native tumor heterogeneity and TME. Freshly isolated tumor cells from ovarian, breast cancer and non small cell lung cancer (NSCLC) patients were embedded in a protein-rich hydrogel and exposed to panels of single and combination drug treatments at different concentrations in a 384-well format for 5-7 days. Effects of drugs and combination therapies on physiologically relevant morphological features, such as tumor cell killing, growth arrest, invasion and immune cell proliferation, were measured using our proprietary automated HCI analysis platform. **Results** Patient-specific drug sensitivity profiles were generated based on the response to a broad range of drugs including standard of care (e.g., platinum, paclitaxel, gemcitabine), targeted therapies (e.g., PARP and EGFR inhibitors), and activity of immunomodulatory drugs (e.g., ipilimumab, pembrolizumab and STING agonists). Accurate and reproducible response evaluation demonstrates the feasibility of preclinical drug testing on patient primary material within the platform. **Conclusion** Our platform successfully combined proven *ex vivo* drug testing protocols using fresh patient tumor tissue with preserved TME components and advanced 3D HCI analysis. Our approach offers a rapid, reliable and patient-relevant approach to test various candidate compounds (e.g., antibodies, antibody-drug conjugates and small molecules) for various cancer types. It has the potential to significantly improve the preclinical evaluation of drugs, and also to improve the success rate of clinical trials.

P117 Oncogenic Kit induces replication stress and induces Chk1/ATR inhibitor sensitivity in melanoma. Ching-Ni Njauw[1], Zhenyu Ji[2], Hensin Tsao[2], Antoine Simoneau[2], Raj Kumar[2], Keith T. Flaherty[2], Lee Zou[2], Duc Minh Pham[3]. [1]MGH, Boston, MA, [2]Massachusetts General Hospital, Boston, MA, [3]Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of.

Contrary to cutaneous melanoma (CM), Acral and mucosal melanomas (AMM's) lack effective therapy and exact a higher mortality. Since amplifications and point mutations of KIT are common in AMM, we created a multi-stage murine cellular model of human KIT melanomas (i.e. $mKit^{K641E}$ lines) based on the most common KIT mutation in human melanoma (p.K642E). Compared to its vector-controlled cells (mVec), $mKit^{K641E}$ cells exhibit greater chromosomal aberrations, and sustain 3D spheroid forming capability and aggressive tumor growth in C57BL/6J mice. In addition, DNA replication/RNA processing and ribosomal biogenesis pathways associated with Kit^{K641E} transformation. Surprisingly, an unbiased comparative drug screen uncovered a selective vulnerability to Chk1 inhibition in the Kit^{K641E} activated cells. $mKit^{K641E}$ cells displayed >6-fold greater sensitivity compared to mVec cells (GI50=1.5 µM vs 10 µM) for Chk1 inhibitor PF477736 treatment. Furthermore, we proved that Kit^{K641E} induces profound DNA replication stress as evidenced by increased levels of γ-H2AX and p-$RPA32^{Ser33}$ in replicating cells and defective replication fork progression. Thus, $mKit^{K641E}$ represents a novel model to study the biology of KIT-driven melanoma and therapy development.

P118 Analysis of macrophage function and histone deacetylase inhibition in neuroblastoma. Gabrielle L. Brumfield, Shelby M. Knoche, Alaina C. Larson, Brittany J. Poelaert, Benjamin T. Goetz, Poomy Pandey, Donald W. Coulter, Joyce C. Solheim. University of Nebraska Medical Center, Omaha, NE.

Background: Neuroblastoma is the third most common childhood cancer and accounts for 12% of cancer-associated deaths in children under the age of 15. Treatment of neuroblastoma with the histone deacetylase inhibitor (HDACi) vorinostat induces increased infiltration of macrophages with upregulated immune cell-surface receptors. Neuroblastoma cells release VEGF and M-CSF, which may alter intratumoral macrophage populations. VEGF has also been implicated in alteration of amyloid precursor protein family processing. Our lab demonstrated that amyloid precursor protein 2 (APLP2), a member of the amyloid precursor protein family, plays an important role in the migration of tumor cells. APLP2 is known to be expressed by macrophages, but no studies have previously examined macrophage functions that are impacted by APLP2 in the context of neuroblastoma disease and its treatment by HDACi drugs. Because of the high morbidity and mortality associated with neuroblastoma, studies such as this one that are designed to comprehend the interaction of immunity and treatment in neuroblastoma are clinically significant. Methods: We treated neuroblastoma tumor cells (Neuro-2a) in vitro with M344, an HDACi with structural similarity to vorinostat, and assessed viability through MTT assay. In addition, we generated mice with APLP2 knockout in cells expressing the Csf-1 receptor (a protein characteristically expressed by macrophages and dendritic cells). Polarization of macrophages isolated from the macrophage-targeted APLP2-knockout mice was achieved through treatment with IFN-γ and LPS (M1) or IL-4 (M2). Macrophages were then analyzed through western blotting and flow cytometry for APLP2 expression and polarization markers. Results: Following polarization, macrophages collected from the bone marrow of macrophage-targeted APLP2-knockout mice have an altered distribution of M1 and M2 sub-populations, which are macrophage sub-populations that differ in their migratory capabilities, as well as in their abilities to stimulate or suppress anti-tumor immunity. Furthermore, we showed that M1 and M2 subpopulations of bone marrow-derived macrophages from normal mice differ in their expression of APLP2. We also demonstrated that M344 decreased neuroblastoma cell growth. Thus, APLP2 is influential in macrophage biology, and we have created a novel mouse model for defining its specific contributions in mice treated with HDACi drugs that influence macrophage biology. Conclusions: We have made progress in understanding the impact of the HDACi drug M344 on neuroblastoma cells and are ready to analyze its impact on macrophage/dendritic cell populations in a syngeneic neuroblastoma mouse model, as well as to define the role of APLP2 in the function of these cell populations in the context of neuroblastoma. In addition to their potential contribution to the development of new neuroblastoma therapies, the results from this study are expected to expand our comprehension of macrophage function and regulation, and thus will be of broad value in the immunology and oncology fields.

P119 Differential sensitivity to poly(ADP-ribose) polymerase inhibitors in patient-derived cell models of breast cancer. Immaculate Nalubowa, Subir Singh, Yuen Ngan Fan, Rachel Howard-Jones, Albert Bezman, Dominic I. James, Geoff Muckle, Gareth J. Griffiths. Imagen Therapeutics, Manchester, United Kingdom.

Female breast cancer (BC) became the most commonly diagnosed cancer globally in 2020. One of the hallmarks of BC is both intratumor and intertumor heterogeneity. Efforts have been made to categorize this heterogeneity based on the presence or absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/ERBB2). Receptor expression patterns are used to stratify BC and the subsequent treatments. Much of the current knowledge of breast cancer comes from in vitro and in vivo studies using well established cell lines, some of which have been cultured since the 1950s. Given that serial passaging over many years has been reported to alter the mutational profile of cell lines and therefore their relevance to clinical decisions on the primary tumour, new near-tumor cell models need to be generated. In this study, tumour biopsies or ascitic fluids were obtained from a local cancer research biobank, as well as directly from metastatic BC patients across the globe. Using mechanical dissociation and cell separation techniques we isolated tumor cells and grew them in proprietary media. The resultant models were subjected to whole exome and RNA sequencing. In addition, the models were tested in a 3-day cell death assay against a library of more than 50 FDA-approved drugs. The models and four BC cell lines (HCC1937, ZR-75-1, MDA-MB-436 and HCC1428) were also tested in a longer term 7-day assay against a panel of four poly(ADP-ribose) polymerase (PARP) inhibitors. A total of 10 patient-derived cell (PDC) models were successfully established from biopsies or ascitic fluids taken from patients with primary (1/10) or metastatic (9/10) BC. Models were established from patients with ER+ BC, HER2+ BC and triple negative BC (TNBC) within an average of 23 days from sample arrival. All models displayed a greater sensitivity to the non-targeted drugs in the library compared with the targeted drugs. Mutations in breast cancer associated 1 (*BRCA1*) and *BRCA2* genes that were not present in BC cell lines were identified in a subset of the PDCs. All models showed some sensitivity to PARP inhibitors, and most were exquisitely sensitive to talazoparib with IC50s of less than 100 nM. Determining cell death responses in near-tumour PDCs should thus be adopted to enable more informed clinical decisions for treatment pathways and in parallel will be promising tools for oncology drug discovery.

P120 Novel in vitro targeted combination therapies for anaplastic thyroid cancer.

Muthusamy Kunnimalaiyaan, Parag Parekh, Jalyn Golden, Ying Anderson, Stephen Lai, Maria Cabanillas, Marc Zafereo, Ramona Dadu, Marie-Claude Hofmann. The University of Texas MD Anderson Cancer Center, Houston, TX.

Purpose: To identify effective drug combination strategies targeting multiple oncogenic pathways in anaplastic thyroid cancer. **Background**: Treatments with BRAF and MEK inhibitors induce impressive tumor size reduction in most BRAF-mutated anaplastic thyroid carcinoma (ATC) patients. However, these drugs are ultimately ineffective due to resistance development, probably because ATC cells express multiple oncogenic pathways that can be used to bypass the treatments. Therefore, the identification of novel therapeutic strategies is still urgently needed. We had previously identified additional pathways/targets (TWIST1, MMP9, BCL2, TGFβR, and the stress kinase MAPK14/p38α) by reverse-phase protein arrays (RPPA) and microarray analysis. In this study, we report the in vitro effects of inhibitors specific for the identified targets used alone or in combination with BRAF inhibitors. **Methods**: We established mouse ATC cell lines (MCH2.2-Luc and PPA6-Luc) to graft into immunocompetent syngeneic animals. As a first step, these lines were treated in vitro for 72 hours with increasing concentrations of nine different inhibitors for the above targets, and the MTS colorimetric assay determined viability/IC_{50}. We also tested the combinatorial effects of these compounds with BRAF inhibitors (vemurafenib or dabrafenib) and compared them to single agents' effects. Also, we investigated the colony-forming ability and apoptotic response of these cell lines. **Results**: IC_{50} concentrations for nine different compounds were calculated. Both cell lines showed similar IC_{50} values, except that PPA6-Luc was more resistant to the BRAF inhibitor dabrafenib than MCH2.2-Luc. The MMP-9 inhibitor and the PARP inhibitor olaparib did not significantly affect the growth of these lines. Among three different MAPK14/p38α inhibitors tested, ralimetinib was the most effective (IC_{50} @ 15 mM). Importantly, venetoclax (BCL2 inhibitor) and harmine (TWIST1 inhibitor) effectively inhibited cell growth with an IC_{50} of 8 μM and 25 mM, respectively. Treatments using 0.1mM dabrafenib or 1mM vemurafenib in combination with 4 other drugs (5 mM each below the IC_{50}) resulted in significant synergy only with ralimetinib (38% growth decrease) and harmine (37% growth decrease) compared to treatments with single agents. Colony-forming assays confirmed the growth suppression. **Conclusions:** Inhibiting specific targets previously identified by RPPA and microarray analysis demonstrated, for the first time, in differential ATC cell growth suppression. Notably, only a combination of specific inhibitors (dabrafenib combined with the TWIST1 or the MAPK14/p38α inhibitors) resulted in synergy, suggesting that these targeted combinations will be more effective in our preclinical ATC mouse model than the other drugs. By treating these combinations in a mouse model may contribute, in the future, to the development of clinical and therapeutic strategies for aggressive thyroid cancer.

P121 Dual RAF/MEK inhibitor VS-6766 for treatment of solid tumors with diverse MAPK pathway alterations. Silvia Coma[1], Sanjib Chowdhury[1], Monica Musteanu[2], Mariano Barbacid[2], Jonathan A. Pachter[1]. [1]Verastem Oncology, Needham, MA, [2]Centro Nacional de Investigaciones Oncologicas, Madrid, Spain.

The RAS/RAF/MEK/ERK (MAPK) pathway shows frequent mutations in cancer which often confer worse prognosis. Whereas KRAS mutations (mt) are prevalent in pancreatic cancer (PDAC; 98%), colorectal cancer (CRC; 45%) and non-small cell lung cancer (NSCLC; 31%), melanoma shows frequent mutations in BRAF (60%) and NRAS (28%). Although several BRAF and MEK inhibitors (MEKi) are FDA approved, there is still a need for agents with improved response rate, duration of response, and tolerability. VS-6766 is a unique dual RAF/MEK inhibitor. In contrast to MEKi, VS-6766 is a potent allosteric inhibitor of MEK kinase activity, which promotes a dominant negative RAF/MEK complex preventing phosphorylation of MEK by wildtype BRAF, BRAF V600E and CRAF. This unique mechanism allows VS-6766 to block MEK signaling without the compensatory activation of MEK that appears to limit the efficacy of MEKi. In 3D proliferation assays in vitro, VS-6766 inhibited cell proliferation across multiple MAPK pathway alterations, including KRAS (G12C, G12D, G12V, G13D and Q61K), BRAF (V600E and class 2), NRAS and NF1 mt. Cell lines with KRAS G12V mt (n = 10; IC50 = 10 - 400 nM, average = 120 nM) were generally more sensitive than cell lines with KRAS G12D mt (n = 9; IC50 = 150 – 12,000 nM; average = 2.4 mM). Cell lines with NRAS mt also appeared to be especially sensitive to VS-6766 (average IC50 = 80 nM). Overall, VS-6766 inhibited proliferation of cell lines representing multiple cancers including NSCLC, PDAC, CRC and melanoma. VS-6766 effectively suppressed RAS pathway signaling (pMEK, pERK, DUSP4, pEphA2) throughout a 48-hour period among a panel of KRAS mt NSCLC cell lines. In a genetically engineered mouse tumor model of KRAS G12V mt/Trp53 KO NSCLC, which has previously been shown to be CRAF-dependent (Sanclemente et al., 2018), VS-6766 (0.1 mg/kg QD) induced regression of all tumors, whereas the MEKi trametinib at the same dose did not induce significant tumor growth inhibition. In KRAS mt NSCLC (H358) and ovarian cancer (TOV21G) xenograft models, VS-6766 significantly inhibited tumor growth (tumor growth inhibition, TGI = 70% and 69%, respectively), whereas the equivalent dose of trametinib was much less effective in inhibiting tumor growth (TGI = 13% and 29%, respectively). Clinically, intermittent dosing of VS-6766 as monotherapy has shown partial responses in patients with KRAS or BRAF mt gynecological malignancies (3/5) and KRAS mt NSCLC (3/10) (Guo, 2020). Interestingly, in NSCLC, responses occurred especially in patients with KRAS G12V mt tumors, which correlates with the greater anti-proliferative potency of VS-6766 observed across KRAS G12V mt NSCLC, CRC and PDAC cell lines. These data support the recent initiation of two registration-directed studies evaluating VS-6766 ± defactinib (FAK inhibitor) for the treatment of recurrent low-grade serous ovarian cancer (NCT04625270) and recurrent NSCLC with KRAS G12V or other KRAS mutation (NCT04620330), and suggest potential utility of VS-6766 for treatment of additional tumor types and oncogenic mutations in the MAPK pathway.

P123 Antitumor activity of tipifarnib and PI3K pathway inhibitors in HRAS-associated HNSCC. Francis Burrows[1], Shivani Malik[1], Stacia Chan[2], Asako Mccloskey[1], Zhiyong Wang[3], Mara Gilard[3], Silvio Gutkind[3]. [1]Kura Oncology, San Diego, CA, [2]Kura Oncology, San Diego, CA, [3]UCSD, San Diego, CA.

HRAS-MAPK and PI3K-AKT-mTOR are important oncogenic pathways in head and neck squamous cell carcinoma (HNSCC) and other squamous cell carcinomas (SCCs). HRAS is mutated in ~5% and overexpressed in approximately 30% of HNSCC patients, raising the possibility that some HRAS wild-type (WT) HNSCCs may also display a degree of dependence on HRAS. RAS proteins undergo several post-translational modifications, including the addition of a farnesyl isoprenoid moiety by the enzyme farnesyltransferase (FT), which facilitate their attachment to membranes. This dependence prompted the development of selective inhibitors (FTIs) for the treatment of RAS-driven cancers. Tipifarnib is a potent and selective FTI that has demonstrated antitumor activity in recurrent/metastatic HNSCC carrying HRAS mutations. Based on these data, a pivotal study (NCT03719690) evaluating the efficacy of tipifarnib in HRAS mutant HNSCC (AIM-HN) is currently ongoing. PIK3CA (the catalytic subunit of PI3K), another prominent driver in HNSCC, is commonly activated either by gain of function mutations or gene amplification with some overlap between the two subsets. Multiple reports indicate that HRAS and PIK3CA pathways cooperate and crosstalk in driving tumor progression in SCCs and resistance to inhibitors of respective pathways. In this study, we explored whether combined inhibition of HRAS farnesylation (by tipifarnib) and inhibition of PI3K pathway signaling (with inhibitors of PI3K-a, AKT or mTORC1/2) would be more effective in CDX and PDX models of HRAS-associated SCCs relative to the monotherapy approaches. In a panel of HNSCC cell lines harboring HRAS and/or PIK3CA alterations (mutation, overexpression or amplification), tipifarnib reduced cell growth and, in combination with PI3K-a inhibitor alpelisib, induced cytotoxicity. Consistent with in vitro findings, robust inhibition of tumor growth was observed in majority of animals treated with the combination of tipifarnib and alpelisib. Similar activity was noted with the AKT inhibitor uprosertib and the mTORC1/2 inhibitor sapanisertib. Regressions with the FTI-PI3K-a inhibitor doublet were observed both in tumors that were HRAS mutant or HRAS overexpressed with or without mutation or amplification of the PIK3CA gene or carried the latter without alterations in HRAS, suggesting that concomitant blockade of both targets may have surprisingly broad and potent anti-tumor activity in HNSCC. In dose-scheduling experiments in PDX models, simultaneous blockade of both targets was superior to split intermittent dosing of the two drugs, underlining the cooperativity of the two pathways in these models. Mechanistically, tipifarnib repressed the compensatory MAPK pathway activation induced by alpelisib at the level of phosphorylated ribosomal S6 protein. Impact of tipifarnib and alpelisib combination on additional MAPK and AKT signaling mediators will be reported.

P124 JDQ443, a covalent irreversible inhibitor of KRAS G12C, exhibits a novel binding mode and demonstrates potent anti-tumor activity and favorable pharmacokinetic properties in preclinical models. Saskia M. Brachmann[1], Andreas Weiss[1], Daniel A. Guthy[1], Kim Beyer[1], Johannes Voshol[1], Michel Maira[1], Anirudh Prahallad[2], Diana Graus Porta[1], Christian Schnell[1], Nils Ostermann[1], Andrea Vaupel[1], Marc Gerspacher[1], Catherine Leblanc[1], Dirk Erdmann[1], Dario Sterker[1], Grainne Kerr[1], Giovannoni Jerome[1], Victoria Head[1], Rowan Stringer[1], Ruben De Kanter[1], Kearns Jeff[3], Danielle Roman[1], Toni Widmer[1], Peter Wessels[1], Eloisa Jimenez Nunez[1], Richard Sedrani[1], Frederic Zecri[3], Francesco Hofmann[4], Jeff Engleman[5], Edwige Lorthiois[1], Simona Cotesta[1]. [1]NIBR, Basel, Switzerland, [2]Novartis, Basel, Switzerland, [3]NIBR, Cambridge, MA, [4]Pierre Fabre Medical Care, Geneva, Switzerland, [5]Treeline Biosciences, Cambridge, MA.

RAS is the most frequently mutated oncogene in cancer. KRAS *G12C* mutations are most prevalent in lung adenocarcinoma (~13%) and colorectal adenocarcinoma (~4%), and occur less commonly in other solid tumor malignancies. First generation $KRAS^{G12C}$ inhibitors show anti-tumor activity in early phase clinical trials. However, the emergence of resistance, mediated at least in part by RAS gene mutations that disrupt inhibitor binding and reactivation of downstream pathways, limit the duration of response. Here we report the identification of JDQ443 (NVP-JDQ443), a novel $KRAS^{G12C}$ inhibitor which binds under the switch II loop with a novel binding mode, exploiting unique interactions with the $KRAS^{G12C}$ protein compared to sotorasib and adagrasib. JDQ443 potently inhibits $KRAS^{G12C}$ cellular signaling and proliferation in a mutant selective manner by irreversibly trapping the GDP-bound state of $KRAS^{G12C}$ through formation of a covalent bond with cysteine at position 12. Consistent with its mechanism as an irreversible inhibitor, JDQ443 shows sustained target occupancy (TO) *in vivo* ($KRAS^{G12C}$ TO t1/2 ~ 66 h in the MiaPaCa2 model) despite a blood half-life of ~ 2 hours, and exhibits a linear PK/PD relationship. JDQ443 has dose-dependent anti-tumor activity in mice bearing *KRAS G12C* mutated tumor xenografts comparable to sotorasib and adagrasib. In mouse, rat, and dog, JDQ443 is orally bioavailable, achieves exposures in a range predicted to confer anti-tumor activity, and is well-tolerated. Continuous delivery of JDQ443 using mini-pump administration demonstrates that area under the curve (AUC), rather than maximal concentration (Cmax), is the driver of efficacy. Combination of JDQ443 with the SHP2 inhibitor TNO155 further increases KRAS G12C target occupancy *in vivo*, enhanced pre-clinical anti-tumor activity, and delayed the emergence of resistance in xenografts. A genome-wide CRISPR screen in 5 KRAS *G12C* mutated lung cancer cell lines identifies novel mechanisms of resistance to the KRAS/SHP2 drug combination. Furthermore, the characterization of JDQ443 alone and in combination with TNO155 in BaF/3 pools addicted to KRAS alleles that have previously been shown to mediate resistance to adagrasib in clinical samples will be discussed. Collectively, these data show that JDQ443 is a potent, mutant-selective, covalent irreversible $KRAS^{G12C}$ inhibitor with favorable pharmaceutical properties. A phase Ib/II clinical trial of JDQ443 alone and in combination with TNO155 in patients with advanced solid tumors harboring the *KRAS G12C* mutation is ongoing (NCT04699188).

P125 Combined inhibition of SHP2 and CDK4/6 is active in NF1-associated malignant peripheral nerve sheath tumor. Jiawan Wang[1], Ana Calizo[1], Kai Pollard[1], Angela C. Hirbe[2], Christine A. Pratilas[1]. [1]Johns Hopkins University, Baltimore, MD, [2]Washington University in St. Louis, St. Louis, MO.

Background: NF1 is an essential negative regulator of RAS activity and is altered in nearly 90% of malignant peripheral nerve sheath tumors (MPNST). Additional recurrent molecular changes include loss of function alterations in *CDKN2A*, *TP53*, *EED* and *SUZ12*, but molecular targeting of these genomic events represents a unique challenge. We previously reported that the efficacy of MEK inhibitor is limited by adaptive activation of receptor tyrosine kinases and the adaptor protein SHP2, and that combined inhibition of MEK and SHP2 is effective in MPNST. Loss of *CDKN2A*, inactivation of RB1, and hyperactivation of cyclin dependent kinases (CDK) in MPNST also suggest that small-molecule CDK4/6 inhibitors (CDK4/6i) may be a potential therapeutic strategy, but monotherapy with CDK4/6i also demonstrates limited activity. Given the dependency of D-cyclins on RAS signaling, we hypothesize that the anti-tumor effects of CDK4/6i may be potentiated by agents (SHP2i) targeting the upstream activators of RAS. **Methods**: The effects of shRNA-mediated inducible SHP2 knockdown on RAS signaling, short-term and long-term cell growth, and response to CDK4/6i were examined using immunoblotting, high throughput proliferation assays, and colony formation assays. Combined effects of SHP2i plus CDK4/6i on signaling, cell and tumor growth were assessed. Pharmacodynamic (PD) assays were performed on tumors extracted following drug treatment in patient-derived xenograft (PDX) models. **Results**: Despite a modest effect of SHP2 knockdown on ERK signaling, shSHP2 reduced MPNST cell growth. SHP2 knockdown or SHP2i treatment alleviated activation of ERK signaling and cyclin D1 expression induced by CDK4/6i, and enhanced the sensitivity to CDK4/6i. Combination benefit was observed in *in vitro* cell growth and *in vivo* PDX. Although some PDX models demonstrated similar responses to SHP2i alone or SHP2i + CDK4/6i during the initial 4 weeks on treatment, we found more sustained growth inhibition exerted by the combination. PD studies demonstrated a decrease in p-ERK levels in tumors treated with either SHP2i alone or the SHP2i/ CDK4/6i combination. **Conclusions**: Our preliminary data demonstrate that the combined inhibition of SHP2 and CDK4/6 is active and produces durable response in models of NF1-associated MPNST. This combination strategy may represent a novel treatment strategy for patients with MPNST.

P127 Mitochondrial matrix protease ClpP agonists suppress breast cancer stem cell function by downregulating multiple stem cell regulatory mechanisms. Yoshimi E. Greer[1], Lidia Hernandez[1], Donna Voeller[1], Raj Chari[2], Binwu Tang[1], Christina M. Annunziata[1], Sam Gilbert[3], Lalage Wakefield[1], Edwin Iwanowicz[4], Lee M. Graves[5], Stanley Lipkowitz[1]. [1]National Cancer Institute, Bethesda, MD, [2]National Cancer Institute, Frederick, MD, [3]San Diego State University, San Diego, CA, [4]Madera Therapeutics, LLC, Cary, NC, [5]University of North Carolina School of Medicine, Chapel Hill, NC.

Background: We previously reported that small molecule ONC201 induces mitochondrial structural and functional damage, leading to death in breast cancer cells. Subsequent studies demonstrated that ONC201 and the related analogs TR compounds are agonists of mitochondrial caseinolytic protease P (ClpP), an essential protein for maintenance of mitochondrial protein homeostasis. Recent studies have shown that cancer stem cells (CSCs) preferentially use mitochondrial oxidative metabolism for energy production. Here, we report that ClpP agonists inhibit breast CSCs by unique mechanisms targeting pathways vital to maintain CSC function. **Methods**: ONC201 and other ClpP agonists (TR-57, 65), other mitochondria-targeting drugs, such as oligomycin, metformin, CPI-613 were used. MDA-MB231 cell line was used as a primary *in vitro* model system. CLPP knockout (KO) cells were generated by CRISPR/Cas9 technology. Seahorse XF analyzer was used for cellular respiration analysis. Luminescence-based assays were used for cell viability and metabolism assays. Protein expression was examined by Western blotting. Aldefluor assay and SORE6 (OCT4/SOX2 response element)-reporter gene were used to monitor CSC fraction. Mammosphere formation assay was used to evaluate CSC function *in vitro*. *In vivo* limiting dilution analysis was used to evaluate tumor initiation capability of cells injected into mammary fat pad of athymic nude female mice. **Results**: Seahorse XF analyzer showed that mammospheres are more dependent on OxPhos than glycolysis compared with cells grown in 2D, supporting the hypothesis that breast CSCs rely on OxPhos. ClpP agonists reduced the CSC fraction in both Aldefluor and SORE6 reporter assays. ClpP agonists inhibited mammosphere formation in CLPP WT, but not in CLPP KO cells, demonstrating the on-target effects on CSC function. In *in vivo* assays, tumor formation was significantly ($p<0.001$) inhibited in the ClpP agonist-treated group compared with the control group, and the effect was CLPP-dependent. Altogether, these findings support that ClpP agonists inhibit CSC in breast cancers. We found that ClpP agonists downregulate multiple pathways and proteins critical for CSC maintenance including mevalonate pathway, HIF1a, EPAS1, YAP, and Myc. We also observed that other mitochondria targeting drugs such as oligomycin, metformin downregulate these signaling pathways and proteins to some extent. Importantly, however, ClpP agonists showed significantly greater impact in mammosphere formation and cell growth assays, compared with other mitochondria-targeting drugs. Further studies revealed that ClpP agonists uniquely deplete NAD+/NADH and promote reactive oxygen species, both of which are shown as key factors to maintain pluripotency of stem cells. Moreover, ClpP agonists uniquely inhibited enzymes involved with glutamine catabolism and proline biosynthesis, vital to amino acids and nucleotide synthesis. **Conclusion:** ClpP agonists inhibit cell growth and tumor initiation in breast cancer cells by targeting multiple pathways essential to maintain CSC function.

P129 Ketogenic diet (KD) and targeting Warburg effect: Apparent toxicity of KD combination with 2-deoxy-D-glucose. Rafal Zielinski[1], Maria Poimenidou[1], Matthew Khuong[1], Krzysztof Grela[1], Roberto Cardenas-Zuniga[1], Stanislaw Skora[1], Izabela Fokt[1], Michael Gagea[2], Jody Swain[1], Sigmund Zhou[3], Waldemar Priebe[1]. [1]UT MD Anderson Cancer Center, Houston, TX, [2]UT MD Anderson Cancer Center, Houston, TX, [3]University of Texas McGovern School of Medicine, Houston, TX.

Introduction: Cancer dependence on glucose metabolism has been recognized as a potential target for the development of new anticancer therapies. Ketogenic low-carbohydrate diet (KD) and aerobic glycolysis is known as a Wartburg effect are two approaches linked by a common aim to reduce cancer glucose metabolism. 2-Deoxy-D-glucose (2-DG) has been shown to inhibit glycolysis and in effect to inhibit glucose metabolism and tumor growth in vitro and in vivo in a wide range of cancer models. Anticancer effects of 2-DG can be impacted by several factors including competition of glucose from the food intake. Ketogenic diet has been proposed as a strategy to inhibit proliferation of cancer cells that are highly dependent on glucose for growth and survival by reducing glucose access from food intake and switch to lipid metabolism. Combination of both approaches seemed to be a logical step to increase overall antitumor effects. **Objective**: The objective of the study was to determine safe doses of 2-DG in mice fed with KD and determine potential toxic side effects of 2-DG in mice maintained on carbohydrate-reduced vs. regular diet. **Methods:** Female CD-1 mice were maintained on a regular grain-based or carbohydrates-reduced diet (Research Diets (20 % Kcal Protein, 60 % Kcal Fat, 20 % Kcal carbohydrate). Animals were dosed with 2-DG once a day five times a week by oral gavage (PO) or intraperitoneal (IP) injections. Animals were watched daily for the development of toxicity symptoms. **Results:** No toxic side effects in mice fed with a regular, chow-base diet and treated orally with 2-DG were noted during the six-week treatment (doses ranged from 0.25 to 2.0 g/kg, five times/week). In the contrary, 2-DG-treaded animals maintained on KD showed dose-dependent toxicity with median survival (MS) 10 and 11 days for 1.0 and 2.0 g/kg respectively. For mice receiving 2-DG at 0.5 g/kg MS was 28 days and only 20% mice died in the group receiving 0.25 g/kg. In separate experiments mice treated with 2-DG orally or by intraperitoneal injections IP (1.5 g/kg) showed similar MS of 11 days. Complete blood count analysis of 2-DG treated animals fed with KD revealed acute thrombocytopenia. Post-mortem analysis of dissected organs indicated the development of acute interstitial pneumonia in treated mice. **Conclusion:** A highly reproducible toxicity leading to a rapid death of all treated animals maintained on a ketogenic diet was observed for 2-DG at doses 1.0 and 2.0 g/kg administered PO or IP. The same doses appeared to be safe in mice fed with a regular diet. The combination treatment toxic effects include acute thrombocytopenia and acute interstitial pneumonia. Delineation of the exact mechanism of toxicity requires additional studies. This study provides an important insight on the apparent toxicity of 2-DG when combined with the ketogenic diet and should serve as a reference for any future studies aimed to combine the ketogenic diet with inhibitors of glycolysis or other metabolic inhibitors.

P130 P-cadherin activates metabolic coupling in the tumor-mesothelial niche: From glycolysis through lactate to lipogenesis. Kun Wang[1], Jing Ma[1], Carmen C. L. Wong[1], Chi Bun Chan[2], Alice S. T. Wong[1]. [1]The University of Hong Kong, Hong Kong, Hong Kong (Greater China), [2]The University of Hong Kong, Hong Kong, Hong Kong (Greater China).

Peritoneal metastasis affects >70% ovarian cancer patients, and its effective management is an unmet medical goal. Successful adhesion to the peritoneal mesothelium is a key rate-limiting step for metastasis formation, but it is unclear the selective advantage of why ovarian cancer cells colonize specifically to the unique mesothelial environment. Metabolic reprogramming is a hallmark of cancers. Still their role in peritoneal metastasis remains to be fully understood. To overcome the limitations of a lack of tightly controlled experimental model to pinpoint key molecules causing metastasis, we have established an isogenic model of spontaneous human ovarian cancer metastasis. Metabolism is characterized using RNA-seq, RT-PCR, real-time cell metabolic assay, targeted lipidomic analysis and neutral lipid staining. Using coculture, we showed that HM but not NM specifically activated peritoneal mesothelium in a P-cadherin-dependent manner compared to other cadherins, suggesting that there is a unique role for P-cadherin. RNA-seq revealed distinct regulation of lipogenic pathway in non-specific or P-cadherin siRNA treated HM. For these, we have confirmed that P-cadherin could promote the expression of lipogenic genes ACLY, FASN and ACAT2 in HM. Concomitantly, P-cadherin activated glycolysis (HK2, Gpi and PGK1) in the mesothelial cells. Targeted lipidomic analysis further showed that lactate acid that was enriched in the mesothelium was shuttled from the mesothelium to the HM to fuel de novo lipid synthesis in HM, revealing a feed-forward positive loop. Suppressing lactate uptake by using a monocarboxylate transporter 1 inhibitor could abolish the metabolic crosstalk and subsequent metastatic outgrowth. Together, these findings reveal the metabolic differences between metastatic versus non-metastatic tumor cells in understanding ovarian cancer metastasis to the mesothelium, and suggest targeting P-cadherin could be a promising therapeutic approach (RGC17105919).

P131 The role of UDP-6 glucose dehydrogenase (UGDH) in estrogen-mediated phenotypes in both estrogen receptor positive and estrogen receptor negative breast cancer. Meghan Price[1], Catherine Lavau[2], Cesar Baeta[2], Jovita Byemerwa[2], Suzanne Wardell[2], Olivia Brueckner[2], Debarati Mukherjee[2], Corinne Haynes[2], Annee Nguyen[2], Chingyi Chang[2], Donald McDonnell[2], C. Rory Goodwin[2]. [1]Duke University School of Medicine, Durham, NC, [2]Duke University, Durham, NC.

A better understanding of pathologic mechanisms underlying breast cancer progression and metastasis is critical to improve current treatment modalities. UDP-6- glucose dehydrogenase (UGDH) is an enzyme that has become a recent oncologic target of interest across various cancer subtypes for its role in increasing the aggressiveness and migratory capacity of tumor cells both in vitro and in vivo. UGDH is ubiquitously expressed and plays a critical role in forming the extracellular matrix (ECM), producing nucleotide sugars, and processing hormones through glucuronidation. For these reasons, investigating the effect of UGDH on breast cancer in the setting of hormonal stimulation is important. We found that knocking down UGDH in estrogen receptor positive (ER+) breast cancer cell lines (MCF7 marco and T47D) and an estrogen receptor negative (ER-) breast cancer cell line (MDA-MB-231) could abrogate estrogen induced migration-specific phenotypes in vitro. Furthermore, we found that knocking down UGDH mitigated estrogen stimulated primary tumor growth in vivo for both MCF7 macro and MDA-MB-231 mammary fat pad tumor models in mice. Thus, we found that UGDH regulates estrogen stimulated migratory phenotypes in both ER+ and ER- breast cancers in vitro and primary tumor growth in vivo. For this reason, UGDH and its associated pathways are promising targets for future drug development and small molecular targets in the treatment of both metastatic ER positive and ER negative breast cancers.

P132 Promotion of E-cadherin-mediated tumor cell adhesion by COX-2/ GSK3b signaling is a targetable mechanism of metastatic breast cancer. Balamurugan Kuppusamy[1], Saadiya Sehareen[1], Savitri Krishnamurthy[2], Shikha Sharana[3], Wei Tang[4], Naoto T. Ueno[2], Stefan Ambs[4], Dipak K. Poria[1], Esta Sternecka[1]. [1]Laboratory of Cell and Developmental Signaling, Center for Cancer Research (CCR), National Cancer Institute (NCI), Frederick, MD, [2]Morgan Welch Inflammatory Breast Cancer Research Program and Clinic, MD Anderson Cancer Center, Houston, TX, [3]Laboratory of Cell and Developmental Signaling, Center for Cancer Research (CCR), National Cancer Institute (NCI), Frederick, MD, [4]Laboratory of Human Carcinogenesis, CCR, NCI, Bethesda, MD.

Purpose: Metastatic progression and treatment resistance of breast cancer has been associated with epithelial-mesenchymal transition (EMT), including downregulation of E-cadherin (*CDH1*) gene expression, which can be initiated by inflammatory signaling such as by COX-2 (*PTGS2*). However, E-cadherin expression is maintained in many advanced breast cancers, including inflammatory BC (IBC), where it plays an essential role in forming tumor cell emboli within the cancer parenchyma and dermal lymph vasculature and which predict poor outcomes. Thus, the study of IBC offers an opportunity to understand the mechanisms of IBC and other aggressive BCs that lead to E-cadherin-associated cluster-based metastasis, which has recently received heightened recognition. Here, we have investigated the mechanisms that sustain E-cadherin expression in metastatic breast cancer to identify new targeted treatment options. **Study Design and Methods:** In vitro emboli formation assays and gene/protein expression studies with IBC (SUM149, IBC-3) and non-IBC (SUM159, MCF-7) cell lines along with gene silencing, overexpression, and pharmacological interventions. Analysis of experimental (SUM149) and spontaneous (orthotopic patient-derived xenograft, PDX) lung metastases and of circulating tumor cells (CTCs) in xenograft models treated with celecoxib (Cxb) and/or paclitaxel by imaging cytometry, immunohistochemistry, and/or Western analysis. **Results:** By analyzing the transcription factor C/EBPδ (*CEBPD*) and cells derived from inflammatory breast cancer (IBC), an aggressive breast cancer subtype that often presents with E-cadherin-dependent tumor cell emboli, we discovered that COX-2, unexpectedly, sustained E-cadherin protein expression without changing its mRNA levels. Using an *in vitro* tumor cell emboli culture paradigm (3D), we found that COX-2 or its metabolite PGE2 increased AKT activity and the inhibitory phosphorylation on GSK3β that prevents degradation of p120 catenin (*CTNND1*), a stabilizer of E-cadherin adhesion complexes. Conversely, the COX-2 inhibitor celecoxib downregulated E-cadherin specifically at the protein level and caused cell death in 3D. Co-expression of E-cadherin and COX-2 was seen in breast cancer patients with poor outcomes and, along with inhibitory GSK3β phosphorylation, in patient-derived xenografts (PDX) of metastatic triple-negative breast cancers (TNBC). Celecoxib alone decreased E-cadherin protein expression within xenograft primary tumors, reduced circulating tumor cells (CTCs) and clusters, and sensitized lung metastases to paclitaxel treatment. **Conclusions**: Our study uncovered a novel function of COX-2/PGE2 in promoting E-cadherin protein expression and cell-cell adhesions that are relevant for tumor cell cluster-based metastasis. Indeed, COX-2 inhibition reduced CTC clusters in a xenograft model, and sentized established metastases to chemotherapy. These results suggest that patients with COX-2+/E-cadherin+ metastastic BC, including IBC, may specifically benefit from targeting the PGE2 pathway in cobniation therapy approaches.

P133 NF-κB and NRF2 pathways dysregulation is associated with improved outcomes in HPV-associated head and neck cancer. Aditi Kothari, Travis Parke Schrank, Wendell Gray Yarbrough, Natalia Isaeva. University of North Carolina at Chapel Hill, Chapel Hill, NC.

This study aims to better understand the mechanisms of HPV carcinogenesis. Additionally, it helps to improve therapeutic personalization, identify new therapeutic targets in cancer, and aids in understanding the role of NF-κB in HPV-driven carcinogenesis, HPV biology, and tumor response to therapy. The incidence of HPV-associated (HPV+) head and neck cancer (HNSCC) is increasing rapidly, however, the mechanisms of HPV-driven carcinogenesis in head and neck cancers have not been thoroughly investigated. The relatively favorable prognosis of HPV+ HNSCC, along with severe side-effects seen in patients due to radiotherapy, it is pertinent to develop de-intensification strategies. However, a reliable prognostic biomarker is a limiting factor for clinical trials aiming to decrease the morbidity of therapy for HPV+ HNSCC through treatment de-escalation. Using molecular characteristics of HPV+ HNSCC and based on the presence or absence of NF-κB activating mutations in TCGA head and neck cancer cohort, we developed an NF-kB activity classifier and identified two intrinsic subtypes of HPV+ HNSCCs. The subtype harboring mutations in NF-κB regulators is associated with activated NF-κB, maintenance of episomal HPV, and improved patient survival. Identification of a subtype of HPV+ HNSCC with active NF-κB, improved survival, and absence of HPV genome integration, suggested that these tumors were driven by a distinct or alternative mechanism of oncogenesis that is dependent on episomal HPV and NF-κB activity, while classical HPV-driven carcinogenesis relies on increased expression of HPV E6 and E7 oncogenes occurring upon HPV genome integration and loss of HPV E2.However, it is not yet known why this subset of tumors with overactive NF-κB displays better survival. An interesting finding from our study was that these tumors with increased NF-κB activity had reduced NRF2 signaling.NRF2 has been associated with resistance to treatment and significant downregulation of NRF2 signaling in the subtype of HPV+ HNSCC with active NF-κB may contribute to increased treatment sensitivity and improved patient survival. Preliminary experiments confirmed that baseline and inducible (with small molecule NRF2 activator or radiation treatment) NRF2 transcriptional activity is downregulated in HPV-positive head and neck cancer cells with deleted CYLD or TRAF3 and activated NF-κB, and that this effect is most likely independent of NRF2 degradation mediated by Cul3. Moreover, expression of major NRF2 targets was decreased in HPV+ HNSCC cells harboring constitutively active NF-κB. Finding an inverse correlation between NF-κB and NRF2 activities suggests that inhibition of NRF2 in NF-κB active HPV+ HNSCCs drives their sensitivity to radiation and that NF-κB regulates NRF2 transcriptional activity in HPV+ HNSCC. This project uncovers the role of NF-κB in HPV-driven head and neck cancer etiology. Our novel NF-κB activity RNA classifier or mutations in NF-κB pathway distinguishing the 2 subtypes of HPV+ HNSCC may serve as prognostic biomarkers to help clinicians with therapeutic decisions.

P134 Novel EGFRvIII-selective antibody-drug conjugate REGN3124-PBD is strongly efficacious against orthotopic glioblastoma multiforme patient derived xenografts. Marcus P. Kelly, Sosina Makonnen, Carlos Hickey, Shu Mao, Feng Zhao, Arthur Kunz, Frank Delfino, Thomas Nittoli, Dangshe Ma, William C. Olson, Gavin Thurston, Jessica R. Kirshner.
Regeneron Pharmaceuticals, Tarrytown, NY.

Glioblastoma Multiforme (GBM) is a highly aggressive cancer with few specific molecular targets and poor therapeutic outcome. Epidermal Growth Factor Receptor variant III (EGFRvIII) is a promising target for the treatment of GBM due to its exclusive expression in GBM. However, prior therapeutics have failed in part due to the development of EGFRvIII negative tumors cells from heterogenous tumor populations. We therefore developed a potent antibody-drug conjugate with bystander killing capabilities to target heterogeneous EGFRvIII-expressing GBM tumors. REGN3124 is a fully human EGFRvIII-selective antibody that also demonstrates some binding to amplified wild-type EGFR. REGN3124 was conjugated to the pyrrolobenzodiazepine (PBD) linker-payload SG-3249 to form REGN3124-PBD, and the cytotoxicity and cellular bystander killing activity was characterized in vitro. Initial in vivo activity of REGN3124-PBD was assessed in subcutaneous (s.c.) U251/EGFRvIII and U87/EGFRvIII cell line xenografts and then in s.c. EGFRvIII-positive patient derived xenograft (PDX) models (GBM6 and GBM59). Immunohistochemistry demonstrated heterogenous expression of EGFRvIII in GBM59 tumors. Lastly, efficacy was assessed in animals with established intracranial GBM6 or GBM59 tumors to examine the activity of REGN3124-PBD in an orthotopic setting. REGN3124-PBD demonstrated sub-nM cytotoxicity in vitro and clear bystander killing of EGFRvIII negative U251 cells following targeting of U251/EGFRvIII cells. A single dose of 0.38 mg/kg REGN3124-PBD (3.4 drug: antibody ratio) induced sustained regression of both s.c. U251/EGFRvIII and U87/EGFRvIII xenografts. A single dose of 0.53 mg/kg REGN3124-PBD induced complete regression of s.c. GBM6 PDX tumors and sustained regression of GBM59 tumors. Single dose of 0.53 mg/kg REGN3124-PBD significantly prolonged survival of mice with established intracranial GBM6 or GBM59 tumors, with 5/8 and 7/8 animals surviving >90 days post-treatment, respectively. The high unmet need for effective therapies combined with the potent anti-tumor activity observed, including in those with heterogenous expression of EGFRvIII, support continued assessment of REGN3124-PBD as a novel therapy for treatment of GBM.

P135 Systemic targeting of a CNS tumor (medulloblastoma) using a novel cell-penetrating, nucleic acid binding, monoclonal antibody. Elias Quijano[1], Minsoo Khang[1], Bruce C. Turner[2], Stephen Squinto[2], Ranjit Bindra[1], W. Mark Saltzman[1], Peter Glazer[1]. [1]Yale University, New Haven, CT, [2]Gennao, New Haven, CT.

Targeting tumors of the central nervous system remains challenging given the physiological barriers preventing the delivery of therapeutics across the blood brain barrier (BBB). This is particularly true for nucleic acids, which degrade rapidly in serum and often fail to penetrate cells even with proper formulation into AAVs or LNPs. To overcome these barriers, many current efforts rely on invasive methods of delivery, including intrathecal or intraparenchymal injection. Here we report studies evaluating the ability of a novel cell-penetrating antibody, GMAB, to penetrate the BBB and target a CNS model of medulloblastoma (DAOY) following intravenous administration. Studies with fluorescently labeled GMAB demonstrated that the antibody readily penetrated into the CNS following systemic administration, achieving tumor-specific delivery while sparing surrounding normal brain tissue. Subsequent studies with 3p-hpRNA, a known activator of RIG-I, a cytosolic pattern recognition receptor which detects viral RNA and elicits a type-I interferon response, demonstrated that GMAB could deliver RNA ligands to tumors, resulting in significantly reduced intracranial tumor burden 10 days after a single-dose administered systemically while also preventing the development of spinal metastases. These results highlight a novel approach for the systemic delivery of immunostimulatory RNAs in a targeted manner to multiple tumors including often difficult to treat CNS tumors, which may offer treatment advantages over current approaches.

P136 Response to alpelisib in an adolescent with *PIK3CA*-mutated metastatic gastrointestinal stromal tumour. Sarah Cohen-Gogo, Nisha Kanwar, Furqan Shaikh, Reto M. Baertschiger, Adam Shlien, David Malkin, Juan Putra, Ailish Coblentz, Anita Villani, Abha A. Gupta, Daniel A. Morgenstern. The Hospital for Sick Children, Toronto, Canada.

Introduction Gastrointestinal stromal tumours (GISTs) are mesenchymal neoplasms that arise in the gastrointestinal tract. While most GISTs are driven by mutations in *cKIT* or *PDGFR*, approximately 10% lack these variants and are referred to as 'wild-type'. Wild-type GISTs are commonly succinate dehydrogenase (SDH)-deficient and are characterized by indolent, multi-focal disease at presentation. On occasion, patients experience symptomatic disease progression warranting treatment ; imatinib or sunitinib offer minimal benefit. *PIK3CA* mutations have recently been described in a small proportion of GIST. **Case report** A previously healthy 16-year-old female presented with a five-month history of vomiting and weight loss, compatible with gastric outlet obstruction. Computerized tomography demonstrated a large gastric mass, and multiple liver and peritoneal metastases. Biopsy showed nests of epithelioid cells with round nuclei, vesicular chromatin, and eosinophilic cytoplasm. The lesional cells were immunoreactive for CD117, DOG1, CD34, and smooth muscle actin, while negative for SDH-B immunohistochemistry, in keeping with SDH-deficient GIST. Germline and somatic panel sequencing were performed through the SickKids Cancer Sequencing Program along with RNA sequencing by Illumina TruSight RNA Pan-Cancer NGS analysis. Somatic analysis revealed a *PIK3CA* p.Glu545Lys (E545K) mutation at 0.12 variant allele fraction (VAF). The patient was also found to have germline heterozygous pathogenic splice variant in the *SDHB* gene c.75+1 G>T (p?). In the light of the molecular findings and no alternative curative options, compassionate access to alpelisib was sought. The patient started drug at the recommended adult dose of 300 mg by mouth daily, and after completing two 28-day cycles, showed marked improvement in her abdominal distension and discomfort. Her appetite improved with documented nutritional weight gain. Reimaging at this time showed a 20% reduction in the sum of maximum dimensions of target lesions (per RECIST 1.1). Repeat imaging after five months on treatment confirmed continued improvement with 25% reduction compared to baseline.The patient did not experience hypersensitivity, severe cutaneous adverse reactions, hyperglycemia or pneumonitis. She currently continues on alpelisib monotherapy. **Conclusion** We describe the first report of a clinically meaningful response to a PIK3CA inhibitor in an adolescent patient with advanced metastatic SDH-deficient GIST harbouring a somatic activating *PIK3CA* hotspot mutation. There is still much to be learned about the biology of pediatric/wild-type GIST. In these rare cases, we recommend referral to specialized centers and incorporation of comprehensive next generation sequencing into care. Sequencing should be performed both on germline and somatic DNA to evaluate for a cancer predisposition syndrome, along with possible druggable targets in *PIK3CA*, *cKIT* or *PDGFRA*. We believe that the lack of routine comprehensive genomic analysis may underestimate the frequency of such rare variants.

P137 SGLT2 inhibition improves BYL719-induced hyperglycemia and hyperinsulinemia in rat pre-clinical models. Christian R. Schnell[1], Daniel Wyss[1], Walter Tinetto[1], Thomas Ferrat[1], Jiaping Gao[2], Panza Darrell[2], Josh Gold[3], Valerie Beaulieu[2], John Diener[2], Christine Fritsch[1]. [1]NOVARTIS Pharma AG, Basel, Switzerland, [2]Novartis Institutes for Biomedical Research, Cambridge, MA, [3]Novartis Institutes for Biomedical Research, Cambidge, MA.

Background In phase III SOLAR-1 trial (NCT02437318), the PI3K alpha selective inhibitor alpelisib (ALP) + fulvestrant significantly improved progression-free survival vs. fulvestrant alone in patients with HR+/HER2- advanced breast cancer with *PIK3CA* mutations. Hyperglycemia is an on-target adverse effect of ALP that led to 6% of patient discontinuation in the ALP arm. Recently, Sodium-glucose co-transporter 2 (SGLT2) inhibition was reported to reduce PI3K inhibition-induced glucose and insulin increase. For 6 SOLAR-1 patients, the addition of an SGLT2 inhibitor to metformin (MET) and ALP stabilized blood glucose levels, allowing them to continue ALP treatment. **Methods** Brown Norway (BN) rat and Rat1-myr-p110α tumor bearing nude rat *in vivo* models were used to further investigate the degree of glucose and insulin control achievable upon treatment with ALP and a SGLT2 inhibitor dapagliflozin (DAPA) +/- MET and effects on ALP tolerability and efficacy. **Results** In both rat models tested, the addition of DAPA to ALP nearly suppressed ALP-induced hyperglycemia, was associated with insulin level reduction and insulin sensitivity improvement and no signs for ketoacidosis upon single agent (S.A) nor combination were observed under fed conditions. ALP S.A efficacy in the Rat1-myr-p110α tumor bearing nude rats was maintained when used in combination with DAPA and there was no influence of DAPA on ALP-induced body weight loss (BWL). In BN rats, when combining MET with ALP, a delay in blood glucose reduction was observed vs. DAPA + ALP combination. The triple combination of MET + DAPA + ALP improved further blood glucose levels reduction with the same kinetic as DAPA + ALP. MET + DAPA + ALP triple combination was more effective in reducing plasma insulin levels when compared to MET + ALP or DAPA + ALP double combinations. All combinations tested with MET slightly increased BYL719-induced BWL in BN rats. **Conclusions** SGLT2 inhibitors such as DAPA significantly reduced hyperglycemia and improved hyperinsulinemia induced by ALP in rat models without further BWL induced by ALP. These results warrant further clinical investigation of adding SGLT2 inhibitors +/- metformin to treat ALP-induced hyperglycemia.

P138 *nab*-Sirolimus improves mTOR pathway suppression and antitumor activity versus oral mTOR inhibitors in *PTEN* null bladder cancer (UMUC3) and *TSC2* null liver cancer (SNU398) xenografts. Shihe Hou[1], Heng Du[2], Anita N. Schmid[1], David J. Kwiatkowski[2], Neil P. Desai[1]. [1]Aadi Bioscience, Pacific Palisades, CA, [2]Brigham and Women's Hospital, Boston, MA.

Background: *TSC1, TSC2,* and *PTEN* genes are tumor suppressors in the mTOR pathway and can be inactivated or deleted across many cancers (Kwiatkowski, Clin Cancer Res 2016). The mTORC1 pathway is frequently activated in cancer and causes phosphorylation of downstream targets S6K (activation) and 4EBP1 (inactivation). Nanomolar concentrations of mTOR inhibitors (mTORi) sirolimus and everolimus can effectively inhibit S6K but not 4EBP1 and may lead to therapeutic resistance (Kang, Science 2013). In a registrational phase 2 trial (AMPECT) with malignant PEComa, *nab*-sirolimus (ABI-009) had a response rate of 64% (9/14) in patients with *TSC1* or *TSC2* mutations. In an expanded access program (NCT03817515), of 8 patients with *TSC1* or *TSC2* mutations (2 had prior mTORi) treated with *nab*-sirolimus, 5 had partial responses (all mTORi naïve). This study investigated mTOR pathway inhibition, tumor drug levels, and antitumor activity of *nab*-sirolimus vs equal doses of oral mTORi in *PTEN*-null and *TSC2*-null xenograft models. **Methods:** Athymic mice bearing subcutaneous *PTEN*-null UMUC3 bladder cancer xenografts were treated with either saline or equal weekly doses (15 mg/kg) of *nab*-sirolimus (IV, 7.5 mg/kg, 2x/wk), and sirolimus or everolimus (PO, 3 mg/kg/day, 5 days/wk). Tumors were harvested and analyzed for tumor drug levels (LC-MS/MS) and pS6 inhibition by immunohistochemistry (IHC). The same treatment conditions were repeated in a subsequent experiment with *TSC2*-null SNU-398 hepatocellular carcinoma xenografts, which further analyzed pS6K, pS6, and p4EBP1 via western blot (WB).**Results:** In UMUC3 xenografts, compared with oral mTORi, IV *nab*-sirolimus resulted in significantly higher drug exposure (AUC 7d) in the tumor ($P<0.0001$) and greater pS6 inhibition as measured by IHC ($P=0.0001$ vs sirolimus, $P=0.0034$ vs everolimus). Correspondingly, *nab*-sirolimus resulted in significantly greater tumor growth inhibition (TGI) than sirolimus (69.6% vs 24.3%, $P<0.0001$) and everolimus (36.2%, $P=0.0023$), and prolonged animal survival vs both oral mTORi ($P<0.05$ log-rank). Based on WB in SNU-398 xenografts, IV *nab*-sirolimus consistently inhibited mTOR targets pS6K, pS6, and p4EBP1, whereas oral sirolimus only partially decreased pS6K and pS6 and did not appear to reduce p4EBP1 levels. Correspondingly, *nab*-sirolimus resulted in significantly greater TGI than sirolimus (67.8% vs 36.2%, $P<0.05$) and prolonged animal survival ($P<0.05$ log-rank).**Conclusions:** The relatively low tumor concentrations achieved with oral mTORi may limit their effectiveness as anticancer therapies. IV *nab*-sirolimus at equal dose showed significantly higher tumor accumulation and inhibition of pS6 in a *PTEN*-null bladder cancer xenograft and increased inhibition of mTOR targets pS6K, pS6, and p4EBP1 in a *TSC2*-null hepatocellular carcinoma xenograft. This was accompanied with significantly greater antitumor activity, suggesting that *nab*-sirolimus may have a more optimal pharmacologic profile than the oral mTORi. Clinical studies in cancers harboring these alterations are planned.

P139 An estrogen receptor beta agonist liquiritigenin potentiates inhibition of hormone-dependent breast-cancer growth by cholesterol biosynthesis inhibitor RO 48-8071. Yayun Liang, Salman Hyder. University of Missouri, Columbia, MO.

Hormone-dependent breast cancer represents approximately 70% of all diagnosed breast cancers. Estrogens and progestins stimulate breast cancer cell proliferation, angiogenesis and metastasis. Estrogen receptor (ER)-alpha positive breast cancers are treated with anti-estrogens and aromatase inhibitors, but resistance to these agents invariably develops during the course of therapy. Our goal was to identify novel treatment strategies to suppress breast cancer growth and avoid drug resistance. Cholesterol biosynthesis plays a vital role in tumor cell growth. Cholesterol is an integral part of membrane structure and is also a precursor of steroid hormones that are synthesized by tumor cells and cause cellular proliferation by interacting with specific receptors. Cancer cells have elevated levels of cholesterol, which could be responsible for anti-hormone resistance. During studies to determine the effects of the cholesterol biosynthesis inhibitor RO 48-8071 ([4'-[6-(Allylmethylamino)hexyloxy]-4-bromo-2'-fluorobenzophenone fumarate] (RO), which inhibits oxidosqualene cyclase (OSC), an enzyme that acts downstream of HMG-CoA reductase to convert 2, 3-monoepoxysqualene to lanosterol (a key step in the biosynthesis of cholesterol), it was serendipitously discovered that RO degraded ER-alpha (ERa), and elevated levels of ER-beta (ERb), a known tumor suppressor. We tested RO alone and in combination with liquiritigenin (LQ), a selective agonist for ERb, to ascertain their inhibitory effects against tumor cell progression. RO or LQ alone reduced significantly the *in vitro* viability of MCF-7 and BT-474 breast-cancer cells. BT-474 cells were more sensitive than MCF-7 cells to LQ (IC_{50} 0.15 uM vs 0.25 mM, respectively). RO (5-10 uM) + LQ (0.1-0.3 mM) treatment reduced cell viability in an additive manner in MCF-7 cells and in a synergistic manner in BT-474 cells. Combination treatment was significantly more effective than treatment with a single agent. Administration of RO (10 mg/kg/iv) or LQ (20 mg/kg/ip) significantly inhibited growth of tamoxifen-resistant BT-474 tumor xenografts *in vivo*; combination treatment was even more effective. Both agents appeared to be non-toxic *in vivo*. Mechanistically, RO, LQ, or RO + LQ reduced ERa but induced ERb expression in tumor xenografts. Both compounds significantly reduced expression of markers of angiogenesis and increased apoptosis in tumor xenografts; use of RO + LQ significantly enhanced the effects observed with a single agent. The anti-tumor properties of RO may in part be due to an off-target effect that reduces ERa and increases ERb, the latter then interacting with LQ to more effectively promote its anti-proliferative effects. The potent anti-tumor properties of RO and LQ, particularly when used together, suggest that this combination has substantial potential as a means by which to clinically manage breast-cancer growth, including in disease types that are tamoxifen resistant.

P140 Chromomycin A5, a marine-derived antibiotic, targets the oncogenic TBX2 in breast cancer. Claire Bellis[1], Suparna Chakraborty[1], Mihlali Mlaza[1], Bianca Del Bianco Sahm[2], Paula Rezende Teixeira[3], Leticia Costa-Lotufo[3], Sharon Prince[1]. [1]University of Cape Town, Cape Town, South Africa, [2]University of São Paulo, Sao Paulo, Brazil, [3]University of Sao Paulo, Sao Paulo, Brazil.

Breast cancer (BC) continues to pose a major health burden. In 2020 alone, over 2 million people were diagnosed with BC globally and of these 684 996 people succumbed to the disease. Cancer cell drug resistance and the notion of transcription factors as undruggable targets has slowed progress for novel BC therapies. The transcription factor, TBX2, is commonly over-expressed in breast cancer with various hormone receptor statuses where it functions as a powerful pro-proliferative factor by directly repressing *p21, PTEN, CST-6,* and *NDGR1* expression. Furthermore, TBX2 mediates Cisplatin drug resistance by inducing an S-phase cell cycle arrest allowing for cisplatin-induced DNA damage repair and breast cancer cell survival. Targeting TBX2 with DNA-damaging chemotherapies therefore emerges as a promising anti-cancer strategy to treat BC. To this end, we performed a reverse affinity screen to fish for marine-derived compounds with DNA-damaging- and TBX2 binding- activity and identified the antibiotic Chromomycin A5 (CMA5) as a promising agent. This project confirms the binding affinity of CMA5 for TBX2 by thermophoresis and fully characterizes the *in vitro* anticancer activity of CMA5 in TBX2-driven BC cells. Short-term and long-term selective cytotoxicity of CMA5 were investigated in two TBX2-driven BC cell lines (MCF-7 and T47D) and a non-malignant breast epithelial cell line (MCF-12A) using MTT and clonogenic assays. Our results show that CMA5 exhibited potent (IC_{50} ≤6.5 nM) and selective (SI ~ 3.3) cytotoxicity against BC cells. Importantly, CMA5 inhibited TBX2 protein levels and co-treatment of the BC cells with CMA5 and the proteasome 26S inhibitor MG132 rescued TBX2 levels. These results show that CMA5 targets TBX2 for protein degradation. Furthermore, whereas knocking down TBX2 using shRNA blocked the cytotoxicity of CMA5 (IC_{50} increased by 5-fold), ectopically over-expressing TBX2 increased the cytotoxic activity of CMA5 (IC_{50} decreased by 1.2-fold). These results suggest that CMA5 cytotoxicity is dependent, in part, on the presence of TBX2. To further explore the significance of TBX2 inhibition by CMA5 we investigated the impact of CMA5 on expression levels of the TBX2 target genes *p21, PTEN, CST-6* and *NDGR1* by quantitative real-time PCR analyses. The results revealed that all four genes were upregulated in the presence of CMA5 treatment which suggested that CMA5 inhibits BC cell viability and survival by targeting TBX2 and thus releasing its ability to repress these tumor suppressors. To elucidate the mechanism(s) underpinning the cytotoxic activity of CMA5 we investigated its effects on several markers of DNA damage (yH2AX expression) and apoptosis (cleaved caspase 3, 7, 8, 9 and PARP) by western blotting and immunocytochemistry. Our results show that CMA5 induced DNA damage and killed cells, in part, by both the intrinsic and extrinsic apoptotic programmed cell death pathways. Overall, this study identifies CMA5 as a promising anti-breast cancer agent because of its capability to induce DNA damage and to inhibit the oncogenic TBX2.

P141 Preclinical characterization of LOX-24350, a highly potent and isoform-selective FGFR3 inhibitor. Joshua A. Ballard[1], Timothy Kercher[1], David Abraham[1], Ryan Brecht[1], Nathan A. Brooks[1], Thomas Buckles[1], Desta Bume[1], David Busha[1], Ernst Peder Cedervall[1], Kevin Condroski[1], Kevin Ebata[1], Severine Isabelle Gharbi[2], Robert Hazlitt[1], Tony Morales[1], Nisha Patel[1], Jessica Podoll[1], Kaveri Urkalan[1], Sandra Gomez Villalain[2], Shane Walls[1], Faith Watson[1], Peiyi Yang[1], Barbara J. Brandhuber[1], Steven W. Andrews[1]. [1]Loxo Oncology at Lilly, Boulder, CO, [2]Eli Lilly and Company, Alcobendas, Spain.

Alterations in the fibroblast growth factor receptors (FGFRs) have been identified as oncogenic drivers in many human cancers. Specifically, activating FGFR3 gene alterations are found in ~15% of metastatic bladder cancers. One pan-FGFR inhibitor has been approved for patients with FGFR3-altered bladder cancer and others are in clinical development. Importantly, all of these agents inhibit FGFR1-3 with approximate equal potency. Consequently, these agents are associated with toxicities driven by off-target inhibition of FGFR1 and FGFR2, potentially limiting efficacy. Additionally, existing drugs lose potency in the setting of FGFR3 gatekeeper mutations and acquired resistance due to gatekeeper mutations has been described. LOX-24350 is a highly potent and isoform-selective FGFR3 inhibitor with activity against wild-type FGFR3, FGFR3 activating mutations such as S249C, and FGFR3 gatekeeper (V555M) mutations. Here, we describe the preclinical profile of LOX-24350.

Compound potency and selectivity were measured using enzyme fluorescent activity assays, and cell-based assays using in-cell western and cell-titer Glo methods. Tumor growth inhibition and PK/PD studies were performed in mice.

LOX-24350 showed greater than 56-fold selectivity for FGFR3 S249C over wild-type FGFR1 in mechanistic cellular inhibition assays, while maintaining potency for the V555M gatekeeper mutation. In HEK293 cells stably expressing FGFR3 S249C and FGFR3 S249C/V555M, LOX-24350 inhibited FGFR3 phosphorylation with IC50 values of 3.1 and 5.0 nM, respectively, as compared to FGFR1 and FGFR2 IC_{50} values of 174.5 and 90.7 nM, respectively. Similarly, in NIH3T3 cells engineered to express FGFR3 S249C or FGFR3 S249C/V555M, LOX-24350 inhibited cell growth with IC_{50} values of 12.2 and 22.9 nM, respectively. LOX-24350's isoform-selectivity was best exemplified in cancer cell line models, with IC_{50} values of 15.1 and 12.6 nM in RT112 (FGFR3-TACC3) and UMUC14 (FGFR3 S249C) cell lines, respectively, as compared to 4712.6 nM in DMS114 (FGFR1 amp). LOX-24350 demonstrated high oral bioavailability in preclinical species as well as favorable *in vitro* ADME properties. *In vivo*, LOX-24350 demonstrated tumor regressions in FGFR3-driven tumor models on par with pan-FGFR inhibitors, without body weight loss or hyperphosphatemia seen with pan-FGFR inhibitors. This wider therapeutic index is predicted to allow for greater efficacy in patients.

These data demonstrate that LOX-24350 potently and selectively inhibits FGFR3, the S249C activating mutation, and its gatekeeper mutation, V555M, while sparing FGFR1, FGFR2, and other problematic off-targets. We hypothesize that this profile will lead to differentiated efficacy and tolerability for patients with FGFR3-driven cancers. An IND submission is planned for 2022.

P142 Preclinical characterization of LOX-22783, a highly potent, mutant-selective and brain-penetrant allosteric PI3Kα H1047R inhibitor. Anke Klippel[1], Rui Wang[1], Loredana Puca[1], Andrew Lee Faber[2], Weihua Shen[2], Shripad V. Bhagwat[2], Kannan Karukurichi[1], Feiyu Fred Zhang[3], Carmen Perez[4], Ramon Rama[4], Ana Ramos[4], Yi Zheng[2], Zahid Bonday[2], James Thomas[2], Harold B. Brooks[2], Lisa J. Kindler[2], Sarah M. Bogner[2], Parisa Zolfaghari[1], Mark Hicks II[1], Sophie Callies[5], Brian Mattioni[5], Laurie LeBrun[6], Jim Durbin[5], Erin Anderson[6], Chris Mayne[6], Edward Kesicki[1], Gabrielle Kolakowski[6], Steven W. Andrews[6], Barbara J. Brandhuber[6]. [1]Loxo Oncology at Lilly, New York, NY, [2]Loxo Oncology at Lilly, Indianapolis, IN, [3]Eli Lilly and Company, San Diego, CA, [4]Eli Lilly and Company, Alcobendas, Spain, [5]Eli Lilly and Company, Indianapolis, IN, [6]Loxo Oncology at Lilly, Boulder, CO.

Phosphoinositide 3-kinase alpha (PI3Kα) H1047R mutations are activating oncogenic events that occur in ~15% of advanced breast cancers. While there is one PI3Kα inhibitor FDA-approved for patients with PI3Kα-mutated breast cancer, and many others in clinical development, all of these agents inhibit wild-type PI3Kα and its mutated form with approximate equal potency. As a result, their efficacy is limited by toxicities associated with on target wild-type PI3Kα inhibition, notably hyperglycemia as well as cutaneous and GI toxicity. LOX-22783 is a highly potent, mutant-selective and brain-penetrant allosteric PI3Kα H1047R inhibitor. Here, we describe the preclinical profile of LOX-22783. H1047R selectivity was measured using biochemical kinase activity and cell-titer Glo and signal transduction assays. Tumor growth inhibition, pharmacokinetic and pharmacodynamic effects were assessed in *in vivo* studies using xenograft and patient-derived xenograft (PDX)-models. LOX-22783 inhibited growth and signaling responses in multiple H1047R-driven breast cancer cell lines and demonstrated high selectivity for H1047R mutated PI3Kα (EC_{50} values <5 nM) relative to wild-type PI3Kα (EC_{50} >250 nM) as well as the other wild-type PI3K isoforms (beta, gamma, and delta, all EC_{50} >250nM). In enzyme and cell-based assays, LOX-22783 dissociated from PI3Kα H1047R at a slower rate (3-6 hrs) compared to alpelisib (≤10 mins), potentially allowing for extended inhibition of PI3Kα H1047R by LOX-22783. LOX-22783 also normalized the EGF-stimulated membrane-localization of PI3Kα H1047R while alpelisib did not. LOX-22783 was highly kinome-selective when assayed at 3 µM, with no inhibitory activity on 17 lipid kinases or 374 protein kinases. In preclinical species, LOX-22783 demonstrated high oral bioavailability, including exposure in the CNS, a common site of metastases for patients with breast cancer. *In vivo*, LOX-22783 demonstrated dose-dependent tumor regression in H1047R breast cancer models without inducing hyperglycemia or other toxicities. Tumor pharmacodynamic analyses confirmed successful pathway inhibition. At doses resulting in 90% pathway inhibition, tumor regressions of ≥60% were observed. This wide therapeutic index is predicted to allow for maximizing dose intensity and efficacy in patients, without wild-type PI3Kα inhibition limiting target coverage for the H1047R mutant form. These data demonstrate that LOX-22783 potently and selectively inhibits mutant H1047R, but not wild-type PI3Kα, or other PI3K isoforms. LOX-22783 binds to an allosteric pocket distinct from the ATP binding site used by the approved and investigational PI3Kα inhibitors. We hypothesize that this profile will lead to differentiated efficacy and tolerability for patients with PI3Kα H1047R-mutated cancers, with the additional potential to address brain metastases. An IND submission is planned for 2022.

P143 Development of siRNA-loaded lipid nanoparticles targeting long non-coding RNA LINC01257 as a novel and safe therapeutic approach for paediatric acute myeloid leukaemia. Patrick Connerty[1], Ernest Moles[1], Charles deBock[1], Nisitha Jayatilleke[1], Jenny L. Smith[2], Soheil Meshinchi[2], Chelsea Mayoh[1], Maria Kavallaris[1], Richard B. Lock[1]. [1]Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Sydney, Sydney, NSW, Australia, [2]Fred Hutchinson Cancer Research Center, Seattle, WA.

Introduction: Standard of care therapies for children with acute myeloid leukemia (AML) cause potent off-target toxicity to healthy cells, highlighting the need to develop new therapeutic approaches that are safe and specific for leukemia cells. Long non-coding RNAs (lncRNAs) are an emerging and highly attractive therapeutic target in the treatment of cancer due to their oncogenic functions and selective expression in cancer cells. However, lncRNAs have historically been considered 'undruggable' targets because they do not encode for a protein product. Therefore, the discovery of novel lncRNA targets and the development of efficient methods to silence them is critical for developing new therapies for AML. In this study, we describe the development of an siRNA-loaded lipid nanoparticle (LNP) for the therapeutic silencing of the novel oncogenic lncRNA LINC01257. **Materials and Methods:** Transcriptomic analysis of children with AML from both the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative and AAML1031 study was performed to identify AML specific lncRNAs. The subsequently identified lncRNA LINC01257 was knocked down in AML Kasumi-1 cells via electroporation of siRNA targeting LINC01257 (si-LINC01257). Changes in proliferation and cell viability were assessed via cell count and Annexin V/7AAD staining 48 hours post-transfection. LNPs incorporating non-targeting si-SCR or si-LINC01257 were synthesized through NxGen non-turbulent microfluidics. LNPs were formulated reproducing the FDA-approved Onpattro® siRNA delivery vector, D-Lin-MC3-DMA:DSPC:cholesterol:PEG-DMG. LNP-si-LINC01257 uptake was measured by confocal microscopy and flow cytometry. Kasumi-1 cells and healthy peripheral blood mononuclear cells (PBMCs) were treated with 250nM LNP-si-SCR or LNP-si-LINC01257 for 48 hours. Changes in proliferation and cell viability were assessed with cell counts and Annexin V/7AAD staining 48 hours post-treatment. **Results:** LINC01257 was significantly overexpressed in patients carrying the t(8;21) translocation compared to healthy controls ($P = <0.001$). Compared to si-SCR controls, siRNA knockdown of LINC01257 in Kasumi-1 cells resulted in a 62% reduction in the total cell count after 48 hours ($P = <0.001$) , which was accompanied by a 70% decrease in overall cell viability ($P = <0.004$). LNP-siRNA production yielded a mean LNP size of ~65 nm along with a >85% siRNA encapsulation rate. LNP-siRNAs were efficiently taken up by Kasumi-1 cells (>95% of cells) and LNP-si-LINC01257 treatment successfully ablated LINC01257 expression, which was accompanied by a 55% reduction in total cell count following 48 hours of treatment ($P= 0.02$). In contrast, healthy PBMCs, which do not express LINC01257, were unaffected by LNP-si-LINC01257 treatment despite comparable levels of LNP-siRNA uptake. **Conclusion:** Our data demonstrate the effective use of LNP-based RNA interference modalities for the silencing of cancer-driving lncRNAs and highlight their potential as a viable therapeutic and non-toxic approach in the management of AML.

P145 The RNA helicase EIF4A is a therapeutic vulnerability in triple-negative breast cancer. Na Zhao, Jeffrey Mark Rosen. Baylor College of Medicine, Houston, TX.

Background: Translational control of oncoprotein expression has been implicated in the pathogenesis of multiple solid tumors. The eukaryotic translation initiation factor 4A (eIF4A) is an RNA helicase that catalyzes the unwinding of secondary structure in the 5′-untranslated region (5′-UTR) of mRNAs to facilitate translation initiation. eIF4A is an essential gene in many cancer cell lines and multiple oncogenes or anti-apoptotic proteins that contain structured 5'-UTRs have been shown to require enhanced eIF4A activity for translation. Zotatifin (eFT226) is a selective eIF4A inhibitor that has been shown previously to inhibit tumor growth in several immunocompromised mouse models and is currently in Phase I clinical trials as a single agent (NCT04092673). In this study we investigated the effects of Zotatifin both as a single agent and in combination with standard-of-care chemotherapy on tumor progression in several syngeneic triple-negative breast cancer (TNBC) mouse models. **Methods:** The activity of Zotatifin was assessed as single agent in eight different syngeneic *p53*-null mouse TNBC models across several intrinsic molecular subtypes. Synergism of Zotatifin with chemotherapy was evaluated in three responsive models. In addition, a Zotatifin-resistant model was developed following prolonged drug treatment. Drug response mechanisms and the effect on the tumor immune microenvironment were determined by tandem mass tag mass spectrometry, reverse phase protein array (RPPA) and mass cytometry. **Results:** Zotatifin treatment slowed tumor growth in six out of eight syngeneic TNBC models without obvious toxicity. Mass spectrometry of acutely treated tumors revealed that Zotatifin inhibited proliferative and stem cell signaling pathways including E2F targets, G2/M checkpoints, and NOTCH signaling, and induced proteins involved in Interferon-α and Interferon-γ responses. Mass cytometry analyses showed that Zotatifin suppressed the infiltration of M2 macrophages and neutrophils and promoted the infiltration of conventional dendritic cells to the tumor microenvironment. Interestingly, combination therapy of Zotatifin with chemotherapy significantly slowed tumor growth and improved survival. However, drug resistance emerged after prolonged treatment. The resistant tumors strikingly displayed a mesenchymal histology and an increased activity of the PI3K-AKT-mTOR pathway as determined by RPPA analysis. Combination treatment of Zotatifin with the mTOR inhibitor Everolimus prolonged survival. **Conclusions:** eIF4A is a therapeutic vulnerability in TNBC. Zotatifin inhibits tumor progression both through tumor-intrinsic mechanisms and by targeting the tumor immune microenvironment. Synergism between Zotatifin and chemotherapy supports the clinical potential of combination therapies.

P146 Loss of HS2ST1 cooperates with MAPK inhibition to impair growth of mesenchymal KRAS mutant NSCLC. Leanne G. Ahronian, Silvia Fenoglio, Nikitha Das, Daniel Aird, David Guerin, Douglas Whittington, Haris Jahic, Erin Brophy, Patrick McCarren, Brian McMillan, James Tepper, Michaela Mentzer, Fang Li, Hongxiang Zhang, Xuewen Pan, John Maxwell, Jannik Andersen, Alan Huang, Robert Tjin Tham Sjin. Tango Therapeutics, Cambridge, MA.

Activating mutations in the KRAS oncogene occur in approximately 30% of non-small cell lung cancers (NSCLC). Hence, multiple therapeutic strategies have been explored to block RAS including inhibition of downstream effector molecules in the MAPK pathway and direct pharmacological inhibition of the KRAS G12C mutant protein. However, inhibition of the RAS-RAF-MEK-ERK cascade with MEK inhibitor monotherapy has been insufficient to induce robust clinical responses. To identify novel drug targets that are synthetic lethal with MEK inhibition, CRISPR screens were conducted in multiple KRAS mutant NSCLC cell lines with or without trametinib treatment. Consistent with previous reports, several known MAPK-pathway genes, including KRAS, MEK, ERK, and FGFR1, were identified as top sensitizers validating our functional genomics approach. Interestingly, several novel targets were ranked among these top hits, including several members of the heparan sulfate biosynthesis pathway, such as the heparan sulfate 2-O-sulfotransferase (HS2ST1). In cells, HS2ST1 is responsible for transferring a sulfate from PAPS (3-phosphoadenosine-5'-phosphosulfate) to the 2-O position of a growing heparan sulfate chain. These chains partner with receptor tyrosine kinases at the cell surface to facilitate their interactions with growth factors. In this case, the interaction of FGF2 and FGFR1 has been shown to require HS2ST1-mediated 2-O sulfation, making HS2ST1 a novel druggable target in a well-validated FGFR-MAPK adaptive signaling axis. Here, we report that HS2ST1 is required for the feedback activation of the MAPK pathway that occurs downstream and in response to MEK or KRAS G12C inhibition via genetic validations studies. Knockout of HS2ST1 results in reduced feedback activation via FGFR1 and reduced MAPK pathway signaling. This reduced signaling leads to a reduction in cell growth in the presence of a MEK inhibitor, such as trametinib or selumetinib, or a KRAS G12C inhibitor like sotorasib. Our screen results reiterate the findings of others which indicate that effective MAPK suppression is key to inhibiting KRAS-mutant NSCLC cell growth. We find that HS2ST1 blockade would aid in maintaining the suppression of MAPK pathway signaling in KRAS-mutant NSCLC, leading to reduced cell viability and growth suppression. While others have described pairing receptor tyrosine kinase inhibitors with MAPK pathway inhibitors, this would be a novel approach to reducing upstream MAPK pathway feedback that may lead to reduced toxicity in patients.

P148 Glycogen phosphorylase and synthase inhibitors: Novel therapeutic approaches in anaplastic thyroid cancer. Cole Davidson, Jennifer Tomczak, Eyal Amiel, Frances Carr. University of Vermont, Burlington, VT.

Thyroid cancer (TC) is the most common endocrine cancer. Effective treatment options for papillary (PTC) and follicular (FTC) thyroid cancers afford positive patient prognoses. However, there are no effective, long-lasting treatments for anaplastic thyroid cancer (ATC), which has a median survival of five to six months. While the receptor tyrosine kinase inhibitor sorafenib can extend ATC patient survival to eleven months, tumor reoccurrence and drug resistance often develop. Therefore, there is a need for more effective targeted therapies for ATC. While the cell signaling landscape is well described in ATC, little is known about tumorigenic adaptations in ATC metabolism. Tumors exhibit an increased consumption of glucose compared to normal tissues to fuel tumor progression. Some cancers meet this high glucose requirement by storing and breaking down glycogen. While glycogen has been detected in bovine and canine thyroids, no report thus far has investigated glycogen in normal human thyroids or thyroid cancer. Therefore, our objective was to determine if normal thyroid and TC cells metabolize glycogen and to evaluate pharmacological inhibition of glycogen metabolism in ATC cells. We show for the first time that normal thyroid, PTC, FTC, and ATC cells express glycogen synthase and glycogen phosphorylase brain and liver (PYGB, PYGL) isoforms. We confirmed these observations in patient samples of normal thyroid and TC tissues via immunofluorescence. We revealed that the TC samples expressed high levels of PYGB compared to normal thyroid tissue. Electron microscopy revealed that normal thyroid cells organized glycogen in discrete glycosomes, while ATC cells exhibited smaller, dispersed glycogen packets. Importantly, we demonstrated that the glycogen synthase inhibitor guaiacol depleted glycogen content and reduced ATC cell viability. Conversely, the glycogen phosphorylase inhibitor CP-91,149 (CP) increased glycogen levels and induced apoptosis. Importantly, CP also reduced the number of stem cells in the ATC cell population. We further showed that CP synergized with sorafenib to more effectively inhibit ATC proliferation. CP enhanced glycolysis but inhibited oxidative phosphorylation as revealed using Seahorse. We confirmed an increase in glucose transporter expression using RT-qPCR and glucose import following CP treatment. This possibly represents a futile response to PYGB inhibition. We reasoned that glycogen was preferentially metabolized in ATC to fuel the pentose phosphate pathway to protect against reactive oxygen species (ROS). Indeed, we detected significantly higher levels of ROS in ATC cells treated with CP. We demonstrated that thyroid cells are able to metabolize glycogen and identified a potential biomarker for thyroid cancer (PYGB), which was targeted with CP-91,149. Our work establishes glycogen metabolism as a novel metabolic process in thyroid cells that is associated with TC dedifferentiation and provides insight to the effectiveness of inhibiting glycogen metabolism as a novel therapeutic strategy in ATC.

P149 Novel compounds to probe Hippo kinase STK3 noncanonical function in prostate cancer. Amelia U. Schirmer[1], Lucy M. Driver[1], Megan T. Zhao[1], Carrow I. Wells[2], Xuan Yang[2], David H. Drewry[2], Ivan Spasojevic[1], Everardo Macias[1]. [1]Duke University School of Medicine, Durham, NC, [2]Structural Genomics Consortium, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Prostate cancer (PC) is the second cause of cancer related deaths in men. Most therapies target the Androgen Receptor (AR) which can prolong survival, but resistance emerges and patients eventually succumb to the disease. This highlights the need for new PC molecular targets. Our group observed in PC patient datasets, the Serine/threoninekinase3 (STK3) gene is frequently amplified and correlates with worse outcomes. This is against the established dogma that STK3 is a tumor suppressor in the Hippo Tumor suppressor pathway that canonically regulates oncogenic transcription factor YAP1. Counter to this role, we report that in PC, STK3 has a pro-tumorigenic role. Our work shows that genetic and chemical inhibition of STK3 in PC cells in vitro significantly slowed proliferation and protasphere growth. The goal of this study was to identify and validate novel STK3 kinase inhibitors with increased potency and specificity. **Approach.** We screened large assay panels of kinase inhibitors (ChEMBL) for STK3 active molecules, which identified two independent scaffolds. For assessment of STK3 inhibition, we utilized a battery of in cell and biochemical assays including western blot, STK3-NanoBRET assays, radiometric enzymatic assays and a broad KINOMEscan scanMax panels to assess leads and analogs. For phenotypic studies, we utilized Hi-myc ventral prostate (HMVP2) cells to test STK3 inhibition in 3D spheroid growth and spheroid invasion of matrigel by live cell imaging. Pharmacokinetics (PK) and pharmacodynamics (PD) were performed for oral (PO) and intraperitoneal (IP) routes of our top STK3 inhibitor. A subcutaneous HMVP2 allograft model was used to test *in vivo* efficacy of STK3 inhibition by daily IP injections at 10 mg/kg and 30 mg/kg doses vs. vehicle. Tumor volumes were tracked by caliper measurements and at endpoint, we measured tumor, liver, lung, heart and spleen weights. **Results.** The two lead scaffolds and a number of analogs showed significant inhibition of STK3 by western blot analysis, in cell STK3-NanoBRET assay and recombinant STK3 radiometric assays. Phenotypic studies in HMVP2 spheres treated with analog STK3 inhibitors showed dose dependent decrease in proliferation and matrigel invasion. From our assays, compound SOB-5-47 emerged as the most potent. PK and PD studies showed that IP injections of SOB-5-47 led to short time in plasma, but pro-longed half-life in the organs such as liver, heart and notably prostate with a terminal half-life clearance of 8.3hrs. Daily injections of SOB-5-47 at 10 and 30 mgs/kg significantly delayed HMVP2 tumor kinetics compared to vehicle control. Tumor weights in SOB-5-47 groups were significantly lower vs control. Importantly, no differences in organ weights or total body weights were observed between groups. **Conclusions.** Here we report a noncanical role of STK3 in PC and the development of novel STK3 kinase inhibitors that can be used as tools to further examine the role of STK3. Additionally, we show that STK3 can be targeted *in vivo* to slow PC growth without significant unwanted deleterious effects.

P150 PSMD1 and PSMD3 as putative targets for cancer therapy. Andres J. Rubio, Alfonso E. Bencomo-Alvarez, James E. Young, Vanessa V. Velazquez, Anna M. Eiring. Texas Tech University Health Sciences Center at El Paso, El Paso, TX.

Ever since the ubiquitin proteasome system was characterized, efforts have been made to manipulate its function to abrogate the progression of cancer. As a result, the anticancer drugs bortezomib, carfilzomib, and ixazomib targeting the 26S proteasome were developed to treat multiple myeloma, mantle cell lymphoma, diffuse large B-cell lymphoma, colorectal cancer, and thyroid carcinoma. Despite the success demonstrated by these treatments, adverse side effects and drug resistance are prominent, raising the need for alternative therapeutic options. The 26S proteasome is composed of two 19S regulatory subunits and a 20S catalytic core, leaving ample opportunity for new targets. We recently demonstrated that knockdown of the 19S regulatory subunits, proteasome 26S subunit, non-ATPases 1 (*PSMD1*) and 3 (*PSMD3*), resulted in increased apoptosis of tyrosine kinase inhibitor (TKI)-resistant chronic myeloid leukemia (CML) cells, but had no effect on normal cord blood controls, suggesting they may be good targets for therapy. Therefore, we hypothesized that *PSMD1* and *PSMD3* are potential targets for anticancer therapeutics and that their relevance stretches beyond CML to other types of cancers. In the present study, we analyzed *PSMD1* and *PSMD3* expression in other types of cancers versus normal controls using data from The Cancer Genome Atlas (TCGA) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC), comparing expression with overall survival (OS). Data were analyzed using UALCAN (http://ualcan.path.uab.edu) and GEPIA2 (http://gepia2.cancer-pku.cn/). At the mRNA level, *PSMD1* was found to be overexpressed in 14/24 (58%) TCGA cancers compared with normal controls. This was confirmed at the protein level for breast ($p=4.2e^{-4}$), colon ($p=7.1e^{-20}$), clear cell renal carcinoma ($p=1.8e^{-4}$), and endometrial cancer ($p=2.0e^{-22}$). *PSMD3* was found upregulated at the mRNA level in 18/24 (75%) TCGA cancers, which was confirmed at the protein level in breast ($p=8.3e^{-4}$), colon ($p=1.0e^{-27}$), ovarian ($p=4.2e^{-6}$), clear cell renal carcinoma ($p=4.0e^{-19}$), and endometrial cancer ($p=1.5e^{-19}$). Upon correlation of *PSMD1* expression with OS, we saw that when overexpressed, individuals did significantly worse with adrenocortical carcinoma, lower grade glioma, lung adenocarcinoma, mesothelioma, and uveal melanoma. In contrast, diffuse large B cell lymphoma (DLBCL) and stomach adenocarcinoma patients with lower *PSMD1* expression had a worse OS. Similarly, patients with high levels of *PSMD3* mRNA expression had a significantly worse OS in kidney chromophobe, skin cutaneous melanoma, uveal melanoma, and mesothelioma. Our analysis revealed that differential expression of *PSMD1* and *PSMD3* is correlated with survival in several different cancer types. Future directions will identify *PSMD1* and *PSMD3* post-translational modifications that may be novel targets for anticancer therapeutics. In conclusion, we highlight *PSMD1* and *PSMD3* as potential therapeutic targets for the development of novel proteasome inhibitors to treat cancer patients with less toxicity.

P153 Claudin-1 is a therapeutic target for hepatocellular carcinoma. Natascha Roehlen[1], Marion Muller[1], Sara Cherradi[1], Frank Juehling[1], Francois H.T. Duong[1], Nuno Almeida[1], Fabio Del Zompo[1], Mirian Fernandez[2], Tobias Riedl[2], Hussein El Saghire[3], Antonio Saviano[4], Sarah Durand[1], Clara Ponsolles[1], Marine Oudot[1], Emanuele Felli[4], Patrick Pessaux[4], Irwin Davidson[5], Emilie Crouchet[1], Patrick Laquerriere[6], Mathias Heikenwälder[2], Roberto Iacone[3], Markus Meyer[3], Greg Elson[3], Tamas Schweighoffer[3], Catherine Schuster[1], Laurent Mailly[1], Joachim Lupberger[1], Thomas F Baumert[1]. [1]Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg, France, [2]Division of Chronic Inflammation and Cancer, German Cancer Research Center, Heidelberg, Germany, [3]Alentis Therapeutics, Basel, Switzerland, [4]Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Nouvel Hôpital Civil, Strasbourg, France, [5]Department of Functional Genomics and Cancer, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France, [6]CNRS, Institut Pluridisciplinaire Hubert Curien UMR 7178, Strasbourg, France.

Introduction: Hepatocellular carcinoma (HCC) is the fastest rising and fourth leading cause of cancer death worldwide. While new therapeutic modalities have been recently approved, treatment response and survival in patients remain poor. Claudin-1 (CLDN1) is a cell membrane protein mediating cell-cell adhesion, fate and differentiation. While the function of CLDN1 within tight junctions is well characterized, the role of non-junctional CLDN1 in HCC remains unexplored. **Aim and methods**: Using humanized monoclonal antibodies (mAbs) targeting specifically the extracellular loop of human non-junctional CLDN1 and a large series of patient-derived cell-based and animal model systems we aimed to investigate the role of CLDN1 as therapeutic target for treatment of HCC. **Results:** Targeting non-junctional CLDN1 robustly suppressed tumor growth in a large series of patient-derived model systems, including multicellular tumorspheres and patient-derived xenograft (PDX) mouse models. *In vivo* and *ex vivo* studies further suggested synergistic efficacy in combination with sorafenib. Mechanistic studies revealed that CLDN1 mAbs suppressed tumor cell proliferation, invasion and stemness by interfering with Src, Wnt-β-catenin and STAT3 signaling. Treatment with humanized anti-CLDN1 mAbs is considered to be safe, as administration in non-human primates and mouse models did not reveal any major toxicity even when high doses largely exceeding the therapeutic need were repeatedly applied. **Conclusion:** Our results provide robust pre-clinical proof-of-concept for humanized CLDN1-specific mAbs for treatment of HCC. The unique and differentiated mechanism of action has the potential to break the plateau of limited response and survival offered by currently approved therapies.

P154 Genomic profiling and matched therapy for recurrent or metastatic salivary gland neoplasms. Results from the matched cohort of the GEMS-001 clinical trial. Alberto Hernando-Calvo, Eoghan Malone, Daphne Dai, Amy Prawira, Ilan Weinreb, Anneli Eliason, Angela Rodriguez, Katherine Lajkosz, Sarah Jennings, Anna Spreafico, Lillian L. Siu, Aaron Hansen. Princess Margaret Cancer Centre, Toronto, Canada.

Background Salivary gland tumors (SGT) are rare and heterogeneous diseases with limited systemic options available. Results from recent basket trials including SGT cohorts for specific actionable alterations (AA) have described promising outcomes with molecularly targeted approaches (TA). **Methods** The GEMS-001 study (NCT02069730) is an ongoing multi-arm trial with two phases. In part 1, recurrent or metastatic (R/M) SGT patients (pts) undergo comprehensive immunohistochemistry (IHC) including HER2, androgen receptor (AR) and ALK translocation and multigene panel testing (NGS). Based on the molecular profiling results in part 1, R/M SGT pts are matched to available targeted therapies on disease progression. Here, we present the efficacy results for the matched cohort of the GEMS-001 study. **Results** As of July 2021, 100 pts underwent molecular profiling within part 1. According to the SGT subtype (WHO 2017): 49% adenoid cystic carcinoma (ACC), 18% salivary duct carcinoma (SDC), 9% acinic (ACI) and 24% other subtypes (O). Overall, on IHC HER2 overexpression was present in 10%, AR 24% and ALK translocation 0%. On NGS, PIK3CA mutation (mut) was in 10%, HRAS mut 7%, ERBB2/3 alterations (alt) 5%, NOTCH1-3 mut 2% and ETV6-NTRK3 fusion 2%. Up to 45% pts displayed at least 1 AA and 25% had ≥2 AA. AA were enriched in SDC (100% ≥1 AA) as compared to ACI (33% ≥1 AAs), O (58% ≥1 AA) or ACC (22% ≥1 AA) ($p<0.001$ Fisher's exact test). A total of 34 pts (34%) were matched to proteomic or genomic alterations. Of those matched, median age was 62 years (range 47-84), M:F 26:8, 100% ECOG≤1, and their AA-TA included 15 AR (leuprolide+bicalutamide), 4 HER2 or ERBB2 alt (trastuzumab+pertuzumab), 4 PIK3CA mut (PIK3CA inhibitor), 2 cyclin pathway alt (palbociclib), 2 ETV6-NTRK3 fusion (larotrectinib) and 7 other AAs (1 EGFR L858 mut (afatinib), 1 c-KIT mut (imatinib), 1 BAP1 mut (olaparib), 1 Non-V600 BRAF mut (pan-RAF inhibitor), 1 CHEK2 mut (olaparib), 1 ATM mut (olaparib) and 1 PTCH1 (vismodegib)). As per efficacy, overall response rate was 24% and median progression free survival 6.4 months (m) (95%CI 3.4-8.9 m) for the matched population. Complete responses were observed in 2 pts treated with leuprolide+biculatamide (AR). Partial responses were observed in 2 pts treated with larotrectinib (ETV6-NTRK3), 3 pts treated with trastuzumab+pertuzumab (HER2) and 1 pt treated with leuprolide+biculatamide (AR). Notably, among responders median duration of response was 13.9 m (95%CI 4.6-18.0 m). **Conclusions** In our cohort, more than one third of the population were matched to TA with promising efficacy outcomes. Our results support comprehensive molecular and IHC profiling but its clinical utility may vary depending on the SGT subtype.

P155 Heterogeneity of circulating tumor cell neoplastic subpopulations interrogated by single-cell transcriptomics. Dario Marchetti. UNM Health Sciences Center, Albuquerque, NM.

Fatal metastasis occurs when circulating tumor cells (CTCs) disperse through the blood to initiate a new tumor at specific sites distant from their primary tumor. CTCs have been classically defined as nucleated cells positive for epithelial-cell adhesion molecule and select cytokeratins (EpCAM/CK/DAPI), while negative for the common lymphocyte marker CD45. Although the enumeration of CTCs allowed the estimation of overall metastatic burden in breast cancer patients, the thorough interrogation of circulatory neoplastic cells at single-cell level has never been achieved, and is considered critical to improve therapies. We report that CTCs from metastatic breast cancer (mBC) patients were identified using the RareCyte™ Cytefinder II platform. Second, comprehensive transcriptomic analyses at single-cell level of Lin- and Lin+ blood cell populations isolated from patients identified a unique and heterogeneous cluster of cells which expressed EpCAM/CK and an array of genes not previously associated with classical CTCs. The differences in gene expression suggest that the neoplastic cell population in blood is distributed across heterogeneous cell profiles. This study proposes that the identification of these genes and neoplastic states will promote novel areas of analysis dissecting properties underlying CTC survival, proliferation, and interactions with immune cells. It improves abilities to measure and interfere with CTC states, their plasticity, and CTC functionalities for impactful therapeutic interventions. Fatal metastasis occurs when circulating tumor cells (CTCs) disperse through the blood to initiate a new tumor at specific sites distant from their primary tumor. CTCs have been classically defined as nucleated cells positive for epithelial-cell adhesion molecule and select cytokeratins (EpCAM/CK/DAPI), while negative for the common lymphocyte marker CD45. Although the enumeration of CTCs allowed the estimation of overall metastatic burden in breast cancer patients, the thorough interrogation of circulatory neoplastic cells at single-cell level has never been achieved, and is considered critical to improve therapies. Here we report that CTCs from metastatic breast cancer (mBC) patients were identified using the RareCyte™ Cytefinder II platform. Second, comprehensive transcriptomic analyses at single-cell level of Lin- and Lin+ blood cell populations isolated from patients identified a unique and heterogeneous cluster of cells which expressed EpCAM/CK and an array of genes not previously associated with classical CTCs. The differences in gene expression suggest that the neoplastic cell population in blood is distributed across heterogeneous cell profiles. This study proposes that the identification of these genes and neoplastic states will promote novel areas of analysis dissecting properties underlying CTC survival, proliferation, and interactions with immune cells. It improves abilities to measure and interfere with CTC states, their plasticity, and CTC functionalities for impactful therapeutic interventions.

P156 Identification of previously unknown targets for approved small-molecule drugs using chemoproteomic platform IMTAC™. Vivian Zhang[1], Cindy Huang[1], Chao Zhang[2], Ping Cao[1]. [1]BridGene Biosciences, Inc., San Jose, CA, [2]University of Southern California, Los Angeles, CA.

Small-molecule drugs exert their therapeutic effects through binding and modulating functions of their target proteins. While most approved drugs have defined target(s), a comprehensive target map is generally lacking for clinical and experimental drugs. This limits both understandings of a drug's actions and hampers further optimization. Leveraging its proprietary chemoproteomic platform IMTAC™, BridGene Biosciences has developed an approach to generate more comprehensive target maps for approved small-molecule drugs. This approach can also be applied to drug candidates. The approach was successfully applied to a covalent drug ibrutinib and a non-covalent drug sunitinib, revealing previously unknown targets of these drugs. The drug, sunitinib or ibrutinib, was first chemically modified to generate probe compounds, which were then treated on live cells. Quantitative mass spec analysis of the resulting proteomes was performed to identify the binding targets and quantify their apparent binding affinity to the drug. The identified probe-binding proteins were then ranked based on their apparent binding affinity to determine the proteome-wide selectivity to the drug. BridGene's IMTAC™ profiling verified BTK to be the top target and two other kinases (BLK and TEC) to be prominent targets of ibrutinib in Ramos cells. Analogous IMTAC™ profiling confirmed sunitinib's binding to over a dozen of protein kinases with high to moderate affinity in Ramos cells. The kinase targets of sunitinib in Ramos cells include BLK, LYN, PTK2B, etc. Importantly, non-kinase targets included metabolic enzymes were also identified for ibrutinib and sunitinib in the IMTAC™ profiling. The binding of the drugs to select new targets was verified using biochemical or binding assays. Our study demonstrates that IMTAC™ profiling is a powerful approach for generating comprehensive target maps for clinical and experimental drugs, both covalent and non-covalent ones. Having target scope information will be invaluable at various stages of drug discovery and development, such as the repurposing of existing drugs, the optimization of drug candidates, as well as target identification and deconvolution for phenotypic screening hits.

P157 Targeting Trop2 for treatment of prostate and breast cancer. Tanya Stoyanova. Stanford University, Palo Alto, CA.

Background: Prostate cancer and breast cancer are the most commonly diagnosed non-skin cancers and the second leading cause of cancer related mortalities in men and women respectively in the United States. Fifteen percent of breast cancer is TNBC characterized with lack of receptors for estrogen, progesterone, and HER-2/neu making it resistant to hormone therapies and therapies targeting estrogen, progesterone, and HER-2/neu signaling. The first line of treatment for men with advanced prostate cancer is androgen depravation therapy. While initial responses are observed, unfortunately, the disease frequently recurs as a lethal metastatic form referred to as castration-resistant prostate cancer (CRPC). We recently identified that the cell surface receptor, Trop2, is a new regulator of TNBC and CRPC. We demonstrated that Trop2 is highly expressed in CRPC and TNBC. In this study, we set out to test the therapeutic potential of new fully humanized human anti-Trop2 antibody fragments in CRPC and NEPC. **Methods**: We have generated new human anti-Trop2 antibody fragments. Lentiviral infection was used to generate prostate and breast cancer cell lines with over-expression of Trop2 and knock-down of Trop2. *In vivo* functional assays were performed including subcutaneous xenograft growth and metastasis. **Results:** High protein levels of Trop2 are observed in metastatic CRPC and TNBC. Furthermore, loss of Trop2 significantly delays growth, migration and invasion of prostate and breast cancer cells in vitro and delays tumor growth and metastasis of prostate and breast cancer in vivo. Overexpression of Trop2 enhances tumor growth and metastasis of prostate cancer cells and drives CRPC. We generated new fully humanized human anti-Trop2 antibody fragments that have therapeutic efficacy in preclinical models of prostate and breast cancers. **Conclusions:** In a previous study, we identified cell surface receptor, Trop2, as a novel driver of metastatic CRPC. Herein, our new findings reveal that Trop2 represents a promising therapeutic target for prostate, breast, and potentially other epithelial cancers. We identified lead antibody candidates that bind to human Trop2 protein with high affinity and stability. Furthermore, our lead anti-Trop2 Abs significantly delay the tumors growth in CRPC and TNBC without measurable toxicity and provide new therapeutic strategy for metastatic CRPC and TNBC.

P158 Phenotypic analysis of myeloid cells in a 3D image-based repolarization assay with tumor spheroids. Gera Goverse, Nataliia Beztsinna, Benjamin Visser, Tomas Veenendaal, Marjan van de Merbel, Emma Spanjaard, Ashgard Weterings, Kuan Yan, Leo Price, Lidia Daszkiewicz. Crown Bioscience Netherlands B.V., Leiden, Netherlands.

BackgroundThe immunosuppressive tumor microenvironment (TME) involves multiple cell types and a better understanding of the interplay between these cells could potentially unleash the full potential of many different types of immunotherapies. Tumor infiltrating myeloid cells have both cancer-restraining and cancer-promoting functions. Therefore, to further increase the biological relevance of in vitro platform, we incorporated the myeloid cell compartment into 3D co-cultures of tumor cells and T cells to measure the effects of immune-modulators. Using our proprietary image analysis software and machine learning, a set of morphological features was identified that allowed discrimination between undifferentiated monocytes, M1 and M2 macrophages, and dendritic cells. In addition, the phenotypic profiles of the myeloid cells could be analyzed in the presence of the tumor supernatants or in co-cultures with tumor cells. This assay allows a better understanding of the suppressive tumor microenvironment including multiple cell types and is suitable to test different cancer immunotherapies. **Materials and Methods** Different myeloid cell populations were generated from healthy PBMCs. Polarized M1 and M2 macrophages, DCs, and undifferentiated monocytes were then co-cultured with tumor conditioned media, spheroids derived from different cancer cell lines, or colorectal cancer organoids (CRC), growing in protein hydrogel in 384 well-plates for 1-7 days. In addition, purified T cells were also incorporated in these cultures. The cellular interactions were visualized using high-content microscopy and OMiner® software, which was trained to identify phenotypic profiles of different myeloid cell populations in 3D, was used to quantify their similarity to the defined subsets upon treatment. **Results** Myeloid cell populations were classified according to their phenotypic features identified by 3D image analysis, which verified the repolarization of M2 macrophages by their shift into phenotypic space of the M1 type macrophages upon treatment with CSF1r inhibitor or STING agonist. Repolarization was confirmed with an increased similarity score towards M1 macrophages upon treatment. This approach was further used to reveal the different effects of tumor cells and their immunosuppressive TME on myeloid cell phenotypes, showing that each tumor differently influenced the repolarization of the myeloid cells. In addition, triple co-cultures with T cells revealed the suppressive effect of tumor associated myeloid cells on the proliferation and infiltration of T cells. **Conclusions** The phenotypic analysis of different myeloid cells in 3D co-cultures could be visualized and quantified elucidating the bi-directional interplay between tumor and immune cells, and the functional reprograming of the suppressive tumor associated population towards an M1 phenotype induced by drug candidates. This advanced platform for testing cancer immunotherapies combines the ability to examine the complexity of the TME with the robustness of a high-throughput screening platform.

P159 Molecular tumor board impact at two large health systems. Igor I. Rybkin[1], Michael A. Thompson[2], Frank M. Wolf[3], Kristen Collins[1], Louisa Laidlaw[1], Tom Mikkelsen[1], Jennifer Godden[2], Mary Walters[2], James L. Weese[2], Ronda Broome[3], Joe Burkhart[3], Veronica Jones[3], Chenan Zhang[3], Thomas D. Brown[3], Anna Berry[3]. [1]Henry Ford Cancer Institute, Henry Ford Health System, Detroit, MI, [2]Aurora Cancer Care, Advocate Aurora Health, Milwaukee, WI, [3]Syapse, San Francisco, CA.

Background: Molecular tumor boards (MTB) are a key component of most precision oncology programs, designed to provide a structured multidisciplinary approach to evaluating cancer patients (pts) for therapy (Tx), clinical trial (CT) enrollment, and genetic counseling (GC) services. Two health systems separately instituted MTB in 2017, and through a bio-informatics platform have been tracking the impact of these on pt care. We describe the collective experience in tracking MTB impact. **Methods:** This is a retrospective cohort study of pts reviewed for the first time by MTB between September 1, 2017 and September 30, 2020 at either of the two health systems (HS). Follow up data were obtained by a certified tumor registrar at a median time of 109 days after presentation. Demographics and clinical characteristics of MTB pts were obtained. Pts were identified for whom Tx or CTs were recommended and subsequently administered after MTB review, along with pts for whom GC was recommended and subsequently had genetic testing performed. **Results:** 351 pts were evaluated. Pt demographics included: median age of 65 years; 61% female; 83% White, 13% Black, 3% Asian, with 2% of Hispanic/Latino ethnicity. The most common primary sites observed were lung (75 pts; 21%), breast (44 pts; 13%) and central nervous system (26 pts, 7%). 334 pts (95%) had data regarding Tx and CT recommendations. 124 pts (37%) received a Tx recommendation and 36 (29%) of these received Tx. 73 pts (22%) received a CT recommendation and 4 (6%) of these pts were enrolled on CTs. 340 pts (97%) had data regarding GC recommendations. 94 pts (28%) received a GC referral and 28 (30%) of these received GC. 25 (89%) of these received a molecular test for germline cancer risk, and 10 (40%) of these had a risk mutation identified in one of the following: ATM (4), BRCA2 (2), BARD1 (1), CHEK2 (1), RAD51C (1), and RET (1). **Conclusions:** MTB was successful in matching pts to Tx and CT, and in providing appropriate referrals to GC and identifying germline risk mutations. The bio-informatics platform provided a uniform format and structure to collate and analyze data between programs. These parameters will serve as a baseline to monitor future MTB impact and trends as these precision oncology programs continue to grow. The relatively low percentage of patients of color whose cases were reviewed in this MTB analysis merits further understanding.

P160 MatchMiner: An open-source platform for cancer precision medicine. Harry Klein, Tali Mazor, Priti Kumari, James Lindsay, Andrea Ovalle, Pavel Trukhanov, Joyce Yu, Michael Hassett, Ethan Cerami. Dana-Farber Cancer Institute, Boston, MA.

With the advent of next generation sequencing in cancer care, patients' tumors can be genomically profiled and specific genetic alterations can be targeted with precision medicine drugs. However, the abundance of patient sequencing data coupled with complex clinical trial eligibility has made it challenging to match patients to precision medicine trials. To facilitate interpretation of complex tumor sequencing data and clinical trial genomic eligibility criteria, we developed MatchMiner, an open-source platform to computationally match cancer patients to precision medicine clinical trials. MatchMiner **has several modes of clinical use: (1) patient-**centric, where clinicians look up trial matches for their patient, (2) trial-centric, where clinical trial investigators identify patients for their clinical trials, and (3) trial search, where clinicians identify available trials based on any criteria, including external genomic reports. To support users in all three modes, MatchMiner also displays full genomic reports for patients and detailed trial information in user-friendly formats. MatchMiner trial matching is performed via the MatchEngine, an algorithm that computes matches based on patient genomic and clinical data and trial eligibility criteria. The MatchEngine accepts many different data inputs for patient-trial matching, and is easily customized to work with data available at any institution. At Dana-Farber Cancer Institute (DFCI), MatchMiner supports the following data: 1) patient-specific genomic sequencing data, including mutations, copy number alterations, structural variants, tumor mutational burden and mutational signatures including mismatch repair deficiency or microsatellite instability, 2) patient-specific clinical data, including primary cancer type, gender, age, and vital status, and 3) trial eligibility criteria including genomic targets, cancer type, and age. Unique to MatchMiner, each trial's eligibility criteria is encoded in clinical trial markup language (CTML), a structured format that encodes detailed information about a trial and utilizes boolean logic to encode inclusion and exclusion criteria. Although MatchMiner has been operational at DFCI since early 2017, its impact on patient care has not yet been extensively studied. Thus far, MatchMiner has facilitated 181 precision medicine trial consents (MatchMiner consents, MMC) for 159 patients. To quantify MatchMiner's impact on trial consent, we retrospectively measured time from genomic sequencing report date to trial consent date for a subset of the 181 MMC (166 MMC). We compared time to trial consent date for the 166 MMC to a group of 353 consents for the same trials not facilitated by MatchMiner (non-MatchMiner consents, non-MMC). MMC consented to trials 22% faster (P=0.004, median=195 days, IQR=85-34) than non-MMC (median=250 days; IQR=99-491). Thus, clinical use of MatchMiner decreased time to enroll in a precision medicine study, and suggests that use of precision medicine trial matching tools such as MatchMiner are important for the future of patient care.

P161 Impaired serological response to SARS-CoV-2 mRNA vaccination in patients with hematologic malignancies. Lee M. Greenberger[1], Larry A. Saltzman[1], Jonathon W. Senefeld[2], Patrick W. Johnson[3], Louis J. DeGennaro[1], Gwen L. Nichols[1]. [1]The Leukemia & Lymphoma Society, Rye Brook, NY, [2]Mayo Clinic, Rochester, MN, [3]Mayo Clinic, Jacksonville, FL.

Although SARS-CoV-2 vaccines induce a robust immune response in healthy individuals, limited reports indicate that some immunocompromised patients may not produce anti-spike antibodies after vaccination. In this report, we assessed anti-spike antibody levels among 2200 patients with hematologic malignancies after SARS-CoV-2 mRNA vaccination (NCT04794387). Diagnoses and treatments were self-reported using a national online registry. The side-effects profile of the vaccines was not different compared to previous studies in healthy individuals. The serological response >14 days after full vaccination was evaluated using the Roche Elecsys total immunoglobulin anti-spike assay (range: 0-250 U/ml; positive cutoff of > 0.8 U/mL). Approximately 40% and 60% were male and females, respectively, 96% were white or Caucasian, and the median age was 67 (range, 21 to 92 years). Patients received the BNT162b (Pfizer/BioNtech: 55%) or mRNA1246 (Moderna: 45%) vaccines. The largest patient populations had chronic lymphocytic leukemia (CLL: N = 978, 44%), multiple myeloma (MM: 253, 11.4%), follicular lymphoma (FL: 147, 6.6%), Waldenstrom's macroglobulinemia (WM: 115, 5.9%), Hodgkin lymphoma (HL: 79, 3.5%), diffuse large B-cell lymphoma (DLBCL: 83, 3.7%), mantle cell lymphoma (MCL: 58, 2.6%), or marginal zone lymphomas (MZL: 52, 2.3%). Ninety-one patients (3.8%) were positive for the nucleocapsid antibodies and deleted from the initial analysis. Overall, 564 patients (25%) were seronegative after full vaccination. The largest percentages of seronegative patients had B-cell derived hematologic malignancies: MCL (50%), MZL (37%), DLBCL (34%), CLL (32%), FL (28%), and WM (25%). Seronegative rates of 5.9% and 14% were found in patients with multiple myeloma and acute lymphoblastic leukemia, respectively. All but one HL patient and no patients with smoldering multiple myeloma, myeloproliferative neoplasms, myelodysplastic syndrome were seronegative. Anti-spike antibody levels did not markedly change within 35 individual patients >45 days after the initial vaccine assessment except for one patient who seroconverted after a third vaccine dose. High seronegative rates correlated with treatment regimens that contained anti-CD20 mAbs, any Bruton tyrosine kinase inhibitor, and CD19-directed CAR T-therapy. Seronegative rates were higher with the BioNTech/Pfizer vs. Moderna vaccines in an unadjusted analysis (odds ratio, 1.5; 95% confidence interval, 1.05-1.55; p= 0.015). The overall seronegative rate was ~1% among nucleocapsid-positive patients. Among CLL patients, the seronegative rate was lower among nucleocapsid-positive patients compared to nucleocapsid-negative patients (0% vs. 32%, p<0.001). Providers should be aware that a substantial subset of vaccinated blood cancer patients may be at high risk of breakthrough COVID-19 infections. Alternate therapies with monoclonal antibodies or booster vaccination including those that would allow exposure to more than the spike protein, may offer additional protection for this vulnerable population.

P162 Phase 1 trial of selinexor in children and adolescents with recurrent/refractory solid and CNS tumors (ADVL1414): A Children's Oncology Group Phase 1 Consortium trial. Adam L. Green[1], Charles G. Minard[2], Xiaowei Liu[3], Joel M. Reid[4], Kerice Pinkney[5], Stephan Voss[6], Marvin D. Nelson[7], Elizabeth Fox[8], Brenda J. Weigel[9], Julia Glade Bender[10]. [1]University of Colorado School of Medicine, Aurora, CO, [2]Baylor College of Medicine, Houston, TX, [3]Children's Oncology Group, Monrovia, CA, [4]Mayo Clinic, Rochester, MN, [5]Joe DiMaggio Children's Hospital, Hollywood, FL, [6]Boston Children's Hospital, Boston, MA, [7]Children's Hospital Los Angeles, Los Angeles, CA, [8]St. Jude Children's Research Hospital, Memphis, TN, [9]University of Minnesota Medical School, Minneapolis, MN, [10]Memorial Sloan Kettering Cancer Center, New York, NY.

Introduction: Selinexor is a first-in-class, central nervous system (CNS) penetrant, oral inhibitor of exportin 1 (XPO1), the sole nuclear exporter of many key tumor suppressors. Selinexor is FDA-approved for refractory multiple myeloma and DLBCL and has been evaluated in a phase 1 trial in children with leukemia. We report a phase 1 trial of selinexor in children and adolescents with recurrent CNS and solid tumors, including lymphoma (NCT02323880). **Methods:** A rolling-six design was used to evaluate selinexor (10 or 25 mg tablets) administered twice or once weekly during a 28-day cycle. Dose determination was based on protocol-defined dose limiting toxicity (DLT) using CTCAEv4 during cycle 1. First dose pharmacokinetics (PK) were performed. **Results:** 43 subjects were enrolled (17 males); median (range) age was 15 (6-20) years. 27 (63%) had CNS tumors, most commonly high-grade glioma (n=12); 16 (37%) had extracranial solid tumors. 42 were evaluable for DLT. At the starting dose (35 mg/m^2/dose, twice weekly), no DLTs were observed in 6 subjects, however, 2 subjects had unexpected late myelosuppression delaying initiation of cycle 2. The dosing schedule was amended to twice weekly for 3 weeks followed by a 1 week break. 12 subjects received 35 mg/m^2/dose; 4 experienced DLTs [grade 3 fatigue (n=2), grade 3 thrombocytopenia (n=1), or grade 3 ALT increase (n=1)]. The dose was de-escalated to 20 mg/m^2/dose, 3 weeks on, 1 week off. 12 subjects enrolled; 3 experienced a DLT [grade 3 increased AST/ALT, acute reversible neurologic changes, or neutropenia (each n=1)]. At the 20 mg/m^2 (n=12) and 35 mg/m^2 (n=19) dose levels, respectively, the mean ± SD C_{max} (ng/ml) was 324±116 and 535±174, and AUC (hr•ng/ml) was 3092 ± 842 and 5156 ± 1227. This was comparable to PK in adults receiving 35 and 50 mg/m^2. Based on a desire to achieve a higher C_{max} and avoid breaks in schedule, and emerging evidence for similar effectiveness with decreased toxicity in adults receiving continuous once weekly dosing, we evaluated a dosing schedule with once weekly dosing for all 4 weeks of each cycle. At the initial dose level (45 mg/m^2 weekly), 2 of 6 subjects had DLTs [prolonged grade 2 thrombocytopenia or grade 3 seizure in a primary CNS tumor patient]. Six subjects received 35 mg/m^2/dose once weekly; 1 DLT [grade 3 thrombocytopenia] was observed. Non-dose-limiting toxicity (Grade ≥2 occurring in >10% of subjects during cycle 1) included lymphopenia, leukopenia, neutropenia, thrombocytopenia, anorexia, fatigue, hypophosphatemia, nausea, and vomiting. Subjects received a median (range) of 1 (1-9) cycle; 13 received 2-3 cycles, and 6 received 5-9 cycles. **Conclusions:** Selinexor-related toxicities were primarily hematological and gastrointestinal. The maximum tolerated dose (MTD) of selinexor in children and adolescents with recurrent solid and CNS tumors is 20 mg/m^2/dose twice weekly for 3 weeks followed by one week off. On a continuous once weekly schedule, the MTD and recommended phase 2 starting dose of selinexor is 35 mg/m^2/dose.

P164 Identification of the p53 negative feedback loop as a target for enhancing selinexor activity in neuroblastoma. Rosa Nguyen[1], Hong Wang[2], Ming Sun[1], Dong Geun Lee[2], Junmin Peng[2], Carol J. Thiele[1]. [1]NCI, Bethesda, MD, [2]St. Jude Children's Research Hospital, Memphis, TN.

Neuroblastoma (NB) is the most common extra-cranial pediatric solid tumor and accounts for 15% of cancer-related deaths in children, highlighting an unmet need for novel therapies. Selinexor is a small molecule inhibitor of XPO1. XPO1 shuffles cargo proteins with a nuclear export sequence from the nucleus to the cytosol, many of which are essential for cancer growth and cell maintenance. XPO1 also has a prognostic significance in patients with NB since those with high *XPO1* levels have an inferior event-free (P = 1.2e-5, Bonferroni P = 5.4e-3) and overall survival (P = 4.5e-7, Bonferroni P = 2.1e-4) compared to patients with low *XPO1* expression. To characterize the global transcriptomic consequences of XPO1 inhibition, we performed RNA-sequencing in the NB cell line IMR-5 where we performed a knockdown of *XPO1* or treated cells with selinexor. Conducting a gene set enrichment analysis, we found genes encoding MYCN targets were significantly downregulated by selinexor (normalized enrichment score [NES] = -2.7; nominal P = <0.001) or *XPO1* knockdown (NES = 2.2; nominal P = <0.001). Genes associated with the p53 pathway were significantly enriched (NES = 2.7; nominal P = <0.001 [selinexor treatment]; NES = 2.2; nominal P = <0.001 [*XPO1*knockdown]). Given the large number of XPO1-targets, we applied an advanced tandem mass tag (TMT)-liquid chromatography (LC)/LC-MS/MS based pipeline for deep whole-cell proteomic and phospho-proteomic analyses to interrogate unknown mechanisms of selinexor's action. We found that selinexor induced its cytotoxic effects in NB through the nuclear accumulation of p53. Drug sensitivity assays with selinexor against seven human and one murine NB cell lines yielded good (median EC50=28.7nM) and intermediate responses (median EC50=133.5nM) with all intermediate responders harboring a *TP53* mutation while good responders had *TP53* wild type status. Further, phosphoproteomic analysis showed that nuclear accumulation of p53 led to an increase in p53 phosphorylation at site S315 marking p53 for MDM2-mediated ubiquitination. Since this phosphorylation step is undertaken predominantly by aurora kinase A (AURKA), we used a clinically available AURKA inhibitor, alisertib, in combination with selinexor and found synergistic killing activity *in vitro* and enhanced growth inhibition in two orthotopic xenograft mouse models. These findings suggest a potential therapeutic benefit using selinexor and alisertib to synergistically increase p53-mediated cytotoxicity of high-risk NB. Given the known toxicity profiles and potential therapeutic benefits, these findings support a clinical trial of selinexor and alisertib in children with high-risk NB.

P165 MRTX1719: A first-in-class MTA-cooperative PRMT5 inhibitor that selectively elicits antitumor activity in *MTAP/CDKN2A* deleted cancer models. Christopher R. Smith, Lars D. Engstrom, Svitlana Kulyk, Ruth Aranda, Laura Waters, Krystal Moya, Victoria Bowcut, Allan Hebbert, David Trinh, David M. Briere, J. David Lawson, Jeff Clarine, Lisa Rahbaek, James G. Christensen, Matthew A. Marx, Peter Olson. Mirati Therapeutics, San Diego, CA.

Nearly all oncology therapies target proteins that are hyperactivated in cancer; however, developing precision medicines for cancers that harbor a specific tumor suppressor gene loss remains one of the most significant challenges in cancer research. Previous studies demonstrated cancer cell lines harboring homozygous deletion of the *MTAP* gene (*MTAP* del) are selectively vulnerable to shRNA-mediated PRMT5 inhibition based on the concept of synthetic lethality. *MTAP* is adjacent to, and co-deleted with, the most commonly deleted tumor suppressor gene, *CDKN2A*. *MTAP* is deleted in ~10% of all cancers and is enriched in NSCLC, pancreatic cancer, mesothelioma, MPNST, and several other cancers. *MTAP* del cells accumulate the metabolite methylthioadenosine (MTA) which binds to, and partially inhibits, PRMT5 activity; however, current clinical-stage small molecule PRMT5 inhibitors have binding modes that are mutually exclusive with MTA binding and therefore are not selective for *MTAP* del cancers. To develop a potential precision medicine for *MTAP* del cancers, compounds that selectively bind to the PRMT5/MTA complex were discovered via a fragment-based approach using SPR complemented with structural insight utilizing X-ray crystallography. Optimization of the lead series identified MRTX1719, a potent inhibitor of PRMT5 activity (8 nM IC_{50}) and cell viability (12 nM IC_{50}) in HCT116 *MTAP* del cells with greater than 50-fold selectivity compared with HCT116 *MTAP* WT cells. MRTX1719 possesses favorable drug-like characteristics and human predicted oral PK properties. Daily oral administration of MRTX1719 to tumor xenograft-bearing mice demonstrated dose-dependent inhibition of PRMT5-dependent symmetric dimethylarginine (SDMA) protein modification in *MTAP* del tumors that correlated with antitumor activity. In contrast, minimal inhibition of PRMT5-dependent SDMA and tumor growth inhibition was observed in *MTAP* WT tumor xenografts. Repeat dosing in mice also showed reduced effects in bone marrow compared to nonselective PRMT5 inhibitors currently in clinical trials, suggesting MRTX1719 may have an improved therapeutic index relative to these agents. MRTX1719 treatment resulted in dysregulated post-translational modification of key cancer and cell growth-related proteins, dysregulated RNA splicing, decreased proliferation and increased apoptosis in *MTAP* del cancer cells. Screens evaluating the effect of MRTX1719 on cell viability across panels of cancer cell lines and/or patient-derived xenograft tumor models demonstrated broad anti-tumor activity across MTAP-deleted tumor models. Select targeted therapy and chemotherapy combinations augmented the activity of MRTX1719 and in some models converged on phospho-Rb inhibition to block tumor growth. Together, these data demonstrate that MRTX1719, a selective PRMT5/MTA inhibitor, represents a promising therapeutic strategy for patients with *MTAP* del cancers.

P166 The rs35112940 CD33 polymorphism reduces CD33 internalization and efficacy of CD33-directed gemtuzumab ozogamicin. Mohammed O. Gbadamosi[1], Vivek M. Shastri[1], Soheil Meshinchi[2], Jatinder K. Lamba[1]. [1]University of Florida College of Pharmacy, Gainesville, FL, [2]Fred Hutchinson Cancer Research Center, Seattle, WA.

CD33 is a myeloid-specific cell surface protein widely expressed on acute myeloid leukemia (AML) cells making it an excellent immunotherapeutic target. Current CD33-directed immunotherapeutic treatment strategies include gemtuzumab ozogamicin (GO), an antibody-drug conjugate (ADC) approved in 2017 for treatment AML and other ADCs are various stages of development. Previously, studies from our group have identified germline variations in CD33 that are associated with CD33 cell surface expression levels and clinical outcomes in response to GO. Among these germline variations is rs35112940 (G>A; Arg304Gly), a missense polymorphism located in exon five of CD33 which is associated with lower CD33 expression and reduced benefit from treatment using GO. Herein, we functionally validated the effect of the rs35112940 variant by treating $CD33^{KO}$-HL60 engineered to overexpress wildtype CD33 (HL60-$CD33^{FL}$) or CD33 encoding the rs35112940 variant (HL60-$CD33^{FL}$-rs35112940) with GO. After 48-hour treatment with 250 ng/mL of GO, we observed that HL60-$CD33^{FL}$-rs35112940 cells were more resistant to GO than HL60-$CD33^{FL}$ cells (46.5% vs 66.4% cell viability, P = 0.02). Of note, all engineered cells expressed CD33 with less than 1-log fold difference in MFI (HL60-$CD33^{FL}$ median fluorescence intensity (MFI) vs HL60-$CD33^{FL}$-rs35112940 MFI: 22536 vs 24882). One critical aspect of the mechanism of action of GO is the internalization of the GO-CD33 complex which allows free calicheamicin to induce DNA damage leading to cellular apoptosis. Given the proximity of the rs35112940 loci to the cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM) domain of CD33, which is critical for CD33 internalization, we hypothesized that the rs35112940 variation may impact CD33 internalization as well. To assess this, we performed a flow cytometry-based internalization assay to capture the remaining amounts of CD33 present on the cell surface after 4 hours allowing us to determine the internalization of CD33 over time. Over a 4-hour window, we observed that that HL60-$CD33^{FL}$-rs35112940 cells had an approximate 10% reduction in CD33 internalization in comparison to HL60-$CD33^{FL}$ cells. Taken together these results provide insight into the effect of the rs35112940 variant on GO efficacy and CD33 biology, corroborating our previous findings, and support the use of CD33 polymorphisms to guide patient selection for treatment with GO.

P167 Site-specific Dolasynthen ADCs demonstrate consistent exposure across a wide range of drug-to-antibody ratios. Kalli C. Catcott[1], Susan Clardy[1], Jack Sadowsky[2], Rebecca K. Rowntree[3], Naniye Malli Centibas[1], Ling Xu[1], Andy Polson[3], Kenneth Avocetien[4], Tyler Carter[5], Mark Nazzaro[6], Dokyong "DK" Kim[1], Thomas H. Pillow[3], Neelie Zacharias[3], Cong Wu[3], Jeffrey Zurita[1], Elizabeth Ditty[1], Stephen Bradley[1], Alex Uttard[1], Bingfan Du[1], William S. Sawyer[7], Doug Leipold[3], Gail Lewis Phillips[3], LiuLiang Qin[8], Kelly Slocum[1], Geoffrey Del Rosario[3], Ginny Li[3], Shang-Fan Yu[3], David Lee[1], Radha Iyengar[1], Marc Damelin[1], Dorin Toader[1], Timothy B. Lowinger[1]. [1]Mersana Therapeutics, Inc., Cambridge, MA, [2]Carmot Therapeutics, Berkeley, CA, [3]Genentech Inc., South San Francisco, CA, [4]Gritstone Bio, Emeryville, CA, [5]Elektrofi, Newburyport, MA, [6]Takeda Therapeutics, Cambridge, MA, [7]Genentech Inc., South San Francisco, MA, [8]Alnylam Pharmaceuticals, Inc., Cambridge, MA.

Key defining attributes of an antibody-drug conjugate (ADC) include the choice of targeting antibody, linker, and the drug-to-antibody ratio (DAR). The choice of DAR, within the constraints of acceptable physicochemical properties for the given platform, is a function of balancing delivery of sufficient payload to targeted cells with the ability to achieve sustained in vivo exposures. Previous reports have described lower DAR mc-VC-MMAE conjugates, DAR = 1-2, that demonstrated higher in vivo exposure and lower clearance when compared to higher DAR (e.g. 4-8) counterparts. In theory, high DAR conjugates may be especially desirable when targeting low antigen expressing tumors or when lower potency payloads are used, as each binding and internalization event results in greater payload delivery. Here we report a systematic exploration of DAR across a much wider range than has been previously reported, by combining THIOMAB® protein engineering technology with the Dolasynthen platform. Homogeneous, site-specific ADCs spanning a discrete range of DARs – 2, 4, 6, 12, and 18 – were made by conjugation of Trastuzumab IgG_1 THIOMAB constructs with 1, 2, or 3 engineered cysteines to monomeric or trimeric Dolasynthen. The cytotoxicity of the resulting well-defined ADCs was assessed in vitro in cell lines with high or low expression of HER2 antigen. Pharmacokinetic data for all test articles in mice were generated in tumor bearing mice. In high HER2 expressing cell lines, in vitro cytotoxicity by payload was comparable across DARs. In a lower HER2 expressing system, the higher DAR ADCs performed better. In vivo, our data demonstrated comparable pharmacokinetics for the Dolasynthen conjugates across all DARs. These results illustrate the utility of a DAR ranging platform, such as Dolasynthen when evaluating ADCs as it enables the interrogation of a range of antibody and payload dosing regimens.

P168 Pretargeted radioimmunotherapy using ^{225}Ac for intraperitoneal Her2-expressing epithelial ovarian carcinoma xenografts. Sebastian K. Chung, Christopher S. Chandler, Daniela Burnes Vargas, Shin H. Seo, Michael R. McDevitt, Darren Veach, Blesida Punzalan, Xu Hong, Hong-fen Guo, Garrett M. Nash, Andrea Cercek, Nai-Kong V. Cheung, Steven M. Larson, Sarah M. Cheal. Memorial Sloan Kettering Cancer Center, New York, NY.

Objectives Epithelial ovarian carcinoma (EOC) is a common and lethal gynecologic malignancy that frequently presents as advanced staged disease, such as peritoneal carcinomatosis (PC). We previously reported cures in BT-474 murine xenografts with Pretargeted Radioimmunotherapy (PRIT) using ^{177}Lu radiohaptens targeting Her2 via a bispecific antibody (BsAb). Here-in we report the use of PRIT with alpha emitter ^{225}Ac labelled PrDOTA to treat a PC model of SKOV3, a Her2+ cell line of EOC. ***Methods*** 6 wk old female athymic nude mice inoculated IP with 1E5 luciferase/GFP-transfected SKOV3 cells were separated into 5 groups (n=10). Treatment mice received 1 or 2 cycles of Anti-Her2-C825 BsAb + [^{225}Ac]PrDOTA (Her2-Targeted), at 14 and 21 days after inoculation, respectively. Control groups received Anti-Her2 BsAb only, Anti-GPA33 BsAb + [^{225}Ac]PrDOTA (Off-Targeted) or no treatment. On cycle day 1, the mice were injected IP with 0.25mg (1.19nmol) BsAb. On cycle day 2, 25µg (2.76nmol) CCA16-DOTAY clearing agent (CA) was given IV 22h from BsAb. Mice in therapy groups were injected IP with 1µCi (0.74-0.79nmol) [^{225}Ac]PrDOTA-Bn 4h after CA. Weekly weights and BLIs with IP cavity ROIs were obtained and normalized to the respective values for each mouse at week 0 pre-treatment. End points: weight loss >20% baseline, moribund, or severe abdominal distension. At 154 days, 15 surviving treatment and 1 untreated control mice were submitted for hematology and histopathology. ***Results*** Histologic cures and prolonged survival were demonstrated in treatment mice (17/20 at 133 days) as compared to control mice (12/27 at 133 d, Logrank $p<0.04$). 3 mice from control groups were excluded due to BLI values <50% background in the first 3 weeks, suggesting no tumor burden. Tumors, as measured by normalized BLI values (nBLI), regressed in treatment mice when compared to control mice (2-way ANOVA $p<0.01$). nBLI values between treatment mice (1 and 2 cycles) and control mice (BsAb only, Off-target, no treatment) diverged at week 10 (Tukey's test $p<0.01$). There was no difference in nBLI values between mice treated with 1 or 2 cycles of targeted PRIT (Tukey's test $p>0.05$; all weeks). BLI of mice treated with 1 and 2 cycle of Her2 PRIT decreased 47% when compared to baseline within 1 week (T test $p=0.04$), suggesting treatment effects as early as 1 week. There were no differences in weights when compared to baseline (2-way ANOVA $p>0.05$). While the untreated mouse had high peritoneal adenocarcinoma tumor burden, there was no histologic evidence of viable neoplasia in 15/15 submitted treatment mice. Treatment mice had moderate renal tubular degenerative lesions on histology, but this did not affect renal function based on serum BUN or Cr. All hematologic parameters were within normal limits for treated mice. ***Conclusions*** 1 and 2 cycles of [^{225}Ac]PrDOTA-PRIT against Her2 resulted in histologic cures and prolonged survival in IP SKOV3 xenografts with minimal toxicity. The anti-Her2 PrDOTA-PRIT system is a promising theranostic approach for otherwise incurable PC.

P169 Dynamic cell-level modeling of antibody binding and internalization for radiosensitivity assessements in alpha-emitter radiopharmaceutical therapy. Remco Bastiaannet, Ioanna Liatsou, Robert Hobbs, George Sgouros. Johns Hopkins University School of Medicine, Baltimore, MD.

The radiobiological response to alpha-emitter radiopharmaceutical therapy (αRPT) is often studied in cell monolayers. The geometric models which are currently used to estimate the relationship between isotope activity concentration and cell survival probability typically idealize cells as spheres without considering cellular biological processes. This results in inaccurate and non-generalizable results, which could hamper the rigorous study of the underlying radiobiology. The purpose of this study was to create accurate absorbed dose models by combining Monte Carlo simulations with 3D measurements of cell clustering and geometries, as well as dynamic carrier molecule binding and trafficking in individual cells of cell monolayers. This allows for a more accurate way to model cell survival in these αRPT experiments. Experimental conditions of previous cell survival experiments with ^{212}Pb on NT2.5 HER2+ breast cancer cells were replicated. Live cells were imaged on a confocal microscope. Nuclei were stained with Hoechst and the media was stained with labelled dextran, creating a negative template of the cells. A relevant antibody (Ab) was tagged with AF488. 3D time lapses of membrane binding kinetics and internalization were recorded. Photobleaching was modelled and corrected for. All cells were segmented into nuclei, membrane and cytosol compartments using a purpose-build algorithm. The temporal antibody signals were used to fit pharmacokinetic models, which enabled interpolation and validation with experimental binding assays. The segmentations were used in a Monte Carlo code. S-values for every compartment and time frame were calculated using the Ab distribution directly, capturing the effect of Ab trafficking. Absorbed doses were calculated for each cell and were used to model previously obtained cell survival curves. Statistics were calculated for >100 cells. We observed a large range in absorbed doses (coefficient of variation 0.74). Absorbed doses to the nucleus per unit decay on the membrane, which are mainly determined by cellular geometries, agreed with the geometric model (error <6%). S-values for intercellular decays increased >50% over time, which corresponds to perinuclear trafficking of Abs. The dose contribution of neighboring cells was high (46% of total dose; 6x geometric model), highlighting the importance of cell clustering. Applying this to previous cell survival data yielded an estimated radiosensitivity kappa of 7.1 (geometric model: 2.8). Cell clustering has a larger, and cell geometry has a smaller impact than is assumed in current models. Perinuclear trafficking of internalized Ab positively impacts cell nucleus absorbed dose, which is typically ignored. Dose variability should be included in radiosensitivity modeling. We intend to use such rigorous and highly detailed, cell-level analyses to arrive at simplifications that are generalizable and whose accuracy is better understood. For example, based on our findings a better accounting of cell clustering would substantially improve geometric model calculations.

P170 SpliceCore® a platform for identifying aberrant alternative splicing in triple negative breast cancer for novel therapeutic development. Miguel A. Manzanares[1], Dhingra Priyanka[2], Kendall Anderson[1], Vanessa Frederick[1], Adam Geier[1], Alyssa Casill[1], Martin Akerman[1], Gayatri Arun[1]. [1]Envisagenics Inc., New York, NY, [2]Envisagenics Ins., New York, NY.

Splicing dysregulation is a major hallmark of cancer, affecting tumor progression, metastasis, and therapy resistance. Multiple studies have demonstrated the oncogenic activity of specific cis splicing errors and trans-acting splicing factor misregulation in patient tumors. As such, cancer-associated splicing dysregulation is a novel source of clinically actionable biomarkers and therapeutic targets, particularly for treatment insensitive cancers such as TNBC. Envisagenics has developed SpliceCore, an innovative cloud-based software platform that integrates machine learning (ML) algorithms with high performance computing to analyze large RNA-seq datasets to predict biologically relevant, novel, and highly prevalent tumor specific alternative splicing (AS) changes. Using SpliceCore, we have analyzed >2500 RNAseq samples from different breast cancer subtypes as well as normal breast tissue and identified several AS targets with a potential to translate into therapeutic candidates for TNBC. Here, we will discuss one of our most promising AS targets; it is present in 60.5% of TNBC patients and correlates with poor overall survival. Using SpliceCore, we predicted and designed an optimal set of splice switching oligos (SSO) that can efficiently switch the skipping isoform to an inclusion isoform in TNBC cells. Detailed mechanism of action studies of isoform switching by SSO-0205 have demonstrated the critical role of the isoform in a TGFβ dependent tumor progression mechanism. Pretreatment of the TNBC cells with SSO-0205 24 hours before TGFβ pathway activation reversed the cell proliferative response associated with it. This resulted in a strong inhibition of p21 gene expression, accompanied by a 50% decrease on the number of cells in G2, the mitotic phase of the cell cycle, and 40% decrease on cell viability. Additionally, migratory response induced by TGFβ in TNBC cells was also significantly inhibited by SSO-0205 pretreatment, which downregulated ANGPTL4 gene expression followed by a 55% decrease in cell migration. Together, we provide experimental proof of concept to demonstrate SpliceCore's ability to discover novel disease specific AS targets and design splice correcting oligonucleotides for subsequent correction and therapeutic development. Using this approach, we were able to uncover a novel therapeutic target for TNBC, whose aberrant splicing contributes to TNBC pathogenesis by promoting an overactivation of the TGFβ pathway. Reversal of this aberrant TNBC specific splicing using SSOs represent a new and promising therapeutic approach that will have a significant impact on TNBC treatment and clinical care.

P171 Ligand-displaying-exosomes using RNA nanotechnology for targeted delivery of multispecific drugs for liver cancer regression. Xin Li[1], Satheesh Ellipilli[1], Hongzhi Wang[1], Wen-Jui Lee[2], Yuan Soon Ho[2], Peixuan Guo[1]. [1]The Ohio State University, Columbus, OH, [2]Taipei Medical University, Taipei, Taiwan (Greater China).

Liver cancer such as hepatocellular carcinoma (HCC) poorly responds to chemotherapeutics as there are no effective means to deliver drugs to liver cancer due to the intrinsic drug effluxion and detoxification properties of the liver. Exosomes are extracellular vesicles that deliver payload via a fusion mechanism to cell cytosol without endosome trapping. Here we report the use of GalNAc as ligands to decorate exosomes that loaded with anticancer therapeutics Paclitaxel (PTX) and miR122 that silence both the drug efflux pump MDR1 (multidrug-resistant protein-1) and the oncogenic gene ADAM10 (A Disintegrin and metalloproteinase domain-containing protein 10). GalNAc is the ligand that provides exosome targeting ability to bind the hepatocyte surface receptor ASGP-R (asialoglycoprotein-receptor) usually express on liver cancer cell surface abundantly. PTX is highly hydrophobic, when it is conjugated to a polymeric hydrophilic RNA as a prodrug, its water solubility increases 32000-fold. No toxicity was detected. The PTX conjugated RNA strands assembled into a 4-way junction RNA nanoparticle to harbor 24-copies of PTX and 1-copy of miR122. The 4WJ RNA nanoparticles are loaded into the exosome and are decorated with GalNAc as a targeting ligand using RNA nanotechnology. Binding studies demonstrated that the multispecific exosome selectively binds and internalizes into the HepG2 liver cancer cells, and delivers the PTX and miR122 into the cell cytosol, exhibiting the highest efficacy in killing the targeted cancer cells due to the multispecific effect comes from miR122, PTX, GalNAc, and Exos. The liver cancer tumor regressed significantly after systemic injection of the multispecific exosomes to mice xenograft-bearing liver cancer. The results demonstrate that exosomes can serve as a multivalent vector to carry ligands, RNAi, and chemical drugs as a multispecific strategy to treat incurable liver cancers.

P172 Rubber- and amoeba-like RNA nanoparticles facilitate drug delivery for lung cancer. Xin Li[1], Satheesh Ellipilli[1], Hongzhi Wang[1], Wen-Jui Lee[2], Yuan Soon Ho[2], Peixuan Guo[1]. [1]The Ohio State University, Columbus, OH, [2]Taipei Medical University, Taipei, Taiwan (Greater China).

Lung cancer is the leading cause of cancer death in the United States. Here we report the utilization of the special rubber- and amoeba-like property of RNA nanoparticles to deliver two kinds of anticancer drugs for highly efficient regression of lung cancer. Conjugation of the chemical drugs to RNA nanoparticle enhance the solubility of the drug by 32,000-fold. 5% of the RNA/drug complexes reached the tumor within 30 minutes after systemic injection, and those that did not reach the tumor were excreted from the kidney and found in urine 30 minutes after IV injection. RNA nanoparticles loaded with drugs significantly inhibited the tumor growth in the A549 xenograft mouse model without the need for targeting ligand. This could be explained by several factors, such as the deformative property, the longer circulation time, the passive accumulation, and the quick renal excretion time of the RNA/drug complex.

P174 YAP1 drives immune suppression in urothelial carcinoma of bladder. Pritam Sadhukhan[1], Mingxiao Feng[1], Emily Illingworth[1], Ido Sloma[2], M. Talha Ugurlu[1], Fenna Sille[1], Woonyoung Choi[1], David J. McConkey[1], Mohammad Hoque[1]. [1]Johns Hopkins University, Baltimore, MD, [2]Champions Oncology, Inc., Hackensack, NJ.

YAP1 is a principal component of the Hippo pathway and is considered as one of the critical regulators of tumorigenesis. Recent studies indicate that YAP1 can suppress the antitumor immunity and influence the differentiation of regulatory T cells (Tregs) by inducing TGFβ/ SMAD pathway, but the mechanisms of YAP1 regulated modulation of tumor immune microenvironment is still in its infancy. Most importantly no study has been reported in YAP1 associated immune suppression in UCB. Here, we used syngeneic mouse models with three different mouse bladder cancer cells (MB49, UPPL595 and BBN 975) and demonstrated that YAP1 amplification upregulates the expression of IL-6 in tumor cells *in vitro* and *in vivo*, which in turn directly induces immunosuppressive tumor microenvironment (TME). YAP1 expression in cancer cells significantly induces the infiltration of MDSCs and attenuates the CD8 T cells in tumor tissue. YAP1 upregulation also induces different CXCR2 associated ligands and influences the polarization of naive macrophages into M2 type or tumor associated macrophages. Additionally, YAP1 expression enhances cancer stemness properties in the cancer cells and induces the intracellular accumulation of lipid droplets, which may also be a major factor in the development of immunosuppressive TME. Our pilot in vivo studies also suggest that YAP1 downregulation in the tumor cells can stimulate antitumor adaptive immunity in the host and improve tumor immunogenicity, leading to tumor regression. Overall, our results demonstrated the key role of YAP1 in modulating the TME and establish a potential rationale for targeting YAP1 in combination with checkpoint inhibitors for treating UCB.

P175 Investigating inositol polyphosphate-4-phosphatase, type II (INPP4B) signaling and role in acute myeloid leukemia. Keyue Chen[1], Gizem E. Genc[2], John F. Woolley[1], Daniel K.C. Lee[1], Roberto J. Botelho[2], Leonardo Salmena[1]. [1]University of Toronto, Toronto, ON, Canada, [2]Ryerson University, Toronto, ON, Canada.

Acute myeloid leukemia (AML) is an aggressive clonal stem cell disorder characterized by invasion of bone marrow, impaired hematopoiesis and accumulation of functionally immature myeloblasts. Our previous studies demonstrated that the expression level of the lipid phosphatase, INPP4B, is associated with poor outcomes in AML. To gain an understanding of the role of *Inpp4b* in AML progression, we performed RNA sequencing on *Inpp4b*$^{+/+}$ and *Inpp4b*$^{-/-}$ MLL-AF9 leukemias. Analysis of RNA sequencing data revealed that a disproportionately large number of lysosomal gene transcripts are decreased in *Inpp4b*$^{-/-}$ leukemias; these include cathepsins and lysosomal specific proteases responsible for proteolysis. This discovery provided evidence supporting a potential role for INPP4B in the lysosomal pathway. To validate these findings, immunofluorescence staining of the lysosomal protein LAMP1 was performed, which revealed a greater number of lysosomes in U2OS cells overexpressing INPP4B, and a decrease in lysosome number upon siRNA knockdown of INPP4B. Subsequently the DQ-BSA staining was used to assess INPP4B's effect on lysosomal function. In both U2OS and the leukemia cells, we observed that INPP4B expression is positively associated with the proteolytic activity of lysosomes. Currently we are testing the role of INPP4B expression on sensitivity to lysosomal inhibitors; Lys05, Chloroquine, and Mefloquine. Overall, we aim to uncover how INPP4B expression in AML controls lysosomal mechanisms and biology and to test putative therapeutic strategies to exploit this pathway.

P176 Discovery and development of novel covalent inhibitors of the YAP-TEAD transcription activity. Shirley Guo, Cindy Huang, C. Glenn Begley, Michael J. Bishop, Ping Cao. BridGene Biosciences, Inc., San Jose, CA.

TEAD (TEA domain transcription factor) is a transcription factor. As a major effector of the Hippo signaling pathway, TEAD is reported to be responsible for cell proliferation, apoptosis, and stem cell self-renewal. Emerging evidence has shown that TEADs play a prominent role in the development, progression, and metastasis of cancers such as mesothelioma, liposarcoma, pancreatic, etc. Small-molecule inhibitors of TEADs could be a novel targeted therapy for multiple cancers. Using its proprietary chemoproteomic platform IMTAC^{TM} (Isobaric Mass Tagged Affinity Characterization), BridGene has screened its unique covalent library against live-cell proteomes. Three distinct series of hits, with MCS Tanimoto Coefficients at ~0.3 between any two series, were identified for TEAD proteins with nanomolar affinity and high proteomic selectivity. The covalent interactions between the hit and TEAD proteins were confirmed using orthogonal fluorescence-based experiments. Mass Spec analysis and mutagenesis experiments identified the site of modification within the central lipid (palmitate) binding pocket of TEAD1. Multiple novel and potent small molecule inhibitors were obtained with good druggability. Several functional assays were established to measure inhibition of TEAD-YAP transcription activity/disruption including Luciferase assay in MCF7-Luc, CTG (cell-titer Glo), and ELISA. When evaluated *in vitro*, BridGene's TEAD inhibitors exhibited anti-proliferative properties against NF2-deficient mesothelioma cell lines (NCI-H226, NF2-/-) with low nM IC_{50}, but had little effects on in NF2 wildtype cancer cell lines (NCI-H2452, NF2+/+) or HepG2 cell line. In an ELISA assay, BridGene's small molecules can potently disrupt TEAD-YAP protein-protein interaction in cells. In MCF7-TEAD1 luciferase reporter cells, compounds showed single-digit nM potency in disrupting TEAD downstream gene expression. BridGene is developing covalent TEAD inhibitors to treat multiple cancers that are dependent on TEAD activity, including mesothelioma, cholangiocarcinoma, liposarcoma, pancreatic cancer, etc. With their high potency and proteomic selectivity, these TEAD inhibitors can potentially act as single-agent therapy, or work in combination with other care agents to treat multiple cancers.

P177 NPEPPS regulates cisplatin-resistance and can be targeted to overcome treatment resistance in patient-derived bladder cancer tumoroids. Mathijs P. Scholtes[1], Maryam Akbarzadeh[2], Dan Theodorescu[3], James C. Costello[4], Tokameh Mahmoudi[5], Tahlita C.M. Zuiverloon[1]. [1]Department of Urology, Erasmus MC Cancer Institute, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands, [2]1) Department of Urology, Erasmus MC Cancer Institute, Erasmus University Medical Center Rotterdam 2) Stem Cell and Regenerative Medicine Center of Excellence, Tehran University of Medical Sciences, Rotterdam/Tehran, Netherlands, [3]1) Department of Surgery, Cedars-Sinai Medical Center, 2) Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, 3) Cedars-Sinai Samuel Oschin Comprehensive Cancer Institute, Los Angeles, CA, [4]1) Department of Pharmacology, University of Colorado Anschutz Medical Campus, 2) University of Colorado Comprehensive Cancer Center, University of Colorado Anschutz Medical Campus, Aurora, CO, [5]1) Department of Urology, Erasmus MC Cancer Institute, Erasmus University Medical Center Rotterdam, 2) Department of Pathology, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands.

Gemcitabine/cisplatin combination is the recommended neoadjuvant chemotherapy (NAC) for muscle-invasive bladder cancer (MIBC) patients undergoing surgical removal of the bladder (cystectomy). Patients without residual tumor at cystectomy ,complete pathological response (pCR), have a good 5-yr OS of 80%. However, due to NAC resistance, only 25% of the patients achieve a pCR. This study aims to increase the number of patients reaching pCR by discovery and functional interrogation of novel and pharmacologically targetable mechanisms causal to NAC resistance. We have identified an M1 aminopeptidase, NPEPPS, to regulate intracellular cisplatin import through volume regulated anion channels (VRACs). We show that genetic inhibition of NPEPPS increases the intracellular concentrations of cisplatin, hereby re-sensitizing gem/cis-resistant BC cell lines. On the other side, NPEPPS over-expression enhanced resistance to cisplatin. Pharmacological inhibition of NPEPPS with tosedostat results re-sensitization of gem/cis resistant BC cells *in vitro* and *in vivo*. Additional therapeutically validation was performed in gem/cis resistant patient-derived tumoroids. For this purpose, tumoroid cultures were generated from bladder cancer patients whom did not respond to pre-operative gem/cis, molecularly characterized and compared to the tumor of origin. Comparison of bladder cancer specific hotspot mutations, copy-number aberrations and H&E staining confirmed that patient-specific tumor traits were maintained in *ex vivo* tumoroid cultures. Moreover, tumoroid cultures generated from NAC-resistant patients were also resistant to cisplatin concentrations that exceeded physiological serum concentrations. Interestingly, addition of NPEPPS-inhibitor tosedostat sensitized these NAC resistant tumoroids to serum concentrations of cisplatin. These findings have potential for rapid translation into the clinic and invite trials investigating tosedostat to overcome chemoresistance.

P178 RBM39 degradation leads to exceptional responses in high-risk neuroblastoma by targeting the spliceosome. Jun Yang. St Jude Children's Research Hospital, Memphis, TN.

Genome-wide studies of thousands of human tumors have revealed dysregulated pre-mRNA splicing as a critical pathway in tumorigenesis, which may consequently create vulnerabilities for cancer cells. Indeed, C-MYC-dependent cancers are specifically susceptible to perturbation of spliceosome function. The MYC oncogenes (*C-MYC*, *MYCN*, and *MYCL1*), which are known transcriptional drivers of large numbers of genes, are among the most common genetic abnormalities in human cancer. We found that neuroblastoma, a MYC-driven cancer characterized by splicing dysregulation and spliceosomal dependency, requires splicing factor RBM39 for survival. Indisulam, a "molecular glue" that selectively recruits RBM39 to the CRL4-DCAF15 E3 ubiquitin ligase for proteasomal degradation, is highly efficacious against neuroblastoma, leading to exceptional responses in multiple high-risk disease models, without overt toxicity. Genetic depletion or indisulam-mediated degradation of RBM39 induces significant genome-wide splicing anomalies and cell death. Mechanistically, the dependency on RBM39 and high-level expression of DCAF15 determine the exquisite sensitivity of neuroblastoma to indisulam. Our data indicate that targeting the dysregulated spliceosome by precisely inhibiting RBM39, a vulnerability in neuroblastoma, is a valid therapeutic strategy. In summary, our study has validated the mechanism by which "molecular glues" targeting RNA binding proteins can be exploited for drug development in cancer, and has a broad impact on understanding of the splicing dependency of MYC-driven cancers and the identification of novel therapeutic approaches against them.

P180 Genetic screen identifies PDPK1 as a synergistic target to enhancing the efficacy of MEK1/2 inhibitors in *NRAS* mutant melanoma. Weijia Cai, Mai Nguyen, nicole wilski, timothy purwin, Manoela TiagodosSantos, Andrew Aplin. Sidney Kimmel Cancer Center, Philadelphia, PA.

Melanomas frequently harbor activating *NRAS* mutations; however, there has been little advance in targeted therapy options for *NRAS* mutant melanoma patients. MEK inhibitors (MEKi) showed modest efficacy in clinic, which is insufficient to be approved by FDA. In this study, we performed a genome-wide CRISPR/Cas9-based screening, identified PDPK1 (Phosphoinositide-dependent kinase-1) as a therapeutic target to enhancing the efficacy of MEKi, and validated it in various *NRAS* mutant melanoma cell lines via pharmacological and genetic approaches. Combined inhibition of PDPK1 and MEK (PDPK1i+MEKi) profoundly inhibited *NRAS* mutant tumor growth in a xenograft model. Notably, the combinatorial treatment induced pyroptosis, and increased ratio of intratumoral CD8+ T cells, delayed tumor growth and prolonged survival in an immune competent allograft model whereas it showed a significantly weaker potency in an isogenic immune deficient model. These data suggest PDPK1i + MEKi is an efficient strategy against *NRAS* mutant melanoma in an immune-response-dependent manner. **Significance:** NRAS is still an 'undruggable' target in melanoma. Our discovery rationalizes the clinical development of PDPK1i plus MEKi in NRAS mutant melanoma patients and suggests further synergy of targeted therapy and immunotherapy.

P182 VRK1 is a novel synthetic lethal target in VRK2-methylated glioblastoma. Julie Shields[1], Samuel R. Meier[1], Justin Engel[1], Madhavi Bandi[1], Maria L. Dam Ferdinez[1], Wenhai Zhang[1], Shan-chuan Zhao[1], Minjie Zhang[1], Ashley Choi[1], Yi Yu[1], Xuewen Pan[1], Brian McMillan[1], Brett Williams[1], Robert Tjin Tham Sjin[1], Douglas Whittington[1], Erik Wilker[1], Alan Huang[1], Fang Li[2], Natasha Emmanuel[1]. [1]Tango Therapeutics, Cambridge, MA, [2]Allorion Therapeutics, Natick, MA.

Synthetic lethality — the genetic interaction that causes cell death when two genetic deficiencies co-occur but cell survival when each deficiency occurs alone — can be co-opted for cancer therapeutics. A pair of paralog genes is among the most straightforward synthetic lethal interactions by virtue of their redundant function in the cell. Here we demonstrate a paralog synthetic lethality by targeting VRK1 in VRK2-methylated glioblastoma multiforme (GBM). VRK2 is silenced by methylation in about two-thirds of GBM, an aggressive cancer with few available targeted therapies. Genetic knockdown of VRK1 in VRK2-null or VRK2-methylated cells leads to cell death via cell cycle arrest in G2M and subsequent DNA damage. The lethality is dependent on the kinase activity of VRK1 and is rescued by ectopic VRK2 expression. Knockdown of VRK1 leads to robust tumor growth inhibition in VRK2-methylated glioblastoma xenografts *in vivo*. These results suggest that inhibiting VRK1 kinase activity could be a viable cancer therapeutic in VRK2-methylated glioblastoma.

P183 CRISPR screens identify sensitizers to trametinib in KRAS mutant cancer cell lines. Silvia Fenoglio, Aileen M. Cristo, James Tepper, Teng Teng, Samuel R. Meier, Ashley Choi, Hongxiang Zhang, Shan-chuan Zhao, Shangtao Liu, Leanne G. Ahronian, Daniel Aird, Nikitha M. Das, Yi Yu, Robert Tjin Tham Sjin, Jannik N. Andersen, Alan Huang, Fang Li, Xuewen Pan. Tango Therapeutics, Cambridge, MA.

KRAS is the most frequently mutated oncogene in human tumors and drives tumorigenesis across multiple lineages. Specifically, KRAS is mutated in about 30% of lung cancer and over 90% of pancreatic cancer. Despite recent progress in developing mutant selective $KRAS^{G12C}$ inhibitors, patient treatment options for RAS activated cancers remain very limited. Targeting the MAPK pathway by means of MEK and ERK inhibitors has been explored as an alternative strategy for KRAS mutant cancer. However, the clinical benefit is modest due to drug resistance caused by reactivation of the MAPK pathway and potentially other pathways and/or bypass mechanisms. Here we used CRISPR-based screens to identify potential combination therapy targets to enhance trametinib response in KRAS mutant cancers. We screened in five KRAS mutant cell lines of lung and pancreatic lineages and identified both known as well as novel modulators of MEK inhibitor response. Consistent with previous reports, gene knockouts that impair the reactivation of ERK downstream of MEK inhibition scored as top hits in our drop-out screens. Knocking out MAPK1, RAF1, BRAF and PTPN11 sensitized all five cell lines to trametinib treatment supporting these as candidate drug combination targets for MAPK pathway inhibition. Our screens also identified multiple genes within the heparan sulfate pathway (EXT1, EXT2, EXTL3, XYLT2, ALG6, B3GAT3, B4GALT7 and HS2ST1) and the MAPK7 pathway (MAPK7 and MAP2K5) that sensitize multiple but not all cell lines to trametinib. As part of our target discovery platform, we further validated these screening results using various phenotypic assays. Altogether, our results suggest that resistance to MEK inhibitors is driven by reactivation of the MAPK pathway as previously demonstrated and that impairing such reactivation restores the sensitivity of KRAS mutant cancer cells to trametinib. The genetic mechanisms driving the MAPK pathway rebound are likely different in different cancers and understanding such mechanisms will be key for achieving clinical success.

P184 Anti-tumor efficacy of an MMAE conjugated antibody targeting cell surface TACE/ADAM17-cleaved Amphiregulin in breast cancer. Kristopher A. Lofgren[1], Sreeja Sreekumar[1], Edmund C. Jenkins[2], Kyle J. Ernzen[1], Paraic A. Kenny[1]. [1]Gundersen Medical Foundation, La Crosse, WI, [2]Albert Einstein College of Medicine, Bronx, NY.

The Epidermal Growth Factor Receptor ligand, Amphiregulin, is a key proliferative effector of estrogen receptor signaling in breast cancer and also plays a role in other malignancies. Amphiregulin is a single-pass transmembrane protein proteolytically processed by TACE/ADAM17 to release the soluble EGFR ligand, leaving a residual transmembrane stalk that is subsequently internalized. Here, we report the development of an antibody drug conjugate, GMF-1A3-MMAE, targeting an AREG neo-epitope revealed following ADAM17-mediated cleavage. The antibody does not interact with uncleaved Amphiregulin, providing a novel means of targeting cells with high rates of Amphiregulin shedding. Using fluorescent dye conjugation, we demonstrated that the antibody is internalized by cancer cells in a manner dependent on the presence of cell surface cleaved Amphiregulin. Antibodies conjugated with monomethyl auristatin E (MMAE) were cytotoxic in vitro and induced rapid regression of established breast tumor xenografts in immunocompromised mice. We further demonstrate that these antibodies recognize the Amphiregulin neo-epitope in formalin fixed paraffin embedded tumor tissue, suggesting their utility as a companion diagnostic for patient selection.

P185 Preliminary antitumor activity of MCLA-158, an IgG1 bispecific antibody targeting EGFR and LGR5, in advanced head and neck squamous cell carcinoma. Antoine Hollebecque[1], Irene Brana[2], Lara Iglesias[3], Caroline Even[1], Kato Shumei[4], Marc Díez García[2], Mateo Bover[5], Patricia Martin-Romano[1], Rocio Garcia-Carbonero[6], Guillen Argilés[7], Josep Tabernero[2], Rajan Khanna[8], Viktoriya Stalbovskaya[8], Jeroen Lammerts van Bueren[8], Kees Bol[8], Mohamed Bekradda[9], Andrew Joe[8], Ernesto Wasserman[8], Ezra E.W. Cohen[10]. [1]Gustave Roussy Cancer Campus, Villejuif, France, [2]Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain, [3]Hospital Universitario 12 de Octubre, Imas12, Madrid, Spain, [4]UC San Diego Health, San Diego, CA, [5]Hospital Universitario 12 de Octubre, imas12, Madrid, Spain, [6]Hospital Universitario 12 de Octubre, imas12, UCM, Madrid, Spain, [7]Vall d'Hebron Institute of Oncology (VHIO), Barcelona, France, [8]Merus NV, Utrecht, Netherlands, [9]Oncology Therapeutic Development, Clichy, France, [10]Moores Cancer Center, UCSD, San Diego, CA.

In the expansion part of an ongoing phase 1 study, MCLA-158 is being investigated at the recommended phase 2 dose (RP2D) in patients (pts) with advanced solid tumors, including head and neck squamous cell carcinoma (HNSCC). Epidermal growth factor receptor (EGFR) and WNT signaling are known oncogenic and mitogenic drivers in several cancers, including HNSCC. MCLA-158 is a human common light chain IgG1 bispecific antibody with enhanced antibody-dependent cellular cytotoxicity (ADCC) activity. It targets EGFR and the leucine-rich, repeat-containing, G-protein coupled receptor 5 (LGR5), a transmembrane receptor associated with tumor initiating cells, particularly cancer stem cells. Potent antitumor activity was seen with MCLA-158 in pt-derived HNSCC xenograft models. The RP2D of MCLA-158 was determined to be 1500 mg every 2 weeks (q2w), with 4-week cycles, during the dose escalation part of the study, based on safety, PK and receptor occupancy prediction. A maximum tolerated dose was not reached. The primary objective of the expansion part is to characterize the safety and tolerability of single-agent MCLA-158 and confirm the RP2D. Secondary objectives include assessment of antitumor activity (investigator-assessed overall response rate [ORR] per RECIST 1.1 and duration of response). Key eligibility criteria include prior exposure to standard therapy, ECOG performance status (PS) 0-1, measurable disease (RECIST 1.1), and availability of a baseline tumor biopsy. At the interim data cutoff date of 15 June 2021, 7 pts with advanced recurrent/metastatic HNSCC were enrolled and treated in the expansion phase. Median age was 63 years (range 50-74), ECOG PS 0/1: 2/5. Primary tumor locations were oropharynx (2 pts), hypopharynx (1 pts), larynx (3 pts), and unknown primary (1 pt). All pts had a histology of squamous cell carcinoma. Prior treatment included platinum-based chemotherapy in all pts, and anti-PD-1/PD-L1 in 6 pts. No pts received prior cetuximab. A median of 3 treatment cycles (range 1-8) were administered to the 7 pts, 4 of whom were continuing with therapy at the cutoff. Of the 5 pts who had a postbaseline assessment, 2 had confirmed partial responses (5+ and 8 cycles initiated), and 2 pts had stable disease (reduction in the sum of target lesions of 7% and 17%; 4+ and 5 cycles initiated, respectively). Scheduled first postbaseline tumor assessments for 2 pts occurred after the data cutoff date. Among 26 pts who were treated at the RP2D in the dose escalation and expansion cohorts, the most frequent adverse events regardless of causality (all grades/grade 3) were infusion-related reactions (73%/8%), rash (39%/0%), asthenia (35%/4%), decreased appetite (27%/4%), nausea (27%/4%), and acneiform dermatitis (23%/4%). There were no treatment-related grade 4 or 5 adverse events. In conclusion, MCLA-158 shows promising signs of antitumor activity in pretreated HNSCC, and a well-tolerated and favorable safety profile.

P186 Preclinical evaluation of the proteasome inhibitor ixazomib (MLN2238) on nasopharyngeal cacinoma (NPC). Ka Yee Li, Victor Ho Fun Lee, George Sai Wah Tsao. The University of Hong Kong, Hong Kong, Hong Kong (Greater China).

Background: Nasopharyngeal carcinoma (NPC) is a common cancer in the South Asia and is closely associated with EBV infection. The standard treatment for newly diagnosed NPC is radiotherapy alone or in combination with adjuvant chemotherapy and there is currently no targeted therapy available for NPC. About 30% of the NPC patient will progress within 5 years after treatment, so study on new therapeutic agents is in need. Proteasome inhibitor is emerged as an effective antitumor therapeutic agent in both haematological and solid tumour. Studies have reported that proteasome inhibitor can inhibit nuclear factor-kB (NF-kB) signalling pathway which was found constitutively activated in NPC. Here, we examined the antiproliferation effect and molecular mechanisms of the second-generation, selective and reversible proteasome inhibitor ixazomib (MLN2238) on NPC *in-vitro* and *in-vivo*. Ixazomib is the first oral proteasome inhibitor approved by the FDA for multiple myeloma treatment and has shown less side effect and more selective to proteasome than the first-generation proteasome inhibitor, bortezomib. **Method**: Three human EBV positive NPC cell lines (C666-1, NPC43 and C17) and two PDXs (Xeno113 and Xeno76) PDXs were used. **Results**: In our study, we showed that Ixazomib can inhibit cell viability and proliferation in EBV-associated NPC cells C666-1, NPC43 and C17 in a time- and dose-dependent manner. The IC50 of 72h treatment of ixazomib in NPC cells is 11-40nM which is more sensitive than other solid tumour, e.g. hepatocellular carcinoma (260-428nM) and breast cancer (43-1007nM). Induction of apoptosis by ixazomib was confirmed by flow cytometry analysis of Annexin V staining and western blotting of caspase-3/9 activation and PARP cleavage. S and G2/M cell cycle arrest was observed after Ixazomib treatment, which is demonstrated by flow cytometry analysis of PI staining. Evaluation of the expression level of EBV lytic genes by RT-qPCR and western blot showed that Ixazomib associates with EBV lytic cycle activation in NPC. Consistent with the previous studies, inhibition of canonical NF-kB signalling pathway was seen after 12h of ixazomib treatment in NPC cells. In animal study, 4 or 8mg/kg of ixazomib was administered orally 2 times per week for 3 weeks on NPC cells/PDX transplanted BALD/c-nu/nu and NOD/SCID mice and all showed statistical significant inhibition on NPC growth. Combination treatment of ixazomib and cisplatin showed additive effect in both *in-vitro* and *in-vivo* studies. **Conclusions**: Ixazomib is a potential therapeutic agent for NPC treatment and the preclinical data supports further evaluation and clinical trial of ixazomib for NPC.

P190 Safety and efficacy of neoadjuvant intravesical oncolytic MV-NIS in patients with urothelial carcinoma. Tanner S. Miest[1], Bradley Leibovich[2], Stephen Bardot[3], Paul R. Young[4], Stephen A. Boorjian[2], Mark Gonzalgo[5], Loren Herrera-Hernandez[2], Matthew K. Tollefson[2], Jeffrey Karnes[2], Paige Nichols[2], Tessa Kroeninger[2], Rachel Graham[3], Carole Lahana[5], Monica Reckner[6], Alysha Newsom[2], Nandakumar Packiriswamy[7], Janice Anoka[6], Kah Whye Peng[2], Erol Wiegert[6], Alice Bexon[8], Shruthi Naik[7]. [1]Mayo Clinic Rochester and University of Texas, Houston, TX, [2]Mayo Clinic Rochester, Rochester, MN, [3]Ochsner Clinic, New Orleans, LA, [4]Mayo Clinic Jacksonville, Jacksonville, FL, [5]University of Miami, Miami, FL, [6]Vyriad Inc., Rochester, MN, [7]Vyriad Inc. and Mayo Clinic Rochester, Rochester, MN, [8]Vyriad, Inc., Rochester, MN.

Background: Bladder cancer is a leading cause of cancer death in the United States[1]. The histology in > 90% of cases is urothelial carcinoma (UC). Tumors may present either as non-muscle-invasive (NMIBC) or muscle-invasive disease (MIBC). Current standard of care for patients with high risk NMIBC includes transurethral resection of bladder tumor (TURBT) followed by intravesical immunotherapy with Bacillus Calmette-Guerin (BCG)[2]. Meanwhile, patients with BCG unresponsive NMIBC or MIBC are recommended to undergo radical cystectomy (RC), which adversely impacts quality of life and is associated with significant morbidity[3]. MV-NIS is an investigational oncolytic measles virus with an excellent clinical safety profile[4]. This ongoing phase I clinical study is designed to test the safety, efficacy and identify the recommended phase 2 dose (RP2D) of intravesical MV-NIS in patients with NMIBC or MIBC who are scheduled for RC and not eligible for neoadjuvant chemotherapy. **Methods**: Bladder UC patients were evaluated for eligibility and provided informed consent prior to enrolling. To date 8 patients have been enrolled: 4 to the single dose safety cohort, and 4 to the multi-dose expansion cohort. Patients were administered intravesical ~$1x10^9$ $TCID_{50}$ MV-NIS once at least 1 week prior to RC (safety cohort), or twice at 4 and 2 weeks prior to RC (expansion cohort). Patients were closely monitored during the 2-hour instillation period. Tumor specimens from the pre-treatment TURBT and post-treatment RC were analyzed to determine pre- and post-treatment pathological stage and evaluate tumor killing and immune cell infiltrate. **Results**: Intravesical MV-NIS treatment was well tolerated in all patients. Only a single Adverse Event (AE) attributable to MV-NIS treatment (Grade 1 hematuria). AEs Grade>2 were related to post-surgical complications. Tumor downstaging was observed in 4 of 8 patients. Among 4 patients in the expansion cohort, 2 had no residual disease (pT0). Central assessment of RC tissues showed significant inflammatory infiltrate in all treated bladder specimens. Detailed analyses are ongoing to characterize MV infection and immune infiltrate in bladder tissue. **Conclusions**: The higher-than-expected rate of tumor downstaging and pT0 pathology, paired with the significant immune infiltrate observed in post-treatment bladder tissue, provide compelling evidence that intravesical MV-NIS has clinical activity against UC. These results support the use of two doses of ~$1x10^9$ $TCID_{50}$ as the RP2D in future clinical studies for BCG unresponsive NMIBC or MIBC patients. MV-NIS induced inflammation may act synergistically with checkpoint blockade therapies. **References**1. Siegel, R.L., Miller, K.D. & Jemal, A. *CA Cancer J Clin* **69**, 7-34 (2019). 2. Knowles, M.A. & Hurst, C.D. *Nature reviews. Cancer* **15**, 25-41 (2015). 3. Zakaria, A.S., *et al. Can Urol Assoc J* **8**, 259-267 (2014). 4. Galanis, E., *et al. Cancer research* **75**, 22-30 (2015).

P191 The combination of CD16A/EGFR bispecific innate cell engager AFM24 with SNK01 NK cells promotes efficacious targeting and killing of EGFR$^+$ tumor cells. Jens Pahl[1], Ting-Ying (Hazel) Cheng[2], Uwe Reusch[1], Arndt Schottelius[1], Wolfgang Fischer[1], Yong Man Kim[2], Stephen Chen[2], Joachim Koch[1]. [1]Affimed GmbH, Heidelberg, Germany, [2]NKGen Biotech Inc, Santa Ana, CA.

The epidermal growth factor receptor (EGFR) is an overexpressed antigen on several solid cancers resulting in uncontrolled cell proliferation. Current EGFR-targeting agents such as tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs) mainly rely on EGFR signaling inhibition which is challenged by limited efficacy due to dose limiting toxicity, and/or intrinsic or acquired resistance of the tumor by e.g., KRAS or BRAF mutations. To overcome these limitations and to address the high medical need across EGFR-positive cancers, we investigate a unique approach of combining AFM24, a tetravalent bispecific EGFR- and CD16A-binding innate cell engager (ICE), with the patient-specific NK cell product SNK01. AFM24 mediates high affinity targeting and ADCC of EGFR+ tumor cells by NK cells, independent of EGFR signaling inhibition and irrespective of the mutational status of the tumor. AFM24 is currently being tested as monotherapy in a Phase 1/2a study in patients with EGFR-positive tumors. SNK01 is a first-in-kind, autologous non genetically modified NK cell product with significant anti-tumor cytotoxicity and high and homogeneous expression of CD16A, NKG2D, NKp46 and DNAM-1 , that can be consistently produced even from heavily pre-treated cancer patients. SNK01 showed activity against numerous solid tumors in preclinical studies and in the phase I trials. In order to investigate the synergistic potential of this unique combination, SNK01 cells were generated from PBMCs of healthy individuals and their functional activation by AFM24 in response to EGFR-positive A-431 cells was assessed in cytotoxicity assays and flow cytometry-based CD107a (marker for NK cell degranulation) and IFN-γ mobilization assays. AFM24 binding to and CD16A expression on SNK01 cells was measured by flow cytometry. Both, binding of AFM24 and expression of CD16A were detected on nearly 100% of SNK01 cells, indicating saturation of CD16A-positive SNK01 cells with AFM24. In the presence of AFM24, lysis of EGFR-positive A-431 cells by SNK01 cells was significantly enhanced. Likewise, in response to AFM24, upregulation of CD107a and IFN-γ expression by SNK01 cells was significantly increased upon cross-linking to A-431 cells. By contrast, AFM24 alone did not lead to significant NK cell activation in the absence of target cells or vice versa. Hence, activation of SNK01 cells was strictly dependent on AFM24-mediated cross-linking of SNK01 cells with target cells. In conclusion, this combination holds promise to direct the AFM24-augmented anti-tumor activity of SNK01 cells to EGFR-positive tumor cells independent of EGFR signaling pathway mutations thereby providing a potential new treatment option for underserved patient populations. An IND for a Phase I/II combination trial of AFM24 and SNK01 has been cleared and first-patient-in is on track for the second half of 2021.

P192 Comprehensive preclinical characterization of the mechanism of action of EPI-7386, an androgen receptor N-terminal domain inhibitor. Nan Hyung Hong[1], Shihua Sun[2], Peter Virsik[1], Alessandra Cesano[1], Elahe A. Mostaghel[3], Stephen R. Plymate[2], Berenger Biannic[1], Han-Jie Zhou[1], Ronan Le Moigne[1]. [1]ESSA Pharma, South San Francisco, CA, [2]University of Washington, Seattle, WA, [3]VA Puget Sound Health Care System, Seattle, WA.

Background: Androgen receptor (AR) signaling is a main driver of prostate cancer progression and remains a crucial target for therapeutic intervention even in late stages of the disease. While current anti-androgen therapies targeting directly or indirectly the AR ligand binding domain (LBD) are initially effective, resistance ultimately develops, and new methods of inhibiting the AR pathway are needed. The selective targeting of the N-terminal domain (NTD) of the AR represents a novel method of blocking AR signaling to by-pass LBD-related resistance. EPI-7386 is a potent and metabolically stable NTD inhibitor (aniten) currently in a phase 1 dose-escalation study in mCRPC patients (NCT04421222). Here we further characterized the binding to AR NTD and the mechanism of action of EPI-7386. **Methods:** Target engagement was measured by Cellular Thermal Shift Assay (CETSA) and two-dimensional Nuclear Magnetic Resonance (2D NMR) spectroscopy. The potency and selectivity of EPI-7386 was determined in cellular models expressing different forms of AR using reporter and cell viability assays. qPCR, NanoString, and RNA sequencing were used to explore the activity of EPI-7386 on the AR transcriptome. To determine the effect of EPI-7386 on AR genomic occupancy, Chromatin immunoprecipitation sequencing (ChIP-seq) was performed. **Results:** We confirmed target engagement of EPI-7386 with an LBD truncated AR variant by CETSA using a cell line which expresses only AR-V567es, suggesting the interaction of EPI-7386 with AR NTD. In the same cell line, AR antagonist enzalutamide that binds to AR LBD showed no target engagement with AR-V567es. Furthermore, 2D NMR study results demonstrate an interaction of EPI-7386 with amino acid residues located in the transcription activation unit 5 (Tau-5) region of the AR NTD, a region which has been described to be involved in interactions with transcriptional cofactors such as CBP/p300. EPI-7386 strongly impaired the transcriptional activity and gene expression driven exclusively by LBD truncated AR variants including AR-V567es and AR-V7 and decreased cell viability. EPI-7386 has been shown to suppress the AR regulated transcriptome and the combination of EPI-7386 with lutamides resulted in broader and deeper inhibition of AR-regulated gene expression. The analysis of the AR cistrome by ChIP-seq showed that EPI-7386 displaces genome-wide androgen induced AR binding and the combination with enzalutamide completely abrogated AR binding. **Conclusion:** EPI-7386 is a potent AR NTD inhibitor that has the capacity to by-pass AR LBD resistance mechanisms to current anti-androgen therapies by uniquely inhibiting AR-mediated signaling. The agent has the potential for providing clinical benefit as a single agent in patients whose tumors are progressing on anti-androgens or in combination with current anti-androgens in earlier line patients.

P193 AMX-818, a novel prodrug HER2-XPAT T-cell engager (TCE) with potent T cell activation, proteolytic cleavage and efficacy in xenograft tumors, and wide safety margins in NHP (Non Human Primate). Milton To, Pete Yeung, Michael Fox, Mikhail Hammond, Fiore Cattaruzza, Ayesha Nazeer, Caitlin Koski, Lucas Liu, Sina Khorsand, Deena Rennerfeldt, Kari Morrissey, Zachary Lange, Ming Dong, Sharon Lam, Mika K. Derynck, Bryan A. Irving, Volker Schellenberger. Amunix, South San Francisco, CA.

Background: TCEs are effective in leukemias, but have been limited in solid tumors due to *on*-target, *off*-tumor toxicity. Attempts to circumvent cytokine release syndrome (CRS), including step-up dosing and complex designs, have had limited success due to toxicity and immunogenicity. AMX-818, HER2-**XPAT,** or **X**TENylated **P**rotease-**A**ctivated **T** Cell Engager, is a prodrug TCE that exploits dysregulated protease activity present in tumors to expand the therapeutic index (TI). The core of the molecule (PAT) consists of 2 tandem scFvs targeting CD3 and HER2. Attached to the core are two unstructured polypeptides (XTEN) that function as universal masks, sterically reduce target engagement and extend T1/2. Protease cleavage sites at the base of the XTEN masks enable proteolytic activation of XPATs in the tumor microenvironment, releasing a potent TCE with short T1/2. Methods: Nonclinical studies were conducted with HER2-**XPAT** or AMX-818, unmasked HER2-**PAT**, HER2-NoClvSite (no protease linkers) or the fluorescent labeled counterparts *in vitro* and *in vivo* xenografts and NHP. Results: The unmasked HER2-**PAT** demonstrated potent *in vitro* T cell cytotoxicity (EC50 1-2pM), target-dependent T cell activation and cytokine production by hPBMCs (human mononuclear cells). HER2-**XPAT** demonstrated 14,000-fold protection against killing of HER2 tumor cells, and minimal cytotoxicity against cardiomyocytes up to 1uM. *In vivo*, HER2-**XPAT** or AMX-818 induced complete tumor regressions (CRs) in HER2+ BT-474 tumors (similar to effect with equimolar doses of HER2-**PAT)**, whereas HER2-NoClvSite lacked efficacy, supporting the need for protease cleavage for activity. HER2-**XPAT** was also highly efficacious in the HER2 low-expressing HT-55 colorectal model. Labeled XPATs with the same protease cleavage sites in 9 different in vivo xenograft and PDX models demonstrated preferential unmasking into cleaved products in tumors in contrast to healthy organs, with average ~20% of drug in tumor cleaved to the fully active TCE form. In NHPs, AMX-818 caused early T-cell margination as low as 2mg/kg, but has been dose-escalated safely up to 42mg/kg (MTD) with no CRS. Doses of 0.5 to 42 mg/kg demonstrated dose proportional increases in HER2-XPAT exposure, with low to negligible levels of cleavage products present in systemic circulation. Continuous infusion of unmasked HER2-**PAT** induced lethal CRS and cytokine spikes at 0.3mg/kg/d but was tolerated at 0.2 mg/kg/d, providing AMX-818 with ~450-fold improvement in safety compared to HER2-**PAT**. Repeat-dose GLP toxicology studies have demonstrated that the highest dose evaluated (6 mg/kg) was the NOAEL, suggesting that HER2-XPAT has a wide TI based on predicted human PK and predicted efficacious exposures. Conclusions: AMX-818 is a potent, protease-activated prodrug TCE exhibiting preferential tumor unmasking, with minimal systemic unmasking and no CRS at high doses in NHP. AMX-818 represents a promising solution to widen the TI for TCE activity in HER2-high and HER2-low expressing tumors.

P195 Omacetaxine mepessucinate plus venetoclax show strong synergistic anti-leukemic activity in *nucleophosmin* (*NPM1*)-mutated AML patient-derived xenograft (PDX) models to support a phase 1 clinical trial. Federica Mezzasoma, Valeria Cardinali, Ilaria Gionfriddo, Francesca Milano, Sofia Sciabolacci, Alessio Ferrari, Marcella Sabino, Serenella Silvestri, Serena Donini, Valentina Tini, Giulio Spinozzi, Brunangelo Falini, Maria Paola Martelli. Hematology, University of Perugia, Perugia, Italy.

Despite different novel targeted drugs against specific genetic lesions have been developed for acute myeloid leukemia (AML) treatment, therapy in many AML subtypes should still rely on drugs possibly targetting alternative pathways essential for leukemia survival. Different biological and clinical evidences point to *nucleophosmin* (*NPM1*) gene mutations (the most frequent genetic lesion in AML) among strong predictors of sensitivity to venetoclax, a small molecule drug inhibitor of the B-cell leukemia/lymphoma-2 (Bcl-2) anti-apoptotic protein, highly expressed in most AML. Venetoclax appeared to be extremely efficacious in combination with hypomethylating agents (HMA) as first-line therapy, particularly in *NPM1*-mutated AML. Omacetaxine mepessucinate is a synthetic form of the plant cephalotaxine alkaloid homoharringtonine (HHT). HHT binds to the A-site cleft of ribosomes preventing the initial elongation step of protein synthesis and leading to a transient but profound inhibition of protein synthesis especially of those with a short half-life, including the antiapoptotic protein myeloid cell leukemia-1 Mcl-1, acting as mechanism of resistance following Bcl-2 inhibition. Besides its action as 'Mcl-1 inhibitor' (targeting its protein levels), we focused on HHT because i) it is a FDA-approved drug, in the setting of chronic myeloid leukemia (CML); ii) it showed some activity in clinical trials in AML; iii) it was shown to be a safe treatment, when evaluated also in association with chemotherapy, either standard intensive or low-dose regimen, in AML; iv) it is administered subcutaneously in its FDA-approved formulation; v) it has never been used in combination with venetoclax. Here, we sought to explore the therapeutic potential of omacetaxine mepessucinate and venetoclax in pre-clinical *in vivo* models, and used 2 different *NPM1*-mutated AML PDX (one, NPM1/FLT3-ITD double mutant; the other, DNMT3A/NPM1/FLT3-ITD triple mutant) edited to express luciferase allowing to track the disease by *in vivo* bioluminescence imaging (BLI) during treatment and evaluate drug response. HHT was given at 1 mg/Kg by subcutaneous administration for 2 consecutive weeks (5 days/week) and venetoclax at 100 mg/kg by gavage for 4 weeks (5 days/week), in cycles of 28 days. The anti-leukemic activity of the treatment was measured by evaluating AML growth/tissue infiltration on sacrificed animals by immunohistochemistry or flow cytometry and *in vivo* by BLI. Strikingly, on overt disease combinatorial HHT and venetoclax treatment resulted in a strong and synergic anti-leukemic effect with no leukemia detection in a cohort of mice sacrificed and analyzed after the first cycle, and in a significant advantage in a cohort analyzed for survival compared with single-drug or vehicle-treated animals. These data gave the support to design a pilot phase 1 clinical trial aimed at assessing safety and preliminary efficacy of omacetaxine mepessucinate plus venetoclax in relapsed/refractory NPM1-mutated AML patients. The trial is now open to patients accrual at our Hematology department.

P196 Novel hydrophilic drug linkers enable exatecan-based antibody-drug conjugates with promising physiochemical properties and in vivo activity. Haidong Liu[1], Lei Wang[2], Julia Gavrilyuk[3], Tae Han[3], Baiteng Zhao[4], Xiao Shang[3]. [1]ProfoundBio (Suzhou) Co., Ltd., Suzhou, China (Mainland), [2]ProfoundBio (Suzhou) Co., Ltd, Suzhou, China (Mainland), [3]ProfoundBio US Co., Woodinville, WA, [4]ProfoundBio (Suzhou) Co., Ltd; ProfoundBio US Co., Woodinville, WA.

The physiochemical properties of an antibody-drug conjugate (ADC) can impact its stability and pharmacokinetics/pharmacodynamics and are one of the key design attributes. Typically, ADCs with better hydrophilicity are less prone to aggregation and have lower systemic clearance and greater anti-tumor activities. However, the need of incorporating lipophilic payloads with enhanced bystander activity has posed significant challenges to ADC and linker design, especially at higher drug-antibody ratios (DAR). Here we present novel hydrophilic linkers that can greatly improve the hydrophilicity of ADCs conjugated to lipophilic payloads such as exatecan. Exatecan is camptothecin analogue that has failed as a small molecule drug in clinical trials due to lack of therapeutic window but retains considerable promise as an ADC payload because of its high potency and resistance to Pgp efflux. By introducing highly polar PEG, polyhydroxyl and/or polycarboxyl groups, we generated hydrophilic linkers that enable site specific, highly homogeneous conjugation of exatecan to multiple prototypical monoclonal antibodies at DAR8. The linkers may also allow for other DARs such as DAR4 and DAR16. These exatecan-based ADCs with novel hydrophilic linkers were evaluated for their binding affinity and stability and compared with corresponding naked antibodies and deruxtecan-based and conventional vedotin-based ADCs for hydrophilicity, pharmacokinetics, in vitro and in vivo anti-tumor activities. Safety assessments in cynomolgus monkeys have also been planned. ADCs conjugated with the novel hydrophilic linkers were stable at 37^{o}C for 15 days, after 5 cycles of freeze-thaw, and at concentrations as high as 100 mg/mL, as assessed by visual inspection, hydrophobic interaction chromatography, and size-exclusion chromatography. Binding affinity to target-positive cell lines was similar to corresponding naked antibodies. At DAR8, these ADCs were more hydrophilic than deruxtecan-based ADCs and DAR2 species of conventional vedotin-based ADCs. These ADCs also demonstrated potent in vitro cell growth inhibition and induced comparable or stronger tumor regression with single or multiple dosing in multiple mouse xenograft models. The pharmacokinetics profiles of these ADCs are similar to those of the naked antibodies. In summary, our novel hydrophilic linkers can enable conjugation of exatecan-based ADCs at high DARs with favorable physiochemical properties, which result in robust stability, pharmacokinetics, potency, and the potential for a meaningful therapeutic window.

P197 An anti-HER3 antibody, HMBD-001, that uniquely binds to and blocks the HER3 heterodimerization interface, shows superior tumor growth inhibition in biomarker-defined preclinical cancer models including NRG1-fusion driven cancers. Dipti Thakkar[1], Shalini Paliwal[1], Shreya Kar[1], Namita Gandhi[2], Konrad Paszkiewicz[1], Piers Ingram[2], Jerome Boyd-Kirkup[2]. [1]Hummingbird Bioscience, Singapore, Singapore, [2]Hummingbird Bioscience, Houston, TX.

HER3 activation, through NRG1 ligand-dependent and independent heterodimerization with HER2 or EGFR, can drive tumor growth and survival via potent PI3K pathway signaling. HER3 activation has emerged as an important mechanism for both tumor progression and acquired resistance to standard of care therapies in multiple indications. With an effective biomarker strategy to select for patients with HER3 driven cancer, potent inhibition of HER3-driven heterodimerization has the potential to confer profound clinical impact. HER3 targeting approaches to date have not shown expected clinical efficacy. Suboptimal HER3 inhibition is one possible explanation; to prevent downstream PI3K signalling, it is critical to fully block both ligand-dependent and independent HER3 activation. Further, despite the clinical evaluation of several anti-HER3 antibodies, only limited progress has been made to establish predictive biomarkers of response to HER3 inhibition. The most thoroughly studied biomarkers predicting response have been increased expression and genomic rearrangements of the HER3 ligand, NRG1. For example, oncogenic NRG1-fusions have been identified as tumor drivers in up to 0.2% of all solid cancers. Few biomarkers predicting lack of response have been reported to date. HMBD-001, is an unique anti-HER3 antibody rationally developed to bind the dimerization interface of HER3 in order to block all HER3 heterodimerization. In contrast to other anti-HER3 antibodies that predominantly bind to the NRG1 binding domain, we observed potent and superior *in vitro* and *in vivo* tumor growth inhibition for HMBD-001 in multiple cancer models, including NRG1-fusion driven models. We show that HMBD-001 binds to HER3 with high affinity even in the presence of high concentrations of NRG1, in contrast to other antibodies in which NRG1 competes for the HER3 ligand binding site, thereby decreasing affinity and likely potency. Notably, HMBD-001 treatment of an NRG1-fusion ovarian PDX model showed superior tumor growth inhibition compared with anti-HER3 and anti-HER2/HER3 bispecific antibodies targeting the NRG1 binding domain of HER3. To develop a robust biomarker signature for patient selection beyond NRG1-fusions, we interrogated the genomic and transcriptomic data of all preclinical models for which HMBD-001 efficacy data was available, including *in vitro* and *in vivo* models representing multiple HER3 associated cancer sub-types. We identified a novel gene signature that was robustly predictive of HMBD-001 response across a wide range of cancers, including loss of function and gain of function mutations in specific downstream mediators of the MAPK and PI3K signaling pathways. In conclusion, the superior potency of HMBD-001 in preclinical models predicts more complete inhibition of HER3 and better responses in HER3 driven cancers that can be identified using a novel gene signature biomarker. Clinical trials of HMBD-001 in HER3 driven cancers, including those with NRG1-fusions, are expected to commence in 2021.

P198 MDNA11 is a long-acting 'beta-only' IL-2 agonist that demonstrates a safe and durable anti-tumor immune response. Minh D. To[1], Fahar Merchant[1], Ma'an Muhsin[1], Carole Galligan[1], L. Bruce Pearce[2], Peter Lloyd[3], Rosemina Merchant[1]. [1]Medicenna Therapeutics Inc., Toronto, Canada, [2]Biologics Consulting Group Inc., Alexandria, VA, [3]KinDyn Consulting Ltd., Warnham, United Kingdom.

Background: MDNA11 is an engineered 'beta-only' IL-2 superkine with (1) enhanced affinity for IL-2Rb (CD122) to preferentially activate anti-cancer effector immune cells and (2) extended half-life by fusion to human albumin, avoiding the need for frequent administration. **Methodology:** MDNA11 was characterized in *in vitro* and *in vivo* studies including assessment of efficacy in syngeneic tumor models as single agent and in combination with immune checkpoint inhibitors (CPIs). A GLP study in non-human primate was conducted to evaluate the safety, pharmacodynamic and pharmacokinetic profiles of MDNA11. **Results:** MDNA11 does not bind IL-2Ra (CD25) but demonstrates a 30-fold higher affinity for IL-2Rb (CD122), resulting in enhanced *in vitro* STAT5 signaling in human NK and $CD8^+$ T-cells with diminished activation in Tregs when compared to rhIL-2. In CT26 and MC38 syngeneic tumor models, MDNA11 demonstrated potent and durable anti-tumor activity as monotherapy (Q1W x 2) and synergized with anti-PD1 and anti-CTLA4 to achieve complete response and tumor clearance. These mice were protected against relapse and subsequent tumor re-challenges without any further treatment by inducing long-term antigen-specific $CD8^+$ T-cells. MDNA11 binds to human and cynomolgus monkey IL-2 receptors with near-identical affinity. A GLP toxicology study involving intravenous administration on a Q2W x 3 schedule showed that MDNA11 was well tolerated up to the highest planned dose of 600 mcg/kg. The most common clinical observations were dehydration and diarrhea, which generally occurred following the first but not subsequent dose administration. There was a dose-proportional increase in C_{max} and AUC. MDNA11 induced dose-dependent proliferation and expansion of peripheral $CD4^+$ and $CD8^+$ T-cells lasting beyond 7-days following each administration. Similar stimulatory effects on NK cells were observed but effects on Tregs were limited. Despite the robust immune response, there was no sign of cytokine release syndrome nor evidence of ADA. At the highest dose of MDNA11 (600 mcg/kg), an increase in eosinophil counts was observed following the second dose, but histopathological evaluation showed no evidence of pulmonary edema nor vascular leak syndrome in all organs examined. These data constitute a strong framework for the conduct of a phase 1/2 study in patients with advanced solid tumors that encompasses dose escalation, dose expansion, and combination with CPIs. **Conclusions:** MDNA11 is a long-acting 'beta-only' IL-2 superkine that exhibits robust anti-tumor activity in mouse tumor models as a single agent and in combination with anti-PD1 and anti-CTLA4. In a non-human primate GLP toxicology study, MDNA11 demonstrated durable and selective activation of immune effector cells (NK, $CD4^+$ and $CD8^+$ T-cells), and a favorable safety profile.

P199 Tenfibgen nanoencapsulated RNAi feCK2 inhibits protein kinase CK2 and induces apoptosis in feline oral squamous cell carcinomas in vivo. Frank G. Ondrey[1], Gretchen M. Unger[2], Claire M. Cannon[3], Janeen H. Trembley[4], BT Kren[1], Jaime F. Modiano[3], Khalil Ahmed[1]. [1]University of Minnesota, Masonic Cancer Center, Minneapolis, MN, [2]GeneSegues, Chaska, MN, [3]University of Minnesota, College of Veterinary Medicine, Minneapolis, MN, [4]University of Minnesota, Dept of Laboratory Medicine, Minneapolis, MN.

In an initial safety evaluation in domestic cats suffering from oral squamous cell carcinomas (FOSCC), nine cat subjects were treated IV with 5-6 doses given twice weekly of s50 RNAi feCK2 at either 2 or 20 ug/kg (Cannon et. al., Hu Gene Ther Clin Devel 28(2):80-6, 2017). Treatment was generally well tolerated. In one case, a clear drug-related Grade 3 side effect was rapid tumor death (tumor lysis) resulting in an open wound. This issue has been addressed successfully in separate mice studies with longer, lower-dose SQ regimens. Protein kinase CK2, as a promising target in oncology, plays vital roles in cell growth, proliferation, and suppression of apoptosis (Ahmed et al., Trends Cell Biol 12: 226-30, 2002). Anti-feCK2aa' is a tumor-targeted 20 nm crystalline capsule bearing single-stranded RNAi oligos against both CK2 catalytic kinase subunits with a ligand-coated shell derived from Tenascin-C (tenfibgen). Tenascin-C is upregulated in multiple solid tumors and is present in tumors throughout their lifecycle. The capsule's ultrasmall size enables efficient raft-mediated delivery of oligos to the perinuclear space of the target cell, and the ability to reach metastases without reliance on EPR (Unger et. al, Mol Cancer Ther 13:2018-29, 2014). Initial mechanistic work focused on changes in clinically scored IHC of the CK2a subunit in study tumors. In an effort to develop a future working hypothesis, remaining paraffin-embedded tissues were retrospectively examined for both kinase subunits of CK2 as a mixture (CK2aa'), cleaved PARP (CP, apoptosis marker) and Ago2 (critical enzyme required for RNAi drug activity). Previous work in murine models showed variation of Ago2 levels in primary human and xenograft tumors. Further, model xenograft and syngeneic tumors responded with increased Ago2 levels and drug activity upon 10 mg/ml IV priming followed by low-dose SQ treatment (Cancer Res 2016;76(14 Suppl):3746). Using confocal fluorescence microscopy, we observed that decreased post-treatment CK2aa' and increased CP corresponded positively with clinical tumor responses while non-responders showed an inverse relationship. Examination of arrayed human HNC samples also showed a variation in Ago2 levels but premalignant oral lesions from a human chemoprevention trial did not. These observations suggest a possible future path for feline patient therapy of advanced lesions using Ago2 stratification, potential IV priming and more convenient, longer-term SQ dosing administered by feline caregivers. A path for human therapy may lie through chemoprevention with much to be learned from feline studies. Funding: University of Minnesota Clinical and Translational Sciences Award (1UL1 RR033183-01), NIH SBIR Contract HHSN26120 1300030C, N01-CN-15000, Mod#5, V.A. Merit Review research funds (BX00173 and BX003282) and NCI Award CA150182.

P200 Pre-clinical development of a dopamine receptor 2, PD-1 and CD47 trispecific antibody for treatment of small cell lung cancer. Shugang Yao[1], Yun Cui[2], Anna Kazanats[1], Liying Gong[1], Claire Bonfils[3], Dominic Hou[4], Emily Chen[5], Elijus Undzys[1], Jacynthe Toulouse[6], Milica Krstic[7], Hiba Zahreddine[1], Israel Matos[5], Alex Zhou[5], Aniel Moya-Torres[3], Richard Wargachuk[4], Carl Gay[8], Lauren Byers[9], Gordon Ngan[10], Luis da Cruz[3], David Young[1]. [1]KisoJi Biotechnology Inc., Montreal, QC, Canada, [2]KsioJi Biotechnology Inc., Montreal, QC, Canada, [3]KisoJi Biotechnology Inc, Montreal, QC, Canada, [4]KisoJI Biotechnology Inc., Montreal, QC, Canada, [5]KisoJi Biotechnology Inc., Toronto, ON, Canada, [6]KisoJi Biotechnology inc., Montreal, QC, Canada, [7]KisoJi Biotechnology Inc., Montrel, QC, Canada, [8]MD Anderson Cancer Center, Houston, TX, [9]MD Anderson Cancer Center, Houston, TX, [10]gordon.ngan@kbimail.com, Toronto, ON, Canada.

We developed a single domain VHH multi-specific antibody format. Multispecific antibodies have multiple mechanisms of action which may work independently or together, to achieve better clinical outcomes in cancers with high unmet medical need such as SCLC. Here we describe the preclinical development of a trispecific antibody (KB-436) that targets Dopamine Receptor 2 (DRD2), PD-1 and CD47. DRD2 is a G protein-coupled receptor upregulated in many cancer types where it correlates with decreased patient survival. In pre-clinical studies DRD2 is associated with cancer cell stemness and tumor growth. Clinical responses were achieved with small molecules targeting DRD2 and dopaminergic drugs. In SCLC, representing 15% of lung cancers, 60-70% of patients showed high expression of DRD2. Checkpoint inhibition has shown some efficacy in lung cancer where PD-L1 inhibitors were approved as first line therapy in SCLC . SCLC patients rapidly fail chemotherapy, develop resistance to treatment including to immunotherapy associated with lack of tumor infiltrating immune cells, appearance of metastases and large numbers of circulating tumor cells. These observations suggest a link between DRD2 expression and resistance to treatment, making this receptor an attractive target for a multispecific therapy. CD47 is overexpressed by many cancers and is also expressed on lymphocytes. Here, targeting CD47 is integral to T-cell engagement and redirection, in a mechanism distinct from CD3 mediated T-cell engagement. The VHH modules of KB-436 (anti-DRD2, anti-PD-1 and anti-CD47) mediate multiple mechanisms of action to achieve anti-tumor effect. The anti-DRD2 VHH induces intracellular signaling, the anti-PD-1 VHH restores T cell function, and the anti-CD47 VHH recruits T cells without their generalized activation and blocks interaction of CD47 with SIRPa. Treatment with anti-DRD2 antibody significantly suppressed tumor growth in the DRD2-positive NCI-H510A SCLC model in SCID mice. KB-436 anti-tumor efficacy was tested in several *in vivo* immuno-oncology xenograft models of human SCLC and other solid tumors , reconstituted with human PBMC or with CD34+ hemopoietic stem cells. Treatment suppressed tumor growth, enhanced the *in vivo* effect of cisplatin-treatment in a less chemosensitive NCI-H69 variant, blocked metastases formation in CD34+ humanized NCG mice bearing established NCI-H69 tumors, and blocked metastases formation and increased survival in tail vein metastatic models. Trispecific KB-436 has a half-life in mice of around 5 days similar to that of other antibodies. It is produced at high yield (6 g/L) in a manufacturing cell line, conventional purification yields 99% purity and notably displays high stability under accelerated stability testing. In conclusion, the trispecific KB-436 antibody, has strong *in vivo* anti-tumor activity mediated via multiple mechanisms of action, is easily expressed and purified and is very stable. Together, this data supports the clinical development of KB-436 in advanced metastatic solid cancer indications, including SCLC.

P201 Zenocutuzumab is an effective HER2/HER3 Biclonics® antibody in cancers with *NRG1* fusions. Jan Gerlach[1], Igor Odintsov[2], Ron Schackmann[1], Marc Ladanyi[2], Jeroen Lammerts van Bueren[1], Romel Somwar[3], Cecile Geuijen[1]. [1]Merus NV, Utrecht, Netherlands, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Memorial Sloan Kettering Cancer Center, New York, NY.

Neuregulin 1 (NRG1) fusion proteins are oncogenic drivers in multiple cancer types, including non-small cell lung cancer, pancreas adenocarcinoma, and other solid tumors. NRG1 fusion proteins bind to HER3 and signal through HER2/HER3 heterodimers, leading to oncogenic transformation. Zenocutuzumab is an HER2/HER3 humanized IgG1 bispecific antibody that specifically and potently blocks NRG1 fusion-driven signaling of the heterodimeric complex. Zenocutuzumab binds via its anti-HER2 monovalent Fab arm to domain I of HER2. Docking of zenocutuzumab in this position increases the effective affinity of the anti-HER3 Fab arm, which binds to domain 3, a critical epitope that blocks binding of the HER3 ligand NRG1. Consequently, zenocutuzumab displaces NRG1 binding to HER3, even at high ligand concentrations, keeping HER3 in an inactive state and blocking HER2/HER3 heterodimerization and NRG1-mediated signaling. The clinical efficacy and safety of zenocutuzumab were recently demonstrated in patients with *NRG1* fusion-driven cancers who were enrolled in the ongoing global multicenter eNRGy study and a global early access program NCT02912949 (J Clin Oncol 39;15_suppl; abstract 3003). Treatment with zenocutuzumab led to rapid and major radiologic tumor regression and biomarker responses in heavily pretreated patients with multiple NRG1 fusion-positive cancers with several different N-terminal fusion partners. Zenocutuzumab has been enhanced for antibody-dependent cell-mediated cytotoxicity (ADCC) to recruit and bolster immune effector cells to aid in tumor elimination. Here we explored the different Fc-mediated immune effector functions of zenocutuzumab. In several cancer cell lines, zenocutuzumab induced Fc-mediated dose-dependent ADCC and antibody-dependent cellular phagocytosis. Insertion of *ATP1B1-NRG1* and *SLC3A2-NRG1* into immortalized pancreatic ductal epithelial cells (H6c7) led to activation of the HER3 oncogenic signaling pathways including AKT. The H6c7-*SLC3A2-NRG1* cells formed tumors when implanted in the subcutaneous flank of immune compromised mice (after about 10 weeks). Furthermore, a dependency of the transformed cell on this oncogene for growth and survival was also observed. Zenocutuzumab effectively inhibited proliferation of these *NRG1* fusion-expressing, transformed cells. In addition, proteomic analysis showed that zenocutuzumab inhibited the HER3 and AKT-related downstream signaling pathways. In conclusion, these data provide strong mechanistic evidence supporting the clinical efficacy of zenocutuzumab in patients with NRG1 fusion-driven cancers.

P202 Initial findings from an ongoing first-in-human phase 1 study of the CBP/p300 inhibitor FT-7051 in men with metastatic castration-resistant prostate cancer. Andrew J. Armstrong[1], Michael S. Gordon[2], Melissa A. Reimers[3], Arif Hussain[4], Vaibhav G. Patel[5], Elaine T. Lam[6], Alex Sedkov[7], Von Potter[7], Neal Shore[8]. [1]Duke Cancer Institute Center for Prostate and Urologic Cancers, Duke University, Durham, NC, [2]HonorHealth Research Institute, Scottsdale, AZ, [3]Washington University in St. Louis, St. Louis, MO, [4]University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD, [5]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, [6]University of Colorado Cancer Center, Aurora, CO, [7]Forma Therapeutics, Inc., Watertown, MA, [8]Carolina Urologic Research Center, Myrtle Beach, SC.

Background: Prostate cancer is the 2nd leading cause of cancer-related deaths among men in the US. CREB binding protein (CBP) and paralog p300 are co-activators of androgen receptor (AR) relevant to metastatic castration-resistant prostate cancer (mCRPC) progression and AR therapy resistance. FT-7051 is an oral, potent and selective inhibitor of CBP/p300 bromodomain with activity in preclinical prostate cancer models, including those resistant to enzalutamide. **Methods:** The Courage Study (NCT04575766) is a first-in-human, multicenter, phase 1, open-label study to examine the safety, PK/PD, and preliminary anti-tumor activity of FT-7051 in mCRPC patients (pts) who have progressed despite prior therapy, including at least one AR pathway inhibitor. The study uses a Bayesian optimal interval (BOIN) design with an accelerated titration phase. FT-7051 is dosed on a 28-d cycle (21-d on/7-d off). Following accelerated titration, dose escalation/de-escalation decisions are made by comparing the observed dose-limiting toxicity (DLT) rate at the current dose with pre-specified dose escalation/de-escalation boundaries. The primary objectives are to evaluate safety and tolerability of FT-7051 and determine the recommended phase 2 dose. Key secondary endpoints include PSA at 12 wks, time to PSA progression, time to radiographic progression, overall response rate, and PK parameters. Biomarker analyses include PD assessments of CBP/p300 inhibition in surrogate tissues and genetic analyses in circulating tumor cells (AR, AR-v7) and peripheral blood. **Results:** As of 18-June-2021, a total of 5 pts were enrolled with exposure data entered into the database, with 2 pts ongoing and 3 pts discontinued (disease progression, n=1; no longer clinically benefitting, n=1; subject withdrawal, n=1). Pts had a median age of 71 yrs (range: 66-82) with a median time since first mCRPC diagnosis of 2.3 yrs (0.4-4.7) and a median of 3 (1-5) prior lines of mCRPC therapy. Mutations reported by the investigator for 3 pts included MYC, p53, RB1, AR, and PTEN loss. Preliminary PK analyses indicated that FT-7051 exposure increased with dose in a greater than dose-proportional manner. Importantly, observed FT-7051 exposures were consistent with the predicted efficacious exposure threshold derived from PK/efficacy modeling in preclinical studies. TEAEs were reported in 4 (80%) pts. Most TEAEs were mild (Gr1) or moderate (Gr2), ≥Gr3 TEAEs included one event of possibly related Gr3 hyperglycemia and one event of unrelated Gr5 disease progression. **Conclusions**: Preliminary safety and PK data from the accelerated titration phase of this BOIN study support the continued investigation of FT-7051 in men with mCRPC. Initial PK data confirm that FT-7051 exposure is consistent with the predicted efficacious exposure threshold determined by PK/efficacy modeling. Additional analyses of PSA, PD assessments, and genetic analyses, including AR-v7 status, will be reported.

P203 Milademetan is a potent, murine double minute 2 (MDM2) inhibitor, highly active in TP53 wild-type ($p53^{WT}$) Merkel cell carcinoma (MCC) cell lines. Varsha Ananthapadmanabhan[1], Aine Knott[2], Kara M. Soroko[2], Prafulla C. Gokhale[2], Vijaya Tirunagaru[3], Robert Doebele[3], James A. DeCaprio[1]. [1]Dana Farber Cancer Institute, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, [2]Dana Farber Cancer Institute, Boston, MA, [3]Rain Therapeutics, Newark, CA.

Background: MCC is a highly aggressive neuroendocrine carcinoma of the skin with a poor overall prognosis. Current treatment options include surgery and radiation therapy for local MCC tumor and checkpoint blockade therapy for advanced disease. However, primary and acquired resistance can reduce response to therapy. Around 80% of all MCCs have integrated copies of Merkel cell polyomavirus (MCV). Virus-Positive MCC (MCCP) tumors typically contain few somatic mutations and express wild type (WT) p53 (*TP53*). In contrast, Virus-Negative MCC (MCCN) tumors have a high mutational burden, with a predominantly UV mutational signature. MCCP express two viral proteins: MCV small T antigen (ST) and a truncated form of large T antigen (LT). The MCV ST recruits MYCL and EP400 to form the SLaP complex that specifically activates several genes contributing to oncogenesis. A direct target of the SLaP complex is MDM2, an E3 ligase for p53. In $p53^{WT}$ MCCP, SLaP-dependent activation of MDM2 inhibits the tumor suppressive functions of p53. Here, we analyzed the efficacy of milademetan (RAIN-32), a potent, selective, and orally available MDM2 inhibitor, in MCC. **Experimental procedures**: Established MCCP cell lines with WT (MKL-1, WaGa, PeTa) or mutant p53 (MS-1), were treated with vehicle or several concentrations of milademetan or another MDM2 inhibitor AMG232 and cell viability was analyzed. Similar viability assays were also performed using MKL-1 p53 knockout (KO) cells and two newly established $p53^{WT}$ primary MCC cell lines. The p53 response in MCC cells treated with vehicle, milademetan or AMG232 was assessed by western blot (WB) analysis of p53 and its downstream effectors p21, PUMA and PARP-1. For *in vivo* testing, an initial tolerability study was conducted with once daily administration of milademetan by oral gavage in NSG mice. Milademetan activity was evaluated in MKL-1 xenograft and patient-derived xenograft (PDX) models in NSG mice. Pharmacodynamic markers of response in tumor samples from mice treated with vehicle or milademetan is analyzed by q-PCR and WB analysis. **Summary of Results**: Nanomolar concentrations of milademetan reduced cell viability of $p53^{WT}$ but not $p53^{mutant}$MCCP MCC cell lines. Milademetan treatment increased levels of p21, PUMA and cleaved PARP-1 in MCCP cell lines MKL-1 and WaGa. Using p53 KO MKL-1 cells, we show that the effect of milademetan on MCC cell viability is p53 dependent. *In vitro* data show that milademetan is more potent than AMG232 in the context of MCC. Tolerability studies show that mice safely tolerate 100 mg/kg of milademetan. Milademetan treatment in the MKL-1 xenograft tumor model shows a dose-dependent response in tumor growth inhibition. In the DFMC-33043 PDX model, milademetan significantly inhibited tumor growth. **Conclusion:** Milademetan is a promising drug effective against $p53^{WT}$ MCC cell lines, xenograft, and PDX models. Ongoing *in vivo* testing of the anti-cancer cell activity of milademetan will provide evidence for clinical exploration of milademetan in MCC refractory to current therapies.

P204 Targeting the p300/CBP epigenetic pathway to overcome hormone therapy resistance in advanced prostate cancer. Emily L. Chen[1], Nathan Hawkey[2], Beatrice C. Thomas[2], Kathryn E. Ware[2], Daniella Runyambo[2], Maureen Caligiuri[3], Erik Wilker[3], Erik J. Soderblom[4], M. Arthur Moseley III[4], Sylvie M. Guichard[3], Andrew J. Armstrong[2], Jason A. Somarelli[2]. [1]Duke University School of Medicine, Durham, NC, [2]Duke University Medical Center, Durham, NC, [3]Forma Therapeutics, Watertown, MA, [4]Duke University, Durham, NC.

Purpose/Objective(s): Despite improvements in the treatment of advanced prostate cancer, there remains a major unmet need for further treatment options in the setting of metastatic castration-resistant prostate cancer (mCRPC). Recent evidence has pinpointed androgen receptor (AR) co-activators, such as p300/CREB binding protein (CBP), as potential therapeutic targets. Here, we describe the impact of a novel small molecule inhibitor of the p300/CBP bromodomain, FT-6876, in preclinical models of mCRPC. **Materials/Methods:** FT-6876 is a potent inhibitor of the bromodomains of CBP and p300 with high selectivity against other bromodomain-containing proteins (Caligiuri *et al*. Proceedings: AACR Annual Meeting 2020; *Cancer Research*. DOI: 10.1158/1538-7445.AM2020-3079). FT-6876 was tested, both alone and in combination with enzalutamide, in a panel of enzalutamide-sensitive and enzalutamide-resistant human prostate cancer cell lines, including LNCaP, LN95, and CS2. A Snail-inducible system, which confers enzalutamide resistance, in both LNCaP and LN95 lines, was also used. To test the effect of FT-6876 in inhibiting prostate cancer growth *in vitro*, 2D colony forming assays and 3D spheroid assays were performed. RT-qPCR and multi-omics analysis were performed to assess changes in gene and protein expression. To test the effects of FT-6876 in physiologic settings, an *ex vivo* murine pulmonary metastasis assay (PuMA) as well as a patient-derived xenograft (PDX) model were used. **Results:** FT-6876 treatment resulted in concentration-dependent growth inhibition of all tested enzalutamide-sensitive and -resistant cell lines. Treated cells showed decreased expression of several key prostate cancer genes, including *KLK3* (PSA), *TMPRSS2*, and *MYC*. A multi-omics approach using proteomics, acetylomics, and RNA-Seq illuminated the global landscape of alterations in epigenetic and downstream signaling pathways induced by FT-6876. Changes were observed in both enzalutamide-sensitive and -resistant models. Finally, FT-6876 reduced metastatic growth in the PuMA model and induced tumor stasis in a patient-derived xenograft model of prostate cancer resistant to enzalutamide. **Conclusion:** Overall, we demonstrate that inhibiting the p300/CBP axis with the novel bromodomain inhibitor FT-6876 could be an effective therapeutic approach for both enzalutamide-sensitive and enzalutamide-resistant prostate cancer models. The safety and tolerability of FT-7051, an oral p300/CBP inhibitor related to FT-6876, is currently being evaluated in the Courage Study (NCT04575766), a first-in-human phase 1 study in men with mCRPC.

P205 Novel 1,1-diarylethylene compounds degrade FOXM1 and selectively and potently reduce survival of high-grade serous ovarian cancer cells. Cassie Liu[1], Catalina Muñoz-Trujillo[1], John A. Katzenellenbogen[2], Benita S. Katzenellenbogen[3], Adam R. Karpf[1]. [1]Eppley Institute for Research in Cancer & Allied Diseases and the Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE, [2]Department of Chemistry and Cancer Center, University of Illinois at Urbana-Champaign, Urbana, IL, [3]Department of Molecular and Integrative Physiology and Cancer Center, University of Illinois at Urbana-Champaign, Urbana, IL.

Background: Forkhead box M1 (FOXM1) is a master transcriptional regulator and multifunctional oncoprotein that is widely overexpressed in ovarian cancer and associated with poor prognosis. Due to its promotion of numerous oncogenic phenotypes in ovarian cancer, FOXM1 has emerged as an attractive therapeutic target for ovarian cancer treatment. Recently, we reported that 1,1-diarylethylene compounds, NB-55, NB-73, and NB-115 (NB compounds), potently inhibit FOXM1 in breast cancer cells and mediate anti-cancer effects. Given the high molecular similarity between triple-negative breast cancer (TNBC) and high-grade serous ovarian cancer (HGSC), we characterized this new class of FOXM1 inhibitors in HGSC cells in the present study and compared their effects to other reported FOXM1 inhibitors. **Methods:** We conducted dose-response curves of NB compounds and other FOXM1 inhibitors in HGSC cells (CAOV3 and OVCAR4) and normal control (FT282 C11) cells. Western blot analyses probed for FOXM1, p-FOXM1, CCNB1, FOXA1, and FOXO3A in HGSC cells treated with NB compounds. We used the proteosome inhibitor MG132 to investigate the mechanism of FOXM1 inhibition by NB-73 and NB-115. We investigated FOXM1 transcriptional activity, colony formation, cell cycle, and apoptosis in HGSC cells treated with NB-73 and NB-115. **Results:** Dose-response curves in HGSC cells revealed IC50 values in the low nM for monensin; mid-nM for NB-73 and NB-115; high nM for thiostrepton; and low µM for NB-55, FDI-6, and n-phenylphenanthren-9-amine. RCM-1 did not affect HGSC cell proliferation. Dose-response curves in normal control fallopian tube epithelial (FTE) cells revealed IC50 values of high nM for thiostrepton and low µM for NB compounds and FDI-6. HGSC cells treated with NB compounds demonstrated decreased levels of FOXM1 and p-FOXM1 but not FOXA1 and FOXO3A. NB-73 and NB-115 decreased FOXM1 promoter activity and gene target expression but were no longer able to decrease FOXM1 levels when combined with MG132 in HGSC cells. Clonogenic assays of HGSC cells treated with NB-73 and NB-115 revealed decreased colony formation at low nM concentrations. HGSC cells treated with NB-73 and NB-115 have higher cell populations in G2/M and undergo higher rates of apoptosis compared to non-treated HGSC cells. **Conclusions:** NB-73 and NB-115 promote proteasomal degradation of FOXM1 and are more potent FOXM1 inhibitors of cell proliferation than previously characterized FOXM1 inhibitors in HGSC cells. NB-73 and NB-115 potently reduce HGSC survival and selectively inhibit HGSC cells as compared to normal control FTE cells. Our data encourage further assessment of NB compounds as a novel therapeutic strategy in the vast majority of HGSC that overexpress FOXM1. **Support:** NIH T32CA009476(CL), research grants 083(BSK) and 084(JAK) from the Breast Cancer Research Foundation, the Landfield Cancer Research Fund(BSK), NIH R03CA224339(ARK), The Marsha Rivkin Center(ARK), The Betty J. and Charles D. McKinsey Ovarian Cancer Research Fund(ARK), and The Fred & Pamela Buffett Cancer Center (NCI P30CA036727)(ARK).

P206 AB521 potently and selectively inhibits pro-tumorigenic gene transcription by Hypoxia-Inducible Factor (HIF)-2α *in vitro* and *in vivo*. Kelsey E. Sivick Gauthier, Dana Piovesan, Soonweng Cho, Kenneth V. Lawson, Patrick G. Schweickert, Alejandra Lopez, Suan Liu, Timothy Park, Artur Mailyan, Jeremy T. A. Fournier, Joel W. Beatty, Samuel L. Drew, Jarek Kalisiak, Balint Gal, Guillaume Mata, Zhang Wang, Brandon R. Rosen, Clayton Hardman, Matthaw P. Epplin, Kai Yu, Karl T. Haelsig, Lixia Jin, Elaine Ginn, Jennie Au, Cesar A. Meleza, Joel Tencer, Amber Pham, Hyock J. Kwon, Stephen W. Young, Manmohan Leleti, Jay P. Powers, Matthew J. Walters. Arcus Biosciences, Hayward, CA.

Cells in the solid tumor microenvironment are frequently exposed to hypoxic conditions, necessitating molecular adaptations for survival. Of particular importance are transcriptional changes mediated by heterodimeric Hypoxia-Inducible Factor (HIF) proteins that consist of an oxygen-regulated α monomer (either HIF-1α, -2α, and -3α) coupled to a constitutively expressed β monomer (HIF-1β/ARNT). In normal oxygen conditions, HIF-2α is degraded following ubiquitination by the von Hippel-Lindau (pVHL) E3-ubiquitin ligase complex. Exposure to hypoxia, *VHL* mutation, or epigenetic silencing of pVHL leads to HIF-2α stabilization and transcription of pro-tumorigenic gene sets in both cancer and non-cancer cells. Inhibition of HIF-2α has been shown clinically to be an effective strategy to mitigate tumor growth, particularly in patients suffering from VHL disease or clear cell renal cell carcinoma (ccRCC), a cancer that has a particularly high prevalence of pVHL dysfunction. Applying a pharmacophore mapping and structure-based design approach, we identified a novel and potent small molecule HIF-2α inhibitor, AB521. AB521 avidly binds the HIF-2α PAS-B domain, preventing HIF-2α-mediated gene transcription. AB521 is characterized by a favorable preclinical pharmacokinetic profile and is projected to be suitable for once-daily dosing in humans. When delivered orally in mice, AB521 significantly regressed established 786-O xenograft tumors and decreased pharmacodynamic markers associated with HIF-2α in a dose-dependent manner. *In vitro*, AB521 potently inhibited HIF-2α-specific luciferase reporter transcription under high-serum conditions, VEGF protein secretion, colony formation in soft agar, and did not exhibit off-target cytotoxicity in 786-O cells. AB521 selectively inhibited HIF-2α-, but not HIF-1α-, mediated gene expression in hypoxic Hep3B hepatocellular carcinoma cells. AB521 also inhibited the transcriptional activity of endogenous HIF-2α in relevant human primary cell types, including endothelial cells and pro-tumorigenic M2-polarized macrophages. Importantly, inhibiting HIF-2α did not impact functionality of activated hypoxic human T cells, suggesting that AB521 would be favorable combination partner for I-O therapeutic agents. Indeed, expression of CD73, the primary enzyme responsible for synthesis of the immunosuppressive metabolite adenosine, was highly correlated with hypoxic signatures across several indications in publicly available bioinformatic datasets, suggesting combinations with adenosine pathway antagonists in ccRCC and beyond. In summary, AB521 is a novel and selective HIF-2α inhibitor with potent anti-tumor activity. Clinical evaluation of this molecule is expected to begin in the latter part of 2021.

P207 BBP-398, a potent, small molecule inhibitor of SHP2, enhances the response of established NSCLC xenografts to KRAS^{G12C} and mutEGFR inhibitors. James P. Stice[1], Sofia Donovan[1], Yuting Sun[2], Nancy Kohl[2], Barbara Czako[2], Faika Mseeh[2], Paul Leonard[2], Anna Wade[3], Justin Lim[3], Phil Jones[4], Eli Wallace[1], Kerstin Sinkevicius[1], Pedro Beltran[1]. [1]Navire Pharma, Inc., a BridgeBio Pharma affiliate; BridgeBio Oncology Therapeutics, South San Francisco, CA, [2]Institute of Applied Cancer Science, MD Anderson Cancer Center, Houston, TX, [3]Navire Pharma, Inc., a BridgeBio Pharma affiliate, Palo Alto, CA, [4]Institute of Applied Cancer Science, MD Anderson Cancer Center, Houston, TX.

Src homology 2 domain-containing phosphatase (SHP2), a ubiquitously expressed non-receptor tyrosine phosphatase, plays a critical role in the regulation of the MAPK signaling pathway and cellular proliferation. Activating mutations in SHP2 are associated with the development of multiple malignancies including leukemia, lung cancer and neuroblastoma. In addition, SHP2 promotes the conversion of oncogenic KRAS to its active GTP-bound state and it's inhibition can enhance efficacy of GDP-KRAS^{G12C} inhibitors as well as other MAPK pathway inhibitors (RAF, MEK and ERK) which have suboptimal clinical efficacy as single agents. As a result, inhibition of SHP2 through genetic manipulation or pharmacological means has been shown to suppress tumor growth and presents an attractive potential avenue for the treatment of malignancies as monotherapy or in combination with other MAPK/PI3K inhibitors. Here we describe BBP-398, a potent, orally bioavailable allosteric small molecule inhibitor of SHP2. BBP-398 displays high selectivity against other phosphatases, kinases, GPCRs, transporters and hERG. Predicted human PK properties show good oral bioavailability with half-life of ~12-16 hours enabling continuous daily dosing and optimal therapeutic index in combination with other targeted therapeutics. In cellular assays, BBP-398 demonstrates potent pERK/DUSP6 inhibition and loss of viability across a panel of cell lines with active MAPK signaling, such as mutant EGFR and KRAS^{G12C}. In vivo, BBP-398 strongly suppresses RAS-ERK signaling in RTK- or RAS-driven xenografts. In the EGFR-dependent non-small cell lung cancer (NSCLC) HCC827 and esophageal squamous cell carcinoma KYSE-520 xenograft models, BBP-398 drives dose dependent efficacy consistent with the level of target inhibition. Detailed analysis of tumor response shows that efficacy is driven by maintaining better than 50% inhibition of pERK for most of the dosing interval. In addition to its strong single agent activity, BBP-398 also leads to enhanced efficacy in vitro and in vivo when used in combination with targeted therapeutics against driver MAPK genetic alterations, such as KRAS, EGFR or MET. Combination targeting, such as with the GDP-KRAS^{G12C} inhibitor sotorasib in the NSCLC NCI-H358 xenograft model, or with the mutant EGFR inhibitor osimertinib in the HCC827 erlotinib resistant (ER) xenograft model, drives strong suppression of MAPK activity and results in tumor regressions. Collectively, these findings highlight that SHP2 inhibition is a promising molecular therapeutic strategy in cancer which can potentially strongly suppress tumor growth as a single agent or in combination with other MAPK pathway inhibitors. Given its preclinical properties and projected favorable clinical pharmacokinetic profile, BBP-398 is currently being evaluated in a Phase 1/1b trial in patients with advanced solid tumors (NCT04528836).

P208 Synergistic antitumor activity of lisaftoclax (APG-2575) and alrizomadlin (APG-115) through dual targeting of BCL-2/MDM2-P53 apoptotic pathways in preclinical models of acute myeloid leukemia. Douglas D. Fang[1], Qiuqiong Tang[1], Qixin Wang[1], Na Li[1], Feng Zhou[1], Guoqin Zhai[1], Yan Yin[1], Dajun Yang[2], Yifan Zhai[1]. [1]Ascentage Pharma (Suzhou) Co., Ltd, Suzhou, China (Mainland), [2]Department of Experimental Research, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-Sen University Cancer Center, Guangzhou, China (Mainland).

Background: In many hematologic malignancies, BCL-2 is overexpressed and is a viable therapeutic target. In relapsed or refractory acute myeloid leukemia (R/R AML), BCL-2 inhibitor venetoclax as a single agent has limited activity, thereby necessitating combination treatments, some of which are actively under clinical development. In addition, the mutation rate of *TP53* is rare in AML, and the frequency of MDM2 overexpression is high, representing an opportunity for pharmacologic intervention of the MDM2-P53 apoptosis pathway. Synergistic effects of MDM2 inhibitor idasanutlin and venetoclax have been reported in preclinical models of *TP53* wild-type AML (Pan et al, *Cancer Cell* 2017). Lisaftoclax (APG-2575) and alrizomadlin (APG-115) are novel BCL-2 and MDM2 inhibitors, respectively. They show unique pharmacological properties as well as clinical activity and tolerable safety profiles in patients with hematologic or solid malignancies (Tolcher et al, *J Clin Oncol* 2021:2506; Ailawadhi et al, *J Clin Oncol* 2021:7502). Here, we examined the effects of combined lisaftoclax and alrizomadlin in preclinical models of AML. **Materials and Methods**: AML cell lines and mouse xenograft models were employed. Antiproliferative activity was measured by CellTiter-Glo luminescent cell viability assay. Apoptosis was evaluated by annexin V/propidium iodide staining and flow cytometry. Meso-Scale Discovery ELISA assay and western blot analyses were used to characterize the mechanisms of the synergistic effects of this combination. In animal studies, clinically relevant doses of lisaftoclax were employed. A pulsed high-dose regimen of alrizomadlin was used to achieve best efficacy and manage on-target toxicity. **Results**: In *TP53* wild-type AML cell lines MOLM-13, MV-4-11, and OCI-AML-3, both single-agent lisaftoclax and alrizomadlin exerted apoptogenic activity. The combination had synergistic apoptogenic and antiproliferative activity. In subcutaneous xenograft models derived from MV-4-11 and OCI-AML-3 cells, the combination substantially inhibited tumor growth relative to the impact observed with either single agent. In a systemic xenograft model derived from MOLM-13 cells, the combination significantly ($P < .05$) extended the life span of tumor-bearing mice by 135% to 237% compared to that of tumor-bearing mice treated with lisaftoclax or alrizomadlin alone, which resulted in extensions of 4% to 26% and 41% to 95%, respectively. Synergistic anti-leukemic activity was confirmed in a patient-derived systemic AML model. Mechanistically, the combination synergistically downregulated anti-apoptotic proteins MCL-1 and BCL-xL and primed AML cells to apoptosis induced by lisaftoclax. **Conclusions**: We show a significant synergy of lisaftoclax and alrizomadlin and novel mechanisms associated with the combination of these agents in preclinical models of *TP53* wild-type AML. These data support investigation into the clinical outcomes of the combination treatment in patients with *TP53* wild-type AML.

P209 Collaborative crosstalk between two apoptosis pathways drives synergy of dual inhibition of BCL-2/MDM2 in preclinical models of neuroblastoma. Douglas D. Fang[1], Qiuqiong Tang[2], Qixin Wang[1], Yanhui Kong[1], Na Li[1], Feng Zhou[1], Ran Tao[1], Dajun Yang[3], Yifan Zhai[1]. [1]Ascentage Pharma (Suzhou) Co., Ltd, Suzhou, China (Mainland), [2]Ascentage Pharma (Suzhou) Co., Ltd,, Suzhou, China (Mainland), [3]Department of Experimental Research, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China (Mainland).

Introduction: The prognosis of patients with neuroblastoma is poor, and novel therapeutic strategies are needed to improve clinical outcomes. Neuroblastoma often exhibits a low *TP53* mutation rate and overexpresses *MDM2* and *BCL-2* genes. Dual targeting of the MDM2-P53 and BCL-2 apoptotic pathways is an attractive treatment strategy (Vernooij et al, *Mol Cancer Ther* 2021; Dalton et al, *Mol Cancer Ther* 2021) and is under clinical development using idasanutlin and venetoclax. However, the mechanisms of action (MOAs) of this combination in neuroblastoma are not fully understood. Alrizomadlin (APG-115) is a potent, orally bioactive MDM2 inhibitor. Lisaftoclax (APG-2575) is a novel BCL-2 selective inhibitor. Both agents have shown clinical activity in solid and hematologic malignancies (Tolcher et al, *J Clin Oncol* 2021:2506; Ailawadhi et al, *J Clin Oncol* 2021:7502). We investigated the effects of combined alrizomadlin and lisaftoclax treatment in preclinical models of neuroblastoma and examined novel MOAs associated with this combination. **Materials and Methods**: Antiproliferative activity was measured by CellTiter-Glo luminescent cell viability assay. Apoptosis was evaluated by annexin V/propidium iodide staining and flow cytometry. Meso-Scale Discovery ELISA and western blot analyses characterized the mechanisms of this combination's synergistic effects. In animal studies, clinically relevant doses of lisaftoclax were employed. A pulsed high-dose regimen of alrizomadlin was used to achieve best efficacy and manage on-target toxicity. **Results:** In $TP53^{WT}$ neuroblastoma cell lines IMR-32 and SH-SY5Y, the combination of lisaftoclax and alrizomadlin synergistically inhibited proliferation and induced apoptosis, as evidenced by elevations in caspase-3/7, cleaved PARP, and annexin V^+ subpopulations. Tumor growth was synergistically inhibited in 3 distinct subcutaneous mouse xenograft tumor models derived from neuroblastoma cell lines or patient tumors. In the cell lines, alrizomadlin treatment upregulated proapoptotic BCL-2 family member expression (NOXA, BAX, and PUMA) and downregulated antiapoptotic BCL-2 family member MCL-1, likely sensitizing cancer cells to lisaftoclax-induced apoptosis. Treatment of these cells with lisaftoclax and alrizomadlin reduced all 3 major BIM-related complexes, thereby increasing free BIM and triggering cellular apoptosis. Lisaftoclax and alrizomadlin downregulated NMYC and its transcription target MDM2, preventing the development of alrizomadlin resistance mediated by a feedback upregulation of MDM2, and inhibition of the MDM2-P53 apoptotic pathway by alrizomadlin primed cancer cells to BCL-2 inhibition. **Conclusions:** Alrizomadlin synergizes with lisaftoclax to achieve synthetic lethality through a regulated interaction at multiple molecular nodules, and inhibition of BCL-2 apoptotic pathway by lisaftoclax may prevent cancer cells from developing resistance to MDM2-P53 inhibition. These data warrant clinical development of combined alrizomadlin and lisaftoclax treatment in patients with neuroblastoma.

P210 MDM2 gene amplification as a predictive biomarker for the MDM2 inhibitor milademetan. Vijaya G. Tirunagaru[1], Mrinal M. Gounder[2], Prasanna R. Kumar[3], David S. Hong[4], Robert C. Doebele[1]. [1]Rain Therapeutics, Newark, CA, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Daiichi Sankyo, Japan, [4]University of Texas, MD Anderson Cancer Center, Houston, TX.

MDM2 is an E3 ubiquitin ligase that plays a critical role in the degradation of the tumor suppressor p53. Milademetan (RAIN-32) is an orally available, small molecule inhibitor of MDM2 that disrupts the MDM2-p53 protein complex thereby restoring p53 expression and activity. Milademetan has demonstrated anti-tumor activity in de-differentiated liposarcoma, a tumor type characterized by *MDM2* gene amplification (amp) and WT *TP53,* in the phase I study of monotherapy milademetan (NCT01877382). Here we investigate the use of *MDM2* gene amplification as a predictive biomarker for the selection of patients with advanced cancers who might benefit from milademetan. In this study we evaluated milademetan in *MDM2* gene amp cancer models from a variety of tumor types using both *in vitro* assays, including 2D viability assays and organoid models, as well as *in vivo* patient-derived xenografts. We employed a mutual exclusivity analysis between *MDM2* copy number (CN) and *TP53* mutation status using publicly available next generation sequencing data to derive an optimal *MDM2* CN threshold for patient selection and present clinical data from patients with *MDM2* amp tumors treated with milademetan in the phase 1 study (NCT01877382). Three cell lines, CCFSTTG1 (astrocytoma), DKMG (glioblastoma), and SJSA1 (osteosarcoma), with *MDM2* amp were identified and demonstrated inhibition of cell proliferation by milademetan with GI_{50} <100 nM. Evidence of MDM2 target engagement and activation of p53 was demonstrated through induction of p21 and PUMA, transcriptional targets of p53. Four patient-derived organoid models from diverse cancers (2 lung adenocarcinoma, 1 cholangiocarcinoma, and 1 renall cell carcinoma) were identified with *MDM2* amp and WT *TP53* and demonstrated differential selectivity compared to non-*MDM2* amp models also with WT *TP53* (head and neck scquamous cell carcinoma, endometrial carcinoma). Xenograft models from tumor types including gastric, lung adenocarcinoma and osteosarcoma with *MDM2* amp showed dose-dependent anti-tumor activity to milademetan *in vivo*. Induction of MIC-1, a p53 target gene, was observed *in vivo* following milademetan dosing. An *MDM2* CN threshold of ≥ 12 was derived using mutual exclusivity analysis using the AACR Genie dataset across solid tumor types. Using TCGA Pan-Cancer Atlas dataset, we identified 1.1% of cancers that met the criteria of MDM2 CN ≥ 12 and WT *TP53*. Finally, 3 patients (breast cancer, synovial sarcoma, and small cell lung cancer) with *MDM2* CN ≥ 12 were previously enrolled on the U101 study of milademetan, Schedule D (3 days on 11 days off), which was determined to be the dose schedule for future clinical trials, and all 3 patients experienced tumor reduction with 2 patients demonstrating a partial response and 1 confirmed partial response.

Milademetan shows evidence of preclinical and clinical anti-tumor activity in genetically selected tumors using *MDM2* amp and WT *TP53* as selection criteria. A basket study evaluating milademetan in solid tumors with *MDM2* CN ≥ 12 and WT *TP53* (MANTRA-2) is planned.

P211 The investigational chemoprotection drug ALRN-6924, a dual inhibitor of MDMX and MDM2, shows potential for radioprotection. Allen Annis, David Sutton, Manuel Aivado, Vojislav Vukovic. Aileron Therapeutics, Inc., Boston, MA.

Aim: We investigated whether p53 activation with ALRN-6924 can prevent toxicity in mouse models of acute radiation injury. **Materials and methods**:ALRN-6924 is a clinical-stage, first-in-class, stabilized cell-permeating alpha-helical peptide that disrupts the interaction of the p53 tumor suppressor protein with its endogenous inhibitors, MDMX and MDM2. In previous experiments it was shown that ALRN-6924 can induce transient, dose-dependent cell cycle arrest in the bone marrow to protect those cells from chemotherapy while not protecting p53-mutant cancer cells. Because radiation (like chemotherapy) preferentially affects proliferating cells, we hypothesized that ALRN-6924 may also protect proliferating cells in normal tissues from radiation-induced cellular toxicity. Cell cycle arrest was measured in the bone marrow of ALRN-6924-treated C57BL/6 mice by flow cytometry using EdU incorporation. Serum levels of macrophage inhibitory cytokine-1 (MIC-1), a biomarker of p53 activation, were measured by ELISA. Cell proliferation and apoptosis were measured in formalin-fixed mouse bone marrow by immunohistochemistry analysis of Ki67 and cleaved PARP, respectively. C57BL/6 mice (n=7/group) were treated with one or more intravenous 2.4 mg/kg doses of ALRN-6924 at 24, 16, 8, or 1 hour (or combinations thereof) or placebo prior to an abdominally targeted (shielded body) 15 Gy radiation dose and then monitored for body weight (BW). **Results**: MIC-1 was elevated in the serum of ALRN-6924-treated mice in a dose-dependent fashion. Repeated doses of ALRN-6924 every 8 hrs yielded sustained MIC-1 elevation, which correlated with reduced Ki67 positivity in the bone marrow. Treatment-dependent changes in cPARP expression were evident, but minimal in magnitude. In a nonlethal radiation exposure model, ALRN-6924 yielded significant protection from radiation-induced BW loss in a schedule-dependent manner. Placebo-treated mice showed 10% to 15% BW loss five days after irradiation, while mice receiving one or more ALRN-6924 doses 8 hrs prior to irradiation had an average of 4% BW loss ($p=0.008$, two-sided t test). **Conclusions**: ALRN-6924 mitigates toxicity in a mouse model of acute radiation injury. The observed radioprotection effect correlates with pharmacodynamic markers of cell proliferation and cell cycle arrest after one or more doses of ALRN-6924, and further supports previous demonstrations of chemoprotection with ALRN-6924. These results provide a rationale to investigate ALRN-6924 as a radioprotective agent.

P212 Systems biology-guided indication selection to inform the clinical development of a novel TEAD inhibitor. Marta Sanchez-Martin, Sakeena Syed, Hyejin Frosch, Chelsea Turcotte, Benjamin Amidon, Karen McGovern, Jeffrey Ecsedy, Michelle X. Zhang. Ikena Oncology, Boston, MA.

TEAD transcription factors are the final effectors of the Hippo pathway, a signaling cascade comprising multiple tumor suppressors (NF2, MST1/2, LATS1/2) critical in regulating proliferation, survival, and tissue homeostasis. Activated Hippo signaling suppresses TEAD-dependent transcription through the phosphorylation and degradation of TEAD co-activators YAP1 and TAZ in the cytosol. Consistently, genetic alterations in NF2, LATS1/2, YAP1 or TAZ lead to aberrant TEAD activation and are implicated in tumor initiation, progression, and therapeutic resistance in cancer. Here we present a unique systems biology approach integrating genomic, transcriptional and tissue-based analysis, to inform clinical development, indication and patient selection biomarkers for a novel inhibitor of TEAD being developed by Ikena. To identify cancers dependent on TEAD activity, tumor types were evaluated based on the cumulative incidence of genetic alterations in Hippo pathway genes including NF2, LATS1/2, YAP1, TAZ and others. This novel analysis pointed to a subset of tumors with high frequency of genetic alterations driving aberrant Hippo signaling including mesothelioma and NSCLC. This same subset was found to highly express a transcriptional signature indicative of YAP1/TEAD-dependency, confirming increased activation of TEAD transcription and pointing to the dependency of these tumors on TEAD activity. A proprietary IHC method was used to further assess YAP1/TAZ activation by assessing expression of either protein in the nucleus in multiple tumor tissue microarrays. Tissue-based analysis showed high YAP1 nuclear expression in tumors with frequent genetic alterations and high YAP1/TEAD-signature. Mesothelioma in particular ranks top among these indications, due to high frequency of NF2 deficiency and other Hippo pathway alterations. Consistently, TEAD inhibition showed single agent activity in two xenograft models of mesothelioma with NF2 deficiency and LAST1/2 alterations respectively. Hippo pathway alterations frequently co-occur with mutations in other oncogenic signaling pathways, *e.g.* EGFR. Importantly, YAP1/TAZ have been implicated in acquired resistance to targeted therapies in cancer including EGFR inhibitors in EGFR mutant tumors. Indeed, PDX models derived from patients who relapsed on osimertinib treatment showed high YAP1 protein expression in the nucleus. In addition, combination of TEAD and EGFR inhibitors induced apoptosis in vitro and greater antitumor activity than either drug as a single agent in vivo in EGFR mutant cancer models. In summary, the integration of multi-disciplinary bioinformatics, pharmacologic and tissue-based approaches enabled the identification of cancer types with high dependency on Hippo signaling. Moreover, these studies support the for monotherapy and combination of TEAD inhibitors with other targeted therapies including EGFR inhibitors. Altogether this unique approach has identified cancer patients who may benefit from TEAD inhibition and has informed the clinical development plan of a novel TEAD inhibitor.

P214 MTAP^{null}-selective PRMT5 inhibitors drive regressions in *MTAP*-deleted xenograft models across histologies. Kimberly J. Briggs, Kevin M. Cottrell, Matthew R. Tonini, Erik Wilker, Lina Gu, Charles B. Davis, Doug Whittington, Deepali Gotur, Haris Jahic, Matthew J. Goldstein, Alan Huang, John P. Maxwell. Tango Therapeutics, Cambridge, MA.

PRMT5 is a type II arginine methyltransferase that regulates essential cellular functions via symmetric dimethylation of target proteins involved in spliceosome regulation, cell cycle progression, apoptosis, the DNA-damage response, and other functions. PRMT5 dependence in cells with *MTAP* deletions is a strong and prevalent synthetic lethal interaction. Due to their mechanisms of action, existing clinical PRMT5 inhibitors do not recapitulate the selectivity for MTAP^{null} cells demonstrated by genetic perturbation. Given that *MTAP* deletion occurs in approximately 10-15% of all human cancer[i],[ii],[iii], a molecule that selectively kills MTAP^{null} cancer cells provides an important opportunity to deliver a targeted treatment to a significant patient population. We have discovered small molecules that exhibit MTA-cooperative PRMT5 binding and selectively kill MTAP^{null} cancer cells. Striking MTAP-dependent viability effects are demonstrated in MTAP-isogenic cell lines representing multiple lineages, and in a multi-lineage cell line panel comprised of 200 cancer cell lines. Furthermore, oral administration of an MTAP^{null}-selective PRMT5 inhibitor demonstrates dose-dependent antitumor activity and strong regressions across multiple histologies in both cancer cell line and patient-derived xenografts. These data suggest the therapeutic potential of MTAP^{null}-selective PRMT5 inhibitors in *MTAP*-deleted cancers. [i] Cerami et al., 2012 [ii] Gao et al., 2013 [iii] Lee et al., 2014

P215 The MDM2 inhibitor milademetan induces synthetic lethality in GATA3 mutant, ER positive breast cancer. Vijaya G. Tirunagaru[1], Gaia Bianco[2], Charlotte K. Y. Ng[3], Elisabetta Marangoni[4], Salvatore Piscuoglio[2], François-Clément Bidard[4], Robert C. Doebele[1]. [1]Rain Therapeutics, Newark, CA, [2]University of Basel, Basel, Switzerland, [3]University of Bern, Bern, Switzerland, [4]Institut Curie, Paris, France.

GATA3 is mutated, predominantly via frameshift (fs) mutations, in 15-18% of estrogen receptor (ER)-positive breast cancers and *GATA3* mutations define a subset of patients with poor response to hormonal therapy. Importantly, *GATA3* mutations are not currently targetable. Computational analysis of the data from the large-scale, deep RNAi screen project DRIVE led to the identification of *MDM2* as a synthetic lethal partner in a *GATA3* mutant ER+ breast cancer cell line. The objective of this study was to evaluate the activity of milademetan (RAIN-32), an orally available, small molecule inhibitor of MDM2 in GATA3 mutant ER+ breast cancer. Here, we evaluated the effect of *MDM2* silencing in ER+, *GATA3* mutant breast cancer cell line MCF-7 harboring a loss-of-function mutation (p.D335Gfs) that has been recurrently observed in breast cancer patients and tested the antitumoral activity of milademetan *in vitro* (MCF-7 cell line) *ex vivo* (patient derived organoids; PDO) and *in vivo* (cell line-derived xenograft and patient-derived xenograft (PDX) models). Additionally, we hypothesized that the synthetic lethality is p53 dependent and to this aim we explored the p53 dependent effects of milademetan inhibition by determining the effect of MDM2 loss in the absence of p53 and analyzing the expression of p53-target genes. Finally, we evaluated the impact of *GATA3* mutations on survival in breast cancer using TCGA and AACR Genie datasets.*MDM2* silencing significantly reduced cell proliferation in MCF-7. This effect was shown to be p53 dependent as p53 knockdown reversed the effect of MDM2 loss. MCF-7 cell line was also sensitive to milademetan *in vitro*. Finally, we tested milademetan in ER+ breast cancer models (PDO and a fulvestrant-resistant PDX) harboring a GATA3fs mutation and wild type *TP53*. Treatment of these models displayed dose-dependent anti-tumor activity to milademetan *ex vivo* and *in vivo*. Sequencing data analyses of the publicly available datasets revealed lower survival in *GATA3* mutant breast cancer and mutual exclusivity with *TP53* mutations. In conclusion, MDM2 deficiency leads to synthetic lethality in *GATA3* mutant breast cancers. Milademetan shows evidence of antitumor activity in genetically selected ER+ breast tumors with *GATA3* mutations. These data provide evidence for clinical exploration of milademetan in ER+, GATA3 mutant breast cancer refractory to current therapies.

P216 IK-930 mediated TEAD inhibition decreases and delays tumor growth and enhances targeted apoptosis in lung and colon cancer xenografts when combined with MEK or EGFR inhibitors. Benjamin Amidon, Hyejin Frosch, Sakeena Syed, Jill Cavanaugh, Chelsea Turcotte, Katie O'Callaghan, Prabitha Natarajan, Jeffrey Ecsedy, Karen McGovern, Alfredo Castro. Ikena Oncology, Boston, MA.

The Hippo signaling cascade is an important pathway that plays a role in controlling cell proliferation and limiting apoptosis, but when dysregulated can contribute to cancer initiation, progression, and therapeutic resistance. The TEAD family of transcription factors, in conjunction with YAP1 or WWTR1/TAZ, are regulated by Hippo pathway signaling and modulate cell growth and proliferation. IK-930 inhibits the growth of TEAD-dependent human cancer xenografts. By exploiting synthetic lethality, the IK-930 single agent activity in TEAD-dependent cancers could expand to other indications upon inhibition of certain oncogenic pathways. Literature indicates that activation of TEAD-dependent transcription by other oncogenes such as mutant EGFR or KRAS mediates resistance to multiple targeted therapies. Additionally, genetic loss of YAP1 leads to increased apoptosis in osimertinib and trametinib treated mutant EGFR NSCLC cell lines (Kurppa, 2020). Here, we present preclinical data that support the use of IK-930 combination therapies to enhance anti-tumor impact of EGFR and MEK blockade in mutant EGFR NSCLC, or with MEK inhibition in several BRAF and KRAS mutant cancers. In these studies, EGFR and MEK1/2 were inhibited and IK-930 used to enhance anti-tumor activity in *EGFR*-mutant NSCLC cells. These NSCLC cell lines that are largely insensitive to osimertinib and/or trametinib, were observed to have a marked increase in apoptosis *in vitro* when treated with the combination of osimertinib, trametinib, and IK-930. We also observed increased nuclear accumulation of YAP1 after treatment of NSCLC cells with osimertinib alone, or osimertinib and trametinib and upregulation of TEAD-dependent transcription. In NSCLC xenografts, the combination of osimertinib and IK-930 prevented tumor growth and the triple combination of osimertinib, IK-930, and trametinib drove complete tumor regression and demonstrated greater efficacy than any single agent or double combination. A second set of studies tested the synthetic lethality effect of IK-930 and MEK inhibition in *RAF*- and *RAS*-mutant tumors. The combination of TEAD inhibition with MEK inhibition enhanced apoptosis in several *KRAS*-mutant NSCLC, PDAC, CRC *KRAS*-mutant cell lines and BRAF-mutant melanoma. The combination of trametinib and IK-930 prevented tumor growth in these xenografts, where either single agent had either modest or little activity. In summary, the Hippo pathway has been implicated in multiple tumor types, including those with resistance mechanisms to EGFR and MEK blockade. IK-930-mediated TEAD inhibition has been investigated to suppress the bypass pathway activation mechanism to the targeted therapies. These data demonstrated the potential to further expand single agent patient benefit by combining IK-930 with MEK and/or EGFR inhibition in a wide variety of cancer indications, including mutant EGFR-driven NSCLC and mutant KRAS colon, lung, and pancreatic cancers. Taken together, these data are informing the clinical development plan and combination strategy for IK-930, a novel TEAD inhibitor.

P217 The next generation PARP inhibitor AZD5305 is active in a broad range of pre-clinical models of ovarian cancer. Giulia Dellavedova[1], Alessandra Decio[1], Anna Staniszewska[2], Elisabetta Leo[2], Raffaella Giavazzi[1], Maria Rosa Bani[1]. [1]Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy, [2]AstraZeneca, Cambridge, United Kingdom.

Since the approval of poly (ADP-ribose) polymerase inhibitors (PARPi) for BRCA-mutated ovarian cancer patients, different PARPi have been developed and have shown efficacy in homologous recombination deficient (HRD) tumours. These first-generation drugs inhibit both PARP1 and PARP2 (as well as other PARP family members) and present undesirable adverse effects, such as haematological and intestinal toxicity, that restricted their use especially in combination with already poorly tolerated chemotherapeutic agents. Here, patient-derived ovarian cancer xenografts (OC-PDXs) were used i) to evaluate the dose response efficacy of AZD5305, a next generation, potent and selective PARP1 inhibitor and ii) to investigate the effect of the combination with carboplatin, a standard-of-care treatment for ovarian cancer patients. AZD5305 was administered orally (po) once daily (QD) for 8 weeks as monotherapy (0.1, 1 and 10 mg/kg) and in combination with carboplatin (CPT) dosed intravenously (35 mg/kg; Q7x4). Two BRCA1 mutated (HOC106 and HOC107) and a BRCA wild-type (HOC84) OC-PDXs (all TP53 mutated) were implanted subcutis and the effect of treatments on tumour growth evaluated. AZD5305 dosed at 0.1, 1 and 10 mg/kg to mice bearing HOC106 tumours, inhibited tumours growth in a dose dependent manner. Administered in combination at 0.1 and 1 mg/kg, AZD5305 potentiated the effect of carboplatin, causing a significant and sustained regression of HOC106 tumours. The anti-tumour efficacy was also evident against the PARPi-resistant OC-PDX HOC107, whereby the tumour growth was inhibited by AZD5305 at 1 and 10 mg/kg dose. Combined with carboplatin, AZD5305 stabilized the growth of HOC107 tumours at doses as low as 0.1 mg/kg that on its own was not effective. As expected, none of the treatments affected the growth of the BRCA wild-type HOC84 (PARPi and carboplatin resistant OC-PDX). Overall, AZD5305 a next generation PARP1-selective inhibitor and trapper, shows improved efficacy and tolerability in combination with chemotherapy compared to first generation, PARP1/2 inhibitors, making it a promising clinical candidate for the treatment of ovarian cancer.

P218 Annamycin, a novel non-cardiotoxic anthracycline with high activity against sarcomas metastatic to lungs. Rafal Zielinski[1], Krzysztof Grela[1], Roberto Cardenas-Zuniga[1], Stanislaw Skora[1], Izabela Fokt[1], Maria Poimenidou[1], Salah-Eddine Lamhamedi-Cherradi[1], Joseph Ludwig[2], Waldemar Priebe[1]. [1]UT MD Anderson Cancer Center, Houston, TX, [2]UT MD Anderson Cancer Center, Houston, TX.

Introduction. Annamycin (ANN) is a non-cardiotoxic potent topoisomerase II poison structurally resembling doxorubicin (Dox). Specific structural modification altered ANN properties dramatically when compared with Dox. This novel drug also includes high activity against multidrug-resistant (MDR) tumors, unique subcellular distribution, and different tissue-organ distribution. Especially high differences have been noted in the lung uptake when compared with DOX. ANN is formulated in multilamellar liposomes (L-Annamycin, L-ANN), and such formulation additionally contributes to the high ANN lung uptake. **Objective.** The objective of this study was to reconfirm the unique tissue-organ distribution pattern in rats and explore ANN high lung uptake to target pulmonary sarcoma metastases by assessing the efficacy of L-ANN in the *experimental* models of lung metastases. **Methods.** Pharmacokinetics and biodistribution studies of ANN were performed in rats after bolus injection of L-ANN, free ANN, and Dox. Detection of Dox and ANN in plasma and different organs was accomplished using LC/MS/MS. *In vivo* efficacy was tested in two experimental lung metastatic models. The tumors were established by intravenous injection of MCA205 or K7M3 cells. The treatment consisted of weekly intravenous injections of 4 mg/kg of L-ANN or Dox. Metastatic nodules were visualized by computer tomography or bioluminescent imaging (BLI).
Results. Pharmacokinetic and tissue-organ distribution of L-ANN in rats confirmed unusually high uptake of ANN in lungs: the C_{max} of ANN was 30-fold higher than Dox after bolus administration of L-ANN or Dox. A remarkable extension of the survival was observed in two syngeneic sarcoma lung metastasis models. In the MCA205 fibrosarcoma model, the median survival of L-ANN treated mice was 87.5 days vs. 21 days for the vehicle-treated mice ($p<0.0001$), and 40% of animals were still alive when the experiment has been terminated on day 140. L-ANN also appeared highly efficacious in the osteosarcoma K7M3 model. Median survival has not been reached due to the high efficacy of L-ANN treatment. The experiment has been terminated on day 220 (the median survival for the vehicle-treated was 58 days, $p=0.0002$). Additionally, L-ANN and Dox were compared in the K7M3-Luc model. The BLI revealed a potent inhibition of the tumor growth by L-ANN-, but not in Dox-treated animals. Consequently, there was no extension of the survival in DOX-treated animals, while no deaths were recorded in L-ANN treated group as of day 90 (MS for Dox-treated mice was 59 days, ongoing study). **Conclusion.** Annamycin (ANN) is a novel non-cardiotoxic anthracycline that exhibits very high uptake in the lungs compared to Dox. Furthermore, ANN's rapid cellular uptake and preferential nanomolar cytotoxicity against cancer led to major efficacy in sarcoma lung metastasis models. These promising preclinical results led to the initiation of a multicenter sarcoma-specific clinical study (NCT04887298).

P220 Reactivation of the tumor suppressor SPARC in bladder cancer by verteporfin.

Neveen A. Said. Wake Forest University Health Sciences, Winston Salem, NC.

Bladder cancer (BCa) is the most common malignancy of urinary system and is one of the top 10 cancers in terms of new cases and deaths in the United States. While most patients present with non-muscle invasive (NMI) that is treated with bladder preserving approaches, recurrence with a muscle invasive (MI) disease with occult or detectable metastases is frequently encountered with poor prognosis. Because of its protracted natural history, BCa is among the most expensive malignancies from diagnosis to death. Limited advances have been achieved in treatment of MI disease in the past 30 years with a few drugs approved as second line of therapy. This limitation is mainly because the current approaches of drug development do not represent the complex heterogeneous BCa ecosystem that influences tumor behavior, treatment response, and disease outcome. In this respect, we have identified **SPARC** is a potent tumor suppressor in BCa whose expression is downregulated/lost in the cancerous compartment in advanced stage disease. We reported that SPARC inhibits BCa through inhibition of the multistep cascade of carcinogenesis, tumor invasiveness and metastasis, with multifaceted inhibitory effect on BCa cells, and their interactions with stromal cells. To date the mechanism of downregulation of SPARC during the evolution of BCa is still unraveled. To overcome this knowledge gap, we developed a comprehensive approach to identify drugs that reactivate SPARC expression in BCa cell lines, and restore its tumor suppressor effect in the BCa ecosystem. We identified verteporfin (VP) as a potent inducer of SPARC expression in established BCa cells and confirmed that VP exerts its anti-cancer effect through reactivation of SPARC. Specifically, VP inhibited BCa cell proliferation, clonogenic survival as well as repopulation on normal urothelial cells, fibroblasts as well as bladder smooth muscle cells. In addition, VP inhibited invasiveness of BCa cell lines through primary bladder smooth cells. *In vivo,* VP inhibited growth of UMUC3 cell lines in xenografts in nude mice; an effect that was partially and significantly mitigated by knockdown of SPARC in UMUC3 cells. Mechanistically, we found that VP-induces SPARC expression in BCa cells through inhibition of the association of histone modifying enzymes KDM4A, and HDAC1 with SPARC promoter. KDM4A, and HDAC1 are upregulated in BCa patients' tumors, and their nuclear expression inversely correlated with SPARC expression. Our data shine the light not only on novel mechanism of the anti-cancer effect of VP through reactivation of a BCa tumor suppressor "SPARC", but also on novel mechanism of repression of "SPARC" by histone modifying enzymes KDM4A and HDAC1, as well as identification of KDM4A and HDAC1 as targets of VP.

P221 Discovery of a covalent inhibitor for an oncogenic mutant RhoA^{Y42C}. Shirley Guo, Ping Cao. BridGene Biosciences, Inc., San Jose, CA.

Ras homolog family member A (RhoA) is a member of a larger family of Rho proteins of small GTPases, with structural and signaling similarities to Ras proteins. RhoA is involved in multiple cellular processes including cytoskeleton regulation and actin-myosin contractility, etc. Recent studies have shown that the most common RhoA mutation in diffuse gastric cancer (DGC), Y42C, causes a gain-of-function and is associated with the loss of the E-cadherin gene CDH (CDH1). Inhibitors of RhoA^{Y42C} could be promising agents for treating DGC that harbors CDH1 mutation/deletion. BridGene expressed RhoA^{Y42C} in cells, screened our proprietary covalent library against the RhoA^{Y42C} live cells, and employed our chemoproteomic platform IMTAC^{TM} (Isobaric Mass Tagged Affinity Characterization) to detect covalent labeling of the mutant RhoA protein. A pilot screening led to the successful identification of a covalent ligand of RhoA^{Y42C}, BGS1933. Mass spec analysis has confirmed the covalent modification of Cys42 in RhoA by BGS1933. Dose-dependent binding of BGS1933 to RhoA^{Y42C} was measured using an orthogonal fluorescence-based assay. The Cancer Genome Atlas (TCGA) study shows that ~1% DGC patients harbor the oncogenic mutant RhoA^{Y42C}. The discovery of a covalent inhibitor of RhoA^{Y42C} will enable us to validate the role of RhoA^{Y42C} in DGC and facilitate the development of new first-in-class targeted therapies for the treatment of gastric cancer.

P222 Combinations of receptor tyrosine kinase inhibitors targeting the tumor and stromal cells of complex spheroids from the National Cancer Institute's Patient-Derived Models Repository (PDMR; https://pdmr.cancer.gov/). Thomas S. Dexheimer[1], Julie Laudeman[1], Thomas Silvers[1], Rene Delosh[1], Russell Reinhart[1], Chad Ogle[1], Eric Jones[1], Nathan P. Coussens[1], Beverly A. Teicher[2], Naoko Takebe[2], Alice Chen[2], James H. Doroshow[2]. [1]Frederick National Laboratory for Cancer Research, Frederick, MD, [2]National Cancer Institute, NIH, Bethesda, MD.

Tumor stroma is critical in shaping the tumor microenvironment and promoting tumor growth and metastasis. Six stroma-targeted kinase inhibitors (cediranib, vandetanib, axitinib, pazopanib, cabozantinib, and lenvatinib) were tested both as single agents and in combination with five tumor cell-targeted kinase inhibitors (erlotinib, gefitinib, osimertinib, rociletinib, and erdafitinib)using a complex spheroid cell culture model. Complex spheroids, containing 60% tumor cells, 25% endothelial cells (HUVEC) and 15% mesenchymal stem cells (MSC), serve as a cell culture model of human solid tumors incorporating both malignant and stromal cells. Seventeen tumor cell lines were grown as complex spheroids, including varied sarcoma and non-small cell lung cancer types. Drug sensitivities of the HUVEC and MSC were also evaluated, both individually as monolayers and mixed as monolayers and spheroids. Overall, the HUVEC and MSC were more sensitive to the VEGFR inhibitors than to the EGFR inhibitors. Growth as complex spheroids reduced the sensitivity of HUVEC and MSC to several kinase inhibitors compared to each cell type alone or in mixed monolayer culture. A significant correlation was observed between the EGFR mRNA expression in patient-derived tumor lines and their sensitivity to the EGFR inhibitors. In combination regimens, Erlotinib was most effective when combined with the VEGFR inhibitors pazopanib, vandetanib and cediranib, which produced more than one log of cell killing at concentrations less than the clinical C_{max} of each drug. Osimertinib and rociletinib, which irreversibly target EGFR variants, were more cytotoxic towards complex spheroids of NCI-H1975 NSCLC (EGFR L858R, T790M) than NCI-H522 NSCLC (EGFR wildtype). Combinations of the mutant-selective EGFR inhibitors with a VEGFR inhibitor (especially, vandetanib, lenvatinib or cediranib) increased the cytotoxicity in complex spheroids containing EGFR mutant and wildtype tumor lines. Highly effective combinations included erdafitinib with vandetanib and erlotinib with cediranib. The combination of cediranib and erlotinib was also evaluated in nine patient-derived xenograft (PDX) models and resulted in two partial responses in a penile squamous carcinoma and uterine sarcoma, while the remainder showed stable, slowed, or progressive disease. Using >1 log of cell kill at drug concentrations less than the mouse C_{max} concentration for each drug as a predictor for *in vivo* responsiveness, the complex spheroid assay was 88% accurate in predicting response or progressive disease in the PDX models. This criteria for predicting *in vivo* activity from *in vitro* model results will be further explored. This project was funded in part with federal funds from the NCI, NIH, under contract no. HHSN261200800001E.

P223 Phase 1, first-in-human, dose-escalation, safety, pharmacokinetic, and pharmacodynamic study of oral TP-3654, a PIM kinase inhibitor, in patients with advanced solid tumors. Ignacio Garrido-Laguna[1], Patrick M. Dillon[2], Sujan Kabir[3], Jian Mei[3], Mark L. Wade[3], Huyuan Yang[3], Carl Stapinski[3], Jason M. Foulks[3], Steven L. Warner[3], Clifford Whatcott[3], Claudia Lebedinsky[3], Siqing Fu[4]. [1]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, [2]University of Virginia, Charlottesville, VA, [3]Sumitomo Dainippon Pharma Oncology, Inc, Cambridge, MA, [4]The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Elevated expression of PIM kinases in various solid and hematologic malignancies is correlated with increased cell survival and reduced apoptosis, implicating inhibition of PIM kinases as attractive targets for disruptive therapy. TP-3654 is an oral, small molecule, investigational PIM kinase inhibitor with favorable selectivity against PIM-1 over other kinases. TP-3654 is currently being evaluated in a phase 1 first-in-human study in patients (pts) with advance solid tumors (NCT03715504). **Methods:** Pts with advanced metastatic, progressive, or unresectable solid tumors refractory or intolerant of established therapies were treated with TP-3654. Pts took TP-3645 orally once (QD) or twice (BID) daily in a 28-day dosing cycle until unacceptable toxicity or disease progression. Escalating dose levels of TP-3654 were evaluated using a 3+3 design. The primary objective was to determine maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs). Secondary objectives included establishing the pharmacokinetic (PK) profile of oral TP-3654 and any evidence of antitumor activity. An exploratory objective was to study predictive biomarkers in tumor tissues and mechanism-based pharmacodynamic activity in peripheral blood mononuclear cells (PBMCs). **Results:** As of 31 May 2021, 22 pts were treated with TP-3654 at 480 mg QD (n=3), 720 mg QD (n=3), 1080 mg QD (n=4), 1440 mg QD (n=3), 720 mg BID (n=3), and 960 mg BID (n=6). Median age of 58 years (range: 20–72), 59% (13/22) pts male, and 86% (19/22) pts received ≥3 lines of prior therapy. Median treatment duration is 2 cycles (range: 0.1–24) and 6 pts received treatment for ≥4 cycles. Two pts remain on active study treatment. No DLT was observed. The most common treatment-emergent adverse events (TEAEs) reported in ≥20% of pts were vomiting, diarrhea, nausea, and abdominal pain. No related myelosuppressive AEs were reported. Fifty percent (11/22) of pts reported 18 Grade ≥3 TEAEs, of which only one (alanine aminotransferase increased) was considered related to TP-3654. No related, serious AEs were reported. Two TEAEs resulted in a fatal outcome (acute kidney injury, completed suicide); neither were related to TP-3654. Best response is stable disease (SD) in 67% (10/15) evaluable pts, SD for ≥ 16 weeks in 5 pts, best reduction of tumor size from baseline is -22%. TP-3654 plasma exposure increased with doses up to 1080 mg QD; the BID regimen at the 720 mg dose achieved greater plasma exposure than the 1440 mg QD dosing regimen. Reduced phosphorylation of PIM-1 downstream signal protein pBAD was observed in isolated PBMCs, predominantly in pts with prolonged clinical benefit. **Conclusions:** Preliminary data suggest that TP-3654 is tolerated as a monotherapy up to 960 mg BID in pts with heavily pretreated, relapsed, and refractory solid tumors. No DLT was observed and MTD was not reached. PK data indicate that the BID dose regimen achieved better plasma exposure than the QD regimen at higher doses. TP-3654 has shown target-specific inhibition of PIM-1 in pts. Updated data will be presented.

P224 Update from the Phase 2 registrational trial of repotrectinib in TKI-pretreated patients with *ROS1+* advanced non-small cell lung cancer and with *NTRK+* advanced solid tumors (TRIDENT-1). Jessica J. Lin[1], Byoung Chul Cho[2], Christoph Springfeld[3], D. Ross Camidge[4], Benjamin Solomon[5], Christina Baik[6], Vamsidhar Velcheti[7], Young-Chul Kim[8], Victor Moreno[9], Anthonie J. van der Wekken[10], Enriqueta Felip[11], Dipesh Uprety[12], Denise Trone[13], Shanna Stopatschinskaja[13], Alexander Drilon[14]. [1]Massachusetts General Hospital, Harvard Medical School, Boston, MA, [2]Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of, [3]Heidelberg University Hospital, National Center for Tumor Diseases, Department of Medical Oncology, Heidelberg, Germany, [4]University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, [5]Peter MacCallum Cancer Center, Melbourne, Australia, [6]University of Washington School of Medicine, Seattle Cancer Care Alliance, Fred Hutchinson Cancer Research Center, Seattle, WA, [7]NYU Perlmutter Cancer Center, New York, NY, [8]Chonnam National University Medical School, and CNU Hwasun Hospital, Hwasun-gun, Korea, Republic of, [9]Fundación Jiménez Díaz - START Madrid, Madrid, Spain, [10]University of Groningen, University Medical Centre Groningen, Groningen, Netherlands, [11]Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain, [12]Karmanos Cancer Institute, Detroit, MI, [13]Turning Point Therapeutics, San Diego, CA, [14]Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, NY.

Background: Repotrectinib is a next-generation ROS1/TRK tyrosine kinase inhibitor (TKI) that is currently in a registrational Phase 2 trial (TRIDENT-1). A previous update from the TRIDENT-1 trial showed a generally well tolerated safety profile in 185 treated patients. Interim efficacy data were previously presented. In *ROS1+* NSCLC patients with 1 prior TKI and prior platinum-based chemotherapy (EXP-2), confirmed overall response rate (cORR) was 40% (2 of 5 patients); in *ROS1+* NSCLC patients with 1 prior TKI without prior platinum-based chemotherapy (EXP-4), cORR was 67% (4 of 6 patients); in *ROS1+* NSCLC patients with 2 prior TKIs without prior platinum-based chemotherapy (EXP-3), cORR was 40% (2 of 5 patients). In *NTRK+* TKI-pretreated advanced solid tumor patients (EXP-6), cORR was 50% (3 of 6 patients). In addition, efficacy was demonstrated in patients who developed solvent front mutations (SFM) following prior ROS1/TRK TKI treatment. cORR was 67% (4 of 6 patients) and 50% (2 of 4 patients) in *ROS1+* NSCLC and *NTRK+* solid tumors patients with SFMs, respectively. One patient who developed a SFM after entrectinib treatment (EXP-2) had a cCR with 5.6+ months duration of response. **Methods:** TRIDENT-1 (NCT03093116) is an ongoing registrational Phase 2 trial enrolling patients whose cancers harbor a *ROS1* or *NTRK* gene fusion. Patients are enrolled into 6 defined expansion cohorts (EXP 1-6), based on cancer type and prior therapy. The primary endpoint is cORR by Blinded Independent Central Review using RECIST v1.1. **Results:** An updated safety analysis across Phase 1 and Phase 2 patients (n=243) based on a data cut-off date of 4 May 2021 was conducted. Treatment-emergent adverse events (TEAEs) observed in ≥20% of patients were dizziness (62%), dysgeusia (43%), constipation (33%), dyspnea (30%), paresthesia (28%), anemia (26%), and fatigue (26%). Grade ≥3 treatment-related AEs (TRAEs) were observed in 17% of patients; no Grade 5 TRAEs were observed. The majority (77%) of dizziness TEAEs were Grade 1 and 4% were Grade 3; none of the dizziness events led to treatment discontinuation. Dose modifications remained infrequent including 24% of patients with a TEAE that led to a dose reduction and 10% of patients with a TEAE that led to drug discontinuation. Updated efficacy information for TKI-pretreated *ROS1+* NSCLC and *NTRK+* advanced solid tumor patients is being collected and will be available for

presentation. **Conclusions:** Repotrectinib is a next-generation ROS1/TRK inhibitor. In an ongoing registrational Phase 2 trial, repotrectinib was generally well tolerated with low frequency of dose modifications. Updated Phase 2 efficacy data will be available for presentation.

P225 Preliminary interim data of elzovantinib (TPX-0022), a novel inhibitor of MET/SRC/CSF1R, in patients with advanced solid tumors harboring genetic alterations in *MET*: Update from the Phase 1 SHIELD-1 trial. David S. Hong[1], Daniel Catenacci[2], Lyudmila Bazhenova[3], Byoung Chul Cho[4], Mariano Ponz-Sarvise[5], Rebecca Heist[6], Victor Moreno[7], Gerald Falchook[8], Viola W. Zhu[9], Aurélie Swalduz[10], Benjamin Besse[11], Dong-Wan Kim[12], Shinkyo Yoon[13], Xiuning Le[1], Tingting Zhao[14], Alysha Kadva[14], Zachary Zimmerman[14], Jeeyun Lee[15]. [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]University of Chicago Medical Center, Chicago, IL, [3]UC San Diego Moores Cancer Center, La Jolla, CA, [4]Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of, [5]Clinica Universidad de Navarra, Madrid, Spain, [6]Massachusetts General Hospital, Boston, MA, [7]Fundación Jiménez Díaz - START Madrid, Madrid, Spain, [8]Sarah Cannon Research Institute at HealthONE, Denver, CO, [9]University of California Irvine, Orange, CA, [10]Centre de Lutte Contre le Cancer - Centre Leon Berard, Lyon, France, [11]Institut Gustave Roussy, Villejuif Cedex, France, [12]Seoul National University Hospital, Seoul, Korea, Republic of, [13]Asan Medical Center, Seoul, Korea, Republic of, [14]Turning Point Therapeutics, San Diego, CA, [15]Samsung Medical Center, Seoul, Korea, Republic of.

Background: Elzovantinib is a novel, type I tyrosine kinase inhibitor (TKI) that targets MET, SRC, and CSF1R. Genetic alterations in *MET*, including exon 14 skipping (Δex14), amplifications, fusions, and mutations occur in many tumor types. SRC is a key downstream MET effector while CSF1R modulates tumor associated macrophages. Inhibition of SRC and CSF1R can potentially improve the durability of response compared to inhibition of MET alone. Currently there are no approved targeted therapies after progression on a MET TKI. The Phase 1 SHIELD-1 trial (NCT03993873) is evaluating the safety, pharmacokinetics (PK), and preliminary activity of elzovantinib in patients with advanced solid tumors harboring genetic *MET* alterations. A previous interim analysis included 15 efficacy evaluable patients. Among 10 MET TKI-naïve patients, 5 had achieved PRs (3 confirmed), including 3 gastric/GE junction, 1 CRC, and 1 NSCLC. Of the 5 MET TKI-pretreated NSCLC patients, 3 had stable disease (Hong et al. EORTC-NCI-AACR 2020, Abstract nr LBA-01). Here we report updated data from the SHIELD-1 trial. **Patients and methods:** Adults with advanced solid tumors harboring genetic *MET* alterations were enrolled using a 3+3 dose-escalation design. Expansion was allowed at doses where clinical activity was observed. Elzovantinib was given orally in continuous 28-day cycles. **Results:** As of 13 May 2021, 52 patients have been enrolled across 7 dose levels, including 30 NSCLC patients (20 Δex14, 8 amplifications, 2 mutations), 9 gastric cancer patients (8 amplifications, 1 fusion), and 13 patients who had other cancers with *MET* alterations. Median age was 63 (33-84) years. Median number of prior therapies was 2 (range 0-6). 34 of 52 patients (65%: 13 NSCLC; 9 gastric; 12 others) had not received a prior MET TKI and 18 (35%: 17 NSCLC; 1 liver) had a prior MET TKI. The most common adverse events (AEs) were dizziness (65%), lipase increase (33%), anemia (29%), constipation (29%), and fatigue (29%). Most AEs were Grade 1 or 2 with 94% of dizziness AEs being Grade 1 or 2. No events of interstitial lung disease/pneumonitis, Grade 3/4 edema, or treatment-related Grade 3/4 ALT/AST elevation were reported. Two dose-limiting toxicities (Grade 2 dizziness; Grade 3 vertigo) occurred at the highest tested dose of 120 mg QD. Systemic exposure increased in a dose-dependent manner. The steady state trough concentrations were consistently above the IC_{95} for inhibition of MET phosphorylation across all cohorts with a terminal half-life of 13-17 hours. Evaluation of the recommended Phase 2 dose (RP2D) is ongoing and further efficacy analysis will be available for

presentation. **Conclusions:** Elzovantinib is a novel MET/SRC/CSF1R inhibitor with a favorable PK profile. Elzovantinib was generally well tolerated with primarily low-grade dizziness, and no high-grade edema reported. The RP2D is currently under evaluation and updated safety and efficacy data will be available for presentation. A global multi-cohort Phase 2 trial of patients with *MET*-altered tumors is planned.

P226 Design and rationale of a first in human (FIH) phase 1/1b study evaluating KIN-2787, a potent and highly selective pan-RAF inhibitor, in adult patients with BRAF mutation positive solid tumors. Meredith McKean[1], Shumei Kato[2], Alexander Spira[3], Yan Xing[4], Hao Xie[5], Elizabeth Buchbinder[6], Ken Kobayashi[7], Richard Williams[7]. [1]Sarah Cannon Research Institute, Nashville, TN, [2]University of California San Diego (UCSD), San Diego, CA, [3]Virginia Cancer Specialists, Fairfax, VA, [4]City of Hope, Duarte, CA, [5]Moffitt Cancer Center, Tampa, FL, [6]Dana Farber Cancer Institute, Boston, MA, [7]Kinnate Biopharma Inc, San Diego, CA.

Background $BRAF^{V600}$ mutations (BRAF Class I mutations) encode a highly active monomeric oncogenic kinase that can be targeted by approved RAF kinase inhibitors in patients (pts) with melanoma (MEL), NSCLC, colorectal & anaplastic thyroid cancers. In other discrete patient populations, molecularly distinct BRAF alterations drive dimer-dependent oncogenic signaling including BRAF Class II and III alterations. Class II alterations include single nucleotide variants (SNVs), indels and gene fusions that induce BRAF homodimer formation leading to oncogenic kinase activation. Class III mutations, typically SNVs, are low activity kinase mutations that promote RAF dimerization and oncogenic MAPK pathway signaling that is RAS-dependent. Approved BRAF inhibitors have limited clinical activity in diverse solid tumors driven by BRAF Class II or III alterations, highlighting the urgency to develop effective targeted therapies for these patients. KIN-2787 is a potent, highly selective pan-RAF type II kinase inhibitor designed to target both monomeric & dimeric forms of the mutant BRAF kinase and minimize paradoxical activation, a liability often observed with other RAF inhibitors that can adversely impact tolerability & require addition of a MEK inhibitor to suppress pathway activation. KIN-2787 has favorable pharmaceutical properties, achieves substantial systemic exposures in pre-clinical toxicology studies & induces regressions in human cancer xenograft models driven by BRAF Class I, II or III mutations. **Methods** This is a FIH, multicenter, non-randomized, open-label, Ph1 study (NCT04913285) of KIN-2787 in adult patients with BRAF mutant advanced & metastatic solid tumors (AMST). KIN-2787 will be given orally bid continuously in 28-day cycles until drug intolerance or disease progression. Planned sample size is approx. 115 pts in two parts: Part A is a dose-escalation to MTD open to pts with AMST driven by BRAF Class I, II or III genomic alterations. Part B will evaluate a selected dose of KIN-2787 in 3 cohorts of pts with MEL, NSCLC, or other AMST, each driven by BRAF Class II or III alterations. Standard Ph1 enrollment criteria are required (ECOG PS ≤ 2, normal organ function, prior receipt of standard treatment or medical judgment that such is not appropriate). Patients may have measurable or evaluable disease per RECIST v1.1. Key exclusion criteria include known active brain metastases from non-brain tumors, prior receipt of BRAF-, MEK-, or MAPK-directed inhibitor therapy (unless for FDA approved indications), HBV & HCV seropositivity. Primary endpoints are safety/tolerability (Part A), and preliminary antitumor activity: objective response rate (ORR), disease control rate (DCR), duration of response (DOR), & duration of stable disease (Part B). Secondary objectives include pharmacokinetic (PK) and pharmacodynamic (PD) assessments including tissue and blood-based measures of MAPK signaling inhibition. This study recently opened for Part A recruitment in the United States (NCT04913285).

P228 Preclinical pharmacokinetics and pharmacodynamics of KB-0742, a selective, oral CDK9 inhibitor. Melinda A. L. Day, Douglas C. Saffran, Nathalie Rioux, Tom Chen, Christina Lee, David B. Freeman, Crystal MacKenzie, Joseph P. Vacca, Peter B. Rahl, Benjamin Wesley Trotter, Charles Y. Lin, Pavan Kumar, Jorge DiMartino. Kronos Bio, Inc., San Mateo, CA.

Tumorigenesis is driven by the accumulation of adverse genetic changes resulting in dysregulated transcription promoting altered gene expression and the cancer cell state. Thus, tumors can develop dependencies on the transcriptional regulators that promote the reprogrammed gene expression landscape. One such regulator is cyclin-dependent kinase 9 (CDK9), which regulates transcriptional elongation and promotes activation of transcription factors. We developed a potent, selective, and orally bioavailable CDK9 inhibitor, KB-0742. KB-0742 is highly selective for CDK9 as compared to other CDKs and has shown minimal off-target effects in kinase and receptor panel screens. Here we present the nonclinical pharmacologic characterization of KB-0742. The pharmacokinetics (PK) of KB-0742 in rats and dogs showed rapid absorption and high bioavailability with a %F of ≥84 after oral administration in rats and >100 in dogs. KB-0742 exhibited low turnover in human microsome and hepatocyte preparations and is projected to have low clearance and a high volume of distribution in humans based on allometric scaling from nonclinical species. These properties may facilitate achievement of sustained target engagement in patients using intermittent dosing which may mitigate the toxicity of CDK9 inhibition. To test this hypothesis, mice bearing MV4-11 (acute myeloid leukemia) xenografts were treated with KB-0742 at either 60 mg/kg for 3-days on/4-days off or continuous dosing at 25 mg/kg. Intermittent dosing showed similar tumor growth inhibition (81%) as seen with continuous dosing (74%). Target engagement was assessed in the tumors by measuring the inhibition of RNA polymerase II (pSER2). KB-0742 treatment resulted in an over 50% decrease in pSER2 compared with vehicle-treated controls. To support the phase I clinical trial, 2 pharmacodynamic assays were developed to measure drug target engagement in peripheral blood mononuclear cells (PBMCs). The first used RNA-expression profiling to measure alterations in gene expression with treatment, and the second used MSD protein analysis to measure changes in pSER2 levels. Ex vivo experiments using PBMCs from 3 healthy donors showed an over 80% reduction in pSER2 levels at 4 hours post-exposure to 1 µM of KB-0742. Similar results were observed using the RNA-expression profiling assay, in which decreased gene expression was observed in key genes at 1 µM exposures. KB-0742's high specificity, oral bioavailability, and favorable PK properties distinguish it from other available CDK9 inhibitors. We are currently testing the hypothesis in the clinic with a phase I trial (NCT04718675) that the ability to dose intermittently will allow for favorable anti-tumor activity while minimizing adverse effects.

P229 Pre-clinical evaluation of next-generation inhibitor targeting a wide spectrum of oncogenic BRAF dimers. Yoon-Chi Han[1], Pui Yee Ng[2], Ryan Schulz[1], Shao Ning Yang[1], Alana Lelo[1], Luisa Shin[2], Matthew O'Connor[1], Ivan Jewett[2], Noboru Ishiyama[3], Darlene Romashko[1], Shalabh Thakur[3], Andrei Salomatov[1], Sherri Smith[2], Elizabeth Buck[1], Christopher Roberts[2], Matthew Lucas[2], Tai-An Lin[1]. [1]Black Diamond Therapeutics, New York, NY, [2]Black Diamond Therapeutics, Cambridge, MA, [3]Black Diamond Therapeutics, Toronto, Canada.

The canonical BRAF V600E (Class I) mutation is a potent oncogene uniquely active as a RAS-independent monomer, successfully targeted by several FDA-approved inhibitors. However, these first-generation BRAF inhibitors are not active against non-canonical BRAF oncogenic mutations, including BRAF-fusions, that drive RAS-independent (Class II) or RAS-dependent (Class III) dimers. As such, developing inhibitors directed against dimeric BRAF oncogenic mutations that avoid paradoxical activation is a major unmet clinical need. We applied proprietary Mutation-Allostery-Pharmacology (MAP) platform technology developed by Black Diamond Therapeutics to identify and validate a group of previously uncharacterized non-canonical oncogenic Class II and Class III BRAF mutation clusters. We further demonstrate that this ensemble of novel and previously validated non-canonical oncogenic BRAF mutants can form the basis of a differentiated drug discovery program aimed at identifying small molecules that potently and selectively target this family of dimeric BRAF mutations. Herein, we describe a small molecule inhibitor, BDTX BRAF-A, with potent anti-proliferative activity directed against tumor cells expressing a wide spectrum of non-canonical Class II/III mutations. This broad activity ("MasterKey" profile) of BDTX BRAF-A is further demonstrated in cell lines that harbor endogenous oncogenic dimer-inducing BRAF mutations and in various solid tumor patient-derived xenograft (PDX) models *ex vivo*. Importantly, BDTX BRAF-A did not induce paradoxical RAF activation characteristic of Class I BRAF inhibitors. Finally, BDTX BRAF-A achieves robust anti-tumor efficacy and target engagement of dimeric BRAF oncogenes in mouse models. These data support the continued development of rationally designed molecules targeting a broad range of non-canonical BRAF dimer-promoting mutations to extend the prospect of precision medicine in patients.

P230 A phase 1/2 study of BLU-945, a highly potent and selective inhibitor of epidermal growth factor receptor (EGFR) resistance mutations, in patients with *EGFR*-mutant non-small cell lung cancer (NSCLC). David Spigel[1], Koichi Goto[2], D. Ross Camidge[3], Yasir Elamin[4], Adrianus J. de Langen[5], Natasha B. Leighl[6], Anna Minchom[7], Zofia Piotrowska[8], David Planchard[9], Karen Reckamp[10], Faris Albayya[11], Jennifer Green[11], Sean Kim[11], Melinda Louie-Gao[11], Renata Sawtell[11], Alena Zalutskaya[11], Byoung Chul Co[12]. [1]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN, [2]National Cancer Center Hospital East, Kashiwa, Japan, [3]Lung Cancer Clinic, Anschutz Medical Campus, University of Colorado, Aurora, CO, [4]MD Anderson Cancer Center, University of Texas, Houston, TX, [5]Netherlands Cancer Institute, Amsterdam, Netherlands, [6]Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, ON, Canada, [7]Drug Development Unit, Royal Marsden/ Institute of Cancer Research, Sutton, London, United Kingdom, [8]Massachusetts General Hospital, Boston, MA, [9]Department of Medical Oncology, Thoracic Group, Gustave Roussy, Villejuif, Villejuif, France, [10]Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, [11]Blueprint Medicines Corporation, Cambridge, MA, [12]Department of Internal Medicine, Yonsei University, Seoul, Korea, Republic of.

BACKGROUND Activating *EGFR* mutations such as exon 19 deletion (ex19del) and L858R mutations, exon 19 insertions (ex19ins), and point mutations L861Q, G719X, and S768I are oncogenic drivers in lung adenocarcinoma, the most common NSCLC subtype. Although EGFR-targeted therapies, including first-, second-, and third-generation tyrosine kinase inhibitors (TKIs), have improved outcomes in patients (pts) with *EGFR*-mutant (*EGFRm*) NSCLC, resistance to these drugs is inevitable. BLU-945 is an investigational fourth-generation EGFR TKI designed to suppress both activating and on-target resistance EGFR mutations, such as C797S and T790M. Preclinical data showed BLU-945 has nanomolar potency against *EGFRm*/T790M double and *EGFRm*/T790M/C797S triple mutants with >450-fold selectivity for these mutants over wild-type EGFR in biochemical and cellular assays, and demonstrated robust preclinical antitumor activity, including in intracranial tumors harboring these mutations, providing the therapeutic rationale for BLU-945 clinical development. **METHODS** BLU-945-1101 (NCT04862780) is an international, open-label, first-in-human, phase 1/2 study designed to evaluate safety, tolerability, and antitumor activity of BLU-945 in pts with *EGFRm* NSCLC. Key eligibility criteria include adults with pathologically confirmed metastatic NSCLC with an activating *EGFR* mutation per local assessment (including ex19del or L858R); Eastern Cooperative Oncology Group performance status 0–2; and previous treatment with ≥1 EGFR-targeted TKI. Pts with asymptomatic brain metastases and on stable doses of corticosteroids are eligible. Key exclusion criteria are any additional known primary driver alterations, including but not limited to pathologic aberrations of *EGFR* exon 20 (insertions), *KRAS*, *BRAF* V600E, *NTRK1/2/3*, *HER2*, *ALK*, *ROS1*, *MET*, or *RET*. Phase 1 primary endpoints are maximum tolerated dose, recommended phase 2 dose (RP2D), and safety. The phase 2 primary endpoint is overall response rate (ORR) by RECIST 1.1. Phase 1 secondary endpoints include ORR, duration of response (DOR), pharmacokinetics (PK), and pharmacodynamics (PD); phase 2 secondary endpoints include DOR, disease control rate (DCR), clinical benefit rate (CBR), progression free survival (PFS), overall survival (OS), time to intracranial progression and intracranial response rate, PK, PD, and safety. Phase 1 dose escalation (N≈60) will be conducted using Bayesian optimal interval design. In the phase 2 dose expansion (N≈61), pts will be enrolled into 3 groups based on mutation profile and treated at the RP2D: primary group 1 for pts

with *EGFR* T790M and C797S (n≈37); exploratory group 2 for pts with *EGFR* T790M but not C797S (n≈12); exploratory group 3 for pts with *EGFR* C797S but not T790M (n≈12). Group 1 will use Simon's 2-stage design to test the null hypothesis of ORR ≤20% against a 1-sided alternative of ≥40%. Pts may receive treatment until disease progression or unacceptable toxicity. Recruitment has started and approximately 30 sites will be open for enrollment across North America, Europe, and Asia.

P231 Multigenomic characterization of context-dependent alternative splicing in normal and neoplastic cells. Elizabeth A. McMillan[1], Krishna Sriram[1], Emily Creger[1], Carsten Merkwirth[1], Raffaella Pippa[2], Melinda Pedraza[2], Long Do[2], Vishal Deshmukh[3], Carine Bossard[2], Michael A. White[2]. [1]Biosplice Therapeutics, Inc., San Diego, CA, [2]Biosplice Therapeutics Inc., San Diego, CA, [3]Formerly Biosplice Therapeutics Inc., San Diego, CA.

Alternative pre-mRNA splicing (AS) supports the dynamic and regulated diversification of cells by allowing the production of multiple distinct proteins from individual genes. Dysregulated AS is commonly associated with human malignancies, producing pathological proteomes that underpin disease initiation, progression, and emergence of therapy resistance. The CDC2-like kinases (CLKs) and dual-specificity tyrosine-regulated kinases (DYRKs) are thought to govern the efficiency and specificity of AS by directly phosphorylating serine/arginine-rich splicing factors (SRSFs) and thereby influencing pre-mRNA splice junction selection. Cirtuvivint (SM08502) is a first-in-class small molecule ATP-competitive inhibitor of CLK/DYRK kinases. To directly evaluate the contribution of these kinases to AS profiles, changes in AS following treatment with cirtuvivint followed by high-depth RNAseq analysis across >25 cell lines representing 7 tumor lineages were evaluated. Both baseline and drug-induced changes in AS events (ASEs) were measured using a multivariate analysis of transcript splicing (rMATS). Pan-CLK/DYRK inhibition was found to affect a minority of baseline ASEs, leaving the majority of spliceosome activity intact in all samples. However, the magnitude and quantity of detected drug-induced alterations were larger in a sample of tumor cells from a patient compared with adjacent non-tumorigenic cells. Moreover, most ASEs sensitive to pan-CLK/DYRK inhibition were tumor type-specific irrespective of selective presence at the gene level. Multi-omics data integration revealed sensitivity to cirtuvivint was associated with alterations in splicing genes and that drug-induced ASEs were significantly associated with disease-promoting biology across lineage and oncogenic driver contexts. Perturbed splicing of the AR-V7 variant in treatment-resistant prostate cancer and MDM2 in p53 wild-type cancers were prominent examples. These observations indicate vulnerabilities to CLK-DYRK regulated splicing span a wide range of oncogenic contexts with potential to be therapeutically addressed with pan CLK/DYRK inhibitors.

P233 TAS0953/HM06 is effective in preclinical models of diverse tumor types driven by *RET* alterations. Igor Odintsov[1], Renate I. Kurth[1], Kota Ishizawa[2], Lukas Delasos[3], Allan J.W. Lui[4], Inna Khodos[5], Connor J. Hagen[5], Qing Chang[5], Marissa S. Mattar[5], Morana Vojnic[6], Siddharth Kunte[5], Annalisa Bonifacio[7], Claudio Giuliano[7], Elisa De Stanchina[5], Emily Cheng[5], Emanuela Lovati[7], Marc Ladanyi[1], Romel Somwar[1]. [1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Tohoku University, Sendai, Japan, [3]Cleveland Clinic, Cleveland, OH, [4]Cancer Research UK Cambridge Institute, Cambridge, United Kingdom, [5]Memorial Sloan Kettering Cancer Center, New York, NY, [6]Northwell Health, Lennox Hill Hospital, New York, NY, [7]Helsinn Healthcare, Lugano, Switzerland.

Fusions involving RET receptor tyrosine kinase are a common driver of tumors across different tissue types, such as lung, thyroid, colorectal, soft tissue and others. TAS0953/HM06 (hereby referred to as HM06) is a novel 2ndgeneration RET-specific inhibitor that is effective against RET solvent front (G810) and gatekeeper (V804) mutations. Here, we evaluated the efficacy of HM06 in lung and thyroid carcinomas, and soft-tissue sarcoma cell lines and PDXs derived from RET inhibitor-naive tumor samples or from tumors with acquired resistance to selpercatinib. HM06 was more effective than the RET multi-kinase inhibitors cabozantinib and vandetanib, and as effective as selpercatinib and pralsetinib in inhibiting growth of patient-derived and isogenic lung, thyroid and sarcoma cell lines (IC_{50}=0.02-0.1 μM) harboring different RET fusions (KIF5B-RET, CCDC6-RET, TRIM33-RET, SPECCL1-RET) or activating mutations (RET C634W). Growth of non-tumor cells was up to 80-fold less sensitive to HM06 (IC_{50}= 1.6 μM). Treatment of RET fusion-positive lung cancer cells with HM06 resulted in a dose-dependent inhibition of RET phosphorylation (Y905 and Y1062) and the downstream effectors AKT, ERK1/2, p70S6K and S6. Caspase 3/7 activity and markers of apoptosis (BIM, cleaved PARP) were induced by HM06 to a similar extent as pralsetinib and selpercatinib (dose range: 0.05-1 μM). HM06 induced changes in the core mediators of cell cycle regulation (upregulation of p27, downregulation of CCND1) and suppressed expression of MYC and ETV5. *In vivo*, HM06 blocked tumor growth and/or induced regression of up to 65% in seven patient-derived xenograft (PDX) models with RET fusions (five NSCLC PDXs, one sarcoma PDX and one NSCLC cell-line xenograft) to a similar extent as pralsetinib and selpercatinib. However, 6 weeks after cessation of treatment of the SPECCL1-RET-driven sarcoma PDX model, growth of tumors treated with HM06 was suppressed completely, whereas 3/5 pralsetinib-treated tumors and 1/5 selpercatinib-treated tumor regrew. Combination of HM06 and the MET inhibitor capmatinib effectively blocked growth of PDX tumors in a model that was derived from a patient sample that expressed *RET* fusion and *MET*amplification, and was resistant to selpercatinib. These results suggest that HM06 may be an effective therapy for RET-driven tumors in a tissue-type agnostic manner and can effectively address common on-target and off-target resistance mechanisms such as RET G810X and V804X mutations. HM06 is currently in a phase 1 and 2 clinical trial for patients with advanced solid tumors with RET gene abnormalities (margaRET, NCT 04683250).

P234 ORIC-114, an orally bioavailable, irreversible kinase inhibitor, has superior brain penetrant properties and enhanced potency in preclinical studies of HER2-positive breast cancer. Melissa R. Junttila[1], Jason E. Long[2], Robert Warne[2], Sunghwan Kim[3], Younho Lee[4], Hwan Kim[3], Juhee Kang[3], Jiyoon Seok[3], Jihye Yoo[3], Youngyi Lee[3], Dong-Hyuk Seo Seo[3], Jung Beom Son[4], Daekwon Kim[3], Hwan Geun Choi[5], Nam Doo Kim[4], Tatiana Zavorotinskaya[2], Chelsea Chan[2], Matthew Panuwat[2], Jessica Sun[2], Jae H. Chang[2], Lori S. Friedman[2]. [1]ORIC Pharmaceuticals, South San Francisco, CA, [2]ORIC Pharmaceuticals, S San Fran, CA, [3]Voronoi, Incheon, Korea, Republic of, [4]Voronoibio, Incheon, Korea, Republic of, [5]B2Sbio, Incheon, Korea, Republic of.

Amplification of human epidermal growth factor receptor 2 (HER2) is an oncogenic driver found in approximately 25% of breast cancer. Despite the arsenal of HER2-directed therapies available to patients, more than 50% of patients with HER2 amplification eventually develop central nervous system (CNS) metastases over the course of their disease indicating a clear medical need for brain penetrant therapies in this patient population. ORIC-114 is a brain penetrant, orally bioavailable, irreversible small molecule inhibitor that was designed to target exon20 insertions in epidermal growth factor receptor (EGFR) and HER2. ORIC-114 is highly selective for the EGFR/HER2 family of receptors, reducing the risk for off-target kinase liabilities. In biochemical assays, ORIC-114 displayed low nanomolar potency on HER2. To explore the application of ORIC-114 in the HER2-amplified tumor setting, a cell viability screen was performed against a panel of human breast cancer lines containing both HER2-amplified and non HER2-amplified cell lines. ORIC-114 demonstrated greater than 100-fold enhanced cellular potency on HER2-amplified cancer cell lines relative to non-amplified cancer cell lines. Notably, ORIC-114 cellular EC50s in HER2-amplified breast cancer cell lines were below 30 nM and more potent than both lapatinib and tucatinib, two FDA-approved tyrosine kinase inhibitors for the treatment of HER2-positive breast cancer. ORIC-114 was designed to incorporate physicochemical properties suitable to cross the blood-brain barrier and has exhibited good brain penetration across multiple preclinical species. To further investigate the brain penetrant attributes of ORIC-114 in the context of HER2-positive breast cancer with brain metastases, key features were assessed relative to tucatinib, which has demonstrated clinical efficacy in this setting. In contrast to tucatinib, ORIC-114 displayed minimal impact on efflux transporters as it was only a weak substrate for P-glycoprotein (P-gp) and was not a substrate for breast cancer associated protein (BCRP) in vitro. In addition, ORIC-114 demonstrated superior in vivo brain penetration relative to tucatinib as measured by free brain-to-plasma ratio in mouse. Consequently, ORIC-114 free brain concentrations in rodents were greater than tucatinib, even when the active metabolites of tucatinib were considered. Taken together, these data further establish ORIC-114 as a selective, irreversible, and brain penetrant EGFR/HER2 inhibitor, making it a promising therapeutic candidate for development in patients with HER2-positive tumors including those with CNS metastases. A Clinical Trial Application for ORIC-114 is anticipated in the second half of 2021.

P236 Targeting AXL/PDK1 signaling axis activates immunogenic cell death in liver cancer. Yunong Xie[1], Lei Zhou[2], Cheuk Yin Lin[1], Terence Lee[3], Jin Ding[4], Stephanie Ma[1], Man Tong[1]. [1]School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, Hong Kong (Greater China), [2]School of Biomedical Sciences, LKS faculty of Medicine, The University of Hong Kong, Hong Kong, Hong Kong (Greater China), [3]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, Hong Kong (Greater China), [4]National Center for Liver Cancer, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University of China, Shanghai, China (Mainland).

Hepatocellular carcinoma (HCC) is the most malignant form of primary liver cancers with poor prognosis. Majority of the cases are diagnosed in advanced stages where therapeutic options are limited and the response rates are low. There is an unmet clinical need for more effective therapeutic treatment. AXL is a receptor tyrosine kinase which belongs to the family of TAM receptors. Overexpression of AXL has been reported in multiple cancers, including HCC. We and others have also reported an up-regulation of AXL in sorafenib-resistant HCC. However, its role in regulating anti-tumor immunity in HCC is not fully understood. In this study, we observed an immunosuppressive gene signature in sorafenib-resistant HCC by RNA-Seq and pathway enrichment analysis. Genetic manipulation and pharmacological inhibition of AXL promoted immunogenic cell death in sorafenib-resistant HCC cells, as evident by increased surface calreticulin and HMGB1 secretion. PDK1, a key modulatory kinase downstream of AXL in the PI3K/Akt pathway, was also up-regulated in sorafenib-resistant HCC. Knockdown of PDK1 similarly led to an increased immunogenic cell death. In a sorafenib-resistant immunocompetent mouse HCC model, blockade of AXL with a selective AXL inhibitor increased tumor-infiltrating activated and proliferating CD8+ T cells, resulting in tumor suppression and prolonged survival of the mice. Taken together, our findings suggest that targeting the AXL/PDK1 signaling axis could be a viable therapeutic option which enhances anti-tumor immunity for the treatment of HCC.

P237 PRT2527 is a potent and selective CDK9 inhibitor that demonstrates anti-cancer activity in preclinical models of hematological malignancies and solid tumors with *MYC* amplification. Yang W. Zhang[1], Liang Lu[1], Min Wang[2], Dave Rominger[1], Stefan Ruepp[1], Kirsten Gallagher[3], William Gowen-MacDonald[1], Chaofeng Dai[1], Miles Cowart[1], Andrew Combs[1], Bruce Ruggeri[1], Peggy Scherle[1], Kris Vaddi[1]. [1]Prelude Therapeutics, Wilmington, DE, [2]Prelude Therapeutics, Wilmington, DE, [3]Prelude Therapeutics, Wilmington, DE.

Cyclin-dependent kinase 9 (CDK9) is a master regulator of transcription that controls paused RNA polymerase II (RNAP2) release through phosphorylation of its carboxy-terminal domain, resulting in productive transcription elongation. CDK9 has been extensively studied as a potential target for cancer therapy in "transcriptionally addicted" tumors as transient inhibition of CDK9 primarily depletes proteins with short half-lives, such as the oncogenes MCL1 and MYC, making CDK9 a promising target in cancer. Here we show that PRT2527 is a potent and highly selective CDK9 inhibitor with moderate to high clearance that achieves optimal temporal target engagement and exhibits potent in vitro and in vivo activities. PRT2527 inhibited CDK9 enzymatic activity with an IC_{50} of 0.98 nM in a biochemical assay and showed high selectivity in a panel of kinases when tested at physiologically relevant 1 mM ATP concentration. In vitro, PRT2527 inhibited phosphorylation of Ser2RNAP2 in NCI-H929 cells with an IC_{50} of 54 nM, and an IC_{50} of 198 nM in a plasma assay to adjust for human plasma protein binding. Transient treatment of cells with PRT2527 inhibited pSer2RNAP2, depleted MCL1 and MYC proteins, and activated cleaved caspase-3 (CC3) in a concentration-dependent manner. In a proteomic profiling study, MCL1 was identified as one of the major down-regulated proteins following PRT2527 treatment. In a panel of hematological cancer cell lines representing B- and T-ALL, AML, and non-Hodgkin's lymphoma (NHL), as well as subsets of sarcoma, prostate, adenoid cystic carcinoma (ACC), and non-small cell lung cancer (NSCLC) cell lines, PRT2527 treatment consistently led to a potent, concentration-dependent inhibition of proliferation. In a pharmacokinetic/pharmacodynamic (PK/PD) study, intravenous (IV) administration of PRT2527 achieved transient target engagement, depletion of MCL1 and MYC proteins, and induction of apoptosis in tumor tissue. This PK/PD correlation was successfully translated into in vivo efficacy in multiple models. Once weekly dosing of PRT2527 was well-tolerated and significantly inhibited tumor growth in various AML CDX models and induced tumor regressions in double-hit and triple-hit diffuse large B-cell lymphoma (DLBCL) CDX and PDX models carrying the *MYC* translocation. Combining PRT2527 with venetoclax achieved complete tumor regressions in a venetoclax resistant OCI-AML3 model. PRT2527 demonstrated potent ex vivo activity in PDX models of B-ALL and T-ALL, as well as various solid tumor PDX models with high levels of *MYC* amplification and overexpression, including pancreatic carcinoma, gastric and gastroesophageal carcinomas, NSCLC, and sarcoma. In vivo efficacy studies with once weekly IV administration of PRT2527 confirmed significant tumor growth inhibition in select *MYC*-amplified solid tumor PDX models. Taken together, this preclinical characterization supports the advancement of PRT2527 into clinical studies for transcriptionally addicted hematological malignancies and solid tumors with *MYC* amplification and/or dysregulation.

P238 Derazantinib, an inhibitor of fibroblast growth factor receptors 1-3, synergises with paclitaxel in pre-clinical gastric tumor models. Paul M. McSheehy[1], Mahmoud El Shemerly[2], Felix Bachmann[1], Laurenz Kellenberger[1], Heidi Lane[1]. [1]Basilea Pharmaceutica International Ltd, Basel, Switzerland, [2]Basilea Pharmaceutica International Ltd., Basel, Switzerland.

Background: Derazantinib (DZB) is an oral fibroblast growth factor receptor (FGFR) inhibitor with clinical activity in intrahepatic cholangiocarcinoma. Kinase assays indicate activity against other important targets in oncology, including CSF1R and VEGFR2. We have shown that DZB can inhibit phosphorylation of CSF1R upon ligand stimulation in mouse macrophages ex vivo (GI50=100 nM); suggesting tumor-associated macrophages (TAMs) as an important target for DZB. Paclitaxel also reduces M2-TAM function, suggesting a potential synergy when combined with DZB. DZB monotherapy showed strong efficacy in some gastric (GA) PDX tumor models, so we have investigated the combination of DZB and paclitaxel in several GA-models in vivo.
Materials and Methods: Four human tumor GA cell lines were studied in vitro: SNU-16, Fu97, AGS and KATOIII. SNU-16 (FGFR2-fusion) was grown s.c. in Balb/c mice as a xenograft (CDX), and 5 different patient-derived xenografts (PDX) with various FGFR-aberrations (fusion, amplification, over-expression or mutation) were grown in the same host. Mice were treated with different doses of DZB (p.o., qd) alone, and/or with paclitaxel (15 mg/kg, i.v., qw) for 3-4 weeks when tumors were 150 mm^3. At the endpoint, tumors were ablated and snap-frozen or paraffin-embedded (FFPE) for western-blot/qPCR or immunohistochemistry (IHC), respectively. Efficacy was summarized as the endpoint dT/C, and the interaction assessed formally as synergy/additivity/antagonism by the Clarke-Combination-Index (CCI). The statistical significance of M2-TAMs was assessed using a one-tailed Fisher contingency test. The tumor models were run by CrownBio Inc, which also provided data on the FGFR-aberrations.
Results: In vitro, DZB showed synergy with paclitaxel in SNU-16 and Fu97 models at concentrations known to be achievable in mouse plasma. In vivo, three experiments with the SNU-16 model showed reproducible synergy (mean CCI = -0.64) with the combination causing at least stasis and some complete-regressions. A PD-study after 3-days treatment showed a significant decrease in Ki67 and increase in the M1-TAMs in the combination group. Plasma PK showed no indication of a drug-drug interaction between the two compounds. In the 5 PDX-models, the combination showed synergy in three models and additivity in two. IHC analysis of M2-TAM levels in vehicle-treated mice of all 6 models showed that the two additive models had M2-TAM levels ≤0.8%, while synergy was seen in the 4 models with M2-TAMs of 1.1-8.7%. ($p=0.03$, using a cut-off of 1%). **Conclusions:** DZB combined with paclitaxel in vivo showed synergy/additivity in GA-tumor models with FGFR aberrations including FGFR2-fusions/amplifications, and FGFR1-3 over-expression or mutations. Additionally, the number of M2-TAMs may also play a role in sensitivity to the combination, which is consistent with the CSF1R kinase being an important target for DZB.

P239 Safety and efficacy of copanlisib in combination with nivolumab: A phase Ib study in patients with advanced solid tumors. Benedito A. Carneiro[1], Robert Jotte[2], Nashat Gabrail[3], Omid Hamid[4], Funan Huang[5], Shalini Chaturvedi[5], Matthias Herpers[6], Lidia Mongay Soler[5], Barrett H. Childs[5], Aaron Hansen[7]. [1]Cancer Center at Brown University, Lifespan Cancer Institute, Providence, RI, [2]Rocky Mountain Cancer Centers, Denver, CO, [3]Gabrail Cancer Research Center, Canton, OH, [4]The Angeles Clinic and Research Institute, A Cedars-Sinai Affiliate, Los Angeles, CA, [5]Bayer HealthCare Pharmaceuticals, Inc., Whippany, NJ, [6]Bayer AG, Berlin, Germany, [7]Princess Margaret Cancer Centre, Toronto, Canada.

Introduction: Copanlisib (C) is a pan-class I PI3K inhibitor, with predominant activity against the PI3K-α and -δ isoforms, approved for patients (pts) with relapsed follicular lymphoma. The PD-1 inhibitor nivolumab (N) is approved for several advanced or metastatic solid tumors. Following preclinical demonstration of the immunomodulatory activity of C (Glaeske et al. AACR 2018), we report Phase Ib results evaluating the safety and efficacy of C+N in pts with advanced solid tumors (NCT03735628).**Methods:** PD-1 inhibitor-naive adult pts with advanced solid tumors received C 45 mg or 60 mg i.v. (days 1, 8, and 15; 28-day cycle) and N 240 mg (day 15 of cycle 1 and days 1 and 15 of subsequent cycles). The primary objective was determination of the recommended Phase II dose (RP2D) of C in combination with N. Secondary endpoints were safety/tolerability, pharmacokinetics (PK), and efficacy. Exploratory real-time evaluation of 77 pharmacodynamic and predictive immune cell biomarkers by flow cytometry on whole blood was performed. **Results:** 16 pts were treated (C 45 mg + N 240 mg, n=5; C 60 mg + N 240 mg, n=11). Median age was 65 years (range 37–89), 12 pts (75%) were male, and 8 pts (50%) had stage IV disease at diagnosis; the most common tumor types were head and neck squamous cell carcinoma (HNSCC; 7 pts) and bladder cancer (BC; 4 pts). No dose-limiting toxicities were reported. The RP2D of C+N 240 mg was 60 mg. As of 13 May 2020, 4 pts remain on treatment. The most common treatment-emergent adverse events (TEAEs) of any grade were hypertension and diarrhea (7 pts [44%] each, ≤ grade [G] 3) and maculo-papular rash and fatigue (6 pts [38%] each, ≤G3). C-related TEAEs were reported in 88% of pts, all ≤G3. AEs leading to C dose interruption/reduction were reported in 31%/19% of pts; TEAEs led to C discontinuation in 1 pt (60 mg; hematuria). Serious AEs occurred in 5 pts (31%). One G5 TEAE occurred (45 mg; general physical health deterioration, unrelated to C or N). No PK interactions were observed between C and N. Two pts had a partial response: 1 in the C 45 mg group (HNSCC) and 1 in the 60 mg group (BC; benefit sustained after 19 cycles). Stable disease was seen in 10 pts and disease progression in 3 pts; disease control rate (DCR) was 75%. Maximum decrease in circulating monocytic myeloid-derived suppressor cells (M-MDSCs; $p<0.05$) from baseline occurred on day 2 after C, returning to baseline on day 8. A significant increase in activated (HLA-DR+ and CD38+) natural killer and CD8+ T cells was seen 2 weeks post-treatment with C+N. Lower baseline levels of CD8+ $T_{effector\ memory}$ (T_{EM}) subset CD45RA-/CCR7- (CD3+/CD8+) seemed to associate with higher DCR. **Conclusions:** C+N showed acceptable safety and preliminary efficacy in pts with advanced solid tumors. The immunomodulatory effect of C on M-MDSCs was seen 2 days post-treatment, and lower T_{EM} subset levels seemed to associate with better disease control. These results support further investigation of C+N in pts with advanced solid tumors. Funding: Bayer AG. Writing support: Complete HealthVizion.

P240 Benchmarking the novel dual-MEK inhibitor, IMM-1-104, head-to-head and in combination with sotorasib (AMG-510) in the MIA PaCa-2 (KRAS-G12C) pancreatic cancer xenograft model. Peter J. King[1], Amy E. Axel[1], Kevin D. Fowler[1], Sarah E. Kolitz[2], Scott Barrett[3], Benjamin J. Zeskind[3], Brett M. Hall[1]. [1]Immuneering Corporation, San Diego, CA, [2]Immuneering Corporation, Cambridge, MA, [3]Immuneering Corporation, New York, NY.

Background: Approximately 9 out of 10 patients diagnosed with pancreatic cancer have tumors driven by a single activating mutation in KRAS. These tumors remain unaddressable by current therapies and collectively represent a high unmet clinical need. KRAS is the most commonly mutated form of RAS and represents approximately 85% of all RAS mutant human tumors with HRAS and NRAS accounting for the remaining 15%. Sotorasib (AMG-510), a covalent KRAS-G12C inhibitor, recently received accelerated regulatory approval for KRAS-G12C lung cancer in the United States. While the G12C mutation has been documented in up to 15% of all KRAS mutant tumors, it is rarely observed (<1%) in human pancreatic cancer, which could severely limit this type of new inhibitor in certain histologies. IMM-1-104 is a novel dual-MEK inhibitor that resists CRAF-bypass (goal: improve activity against RAS mutant tumors), and is designed to have a short plasma drug half-life, which leads to deep, cyclic inhibition of the MAPK pathway (goal: improved tolerability). **Materials & Methods:** The novel, orally bioavailable dual-MEK inhibitor, IMM-1-104, was evaluated for antitumor activity, as a single agent (50, 100, 150 mg/kg BID p.o.) in a head-to-head format against sotorasib (10, 30, 100 mg/kg QD p.o.) or in combination (50, 100, 150 mg/kg BID p.o. IMM-1-104 with 30 mg/kg QD p.o. sotorasib) in the MIA PaCa-2 KRAS-G12C mutant pancreatic tumor xenograft model. Tumor-bearing animals were treated for 21 days of oral dosing, before drug treatments were terminated and animals monitored for additional 3 weeks. **Results:** IMM-1-104 and sotorasib were well-tolerated as single agents and in combination with each other across all dose levels tested. Both drugs demonstrated dose-dependent antitumor activity with tumor regressions noted at higher dose levels. IMM-1-104 in combination with sotorasib produced deep regressions that were sustained longer than either drug alone. **Conclusions:** The dual-MEK mechanism and short half-life of IMM-1-104 has demonstrated broad activity with low toxicity across multiple RAS and RAF mutant preclinical tumor models. Tumor regressions were observed in NRAS mutant melanoma (SK-MEL-2) and KRAS mutant colorectal (Colon-26) rodent tumor models, and tumor stasis was observed in KRAS mutant lung (A549) and BRAF mutant melanoma (A375) tumor xenograft models. Here, we report that IMM-1-104 treatment resulted in tumor regressions similar to that observed for sotorasib in the recently benchmarked KRAS-G12C mutant pancreatic cancer xenograft model (MIA PaCa-2). IMM-1-104 in combination with sotorasib promoted deep, durable tumor regressions, when compared to either drug alone. Therefore, future drug-drug combinations with upstream inhibitors such as sotorasib may afford greater durability in combination for patients with KRAS-G12C and other select tumor types. In totality, these data suggest the potential for broad, single agent activity of IMM-1-104 in tumors with inappropriately elevated MAPK signaling, including a large percentage of KRAS mutant pancreatic cancer.

P241 A highly potent HPK1 inhibitor augments immune cell activation and anti-tumor immunity in a syngeneic tumor mouse model. Hao Liu[1], Lei Wu[2], Song Feng[2], Wei Huang[2], Huijuan Li[2], Jing Lin[2], Yangyang Liu[2], Liufeng Mei[2], Baoqi Ren[2], Julie Xie[1], Lili Yao[2], Wenge Zhong[2]. [1] Regor Pharmaceuticals Inc., Cambridge, MA, [2] Regor Therapeutics Inc., Shanghai, China

Background: HPK1 (MAP4K1) is a serine/threonine Ste20-related protein kinase that belongs to the mitogen-activated protein kinase (MAPK) family. HPK1 is mainly expressed in hematopoietic cells and serves as a negative regulator of anti-tumor immunity through modulating the activation of lymphocytes and dendritic cells. Upon TCR activation, HPK1 phosphorylates the adaptor protein SLP76 at Ser376 to destabilize the SLP76 microclusters, which leads to the attenuation of the TCR signaling. The reported anti-tumor efficacy data from HPK1 knockout and kinase-dead knock-in mouse models support HPK1 as a novel intracellular I/O target. **Methods:** Regor CARD platform (Computer Accelerated Rational Discovery) was deployed to identify potent and selective inhibitors of HPK1. Biochemical assays and primary human pan T cell-based cellular assays were utilized to support the structure-activity relationship (SAR) analysis and inhibitor optimization. *In vitro* and *in vivo* target engagement studies were conducted in Jurkat T cells and mouse splenocytes, respectively. *In vivo* efficacy study data were generated using CT-26 syngeneic tumor mouse model. **Results:** Highly potent HPK1 inhibitors with good selectivity against the liable immune kinases were identified. RGT-197, one of the lead inhibitors demonstrated a significant increase of IL-2 secretion in primary human pan T cells upon TCR activation. It inhibited TCR-induced phosphorylation of SLP76 at Ser-376 *in vitro* and *in vivo*. Anti-tumor efficacy was observed in CT-26 tumor-bearing mice by oral dosing of RGT-197 as a single agent and in combination with an anti-PD1-monoclonal antibody. Furthermore, RGT-197 increased the level of cytokines important for anti-tumor immunity *in vivo*. **Conclusion:** RGT-197, a potent and selective HPK1 inhibitor, provides a potential opportunity as a small molecule I/O agent to boost anti-tumor immunity either as monotherapy or in combination with immune checkpoint inhibitors.

P242 Preclinical efficacy landscape of the pan-CLK/DYRK inhibitor Cirtuvivint (SM08502). Carine Bossard[1], Emily Creger[1], Elizabeth A. McMillan[1], Carsten Merkwirth[1], Maureen Ibanez[1], Deepti Bhat[1], Josh Stewart[1], Brian Eastman[1], Chi-Ching Mak[1], Vishal Deshmukh[2], Michael A. White[1]. [1]Biosplice Therapeutics Inc., San Diego, CA, [2]Formerly Biosplice Therapeutics Inc., San Diego, CA.

A monumental challenge facing cancer therapy is the vast mechanistic diversity of tumors among and even within cancer patients. This severely limits the number of patients that respond to any given therapy and the durability of responses that do occur. Deep phenotypic profiling of almost 9000 patient samples indicates perturbation of alternative pre-mRNA splicing (AS) is often a root cause of cancer. This offers an extraordinary opportunity to restore health through normalization of AS in diseased tissue. The bottleneck to progress has been finding and drugging the right targets. The CLK/DYRK kinases modulate AS efficiency and specificity, and therefore, targeting them offers the potential to address this bottleneck. The isoquinoline cirtuvivint is a potent ATP-competitive inhibitor of the Cdc2-like kinases (CLK1-4) and the dual-specificity tyrosine phosphorylation-regulated kinases (DYRK1-4), with activity against only a minimal number of the remaining members of the CMGC-family kinases or the kinome as a whole. Cellular target engagement assays indicated biological IC_{50}s below 0.06 μM across the CLK/DYRK target class. Thus, cirtuvivint has utility for robust chemical evaluation of the overarching contribution of CLK/DYRK family activity to tumor biology in general and context-dependent AS in particular. To help assess the breadth and depth of CLK/DYRK dependencies in human cancers, the consequences of pan-CLK/DYRK inhibition on growth and survival of 154 cancer cell line models, 46 PDX models, and 43 CDX models were tested. EC_{50}s in cell viability assays ranged from 0.014 to 0.73 μM in culture with strong effects observed in subsets of cell lines across all lineages tested. Molecular profiles associated with high sensitivity to cirtuvivint included somatic mutations in the RBM10 splicing factor. Tumor growth inhibition assays with daily dosing at exposures about 2X below the MTD resulted in significant disease control (≥75% TGI) in 15/46 PDX and 18/43 CDX models. Together, these results indicate broad cancer relevance, at least in the preclinical setting, and are consistent with a common reliance on CLK/DYRK-dependent alternative splicing among otherwise highly mechanistically heterogeneous disease.

P243 The IRAK4 inhibitor CA-4948 demonstrates antitumor activity in a preclinical model of CNS lymphoma. Christina A. von Roemeling[1], Bently P. Doonan[1], Lan Hoang-Minh[1], Han W. Tun[2], Elizabeth Martinez[3], Raul Soikes[3], Reinhard von Roemeling[3], Duane A. Mitchell[1]. [1]University of Florida, Gainesville, FL, [2]Mayo Clinic, Jacksonville, FL, [3]Curis, Inc., Lexington, MA.

Background: Central nervous system (CNS) lymphoma, both primary and secondary disease, carries the worst prognosis of all Non-Hodgkin's lymphoma with a 5 year survival of less than 30% with current therapeutic options. Recent studies have shown constitutive activation of both NF-κB and MAPK signaling pathways in the maintenance and progression of CNS lymphoma. This often coincides with mutations in *MYD88*. IRAK4 is a serine/threonine kinase that mediates interleukin 1 receptor (IL-1R) and Toll-like receptor (TLR) signaling downstream of MYD88, and is a novel therapeutic target for this disease. CA-4948 is an oral first-in-class small molecule inhibitor of IRAK4 that has demonstrated clinical activity in patients with systemic Non-Hodgkin's Lymphoma. Our study goal was to evaluate CA-4948 efficacy in a syngeneic preclinical model of primary CNS B cell lymphoma (A20), supporting future clinical investigation of this agent in this difficult to treat disease. **Materials and Methods:** Plasma, cerebrospinal fluid (CSF), and brain tissue (both naïve and tumor bearing) were assessed for CA-4948 drug concentration following single high-dose oral administration using UPLC-MS/MS. Biomarker expression, including NF-κB and MAPK, was measured in control and CA-4948 treated brain tissue resected from tumor-bearing animals using multi-parameter immunohistochemistry and 3D modeling of cleared tissue. Dose-dependent survival responses were also measured using the A20 preclinical model of primary CNS lymphoma. **Results:** While brain concentrations of CA-4948 remained low with respect to plasma (~4-5%), therapeutic dose levels were attained in both CSF and brain tissue (>502 nM). No significant difference between naïve and A20 tumor bearing brain tissue was observed, indicating the blood brain barrier remained intact in this model. Biomarker analyses revealed significant downregulation of both MAPK and NF-κB signaling pathways in response to CA-4948, as evidenced by reduced protein levels of phospho-p38$^{Thr180/Tyr182}$, phospho-ERK1/2$^{Thr202/Tyr204}$, and phospho-p65^{Ser536} in both the tumor cells and the tumor microenvironment. Finally, CA-4948 treatment produced a dose-dependent survival response in syngeneic animals harboring CNS A20 tumors. **Conclusions:** CA-4948 is able to reach therapeutic dose levels in the CNS in a preclinical murine model of CNS lymphoma, producing significant and dose-dependent anti-tumor activity and survival advantage. These data support further evaluation of CA-4948 in clinical investigation and treatment of patients with CNS lymphoma.

P244 NVL-655 exhibits antitumor activity in lorlatinib-resistant and intracranial models of ALK-rearranged NSCLC. Anupong Tangpeerachaikul, Amit Deshpande, Nancy E. Kohl, Joshua C. Horan, Henry E. Pelish. Nuvalent, Cambridge, MA.

ALK receptor tyrosine kinase can be aberrantly activated by gene rearrangement or point mutation to drive tumor cell proliferation, survival, and metastasis. ALK rearrangements are detected in up to 5% of advanced non-small cell lung cancer (NSCLC), and up to 40% of patients present with brain metastases at diagnosis. Crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib are FDA-approved tyrosine kinase inhibitors for ALK-positive NSCLC. However, durability of response to these treatments is partly limited by mutations in ALK that confer resistance. The G1202R solvent front mutation is commonly observed in patients that have progressed on crizotinib, ceritinib, alectinib, or brigatinib. Patients with tumors harboring G1202R have responded to lorlatinib, but many have relapsed after acquiring compound mutations such as G1202R/L1196M and G1202R/G1269A. Another challenge with lorlatinib is central nervous system (CNS) adverse events attributed to inhibition of tropomyosin receptor kinase B (TRKB), a kinase structurally related to ALK. NVL-655 is a novel preclinical brain-penetrant ALK inhibitor with selectivity over TRKB. We previously reported that NVL-655 inhibits ALK G1202R, G1202R/L1196M, G1202R/G1269A, and G1202R/L1198F in vitro; has favorable kinome and ALK-vs-TRKB selectivity profiles; and shows antitumor activity in a Ba/F3 EML4-ALK variant 1 (v1) G1202R/L1196M xenograft model. Here we report results of a broader preclinical characterization of NVL-655 across ALK models, including an intracranial model. NVL-655 was observed to induce regression in a HIP1-ALK patient-derived xenograft model, an EML4-ALK v1 human cancer cell line-derived model (NCI-H3122), and Ba/F3 xenograft models expressing EML4-ALK v1 G1202R and G1202R/G1269A. Pharmacodynamic (PD) biomarker analyses supported in vivo ALK inhibition by NVL-655. Furthermore, NVL-655 was observed to reduce tumor size and prolong survival by more than 4-fold in a mouse intracranial tumor model of Ba/F3 EML4-ALK v1 G1202R/L1196M luciferase. In conclusion, NVL-655 has a preclinical profile that we believe supports its potential to address a medical need for patients with ALK-positive disease, including those with G1202R compound mutations. We believe the findings presented here support the evaluation of NVL-655 for the treatment of patients with ALK-driven disease.

P245 Synergistic effect of combination of pemigatinib with enfortumab vedotin (EV) in human bladder cancer models. Rodrigo A. Hess, Lisa Truong, Antony Chadderton, Michelle Frascella, Leslie Hall, Holly Koblish. Incyte Research Institute, Wilmington, DE.

Bladder cancer is among the most prevalent cancers worldwide, with urothelial carcinoma accounting for approximately 90% of cases. Until recently, poor overall survival after chemotherapy combined with immune checkpoint blockade therapies for patients with advanced urothelial carcinoma highlighted the need for new therapies. Although data supports the use of targeted therapies, such as FGFR inhibitors, recent advances in the treatment of bladder cancer are changing the landscape. Enfortumab vedotin (EV) is an antibody drug conjugate (ADC) consisting of an anti-Nectin-4 antibody conjugated to monomethyl auristatin E, which specifically delivers the auristatin payload to Nectin-4 high-expressing urothelial carcinomas. While activity and durability of response from both monotherapy trials as well as combination with pembrolizumab are favorable, additional treatment options might be required for either patients not responding to treatment or those who might develop resistance. Importantly, FGFR activation through rearrangement or mutation is frequently found in the luminal papillary subtype which also expresses high levels of Nectin-4. Thus, we sought to test the potential of combining pemigatinib and EV in models of human bladder cancer which express both activated FGFR3 and Nectin-4. Our initial analysis show that FGFR3 mutant tumors express high levels of Nectin-4. We further demonstrated that two bladder cancer cell lines RT112/84 (FGFR3-TACC3 fusion) and UM-UC-14 ($FGFR3^{S249C}$) are sensitive to both pemigatinib and EV in vitro and in vivo. Notably, synergistic anti-tumor effects were observed when pemigatinib was combined with EV in vivo. In addition, combination of pemigatinib with EV significantly improved overall survival when compared to single treatments in these models. Altogether, our data strongly suggest a potential for combination of these therapies in the clinic.

P246 Discovery and characterization of selective, FGFR1 sparing, inhibitors of FGFR2/3 oncogenic mutations for the treatment of cancers. Etienne Dardenne[1], Fernando Padilla[2], Sara Rasmussen[1], Shao Ning Yang[1], Ahmet Mentes[1], Luisa Shin Ogawa[3], Anthony Trombino[2], Darlene Romashko[1], Maria Chevtsova[2], Shalabh Thakur[4], Elisabeth Buck[1], Christopher Roberts[2], Matthew Lucas[2], Tai-An Lin[1]. [1]BDTX, New York, NY, [2]BDTX, Cambridge, MA, [3]bdtx, Cambridge, MA, [4]BDTX, Toronto, Canada.

Background: Targeting FGFR genetic alterations using small molecule inhibitors is a validated therapeutic strategy for urothelial carcinoma and cholangiocarcinoma. However, the current FDA-approved pan-FGFR inhibitors, erdafitinib and pemigatinib, are subject to FGFR1-mediated dose-limiting toxicities (e.g., hyperphosphatemia). These treatments necessitate a high rate of dose reductions, interruptions, and discontinuations, thereby potentially limiting efficacy. In addition, drug-resistant mutations (e.g., gatekeeper) in FGFR2 and FGFR3 genes rapidly emerge in patients treated with these drugs. Our research goals are to reveal the full spectrum of oncogenic FGFR2 and FGFR3 mutations that drive tumor growth and to discover an inhibitor that selectively targets these mutations together with FGFR2 and FGFR3 gene fusion and drug-resistance mutations, while minimizing FGFR1 activity and associated toxicities. We hypothesize that this will deliver an FGFR precision medicine with enhanced anti-tumor activity, an improved drug resistance profile, and broader mutational coverage. **Methods:** Applying the Mutation-Allostery-Pharmacology (MAP) platform technology developed by Black Diamond Therapeutics, we defined a spectrum of 34 allosteric FGFR2/3 oncogenic mutations, including over 28 previously uncharacterized mutations that we now show to be oncogenic. The MAP platform allowed us to further classify those mutations into functional clusters or families of mutations that can be targeted using a single compound. While located throughout the extracellular and kinase domains, we demonstrated how these functional clusters activate FGFR2 or FGFR3. Among the mechanisms identified, a functional cluster of mutations is activated due to disulfide-bond mediated dimerization. **Results**: Herein, we report the discovery of a series of orally available, selective FGFR2/3 inhibitors that 1) shows antiproliferative potency across all 34 mutations; 2) spares FGFR1-wild-type; 3) is active against gatekeeper mutations and 4) shows favorable selectivity versus a subset of closely related kinases in the human kinome. In addition to being potent against FGFR2 and FGFR3 primary mutations, we demonstrated that our FGFR1 sparing inhibitors retain potency against the most prevalent FGFR2 resistant mutations. When dosed orally, one example was well tolerated and exhibited dose-dependent PK/PD and anti-tumor efficacy and regression in several FGFR2 and FGFR3 driven xenograft models in mice. In addition, when dosed at efficacious doses, no FGFR1-mediated hyperphosphatemia was observed in these animals instead of the animals that were treated with pan FGFR inhibitors. **Conclusion:** Our data support the development of rationally designed selective inhibitors targeting a spectrum of FGFR2/3 mutations while sparing dose limiting FGFR1 activity.

P247 Evaluating TRKB activity of novel preclinical brain-penetrant ROS1 and ALK inhibitors. Anupong Tangpeerachaikul, Joshua C. Horan, Henry E. Pelish. Nuvalent, Cambridge, MA.

Oncogenic ROS1 and ALK rearrangements are detected in up to 3% and up to 5% of advanced non-small cell lung cancer (NSCLC), respectively, with up to 40% of patients presenting with brain metastases at diagnosis. Although there are FDA-approved ROS1 and ALK inhibitors, there is a need for next-generation brain-penetrant inhibitors that have activity against drug-resistance mutations while sparing tropomyosin kinase B (TRKB) function in the central nervous system (CNS). TRKB inhibition in the CNS has been implicated in adverse events such as cognitive impairment, mood disorders, sleep effects or sleep disturbances, dizziness, ataxia or gait/motor disturbances, and weight gain, which have been observed with FDA-approved dual TRK/ROS1 inhibitor entrectinib and FDA-approved ALK inhibitor lorlatinib. TRKB-related CNS adverse events present a key challenge for the development of next-generation ROS1 and ALK therapies. We previously reported the discovery of NVL-520 and NVL-655, preclinical inhibitors of ROS1 and ALK, respectively, which have demonstrated activity against common drug-resistance mutations, selectivity versus TRKB, and brain penetrance in preclinical experiments. An important part of the discovery process was generating reliable and relevant TRKB potency measurements to enable structure-activity relationship (SAR) efforts. Here we describe four in vitro TRKB potency assays and explain their utility in guiding selectivity optimization for new inhibitors. These assays include (1) biochemical, (2) cell viability, (3) cellular coupled-enzyme, and (4) direct cellular phosphorylation readouts. Although biological contexts vary, each assay exhibits good cross-correlations, and we recommend multi-assay testing to improve confidence in evaluating TRKB inhibition. Using these assays, we present a multi-assay analysis of the preclinical TRKB potency and selectivity of NVL-520 and NVL-655 alongside FDA-approved or experimental inhibitors of ROS1 (crizotinib, entrectinib, repotrectinib, and taletrectinib) and ALK (crizotinib, ceritinib, alectinib, brigatinib, lorlatinib, and ensartinib). In these preclinical assays, we observed entrectinib and lorlatinib to exhibit poor ROS1-vs-TRKB and ALK G1202R-vs-TRKB selectivity, respectively, which we believe is consistent with their CNS adverse events. In the same experiment, we observed NVL-520 and NVL-655 to exhibit selectivity across all assays, including for ROS1 G2032R and ALK G1202R/L1196M mutations, respectively. In conclusion, we have devised a multi-assay approach to evaluate TRKB inhibition and guide discovery of selective ROS1 and ALK inhibitors, with the goal of minimizing adverse events and driving durable responses for patients.

P248 The tyrosine kinase inhibitor XL092 promotes an immune-permissive tumor microenvironment and enhances the anti-tumor activity of immune checkpoint inhibitors in preclinical models. Jeff Hsu, Colin Chong, Jeffrey Serrill, Sharon Wu, Kevin Leong, Ted Yun, Lynne Bannen, Peter Lamb, Wei Xu, Peiwen Yu. Exelixis, Inc., Alameda, CA.

Background: Cabozantinib, an inhibitor of MET, VEGFR2, AXL, and MER, has shown significant clinical activity in multiple solid tumors, both alone and in combination with immune checkpoint inhibitors (ICIs). XL092 has a similar target profile to cabozantinib but with a significantly shorter clinical half-life and pharmacokinetic properties suitable for once daily oral dosing (Hsu, Eur J Cancer, 2020). In preclinical studies, the combination of XL092 and anti–PD-1 exhibited greater anti-tumor activity than either agent alone. Here, we describe further preclinical characterization of XL092 in combination with ICIs, including modulation of immune cells in vivo and in vitro. **Methods:** Pharmacodynamic activity was evaluated in the MC38 syngeneic tumor model following treatment with XL092 alone or in combination with anti–PD-1 or anti–PD-L1. Tumor CD31+ microvessel density and CD8+ T cell infiltration were assessed by immunohistochemistry, and circulating immune cells were measured by FACS. Macrophage repolarization and efferocytosis were evaluated in vitro using primary human macrophages. **Results:** Treatment of MC38 tumor-bearing mice with XL092 inhibited tumor angiogenesis in a dose-dependent manner, and combination treatment with XL092 and anti–PD-1 increased CD8+ lymphocyte tumor cell infiltration compared with either agent alone. XL092 and ICIs also promoted significant changes in peripheral immune cell subsets in the MC38 model. Treatment with XL092 alone or in combination with ICIs increased circulating levels of CD4+ and CD8+ T cells and B cells, and combination treatment with anti–PD-1 decreased levels of proliferating and IFNg-producing effector T cells in circulation. While the level of peripheral NK cells was unaffected by these treatments, XL092 alone or in combination with ICIs decreased levels of proliferating and GrzB+ effector cells in circulation. Treatment with XL092 alone or in combination with ICIs reduced the levels of circulating myeloid cells, including myeloid-derived suppressor cells, dendritic cells, and macrophages. In vitro treatment of human macrophages with XL092 inhibited macrophage efferocytosis with IC_{50} ~81 nM and promoted repolarization from an M2 anti-inflammatory to an M1 pro-inflammatory phenotype. **Conclusions:** Immune cell profiling showed that treatment with XL092 and ICIs promotes CD8+ T cell tumor infiltration, increases in peripheral lymphocytes, decreases in peripheral myeloid cells, and macrophage repolarization, suggesting that XL092 promotes an immune-permissive tumor microenvironment. Consistent with these immunomodulatory effects, the combination of XL092 and ICIs has shown greater anti-tumor activity in syngeneic tumor models than either agent alone. XL092 is currently being evaluated in multiple solid tumors as a single agent and in combination with ICIs in the phase 1/1b STELLAR trials (NCT03845166).

P249 Preclinical antitumor activity of NVL-520 in patient-derived models harboring ROS1 fusions, including G2032R solvent front mutation. Amit M. Deshpande[1], Satoshi Yoda[2], Anupong Tangpeerachaikul[1], Nancy E. Kohl[1], Joshua C. Horan[1], Aaron N. Hata[2], Henry E. Pelish[1]. [1]Nuvalent Inc., Cambridge, MA, [2]Massachusetts General Hospital Cancer Center, Charlestown, MA.

ROS1 is a proto-oncogene that encodes the receptor tyrosine kinase ROS1, which can be aberrantly activated by gene rearrangement to drive tumor cell proliferation, survival, and metastasis. In non-small cell lung cancer (NSCLC), ROS1 rearrangements are detected in up to 3% of patients with up to 40% of these patients also presenting with accompanying central nervous system (CNS) metastases. Although various tyrosine kinase inhibitors (TKI) have been approved or are under development for treating ROS1-positive patients, these therapies are associated with one or more of the following significant challenges: 1. emergence of ROS1 mutations that confer disease resistance, including the G2032R solvent front mutation, 2. disease progression into the CNS, and 3. treatment-related adverse events (AEs) associated with off-target kinase inhibition, notably TRKB in the CNS. NVL-520 was developed to address the challenges listed above. Preclinical studies have shown that NVL-520 inhibits wild-type and drug resistance mutants of the ROS1 kinase and demonstrated activity against CNS disease with minimal kinase off-target activity, suggesting an opportunity for durable responses for patients with ROS1-positive disease. Here we report detailed characterization of cellular and antitumor activity of NVL-520 in patient-derived models of ROS1-driven NSCLC. **Methods**: Cellular and antitumor activity of NVL-520 were evaluated in patient-derived models of NSCLC representing ROS1 and ROS1-G2032R driven disease. For in vivo studies, treatments were administered orally. Western blotting, immunohistochemistry, and gene expression analyses were used to measure pharmacodynamic (PD) effects in tumor tissue. Pharmacokinetic (PK) analyses were also performed. **Results**: In patient-derived cell (PDC) models of EZR-ROS1 and CD74-ROS1 G2032R, NVL-520 treatment resulted in antiproliferative activity, with IC_{50} values < 10 nM, and reduced levels of phospho-ROS1 and markers of downstream signaling. Studies in patient-derived xenograft (PDX) models demonstrated that NVL-520 was well-tolerated and resulted in dose-dependent antitumor activity, including tumor regression in all models. Analysis of PD biomarkers showed that NVL-520 suppressed ROS1 phosphorylation and significantly impacted ROS1-dependent signaling pathways. PK analysis demonstrated dose-dependent differences in NVL-520 exposure. PD-Efficacy correlation analysis showed that changes in PD were consistent with dose-dependent antitumor activity of NVL-520 with distinct changes observed at doses that induce tumor stasis versus tumor regression. **Conclusion**: NVL-520 exhibits antiproliferative and antitumor activity in NSCLC models driven by ROS1 and ROS1-G2032R solvent front mutation. We believe the findings presented here support evaluation of NVL-520 for the treatment of patients with ROS1-driven disease.

P250 Evaluation of systemic pan-CLK/DYRK inhibition on organ function and tissue self-renewal. Hadi Falahatpisheh, Maryam Dehghani, Jane Morrow, Catherine Tsao, Steven Dorrow, Josh Stewart, Carine Bossard, Brian Eastman, Chi-Ching Mak, Karamjeet Pandher, Michael A. White. Biosplice, San Diego, CA.

Proteome diversification through extensive pre-mRNA alternative splicing (AS) is a fundamental aspect of cell fate determination and tissue specialization. The CDC2-like kinases (CLK1-4) and the dual specificity tyrosine phosphorylation regulated kinases (DYRK1-4) have been recognized as participating in signal transduction-dependent alternative splice junction selection through phosphorylation of their substrate proteins- the serine/arginine rich splicing factors (SRSF1-12). The use of CLK/DYRK kinase inhibitors, with various selectivity and potency profiles within the CMGC family, has implicated this regulatory process in both development and disease. Furthermore, an emerging appreciation for the key role of AS in support of tumorigenesis indicates the CLK/DYRK kinases may represent a druggable oncology target class. However, careful evaluation of potential toxicities associated with inhibition of these kinases has not yet been reported. To address this need, the preclinical toxicity profile of SM08502, a potent pan-CLK/DYRK inhibitor was characterized in multiple exploratory and GLP studies performed in rats and cynomolgus monkeys. Daily dosing of SM08502 was generally well-tolerated in both rats and monkeys, with limited target organs of toxicities. Major clinically relevant target organ of toxicities included gastrointestinal (GI), bone marrow, and lymphatic organ toxicities in both rats and monkeys. GI toxicity was characterized as villous blunting, single cell necrosis of the epithelium, hemorrhage, inflammation, and edema. These findings in the GI tract manifested clinically as emesis, inappetence, vomiting, and diarrhea. Microscopic findings suggest a distinct mechanism from cytotoxic agents such as chemotherapy. Morphological evaluation suggests SM08502 primarily affects GI villar epithelial cell dynamics without impairing crypt cell replication or survival. Complete recovery of GI tract findings upon dose holiday was demonstrated in both the rat and the monkey. The SM08503-related hematological findings included decreased in reticulocytes, and leukocytes (primarily monocytes and lymphocytes) in both species. These hematological effects contributed to decreased hematopoietic cellularity (all lineages) in the bone marrow and lymphocyte depletion in lymphatic organs (thymus, spleen, lymph nodes and GALT (gut-associated lymphoid tissue). The nonclinical experience with SM08502 suggests an acceptable safety profile, with adverse events limited to transient reversible GI and hematological findings.

P251 Discovery and characterization of RLY-2608: The first allosteric, mutant, and isoform-selective inhibitor of PI3Kα. Ermira Pazolli[1], Randy Kipp[1], Alessandro Boezio[1], Hakan Gunaydin[1], Amanda Iskandar[1], Matthew Zubrowski[1], Bret Williams[1], Kelley Shortsleeves[1], Alexandre Larivee[2], Tom McLean[1], Klaus Michelsen[1], Hongtao Zeng[1], Jonathan LaRochelle[1], Joe Manna[1], Lucian DiPietro[1], Mary Mader[1], Bindu Bennet[1], Jeremy Wilbur[1], Qi Wang[3], Levi Pierce[1], Iain Martin[1], James Watters[1], Pascal Fortin[1], Donald Bergstrom[1]. [1]Relay Therapeutics, Cambridge, MA, [2]Paraza Pharma Inc., Montreal, QC, Canada, [3]DE Shaw Research, New York, NY.

Phosphoinositide 3-kinase alpha (PI3Kα) is the most frequently mutated kinase in solid tumors. Traditionally, the development of PI3Kα inhibitors has focused on the active, or orthosteric site. The therapeutic index of orthosteric inhibitors is limited by the lack of clinically meaningful selectivity for mutant versus wild-type (WT) PI3Kα and off-isoform activity. Alpelisib, the only approved orthosteric PI3Kα inhibitor, is emblematic of the class with toxicity related to inhibition of WT PI3Kα and other PI3K isoforms resulting in sub-optimal inhibition of mutant PI3Kα with reductions in dose intensity and frequent discontinuation. To overcome these limitations, we designed RLY-2608, the first allosteric, mutant, and isoform-selective PI3Kα inhibitor. We solved the full-length cryo-EM structure of PI3Kα, performed long time-scale molecular dynamic simulations to elucidate conformational differences between WT and mutant PI3Kα, and leveraged these insights to enable the design of RLY-2608. RLY-2608 does not compete with orthosteric inhibitors for binding and associates 8x faster with mutant PI3Kα relative to WT. In biochemical assays, RLY-2608 inhibits both kinase domain (H1047R) and helical domain (E542K, and E545K) mutant PI3Kα activity with <10nM potency and 8-12x selectivity relative to WT PI3Kα. RLY-2608 is > 1000-fold selective over the β, δ, and γ PI3K isoforms in biochemical assays and demonstrates exquisite selectivity across a panel of 322 kinases, with no other kinases showing > 50% inhibition. In MCF10A cells engineered to express only mutant or WT PI3Kα, RLY-2608 inhibited phosphorylated AKT (pAKT) in a mutant-selective manner. Furthermore, pAKT and viability were significantly inhibited across a panel of cancer cell lines carrying hotspot *PIK3CA* mutations. RLY-2608 showed anti-tumor activity in both kinase and helical domain *PIK3CA* mutant in vivo xenograft models with marked regressions or stasis observed in all models. RLY-2608 was well tolerated, with pharmacodynamic modulation and efficacy observed in a dose dependent manner. Insulin levels measured as an indicator of glucose homeostasis were significantly lower when compared to orthosteric inhibitors, suggesting that RLY-2608 can achieve maximum efficacy by maintaining PI3Kα mutant target coverage throughout the dosing interval with significantly reduced impact on WT PI3Kα. In higher species, dosing of RLY-2608 for 28 days resulted in exposures exceeding mutant PI3Kα cellular PD IC90 throughout the dosing interval without elevated glucose levels or histopathological or ophthalmic findings associated with hyperglycemia. Compared to orthosteric inhibitors, RLY-2608 demonstrates preferential binding and inhibition of mutant PI3Kα, is highly selective across the kinome, and achieves in vivo efficacy without dysregulating glucose homeostasis. These results support clinical investigation of RLY-2608 as a differentiated mechanism for inhibition of oncogenic PI3Kα in patients with *PIK3CA* mutant tumors.

P252 IMM-1-104: a novel, oral, selective dual-MEK inhibitor that displays broad antitumor activity and high tolerability across RAS and RAF mutant tumors *in vivo*. Peter J. King[1], Kevin D. Fowler[1], Sarah E. Kolitz[2], Scott Barrett[3], Benjamin J. Zeskind[3], Brett M. Hall[1]. [1]Immuneering Corporation, San Diego, CA, [2]Immuneering Corporation, Cambridge, MA, [3]Immuneering Corporation, New York, NY.

Background: Inappropriate activation of the MAPK pathway, often stemming from mutations in RAS or RAF, represents one of the most common oncogenic events in human cancer. Therefore, MEK1 and MEK2 (MEK), which lie downstream of RAS and RAF but upstream of ERK, have represented an attractive drug target for over two decades. Unfortunately, first generation MEK inhibitors have been limited by pathway reactivation events and serious on-target drug toxicities that restrain clinical utility, especially in patients with RAS mutant tumors. We sought to develop a new approach to MEK inhibition that would be more effective in RAS mutant tumors and with improved tolerability. IMM-1-104 is a novel dual-MEK inhibitor that is designed to disrupt phosphorylation of MEK and subsequently prevent activation of ERK1 and ERK2 (ERK). IMM-1-104's dual-MEK mechanism resists RAF activation of MEK, and its short drug half-life allows for chronic dosing while maintaining a near-zero drug trough for improved tolerability.
Materials & Methods: IMM-1-104 was profiled across a series of preclinical experiments to assess its physicochemical and drug-like properties. Cell-free and cell-based in vitro as well as in vivo characteristics were evaluated, including four independent in vivo pharmacology rodent studies in lung, colon and skin tumor models. **Results:** IMM-1-104 is a highly selective, orally bioavailable, non-ATP competitive, allosteric dual-MEK inhibitor. At drug exposures up to 1 micromolar (uM) in cell-free and in situ kinome screens, thermodynamic interactions and altered activity levels were observed for MEK1 and MEK2. At higher drug exposures up to 10 uM, IMM-1-104 also thermodynamically interacted with RAF1 (CRAF) and prompted reduction of KSR1 and KSR2 activation markers. IMM-1-104 displayed dual-MEK activity (i.e., RAS mutant tumor cell reductions in both ERK and MEK phosphorylation) across multiple human tumor cell-based models including A549 (KRAS-G12S) lung, A375 (BRAF-V600E) and SK-MEL-2 (NRAS-Q61R) melanoma. Pharmacokinetic studies in rodent models revealed a drug plasma half-life for IMM-1-104 of approximately 1.3 hours. In vivo tumor pharmacology studies in A549 and A375 achieved tumor stasis (i.e., 85% to 95% Tumor Growth Inhibition) with regressions observed in Colon-26 and SK-MEL-2. **Conclusions:** The dual-MEK mechanism and short half-life of IMM-1-104 has proven to be broadly active and well-tolerated in multiple RAS and RAF mutant preclinical tumor models in rodents. IMM-1-104 drives deep, cyclic inhibition of the MAPK pathway (improving tolerability) while resisting pathway bypass mechanisms (improving activity). Our collective data suggest that RAS and RAF mutant tumor cells are not able to tolerate periods of deep but cyclic MAPK pathway inhibition. Overall, these results are consistent with on and off signaling events that can significantly impact cell fate decisions (i.e. signaling dynamics).

P253 Potent and selective AXL tyrosine kinase inhibition demonstrates significant anti-tumor efficacy in combination with standard of care therapeutics in preclinical models.

Susan L. Paprcka[1], Subhasree Sridhar[1], Irene M. Luu[1], Salema Jafri[1], Dillon H. Miles[1], Suan Liu[1], Ruben Flores[1], Shiwei Qu[1], Manjunath Lamani[1], Sriivas Paladugu[1], Cesar Meleza[2], James Wu[1], Hema Singh[1], Yu Chen[1], Sean Cho[1], Akshata Udyavar[1], Angelo Kaplan[1], Enzo Stargnaro[1], Xiaoning Zhao[1], Lixia Jin[1], Manmohan R. Leleti[1], Stephen W. Young[1], Jay P. Powers[1], Matthew J. Walters[1], Ester Fernandez-Salas[1]. [1]Arcus Biosciences, Hayward, CA, [2]Arcus Bioscienes, Hayward, CA.

Background. AXL receptor tyrosine kinase (AXL) is a transmembrane protein that is overexpressed in a variety of tumors and correlates with poor prognosis in cancer patients. AXL is expressed in cancer and stromal cells and has been implicated in the development of resistance to chemotherapy, targeted therapies & immunotherapies. Activation of AXL by its ligand, growth arrest specific protein 6 (Gas6), or ligand-independent dimerization facilitates AXL phosphorylation, initiates signaling cascades that promote cancer cell proliferation, survival, and an immunosuppressive microenvironment. Here we present the discovery and characterization of a novel, highly potent and selective novel AXL inhibitor. **Materials and Methods.** The potency and specificity of the novel Arcus inhibitor against AXL and other kinases was determined using a panel of HTRF KinEASE-TK assays. Intracellular target engagement was evaluated by monitoring displacement of a competitive fluorescent tracer using an AXL NanoBRET™ intracellular kinase assay. To further assess the inhibitory effect of the novel Arcus inhibitor on AXL kinase activity, AXL autophosphorylation induced SH2 domain translocation was measured in cells using a PathHunterÒ U2OS AXL Functional assay system. Inhibition of AXL phosphorylation in cancer cells was evaluated by Western blot and levels of soluble and surface AXL were assessed by ELISA and flow cytometry, respectively. Pharmacokinetics (PK), pharmacodynamics (PD) and anti-tumor efficacy were evaluated in murine models. **Results.** A novel, potent, reversible, and highly selective AXL kinase inhibitor has been generated by Arcus. The novel inhibitor exhibits single-digit nanomolar potency in both biochemical and cell-based assays, retains significant activity in 100% human serum and does not show significant inhibition of the major CYP450 isoforms nor the hERG potassium channel. The novel Arcus molecule inhibits AXL phosphorylation mediated by both ligand-dependent Gas6 stimulation as well as ligand-independent autophosphorylation. AXL phosphorylation and subsequent signaling leads to receptor internalization, thereby decreasing both surface AXL expression and soluble AXL levels. AXL activity is inhibited in a concentration dependent manner significantly increasing both surface AXL expression and soluble AXL levels. More importantly, significant anti-tumor efficacy is observed in combination with targeted therapies in several *in vivo* models. Furthermore, AXL inhibition significantly reduces tumor growth after relapse to single-agent targeted therapy. **Conclusions.** A novel selective inhibitor of AXL tyrosine kinase activity has been developed that demonstrates single-digit nanomolar potency and inhibition of both ligand-dependent and ligand-independent AXL phosphorylation. Significant anti-tumor activity is observed in combination with targeted therapy and upon acquired resistance in xenograft models. Selective AXL inhibition is a promising therapeutic strategy to overcome resistance to chemotherapy, targeted therapy, and/or immunotherapy.

P254 Transcriptional effects in C26 tumor highlight mechanistic aspects of a novel dual MEK inhibitor, IMM-1-104. Sarah E. Kolitz[1], Kevin D. Fowler[2], Peter J. King[2], Benjamin J. Zeskind[3], Brett M. Hall[2]. [1]Immuneering Corporation, Cambridge, MA, [2]Immuneering Corporation, San Diego, CA, [3]Immuneering Corporation, New York, NY.

We developed a novel dual MEK inhibitor, IMM-1-104, that showed 94% tumor growth inhibition (TGI) in the syngeneic C26 mouse model through 10 days at 150 mg/kg (mpk) BID, with only 3.8% tumor-adjusted body weight loss (BWL) through 24 days. The mechanism differs from that of standard MEK inhibitors (MEKi), and leverages signaling dynamics to decouple efficacy and toxicity. IMM-1-104 achieves deep cyclic inhibition of the pathway rather than constant blockade, and prevents the RAF1 (CRAF) bypass which has hampered standard MEKi. In this study, we examined the transcriptional effects of IMM-1-104 in the C26 model via RNA sequencing. These observations confirmed the pattern of deep cyclic inhibition, demonstrating strong MAPK pathway inhibition in tumor 2h after treatment, and near complete release 12h following treatment. This pattern was observed both after the initial dose as well as following chronic BID dosing (18 days), indicating that deep cyclic inhibition was sustainable across chronic dosing. Filtering the transcriptional signatures to examine effects that followed the pattern of deep cyclic inhibition (strong effect at 2h and release at 12h) revealed effects on mitochondrial and metabolism genes. These genes included Hk2, which was downregulated more than 2-fold after 2h of treatment with 100 mpk IMM-1-104 both after the initial dose and following chronic BID dosing. The effect on Hk2 occurred in tumor but not in muscle tissue from the same mice. These results were independent of adaptive immunity, as they were also observed in nude mice. Top transcriptional effects of IMM-1-104 treatment in tumor also included an increase in expression of Wnt pathway genes at 2h, following either a single dose or chronic BID dosing, at both 25 and 100 mpk. This increase was also observed at 12h following the 100 mpk dose. Activation of Wnt signaling in CRC tumors has previously been reported with standard MEK inhibitors. The transcriptional profile of IMM-1-104 in the C26 model highlighted key aspects of its mechanism related to signaling dynamics, which helps explain its ability to achieve high TGI and tolerability. The profile also highlighted aspects shared with standard MEKi that could shed light on potential resistance pathways.

P255 Alvocidib synergizes with BRD4 inhibitors to improve cytotoxity in an AML cell line. Sal Sommakia, Ethika Tyagi, Yuta Matsumura, Jason M. Foulks, Steven L. Warner. SDP Oncology, Lehi, UT.

The super enhancer complex (SEC) is a group of transcription regulatory proteins that coordinate the expression of genetic programs which determine cell identity and drive diseases such as cancer. In acute myeloid leukemia (AML), SECs have been shown to turn on transcriptional programs that drive tumorigenesis and progression. SEC-regulated transcription begins as Cyclin-Dependent Kinase 9 (CDK9) and Cyclin T1 are recruited from an inhibitory complex by bromodomain 4 (BRD4) and brought to the transcriptional start site of genes. CDK9 phosphorylates RNA polymerase II, releasing it from the SEC and leading to transcriptional elongation and gene expression. Alvocidib is a potent CDK9 inhibitor with clinical activity in AML from multiple Phase II studies. Additionally, BRD4 inhibitors have shown early promise in clinical studies with a focus on AML. Starting with the observation of close association of CDK9 and BRD4 and the central role of the SEC in AML, we previously showed that CDK9 inhibitors combined with bromodomain inhibitors inhibited the SEC more effectively than either of these compounds alone. In this study, we sought to quantify the synergy between alvocidib and each of the BRD4 inhibitors, OTX015, IBET762, and CPI-0610, in the MV-4-11 AML cell line. After determining the single agent IC50 value of each agent, 6x6 matrix combination assays were used to determine synergy between alvocidib and OTX015, IBET762, or CPI-0610. The matrix combination assays consisted of concentrations of each : 4x IC50, 2x IC50, 1x IC50, 0.5x IC50, 0.25x IC50, and 0.125x IC50 value. In the combination assays, the cells were incubated with alvocidib for 48 h, then a BRD4 inhibitor was added, and the cells were incubated with alvocidib and the BRD4 inhibitor for a further 48 h. Combination index (CI) values were calculated using the Chou-Talalay method, where CI values less than 1 indicate synergism and lower CI values indicating stronger synergism. Synergistic interactions were observed in MV-4-11 cells for all three combinations of alvocidib plus a BRD4 inhibitor. For the combination of alvocidib and OTX015, synergy was observed for 81% (29/36) of the tested combinations, with the strongest synergy (CI = 0.155) observed at 20 nM alvocidib and 1.472 μM OTX015. For the combination of alvocidib and IBET762, synergy was observed for 75% (27/36) of the tested combinations, with the strongest synergy (CI = 0.144) observed at 10 nM alvocidib and 6.288 μM IBET762. For the combination of alvocidib and CPI-0610, synergy was observed for 72% (26/36) of the tested combinations, with the strongest synergy (CI = 0.101) observed at 10 nM alvocidib and 5.312 μM CPI-0610. These results suggest that combination strategies might be rationally designed to improve tumor targeting effects while reducing adverse side effects. Clinical studies utilizing these combination strategies are the next steps to further explore this approach.

P256 Pan-ErbB inhibition enhances activity of KRAS^{G12C} inhibitors in preclinical models of KRAS^{G12C} mutant cancers. Jacqulyne P. Robichaux[1], Ana Galan-Cobo[1], Ried T. Powell[2], Kelly A. Gale[1], Jun He[1], Fahao Zhang[1], Monique B. Nilsson[1], Xiang Zhang[1], Mary M. Sobieski[2], Nghi Nguyen[2], Stephan C. Clifford[2], John V. Heymach[1]. [1]MD Anderson Cancer Center, Houston, TX, [2]Texas A&M University Health Science Center, Houston, TX.

Sotorasib (AMG 510) is the first KRAS inhibitor to be FDA-approved for the treatment of KRAS^{G12C} mutant lung adenocarcinomas which comprise ~42% of KRAS mutations in lung adenocarcinomas. Preclinical studies have shown that within hours of KRAS^{G12C} inhibition, synthesis of new KRAS^{G12C} protein and increased KRAS signaling was observed, leading to enhanced tumor cell growth and survival. This appeared to be due, at least in part, to a feeback loop leading to activation of EGFR signaling but the role of other *ErbB* family members including *ErbB2* (HER2), *ErbB3* (HER3), and *ErbB4* (HER4) has not been fully evaluated. We hypothesize that short-term adaptation to KRAS inhibition is driven by multiple *ErbB* family members, and that the combination of pan-ErbB inhibitors with KRAS^{G12C} inhibitors could enhance KRAS^{G12C} inhibitor activity and prolong survival in preclinical models harboring KRAS^{G12C} mutations to a greater extent than more specific EGFR inhibitors. To investigate the role of different ErbB family members in this feedback signaling, we evaluated the impact of an EGFR specific inhibitor, erlotinib, an EGFR/HER2 inhibitor, afatinib, or pan-ErbB inhibitor (EGFR/HER2/3/4), poziotinib on anti-tumor activity of KRAS^{G12C} inhibitors in *KRAS^{G12C}* mutant NSCLC cell lines. We initially tested the different inhibitors in combination with sotorasib and adagrasib (MRTX849) and calculated the BLISS index (BI) for each drug pair matrix, using SynergyFinder. We found that when combined with either sotorasib or adagrasib, poziotinib had a synergistic effect (BI>10) in H23 (BI: 17, p=0.01), HCC44 (BI: 11, p=0.04), and H1792 (BI: 13, p=0.01) and an additive effect (BI= -10–10) in H2122 (BI: 2.8). Afatinib and erlotinib were additive in H23 (BI: 9.2 & 9.4), HCC44 (BI:8.8 & 7.9), H2122 (BI: -4.5 & -3.3) and H1792 (BI: 6.6 & 3.3). Poziotinib yielded a higher BI across all four cell lines compared to afatinib ($p<0.0001$) or erlotinib (p=0.0004). To determine if poziotinib prevented upregulation of ErbB-signaling after treatment with KRAS^{G12C} inhibitors, we treated *KRAS^{G12C}* mutant cell lines (NSCLC: H23 and Bladder Cancer: UM-UC-3) with KRAS^{G12C} inhibitors, sotorasib or adagrasib, for four hours and found by Western blotting that phosphorylated EGFR, HER2, HER3, and HER4 were increased after treatment. The addition of 10 nM poziotinib prevented the upregulation of phosphorylated EGFR, HER2, HER3, and HER4 in both cell lines. Together these data demonstrate that inhibition of EGFR, HER2, HER3, and HER4 signaling by the pan-ErbB inhibitor poziotinib resulted in greater synergy with KRAS^{G12C} inhibitors than EGFR or EGFR/HER2 inhibitors and highlight the potential importance of HER3 and HER4, in addition to EGFR and HER2, in feedback signaling after KRAS G12C inhibition. Additional studies of pan-ErbB and ErbB-specific inhibitors with KRAS^{G12C} inhibitors are warranted to determine tolerability and optimal dosing to prolong survival in *KRAS* mutant cancers.

P257 Preclinical data identifies bezuclastinib as a differentiated KIT inhibitor with unique selectivity to KIT D816V and minimal evidence of brain penetration. Anna Guarnieri, Mark Chicarelli, LouAnn Cable, Francis Sullivan, Howard Ball, Shannon Winski, John Robinson. Cogent Biosciences, Boulder, CO.

KIT is a well characterized oncogenic driver of gastro-intestinal stromal tumors (GIST) and systemic mastocytosis (SM), and targeted therapeutics have offered valuable treatment options for these patients. Durable responses in imatinib-resistant GIST are rarely achieved due to secondary mutations that occur in exons 13/14 and 17/18. Exon 13/14 mutations can be addressed with sunitinib treatment, but identifying inhibitors that additionally target exon 17/18 mutations without incurring off-target toxicities related to broad-spectrum kinase inhibition has been challenging. Similarly, agents targeting KIT D816V mutations have shown activity in the treatment of advanced SM, but toxicities such as cognitive effects and intracranial hemorrhage may limit dosing and availability of these therapies. Bezuclastinib (CGT9486) was designed to inhibit exon 17/18 KIT mutations (including KIT D816V) with selectivity versus other closely-related kinases with known liabilities, such as PDGFRα, PDGFRβ, KIT wt, VEGFR2 (KDR), and CSF1R (FMS). Herein, we are presenting results from cell-based kinase profiling assays, which demonstrate that bezuclastinib has a significant and unique selectivity to KIT D816V vs. these aforementioned kinases when tested head-to-head against other clinically relevant compounds in GIST or SM. Additionally, a similar selectivity profile was observed for a broader panel of kinases, ion channels, transporters, and enzymes, which will be presented here. Importantly, we also show that bezuclastinib has minimal brain penetration. In a tissue distribution study performed in rats, bezuclastinib shows a brain:plasma ratio <0.1 following 3 day administration at 25 mg/kg, a dose that closely correlates with clinical exposure. This was supported functionally by assessing neurobehavioral effects of bezuclastinib at dose levels up to 100 mg/kg which showed no CNS related effects. Minimal brain penetration is a preferred feature of an anti-Kit molecule due to CNS-related adverse events observed in these indications. This attractive selectivity and nonclinical safety profile, combined with early clinical data (1) in advanced solid tumors, supports the potential for a best-in-class KIT inhibitor. Bezuclastinib is currently under clinical investigation in advanced SM with additional clinical studies planned in non-advanced SM and imatinib-resistant GIST. 1. Jonathan Trent, William D. Tap, Rashmi Chugh, Gabriel Tinoco, Athanasios Tsiatis, Paul Severson, Kerry Inokuchi, Chao Zhang, Glenn Michelson, Andrew J. Wagner. The Potent and Selective KIT Inhibitor PLX9486 Dosed in Combination With Sunitinib Demonstrates Promising Progression Free Survival (PFS) in Patients With Advanced Gastrointestinal Stromal Tumor (GIST): Final Results of a Phase 1/2 Study. Abstract Presented at the Connective Tissue Oncology Society Virtual Meeting; November 20, 2020.

P258 CBX-12 (alphalex™-exatecan) sensitizes tumors to immune checkpoint blockade in an antigen agnostic manner by immune activation. Sophia Gayle[1], Juan Vasquez[2], Timothy Paradis[1], Kelli Jones[1], Ranjini Sundaram[3], Jinny van Doorn[3], Viswanathan Muthusamy[4], Ranjit S. Bindra[3], Robert J. Aiello[1], Vishwas Paralkar[1]. [1]Cybrexa Therapeutics, New Haven, CT, [2]Department of Pediatrics, Yale University School of Medicine, New Haven, CT, [3]Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, [4]Yale Center for Precision Cancer Modeling, Yale University School of Medicine, New Haven, CT.

Immune checkpoint blockade (ICB) in combination with chemotherapy is standard of care for several solid tumors. However, potent chemotherapies such as topoisomerase inhibitors can result in severe dose-limiting toxicities requiring dose reduction, limiting their efficacy. Moreover, leukocytopenia from chemotherapy induces immunosuppression that further limits the full potential of combinations with ICB. The use of targeted chemotherapies such as antibody-drug conjugates (ADCs) demonstrate potential synergy with ICB, however are limited to a defined subset of patients with tumors expressing the target antigen.

Cybrexa has reported the development of CBX-12, a peptide-drug conjugate consisting of alphalex™-exatecan (1). Rather than targeting a specific antigen, alphalex™ consists of a unique variant of pH-Low Insertion Peptide (pHLIP®; 2-4) which targets the low pH environment of the tumor, a universal feature characteristic of all tumors due to the Warburg effect. The alphalex™ component of CBX-12 forms an alpha helix only in low pH conditions, allowing for directional insertion of the peptide within the cancer cell membrane, delivery of C-terminally linked exatecan across the membrane, and subsequent intracellular release of active exatecan via glutathione reduction of the linker, thereby allowing for tumor-specific intracellular delivery in an antigen-independent manner.

Here we evaluated the potential for CBX-12 to effectively synergize with PD1 and CTLA4 blockade in multiple syngeneic mouse models without requirement for tumor specific antigens. We found that CBX-12/ICB combination treatment significantly delayed tumor growth, improved survival and led to complete tumor regressions. Mice cured with combined CBX-12/ICB therapy demonstrated formation of long-term immunological memory after *in vivo* and *ex vivo* tumor rechallenge. The ability of CBX-12 to induce immunogenic cell death was confirmed by vaccinating syngeneic mice with CBX-12 treated tumor cells and subsequent tumor rechallenge, which demonstrated near total anti-tumor immunity induced by CBX-12. Together, these preclinical data demonstrate the potential for CBX-12 to enhance tumor immunogenicity and potentiate the efficacy of ICB in patients with solid tumors affording a superior, universal tumor targeting mechanism that bypasses the limitations of ADCs.

1. Gayle S et al. 2021.Tumor-selective, antigen-independent delivery of a pH sensitive peptide-topoisomerase inhibitor conjugate suppresses tumor growth without systemic toxicity. NAR Cancer.
2. Rather than targeting a specific antigen, alphalex™ includes a pHLIP® peptide. pHLIP® peptides are a family of pH-Low Insertion Peptides that target acidic cell surfaces. pHLIP® was developed at Yale University and the University of Rhode Island, and is exclusively licensed to pHLIP, Inc.
3. Wyatt LC et al. 2017. Applications of pHLIP Technology for Cancer Imaging and Therapy.

Trends Biotech.
4. Wyatt LC et al. 2018. Peptides of pHLIP family for targeted intracellular and extracellular delivery of cargo molecules to tumors. PNAS.

P260 Development and validation of a novel T-cell modulating, microbiome-based peptide for combination with immunotherapy. Michelle Lin, Archana S. Nagaraja, Dhwani Haria, Yuliya Katlinskaya, Divya Ravichandar, Preston Williams, Roberta Hannibal, Todd Z. DeSantis, Bum-Yeol Hwang, Michi Wilcoxon, Toshi Takeuchi, Karim Dabbagh, Preeti Lal, Helena Kiefel. Second Genome, Brisbane, CA.

The gut microbiome is an important determinant for the success of anti-tumor therapies including chemotherapeutics and anti-checkpoint inhibitors. In this study we wanted to leverage Second Genome's large and curated microbiome database coupled with its proprietary bioinformatics and machine learning tools to discover bioactive peptides from Bifidobacteria that have the potential to drive response to immunotherapy. The genome of Bifidobacterium (B.) breve and B. longum were analyzed for proteins which were potentially secreted, and had unknown functions. 50 peptides were chemically synthesized and then screened in cell based assays for T cell activation and cytokine secretion. In the present study we describe one such novel B. breve-derived 42-aa peptide (SG-3-0020). The peptide stimulated secretion of effector cytokines by in vitro-cultured T cells (IFNg, TNF-a, IL-10 and IL-2) and increased the expression of PD-1 on both CD4+ and CD8+ T cells when stimulated with low-dose anti-CD3 antibody. To identify the binding partners and mechanism of action of the peptide, Mass Spec and Single-cell RNA-seq was used. Mass spec analysis showed that SG-3-05308, a variant of SG-3-0020 binds to a transmembrane glycoprotein of the immunoglobulin superfamily. Silencing this gene via CRISPR-Cas significantly decreased PD1 levels, cell proliferation and IFNg production in human pan T cells. Single cell RNA-seq data showed that SG-3-0020 activates NF-kB signaling and modulates calcium signaling in T cells. The potency of SG-3-0020 was further optimized for binding to activated T cells by using alanine scanning, saturation, and combinatorial mutation libraries using phage display. The results of protein engineering demonstrated that the 13 amino acids from the C-terminus of SG-3-0020 were not critical for binding and a 29-aa long (lacking the C-term) peptide stimulated increased IFNg production in human T cells across multiple human donors in a dose dependent manner. The peptide with the highest potency SG-3-05429 was selected for further understanding of how it interacts with the identified glycoprotein target and activates downstream T cell signaling pathways . Collectively, these data suggest that SG-3-0020's ability to up-regulate key co-stimulatory and checkpoint molecules on T cells provides a strong rationale for its potential future use in combination with IO. These results validate the capability of the Second Genome drug discovery platform to identify novel microbial peptides/proteins of potential therapeutic relevance in IO and demonstrate a unique approach that can identify microbial derived bioactive molecules involved in modulating immune cell effector functions and/or immune cell differentiation.

P262 Automated nerve identification in histopathology slides enables comprehensive analysis of innervation in cancer and tumor neurobiology. Alison R. Miller[1], Daniel Krasnonosenkikh[1], Monica Thanawala[1], Kai Chih Huang[1], George V. Thomas[2], Alexandra B. Lantermann[1], Hongyue Dai[1], Masoud Sadaghiani[1], John A. Wagner[1], Pearl Huang[1]. [1]Cygnal Therapeutics, Cambridge, MA, [2]Oregon Health and Science University, Portland, OR.

Linking features of the tumor microenvironment (TME) to patient outcome is ambitious yet crucial to understand cancer progression and prognosis in order to develop new therapies. The current method to assess histopathological images is visual inspection by a professional, generally a pathologist. While this is an effective approach for diagnosis, it does not scale well for discovery biology and population-based studies for drug discovery purposes. An automated approach would allow for a more rapid, systematic, and comprehensive analysis of several morphological features of the TME by taking advantage of thousands of already existing slides within databases and biobanks. Recent studies have shown intriguing relationships between innervation in tumors (tumor exoneural biology) and patient outcomes. Here we present an automated tool that can detect and quantify nerve presence in tumors. We manually annotated a set of digital slides from The Cancer Genome Atlas (TCGA) in order to develop a deep learning model to quantify the presence of nerves in head and neck tumors stained with H&E. This tool is generalizable and was applied to identify patterns in the appearance of tumor-infiltrating lymphocytes (TILs) in and around tumors. It may be further applied to other structural features such as blood vessels in order to characterize and correlate these features within the TME in hundreds of images across cancer types. This enables integration of imaging features with multi-omics data to uncover potential biological pathways that are upregulated in groups with dense innervation compared to sparse innervation in cancer. The main advantage of this approach is the ability to utilize many public databases in which the features of interest can be correlated with reported patient comorbidities, treatments, and phenotypes. This platform-based methodology can be expanded to other disease areas and could ultimately provide valuable insight about exoneural biology and its role in disease physiology to identify new avenues for therapies.

P263 Interaction between CD36 and FABP4 regulates the import and metabolism of fatty acid in breast cancer cells. Jones Gyamfi[1], Junjeong Choi[1], Doru Kwon[1], JaSeung Koo[2]. [1]Yonsei University, College of Pharmacy, Songdo, Korea, Republic of, [2]Yonsei University, College of Medicine, Seoul, Korea, Republic of.

The breast cancer microenvironment is unique since the breast tissue within which the tumour originates comprises predominantly adipocytes. Adipocytes secrete various growth factors and cytokines that are reported to influence tumour progression by induction of epithelial-mesenchymal transition (EMT) which enhances cancer cell migration, invasion, and metastasis. Immunohistochemical analysis reveals that the invading breast cancer invades surrounding adipose tissues resulting in the delipidation of adipose tissues. To determine the molecular mechanism underlying these events, we co-cultured human adipocytes and breast cancer cells. Co-cultured cells had increased CD36 expression, with fatty acid import. Genetic ablation of CD36 attenuates adipocyte-induced EMT and stemness. Molecular screening for pathways potentially involved in adipocyte-induced CD36 expression reveals activation of the STAT3 signaling axis. Our study identifies a feedforward loop between CD36 and STAT3; where STAT3 binds to the CD36 promoter, activating its expression and CD36 upregulates STAT3 signaling. Breast cancer cells co-cultured with adipocytes actively accumulate fatty acids and undergo metabolic reprogramming, with a shift towards fatty acid oxidation. Seahorse metabolic analysis reveals that co-cultured CD36-expressing cells assumed an energetic phenotype, indicating enhanced mitochondrial respiration. Thus, breast cancer cells alter their metabolic programs, with increased mitochondrial respiration, to meet their energy needs, with the availability of fatty acids. Analysis of breast cancer patient data reveals that increased CD36 expression occurs with increased FABP4 expression. Mechanistic experiments reveal that CD36 directly interacts with FABP4 to regulate fatty acid import, transport, and metabolism in co-cultured breast cancer cells. In vivo studies in mouse models reveal that combined chemical inhibition of CD36 and FABP4 resulted in a significant reduction in tumour growth rate. The study presents an alternative approach by which adipocytes may enhance cancer progression via the transfer of free fatty acids from tumour-associated adipocytes to breast cancer cells. The uptake of free fatty acid subsequently serves as a secondary source of energy to drive breast cancer cell progression. Targeting CD36 in combination with FABP4 may have a potential for therapies targeting the breast cancer microenvironment.

P264 Targeting oxygen metabolism reduces hypoxia in the tumor microenvironment of a syngeneic mouse model. Daan F. Boreel[1], Paul Span[1], Hans Peters[1], Renske J.E. van den Bijgaart[1], Sandra Heskamp[2], Gosse J. Adema[1], Johan Bussink[1]. [1]Radboudumc, Nijmegen, Netherlands, [2]Radboudumc.nl, Nijmegen, Netherlands.

When tumors outgrow their chaotic vasculature, limited diffusion of oxygen into remote tumor areas combined with increased oxygen consumption leads to hypoxia in most solid tumors. This oxygen scarcity is known to induce radioresistance, but may also interfere with the anti-tumor immune response. Hypoxia inhibits immune effector cell function, while immune cells with a more suppressive phenotype become more active. Using the novel mitochondrial complex I inhibitor IACS-010759, we aim to pharmacologically attenuate oxidative phosphorylation (OXPHOS) and subsequently decrease tumor oxygen consumption, hereby relieving hypoxia and sensitizing tumors to both radio- and immunotherapy. Several syngeneic murine cell lines and tumor models on a C57Bl/6 background were used (B16ova, MOC1, MC38 and GL261). *In vitro* oxygen consumption of these tumor cells was measured using the Agilent XF Seahorse Analyzer and cell growth was measured using the Incucyte ZOOM Live Cell Imaging system. Hypoxia and immune marker expression by these cells *in vitro,* and *in vivo* in the tumor microenvironment (TME), were determined using flow cytometry and immunohistochemistry. Tumor hypoxia was calculated as the fraction of tumor area in different zones at increasing distances around vessels on tumor sections.*In vitro,* IACS-010759 treatment potently inhibits oxygen consumption in a dose dependent manner. Under physiological glucose concentrations (1.5mM) IACS-010759 reduces cell growth at least 2-fold more potently than under high glucose culture conditions. On the other hand, growth is only marginally or not at all inhibited under low oxygen conditions (1.0%). This shows that cancer cells use oxygen when it is available, and tend to rely on glycolysis under hypoxia. This contradicts the longstanding notion that cancer cells primarily use glycolysis, also under aerobic conditions, and suggests that cancer cells are often metabolically plastic. Daily IACS-010759 treatment (10mg/kg) of mice bearing B16ova tumors for 4 or 9 days reduces the hypoxic fraction at increased distance from vessels, presumably by the inhibition of oxygen consumption closer to vessels, allowing oxygen to diffuse further into the tissue. No growth reduction by IACS-010759 treatment was observed in these tumors, showing they are able to use glycolysis for their energy supply. Flow cytometry analysis of these tumors showed no changes in abundance of lymphocyte and myeloid cell populations, suggesting inhibition of OXPHOS has no direct inhibitory effect on immune cell presence. We show that IACS-010759 decreases oxygen consumption in several tumor cell lines *in vitro* while it also causes growth inhibition if cancer cells rely on oxygen. *In vivo,* diffusion limited hypoxia can be reduced by the inhibition of OXPHOS, while the abundance of immune cells within the TME was not affected. The observed changes might lead to a more immune permissive TME when induced by radio- and immunotherapy, potentially increasing the efficacy of this treatment combination.