
# AACR Annual Meeting 2023

Late-Breaking Abstracts

Clinical Trial Abstracts

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# Late-Breaking Abstracts

## Minisymposia



## Poster Sessions

# Clinical Trials Abstracts

## Clinical Trials Plenary Sessions



## Clinical Trials Minisymposia



## Clinical Trials Poster Sessions

**LB001**

**Encapsulation of IL-12 with an ultra pH-sensitive nanoparticle platform improves tolerability and promotes antitumor response in mice.**

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**Background:** Interleukin-12 is a potent proinflammatory cytokine that proliferates and activates T cells, NK cells and differentiates Th1 cells. Translation of IL-12 for cancer treatment has been hindered by lethal toxicities due to cytokine release syndrome and there are currently no approved IL-12 therapies. To minimize the severe toxicities while maintaining potency, we have developed ON-BOARD, an ultra-pH sensitive nanoparticle platform for masked and targeted delivery of payloads to the acidic tumor microenvironment. The clinical feasibility of ON-BOARD has been demonstrated by high tumor specificity of pegsitacianine in multiple tumor types from the Phase I and II clinical trials. Herein we report encapsulation and masked delivery of IL-12 to tumor-bearing mice using ON-BOARD, demonstrating significantly improved tolerability, anti-tumor efficacy, and potential for clinical translation.
**Methods:** A mouse IL-12 fused with Fc was formulated in ON-BOARD nanoparticles. Particle properties were characterized and lead formulations were identified by *in vitro* screening to determine pH-mediated bioactivity in reporter and ELISA assays and stability in mouse plasma. *In vivo* studies were performed to compare the activity of unencapsulated IL-12 to ON-BOARD/IL-12 formulations. PD response was evaluated by measuring systemic cytokine levels in plasma, while clinical chemistry was performed to evaluate liver and kidney functions. Anti-tumor efficacy of ON-BOARD/IL-12 formulations was performed in mice bearing syngeneic MC38 colorectal cancer tumors compared to unencapsulated IL-12.
**Results:** ON-BOARD/IL-12 formulations showed high encapsulation efficiency (>85%) and drug loading up to 20% wt. in uniformly distributed stable particles ($D_h$<50nm). pH-specific payload release was confirmed *in vitro* with >100-fold activation window between the acid-activated and intact formulations. Following incubation in mouse plasma the lead ON-BOARD formulations showed stable IL-12 encapsulation by an ELISA assay. *In vivo*, ON-BOARD/IL-12 formulations demonstrated significantly improved tolerability compared to unencapsulated IL-12. When dosed at 5μg/dose compared to unencapsulated protein at 1 μg/dose, ON-BOARD/IL-12 demonstrated reduced body weight loss (<2% vs 13%) and decreased liver injury markers AST and ALT. Analysis of systemic cytokines (IFNγ, IL-6, IL-10, TNFα, etc) showed significantly lower levels for ON-BOARD formulations including >1,000-fold reduction in plasma IFNγ level which is known to be directly induced by IL-12 signaling. ON-BOARD/IL-12 formulations also demonstrated strong anti-tumor efficacy in MC38 tumor-bearing animals with >95% TGI and complete responders.
**Conclusions:** The ON-BOARD platform demonstrated potential for masking toxicity and facilitating tumor-specific delivery of IL-12 proteins for cancer therapy.

**LB002**

**Extracellular vesicle loading of proteolysis targeting chimeras for targeted therapeutic delivery.**

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Breast cancer is a significant public health issue that remains the second leading cause of cancer death among women. Current treatment strategies for breast cancer can contribute to severe side effects and insufficient efficacy. For example, chemotherapy, radiotherapy, and hormone receptor and HER2 targeted therapies can lead to healthy cell damage, a partial, delayed effect, and cardiac toxicity and acquired immune resistance, respectively. An emerging alternative targeted therapy approach is proteolysis

targeting chimeras (PROTAC), which triggers proteasomal degradation of target proteins implicated in cancer. PROTAC provides precision targeting of low-affinity targets for robust, selective efficacy. However, wide-spread clinical use is limited due to instability and poor cell penetration in vivo. In order to enhance the efficacy of PROTAC, we introduce a novel extracellular vesicle (EV) transfection platform for highly efficient loading of PROTAC. This approach can enhance cellular delivery by utilizing EV's in vivo properties, like high biocompatibility, long circulation times, and specific targeting. Microfluidic droplet-based electroporation (μDES) was developed and optimized for high- efficient EV transfection, which offers continuous flow transfection suited for scaling up and GMP manufacturing. We compared the drug loading efficiency to conventional transfection methods, including simple incubation, chemical transfection through lipofection, and conventional physical transfection through the Neon electrotransfection system (ThermoFisher). The resulting EVs were characterized by nanoparticle tracking analysis for size, concentration, and zeta potential, and by TEM for morphological determinations. PROTAC transfection efficiency was also measured through absorbance spectrometry. The resulting μDES PROTAC-loaded EVs showed little, or no, changes compared to the native EVs and Neon control group, while offering higher recovery and transfection rates. We also tested the in vitro therapeutic functionality of PROTAC loaded EVs for histone deacetylase 3 and 8 degradation in the breast cancer cell line MDA-MB-231 with western blot analysis. We demonstrated high degradation ability of μDES loaded EVs and, thus, significantly higher therapeutic function. We also plan to investigate biodistribution behavior of the therapeutic EVs in vivo. PROTAC delivery by EVs could enhance stability, biocompatibility, transportability, and targeting ability of the drug. This serves as a novel PROTAC drug delivery, formulation, and administrative strategy that fills an important gap in current PROTAC use.

**LB003**

**BI 907828: A highly potent MDM2-p53 antagonist suitable for intermittent dose schedules.**

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The E3 ligase MDM2 controls the tumor suppressor function of p53, which is encoded by the *TP53*gene. Compounds designed to bind to MDM2 preventing its interaction with p53 restore the ability of p53 to combat the pathogenic processes that underly cancer development. It has become clear from early clinical experience that thrombocytopenia represents an on-target, dose-limiting toxicity that may restrict the therapeutic utility of first-generation MDM2 inhibitors. Dosing less frequently, while maintaining efficacious exposure levels, has been proposed as an approach to mitigate side effects and improve the therapeutic window. In this -Late Breaking- session we present the discovery and preclinical evaluation of BI 907828, a highly potent, orally bioavailable and selective molecule binding to MDM2 and preventing its interaction with p53. The compound was designed to have a long half-life to enable intermittent dosing schedules in the clinic where it showed a manageable safety profile in a Phase Ia/Ib, dose-escalation/expansion study and encouraging signs of antitumor activity in patients with advanced DDLPS and WDLPS. BI 907828 is actively investigated in several clinical trials including the Phase II/III Brightline-1 study that aims to evaluate whether it is superior to doxorubicin in the first-line treatment of advanced/metastatic DDLPS. BI 907828 belongs to the class of spiro-oxindole MDM2-p53 antagonists and was discovered starting from an optimized core structure with improved chemical stability. Structure-based medicinal chemistry optimization including rigidification of the scaffold were key to accomplish the desired profile which was tested preclinically in several *in vivo* MDM2-amplified xenograft models where treatment with BI 907828 showed efficient tumor growth inhibition and regression.

**LB004**

**Ultra-sensitive targeted DNA panel for very low-frequency mutation detection in circulating cell-free DNA.**

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Due to its noninvasive nature and being readily available in peripheral blood and other bodily fluids, analysis of cell-free DNA (cfDNA) has become a promising application in cancer diagnosis, prognosis and treatment monitoring. However, disease-relevant cfDNA is present in a very limited amount in the huge background of normal cfDNA. This remains a challenge for any detection technology. Many currently available target enrichment and library preparation methods use regular DNA polymerase and amplification processes that introduce substantial bias and artifacts. This results in artifactual errors that greatly limit the detection of true low-frequency variants below 0.5% in heterogeneous samples, such as cfDNA. Here, we present a new targeted cfDNA workflow that overcomes challenges such as biases and artifacts. The workflow uses a highly optimized, high-fidelity reaction chemistry and incorporates UMIs into a single gene-specific, primer-based targeted enrichment process. Compared to regular DNA polymerase, this high-fidelity chemistry resulted in a five- to ten-fold decrease in base substitution error. An individual cancer panel was designed to specifically cover cancer-relevant hotspots and copy number genes with a dense primer design to accommodate the short length of cfDNA. Due to its high-fidelity chemistry and optimal panel design, our streamlined workflow can be completed in a single day. We also report consistent panel performance across different samples. Detection sensitivity and specificity were evaluated on a reference cfDNA sample and a simulated cfDNA sample by mixing enzyme-digested Genome in a Bottle samples, NA12878 and NA24385. We achieved close to 90% detection sensitivity and above 99.9% specificity for 0.1% variant. In addition, copy number variation could be successfully detected with a 1.5-fold difference over normal control samples. These results demonstrate an efficient targeted cfDNA panel workflow that enables cancer-relevant mutation detection with high sensitivity and accuracy. The applications presented here are for research use only. Not for use in diagnostic procedures.

**LB005**

**Insights of renal cell carcinoma from multiomic perspective.**

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Introduction: Clear cell renal carcinoma is the most frequent form of renal malignancy, with an increasing incidence rate worldwide. In this study we used a multi-omic approach to differentiate normal and tumor tissues in clear cell Renal Cell Carcinoma (ccRCC).
Methods: Using transcriptomic data of patients with malignant and adjacent normal tissue samples from gene chip and RNA-Seq cohorts, we identified the top genes over-expressed in ccRCC. We collected surgically resected ccRCC specimens to further investigate the transcriptomic results on the proteome level. The differential protein abundance was evaluated using targeted mass spectrometry (MS).
Results: We assembled a database of more than 600 renal tissue samples from NCBI GEO and TCGA and used these to uncover the top genes with higher expression in ccRCC. For protein level analysis 162 malignant and normal kidney tissue samples have been acquired. The most consistently upregulated genes were IGFBP3, PLIN2, PLOD2, PFKP, VEGFA, and CCND1 ($p<1E-05$ for each gene). Mass spectrometry further validated the differential protein abundance of these genes (IGFBP3, $p = 7.53E-18$; PLIN2, $p = 3.9E-39$; PLOD2, $p = 6.51E-36$; PFKP, $p = 1.01E-47$; VEGFA, $p = 1.40E-22$; CCND1, $p= 1.04E-24$). We

also identified proteins correlating with overall survival.
Conclusions: We used transcriptomic and proteomic data to identify a minimal panel of proteins highly specific for clear cell renal carcinoma tissues. The introduced gene panel could be used as a promising tool in the clinical setting.

**LB006**
**Interactome analysis identifies signaling pathways activated by ETV6-NTRK3 oncogenic gene fusions.**
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Chromosomal translocations creating fusion genes are among the most common mutation class of known cancer genes, and they have long been identified as driver mutations in certain types of cancer. Recently, oncogenic fusion genes (oncofusions, OFs) have been found in many hematological and solid tumors, demonstrating that translocations are a common cause of malignancy. The frequency of recurrent gene fusions varies depending of the specific type of cancer, but currently identified translocations are estimated to drive up-to 20% of cancer morbidity. In many occasions the oncofusion is the sole driver of oncogenesis when present and certain oncofusion pairs are typically found in one or few specific cancers. ETV6-NTRK3 (EN) oncofusion, is a product of the chromosomal t(12; 15)(p13; q25) translocation, which fuses the N-terminal SAM (sterile alpha motif) domain of ETV6 to the C-terminal protein tyrosine kinase domain of NTRK3 (also known as TrkC). EN is expressed from the ETV6 promoter in the fused chromosome 15. The ETV6 promoter is generally more active, than NTRK3 promoter, and causes EN fusions to be expressed more highly in several tissues and especially in bone marrow and salivary glands. ETV6 forms fusions with many kinases, which are found in hematological malignancies and solid tumors. Except for ETV6-NTRK3, other ETV6-kinase fusions are reported in either hematological or solid malignancies and ETV6-NTRK3 further distinguishes itself by being also reported in several subtypes in both cases. Four EN variants with alternating break points have since been detected in a wide range of human cancers. To provide insight into EN oncogenesis, we employed a proximity labeling mass spectrometry approach to define the molecular context of the EN fusions. We identify 237 high-confidence interactors, which link EN fusions to several key signaling pathways, including ERBB, Insulin and JAK/STAT. We then assess the effects of EN variants on these pathways, and show that the pan NTRK inhibitor selitrectinib (LOXO-195) inhibits the oncogenic activity of EN2, the most common variant. This systems-level analysis of defines the molecular framework in which EN oncofusions operate to promote cancer.

**LB009**
**Notch4 inhibition by a novel neutralizing antibody reduces tumor progression and increases macrophage recruitment within the tumor microenvironment .**
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The Notch signaling pathway, particularly endothelial Notch signaling, plays a key role in tumor formation and angiogenesis. Inhibiting Notch1 or its ligand, Delta-like ligand 4 (Dll4), can induce hypersprouting of the tumor vasculature and thus can reduce tumor perfusion and progression. However, due to the expression of Notch1 in multiple healthy cell types, pharmacologic blockade poses the challenge of inducing tissue hypoxia, vascular neoplasms, and toxicity in non-target tissues. Unlike Notch1, Notch4 expression is largely restricted to endothelium and is upregulated in models of triple negative breast cancer (TNBC). Loss of Notch4 in mice results in delayed developmental angiogenesis

and reduced mammary tumor angiogenesis and perfusion. Therefore, Notch4 is an attractive potential pharmacologic target to interfere with tumorigenesis and minimize nonspecific toxicity.
We determined that Notch4 is expressed primarily in tumor endothelium in both human and mouse mammary tumors, including murine syngeneic Py8119 tumors, as well as a selection of other human and syngeneic mouse tumor types such as B16F10 melanoma tumors.
We explored the effects of Notch4 inhibition on the progression of murine mammary epithelial carcinoma using newly developed anti-Notch4 antibodies, 6-3-A6 and its humanized derivative E7011. Administration of E7011 or 6-3-A6 to orthotopically implanted Py8119 murine breast carcinoma and B16F10 melanoma significantly reduced tumor size in comparison to IgG-treated controls. We performed single cell RNA sequencing of Py8119 tumors treated with E7011 and found that although Notch4 transcripts were found specifically in the endothelium, the most notable effect of E7011 was a dramatic increase in the number of macrophages and cancer-associated fibroblasts, suggesting a non-cell autonomous effect.
To examine the potential role of these immune cell population changes in facilitating E7011's anti-tumor activity, we investigated treatment of Py8119 murine mammary carcinomas in the absence of macrophages. Administration of clodronate liposomes to eliminate macrophages blocked the anti-tumor effects of E7011 treatment. Taken together, our data suggests that tumor endothelial Notch4 promotes TNBC growth by altering the immune landscape and increasing anti-tumor macrophage recruitment, and that blockade via the novel anti-Notch4 E7011 antibody has potential therapeutic efficacy.

**LB010**
**LY6K depletion modulates TGF-β and EGF signaling.**
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Lymphocyte antigen 6 complex locus K (LY6K), a glycosylphosphatidylinositol-anchored protein, plays a dynamic role in cancer metastasis. In the current study, we deciphered the effects of LY6K on transforming growth factor-β (TGF-β) and epidermal growth factor (EGF) signaling through clathrin- and caveolin-1 (CAV-1)-mediated endocytosis. LY6K expression level is elevated in higher grade cervical cancer patients correlating with poor overall survival, progression-free survival, and disease-free survival. LY6K-depletion in HeLa and SiHa cancer cells suppressed EGF-induced proliferation and TGF-β-enhanced migration and invasion. Both TGF-β receptor-I (TβRI) and EGF receptor (EGFR) localized at the plasma membrane regardless of LY6K expression, and LY6K bound TβRI irrespective of the presence of TGF-β; however, LY6K did not bind EGFR. LY6K-depleted cells showed impaired SMAD2 phosphorylation upon TGF-β treatment and lower proliferation rates following long-term treatment with EGF. We revealed the atypical movement of TβRI and EGFR from plasma membrane upon ligand stimulation in LY6K-depleted cells and an impaired movement of the endocytic proteins clathrin and CAV-1. Subsequently, transmission electron microscopy showed no clathrin and CAV-1-coated vesicles in LY6K-depleted cells. Our study demonstrates the key role of LY6K in both clathrin- and CAV-1-mediated endocytic pathways regulated by TGF-β and EGF, and it suggests a correlation between LY6K overexpression in cervical cancer cells and poor overall survival.

**LB011**
**Development of novel protein drug conjugates (PDCs) for the selective targeting of ALPP/ALPPL2 expressing tumors.**
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The placental alkaline phosphatases, ALPP and ALPPL2, are glycosylphosphatidylinositol (GPI)-anchored cell-surface proteins that are expressed in the placenta during fetal development but have very little expression on normal adult tissue. These proteins are over-expressed in a number of different solid tumor indications including ovarian, endometrial, gastric, pancreatic and non-small cell lung cancers. As a result of the highly restricted normal tissue expression and over-expression in cancer, ALPP and ALPPL2 are attractive targets for antibody and protein-drug conjugate (ADC and PDC) approaches. The recent initiation of a Phase I clinical trial utilizing a full-length antibody ADC has fueled further interest in these oncology targets. Small protein domain binders, which have the capacity to penetrate deeper into solid tumors and can be engineered into multiple therapeutic formats in a modular fashion, offer a number of potential benefits over full-length antibodies as the targeting vehicle in PDC therapeutics. We report the identification and characterization of a series of high affinity ALPP/ALPPL2 specific single domain VHH binders which, importantly, show no binding to the closely related ALPI or ALPL isoforms (which have high normal tissue expression). Through application of the Almac Discovery PDC technology platform, these VHH domains have been reformatted into a suite of homogenous site-specifically labelled drug conjugates, with defined drug to antibody ratios, in high yields. These conjugates, which are based on mono- and bi-paratopic Fc fusion formats, employ both clinically established and novel linker-toxin combinations. In pre-clinical studies, the lead bi-paratopic PDCs were well tolerated *in vivo* and showed excellent anti-tumor efficacy in ALPP/ALPPL2 positive cell-line derived xenograft models of gastric and pancreatic carcinoma, with sustained regressions still observed 10 weeks after administration of the final dose of agent. These agents have physicochemical and pharmaceutical properties suitable for further development and we anticipate that the excellent pre-clinical efficacy profile of lead PDCs will translate to a highly differentiated product for the treatment of a variety of solid tumor indications.

**LB012**

**Claudin-1 is a driver and therapeutic target for cholangiocarcinoma.**

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Introduction: Cholangiocarcinoma (CCA) shows an alarming rise in incidence and mortality with unsatisfactory treatment options. Claudin-1 (CLDN1), a member of the tight junction family, is a transmembrane protein mediating cell stemness, plasticity and signaling. The functional role of CLDN1 as a therapeutic target for CCA is unknown. We have previously developed highly specific monoclonal antibodies (mAb) targeting exposed non-junctional CLDN1 exhibiting an excellent safety profile in non-human primates (Roehlen, Saviano et al. Science Translational Medicine 2022). Here, we aimed to explore the role of CLDN1 as an oncogenic driver and therapeutic target for CCA.
Methods: Comprehensive CLDN1 expression analyses in patient tissues were performed to evaluate CLDN1 as a therapeutic target. Proof-of-concept studies using CLDN1 mAbs were performed in state-of-the-art mouse CDX and PDX models including models for advanced metastatic disease. Single-cell RNA sequencing and proteomics were applied to investigate tumor cell fate and signaling in vivo and ex vivo models.

Results: Comprehensive analyses of CLDN1 protein and RNA expression in CCA patient tissues revealed a marked and significant upregulation of CLDN1 in CCA. Single-cell RNA sequencing of the CCA microenvironment revealed strong expression in tumor cells showing EMT, cell cycle and interferon response signature, uncovering CLDN1 as a therapeutic target. Targeting exposed CLDN1 by highly specific mAbs resulted in a significant and robust antitumoral effect in vivo across CDX and PDX models for intra- and extrahepatic CCA including advanced metastatic disease. Functional studies in cell-based models of CCA showed that CLDN1 mAbs markedly and significantly suppressed migration and invasion of tumor cells. Mechanistically, treatment with CLDN1 mAb suppressed Notch1, Src, and Hippo-YAP signaling - key signal transduction pathways implicated in CCA development and progression.
Conclusion: Collectively, these results support an important functional role for CLDN1 in CCA pathogenesis and provide robust pre-clinical proof-of-concept for CLDN1-specific mAbs to treat CCA, setting the stage for its clinical development.

**LB013**
**Integrated in vivo functional screens and multi-omics analyses identify alpha-2,3-sialylation as essential for melanoma survival.**
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Glycosylation is a hallmark of cancer biology, and altered glycosylation influences multiple facets of melanoma growth and progression. To identify glycosyltransferases, glycans, and glycoproteins essential for melanoma survival, we conducted an in vivo growth screen with a pooled shRNA library of glycosyltransferases, lectin microarray profiling of benign nevi and melanoma patient samples, and mass spectrometry-based glycoproteomics. We found that alpha-2,3 sialyltransferases ST3GAL1 and ST3GAL2 and corresponding alpha-2,3-linked sialosides are upregulated in melanoma compared to nevi and are essential for melanoma growth in vivo and in vitro. Glycoproteomics revealed that glycoprotein targets of ST3GAL1 and ST3GAL2 are enriched in transmembrane proteins involved in growth signaling, including the amino acid transporter Solute Carrier Family 3 Member 2 (SLC3A2/CD98hc). CD98hc suppression mimicked the effect of ST3GAL1 and ST3GAL2 silencing, inhibiting melanoma cell proliferation. We found that both CD98hc protein stability and its pro-survival effect in melanoma are dependent upon alpha--2,3 sialylation mediated by ST3GAL1 and ST3GAL2. In summary, our studies reveal that alpha-2,3-sialosides functionally contribute to melanoma maintenance, supporting ST3GAL1 and ST3GAL2 as novel therapeutic targets in these tumors.

**LB014**
**Targeting Stearoyl-coA desaturase (SCD) as a therapeutic strategy in *STK11/KEAP1* co-mutant non-small cell lung cancer.**
Utsav Sen, Triparna Sen. Icahn School of Medicine at Mount Sinai, New York, NY.

**Introduction:** Non-small cell lung cancer (NSCLC) is a highly prevalent subtype of lung cancer. Recent findings in the field have improved the clinical outcomes for only a subset of patients who are responsive to immunotherapy or have targetable oncogenic drivers. We have previously showed that concomitant loss of Serine/Threonine Kinase 11 (*STK11*) and Kelch-like ECH Associated Protein1 (*KEAP1*) detected in up to 10% NSCLC cases, dramatically enhances cell proliferation and invasion *in vitro* and *in vivo*. Bulk RNA sequencing identified upregulation of genes involved in ferroptosis, which is an iron dependent form of programmed cell death, in *STK11/KEAP1* double mutant models, nominating ferroptosis as a potential vulnerability in these tumors. However, the mechanism of ferroptosis evasion in this subset in not well understood.
**Methods:** We performed an *in vitro* CRISPR screen in *STK11/KEAP1* co-mutant, single mutant and wild type cell lines. To further characterize the role of ferroptosis regulators in *STK11/KEAP1* co-mutant

setting, we performed phospho-kinase arrays and RNA sequencing in *STK11/KEAP1* cell lines followed by validation through western blotting. Additionally, we performed gene expression analysis in patient derived xenografts (PDX) models treated with the SCD inhibitor to further characterize the mechanisms by which SCD inhibition has synthetic lethal effects in *STK11/KEAP1* co-mutant background.
**Results:** CRISPR/Cas9 based genetic screening identified stearoyl-CoA desaturase (SCD), a gene involved in ferroptosis protection, as a potential therapeutic target in the *STK11/KEAP1* double mutant tumors. We further demonstrate that SCD overexpression protects *STK11/KEAP1* co-mutant NSCLCs from undergoing ferroptosis. Pharmacological inhibition of SCD significantly reduced viability of STK11/KEAP1 co-mutant NSCLCs and made the co-mutant cells sensitive to ferroptosis induction. Phospho-kinase array showed downregulation of JAK-STAT and AKT signaling, in *STK11/KEAP1* co-mutant NSCLCs as compared to both *STK11* and *KEAP1* single mutant isogenic conditions. The downregulation of these pathways was confirmed by gene expression profiling. To further understand the role of SCD in regulating ferroptosis we are studying the effect of concurrent loss of STK11 and KEAP1 on SCD on lipid oxidizing capacity and ferroptosis in a comprehensive panel of NSCLC cell lines and testing whether ferroptosis protection due to SCD overexpression leads to enhanced tumorigenesis in this co-mutant subtype of NSCLC *in vivo* and *in vitro*.
**Conclusions:** Our study demonstrates the biological differences between *STK11/KEAP1* co-mutant NSCLC as compared to the single mutants or wildtype counterparts in a KRAS mutation agnostic manner. In this study, we further establish SCD as a potential therapeutic strategy in *STK11/KEAP1* co-mutant NSCLCs. We also show that SCD-mediated ferroptosis evasion is linked to multiple oncogenic signaling pathways which could be associated with ferroptosis. In summary we define a new therapeutic approach to *STK11/KEAP1* co-mutant NSCLC to improve survival outcomes for patients diagnosed with this devastating disease.

**LB015**

**Bi-steric mTORC1-selective inhibitors activate 4EBP1, suppress MYC, restore anti-tumor immunity, and cooperate with immune checkpoint inhibition to elicit tumor regression.**

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The mammalian target of rapamycin (mTOR) pathway is frequently involved in the pathogenesis of human tumorigenesis. mTOR forms two protein complexes: mTORC1 which responds to growth and stress factors resulting in the phosphorylation of 4EBP1 and S6K for their deactivation and activation, respectively; and mTORC2 which responds to growth factors resulting in pAKT activation. Through 4EBP1 and S6K, the mTORC1 complex regulates translation of oncogenes including MYC.
The *MYC* oncogene is causally involved in the pathogenesis of many human cancers but remains as a challenging therapeutic target. Experimentally, inhibiting *MYC* results in tumor regression associated with immune reactivation. Several lines of evidence suggest that inhibition of the mTORC1 pathway may result in synthetic lethality of MYC-driven cancers. However, to date, existing mTOR pathway inhibitors such as everolimus or sapanisertib fail to sufficiently decrease MYC protein levels *in vivo* because of incomplete inhibition of p4EBP1 or toxicities that limit the activity, respectively. Revolution Medicines has developed third-generation bi-steric selective mTORC1 inhibitors with a rapamycin-like core moiety covalently linked to an mTOR active-site inhibitor. RMC-5552, a representative of the bi-steric class of selective mTORC1 inhibitor, is currently in Phase 1 clinical trials (NCT04774952). Here, we show this new class of inhibitors suppresses both S6K and 4EBP1 phosphorylation and depletes MYC protein expression *in vivo* in an autochthonous conditional transgenic mouse model of *MYC*-driven HCC and across several human patient-derived xenograft models with MYC amplification. Furthermore, bi-steric

mTORC1-selective inhibitors restore anti-tumor immune surveillance and synergize with α-PD-1 immune checkpoint therapy in the *MYC*-driven HCC mouse model. Overall, we provide proof-of-principle that selective mTORC1 pharmacological inhibition can effectively target *MYC*-driven cancers by reducing MYC levels, combine with immune checkpoint inhibition, re-establish anti-tumor immunity, and induce sustained tumor regression in the preclinical setting. These preclinical findings provide a rationale for clinical trials with selective mTORC1 inhibitors in patients with MYC-driven cancers and moreover, support clinical testing of combination with immune checkpoint inhibition.

**LB016**

**Immunotherapeutic potential of ST316, a peptide antagonist of β-catenin.**

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The transcription factor β-catenin is a key player in many cellular processes, including stem cell renewal, cellular homeostasis and inflammation. Deregulation of β-catenin occurs frequently in several cancer types by mutation or overexpression of the β-catenin gene or mutation of negative regulators such as Adenomatous Polyposis Coli (APC). ST316 is a novel peptide antagonist that specifically interferes with the association of β-catenin with its co-activator BCL9, disrupting β-catenin nuclear localization and attenuating target gene expression. In an orthotopic triple-negative breast cancer (TNBC) model, ST316 demonstrates potent anti-tumor activity, resulting in 84% tumor growth inhibition (TGI) ($p<0.001$ vs. vehicle control). In addition to its cell-autonomous mechanisms, deregulated Wnt/β-catenin signaling can promote tumorigenesis by impacting the tumor microenvironment. Reprogramming of immunosuppressive M2-like Tumor Associated Macrophages (TAMs) toward an immune-promoting program (M1-like) is an attractive cancer immunotherapeutic strategy. Here we explored ST316 potential for macrophage repolarization toward the M1-like phenotype, activation of cytotoxic T-cells in macrophage/T-cell co-culture assays and cooperation of ST316 with anti-PD-1 to enhance anti-tumor activity in vivo. Initial studies demonstrate that human macrophages derived from Peripheral Blood Mononuclear Cells (hPBMCs) and subsequently committed to the M2-like identity are reprogrammed toward an M1-like phenotype upon ST316 exposure. ST316 treatment dose-dependently suppressed expression of the M2 marker CD163 by flow cytometry and quantitative PCR, resulting in 100-fold increase in the M1/M2 ratio without substantial impact on cell viability. Importantly, T-cell viability and activation markers associated with M1-state (CD80, CD86) were not affected by ST316 at the concentrations used in these studies. Further, in co-cultures of M2 macrophage with T cells, ST316 exposure resulted in a three-fold increase in T-cell activation compared to control M2/T cell co-cultures, as measured by intracellular IFN-γ staining. Finally, in an orthotopic TNBC model in vivo, subtherapeutic ST316 enhanced the anti-tumor activity of anti-PD-1 [85% TGI with combination, compared to 51% TGI with anti-PD1 alone ($p<0.01$) and -9% TGI with subtherapeutic ST316 alone ($p<0.001$)]. Anti-tumor activity was accompanied by an increase the M1/M2 ratio. Overall, these results support the immuno-therapeutic potential of ST316 and extend the application range of ST316 to include Wnt-driven cancers with poor clinical response to immune checkpoint blockade and other immunotherapeutic agents.

**LB017**

**Potential role of kinesin family member C1 (KIFC1) in melanoma.**

Davis S. Mau, Mary A. Ndiaye, Gabriella R. Zaemisch, Chandra K. Singh, Jess Vera, Sean R. McIlwain, Nihal Ahmad. University of Wisconsin - Madison, Madison, WI.

According to the Surveillance, Epidemiology, and End Results (SEER) analyses, based on 2017-2019 data, approximately ~2.1 percent of men and women will be diagnosed with melanoma at some point

during their lifetime. Melanoma can be fatal if not surgically removed at an early stage, with the 5-year survival being a dismal 29.8% for a stage IV melanoma. Melanoma mortality is primarily due to the distant organ metastases and its resistance to treatment, even with newly developed immunotherapy and/or targeted therapies (BRAF and MEK inhibitors). Thus, the discovery of novel melanoma targets is crucial as they may be able to provide alternative treatment options or be used alone or in combination with current therapies. KIFC1 (kinesin family member C1; also known as HSET, an ortholog in *Drosophila melanogaster*), a nonessential kinesin motor protein, has an established role in centrosome clustering which is utilized by cancer cells to evade apoptosis and mitotic arrest. This occurs when replicating cells harbor additional centrosomes, a hallmark of cancer. The requirement of KIFC1 in mitosis of cancer cells harboring abnormal centrosomes, but not that of normal cells, makes it a potential drug target for cancer management. The goal of this study was to determine the role and significance of KIFC1 in melanoma. Differential expression analysis of GTEX and TCGA RNA-seq data identified significantly higher levels of KIFC1 in both primary and metastatic melanoma as compared to normal skin. In addition, higher expression of KIFC1 was found to be associated with poorer overall survival of melanoma patients. Furthermore, a quantitative protein estimation with the automated ProteinSimple Jess capillary western blotting system demonstrated higher KIFC1 expression in several melanoma cell lines when compared to normal melanocytes. In addition, we also found significantly increased expression of KIFC1 mRNA in mouse melanoma tumors obtained from the genetically engineered $Braf^{V600E}/Pten^{NULL}$ mice, as compared to the normal skin. Next, we determined the effect of a small molecule KIFC1 inhibitor, AZ82 on A375, G361 and Hs294T melanoma cells. AZ82 is an ATP-competitive inhibitor that binds the KIFC1-microtubule complex, blocking the release of ADP and negating its role as a motor protein. Employing RealTime-Glo MT cell viability assay as well as the endpoint XTT growth assay, we found that AZ82 treatment resulted in a significant growth inhibition of human melanoma cells at 2-3.5 μM concentrations. Additionally, AZ82 treatments resulted in a significant decrease in clonogenic survival in all three human melanoma cell lines. Collectively, our data suggests a potential role of KIFC1 in melanoma progression, and merits further investigation to determine the roles and functional and therapeutic significance of KIFC1 in melanoma.

**LB018**

**Therapeutic efficacy of inhibition of specific sirtuins against melanoma in $Braf^{V600E}/Pten^{NULL}$ and PDX models.**

Karla Anaya Aldrete, Gabriella R. Zaemisch, Mary A. Ndiaye, Gagan Chhabra, Nihal Ahmad. University of Wisconsin-Madison, Madison, WI.

Melanoma is a highly immunogenic malignancy and one of the deadliest skin cancers due to its ability to metastasize and its resistance to existing therapies. Current treatment regimens include immunotherapies as well as targeted therapies (e.g. BRAF and MEK inhibitors). However, these immuno- or targeted therapies have been associated with acquired resistance and, therefore, failure of therapy. Therefore, development of novel mechanistically-targeted therapies is of paramount importance for an efficient management of this neoplasm. Sirtuins, a seven-member family of class III histone deacetylases, have been implicated in the development/progression of multiple cancers, including melanoma. Previous studies from our laboratory and elsewhere have shown the pro-proliferative functions of sirtuins SIRT1 and SIRT3 in melanoma. We have also shown that the small molecule inhibitor 4'-bromoresveratrol (4'-BR; co-inhibitor of SIRT1 and SIRT3) has antiproliferative effects in melanoma cell lines as well as in a $Braf^{V600E}/Pten^{NULL}$ mouse model of melanoma. This study was designed to further expand on our previous findings and to determine the therapeutic significance of SIRT1 and SIRT3 in melanoma. To determine the effect of specific inhibition of SIRT3 on melanoma progression, we treated $Braf^{V600E}/Pten^{NULL}$ mice with 100 μg small interfering RNA (siRNA) against SIRT3 (siSIRT3) or its non-targeting control (siNTC) twice weekly (intraperitoneal injections for 5 weeks). We found a trend of decreased tumor volumes in the siSIRT3-treated mice, though not significant. A similar trend was seen

in the final tumor weight of these mice. In the next series of experiments, we used two human melanoma patient-derived xenografts (PDXes) to compare the effect of 4'-BR or siSIRT3 on melanoma tumor growth. Similar to our published study in the *Braf*$^{V600E}$*/Pten*$^{NULL}$ mice, we found that 4'-BR treatment (50 mg/kg, via intraperitoneal injection, twice weekly for 5 weeks) resulted in a significant decrease in tumor growth when compared to vehicle control mice. Additionally, the final tumor weight was significantly decreased in the 4'-BR group. However, the siSIRT3 treatments resulted in a decrease but non-significant decrease in melanoma tumor growth or final weights, supporting our observations in *Braf*$^{V600E}$*/Pten*$^{NULL}$ mice. Considering the lung is a common site for metastasis in melanoma, metastatic nodules in the lungs were quantified in one of our PDX models. We observed a decreased number in both the siSIRT3 and 4'-BR groups vs their respective controls. Overall, our data suggests that targeting SIRT3 alone may not be sufficient for melanoma treatment, and that the concomitant inhibition of SIRT1 and SIRT3 (and potential other sirtuins with pro-proliferative functions in melanocytic cells) may be better for melanoma management. Indeed, additional research is needed to validate our findings in relevant models.

**LB019**

**DUSP4 overexpression exerts anti-tumor effects *in vivo* in pancreatic cancer xenografts in nu/nu mice.**

Chandra K. Singh, Mary A. Ndiaye, Gagan Chhabra, Minakshi Nihal, Nihal Ahmad. University of Wisconsin-Madison, Madison, WI.

Pancreatic cancer (PC) remains one of the most lethal of all human malignancies, being highly aggressive and difficult to treat if diagnosed at late stages. Therefore, it is important to dissect molecular mechanisms implicated in PC pathophysiology. Dual specificity phosphatase-4 (DUSP4) is a phosphatase known to negatively regulate the mitogen-activated protein (MAP) kinase family (MAPK/ERK, SAPK/JNK, P38), which are involved in multiple cancers, including PC. Previously, using tissue microarray containing a variety of pancreatic cancers and normal pancreatic tissues, we found that DUSP4 was significantly downregulated in human PC. We also demonstrated that forced overexpression of DUSP4 resulted in anti-proliferative responses in MIA PaCa-2 human PC cells (*Cancer Res 2016; 76 (14_Supplement): 3667*). Here, we expanded our study to determine, i) the effects of DUSP4 overexpression on tumorigenicity *in vivo*, and ii) the molecular mechanisms associated with DUSP4 overexpression-mediated anti-proliferative responses. MIA PaCa-2 cells were stably transfected with DUSP4 overexpression (OE) plasmid or its empty vector (pCMV6) control, followed by subcutaneous implantation into nu/nu mice (n=8 each group). Tumor growth was measured weekly for 6 weeks followed by excision of tumors and molecular analyses. We found significant reduction in tumor volume and weight in DUSP4 OE group. Further, we found marked reduction in p-ERK1/2 and p-P38 proteins in tumors from DUSP4 OE group. To determine the global mechanisms of DUSP4, we analyzed DUSP4-overexpressing MIA PaCa-2 cell lysates using quantitative global proteomics. Overall, 1678 proteins were identified with ≥2 unique peptides. Out of these, 54 proteins were significantly modulated ≥1.6-fold. Using Ingenuity Pathway Analysis (IPA), we found the association of modulated proteins with increased apoptosis and inhibited invasive and malignant tumors. The top five modulated proteins identified in response to DUSP4 OE were ADD3, PDLIM1, COL2A1, SLC20A1 and CALB2, with the first three being upregulated and the remaining two downregulated. Based on published studies in other cancers, these data support the tumor suppressor role of DUSP4 in PC since i) the loss of ADD3 and PDLIM1 (both cytoskeleton-related proteins) have been found to promote tumor growth, ii) higher expression of COL2A1 has been associated with delayed tumor recurrence, iii) higher expression of SLC20A1 (sodium-phosphate symporter) and CALB2 (calcium-binding protein) are known to be associated with cancer progression. The specific roles of these proteins in PC are not well known, providing us novel opportunities for future investigations. Overall, our data support the tumor suppressor role of DUSP4 in PC and warrants further research to validate our findings.

**LB021**

**KN-052, a novel PDL1/OX40 bispecific antibody, exhibits potent antitumor efficacy.**

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KN052 is a recombinant PDL1/OX40 bispecific antibody which can block PDL1 and PD1/CD80 pathway and active the OX40 signal pathway at the same time. Inhibited PDL1 can block cancer cells to evade T-cell-mediated immune responses and restores T-cell activation and antitumor responses. On the other hand, biding to OX40 which normally expressed on Treg cells can induce ADCC effect and kill the Treg cells. There is a synergistic effect between the blocking of PDL1 pathway and the activation of OX40 signal pathway in cell base assay. PBMCs from four healthy donors showed significant T cell activation effect under the stimulation of superantigen SEB, and the activity of PBMCs from three healthy donors was significantly stronger than that of two single target control antibodies alone and in combination. The anti-tumor activity of KN052 was evaluated by using hPD-L1/hOX40 humanized mouse with MC38 MCA205 syngeneic model. Significant anti-tumor effects were observed in both models in a dose-dependent manner. The HNSTD (highest non-severely toxic dose) of KN052 was determined as 30mg/kg in cynomolgus monkeys. These preclinical data demonstrated acceptable PK and safety profile of KN052 and indicated its potential in a variety of tumors.

**LB022**

**A novel first-in-class USP19 inhibitor for the treatment of cancer-induced muscle atrophy.**

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Cancer cachexia is a metabolic wasting syndrome characterized by weight loss, anorexia and anemia as a result of tumor burden, and affects up to 80% of advanced cancer patients [#1]. Cachexia is particularly prevalent in pancreatic, lung, colorectal and gastro-intestinal cancers and can lead to reduced tolerance and responsiveness to chemotherapy, increased treatment-related toxicity and morbidity, and poor overall quality of life. There are currently no approved therapies for cancer cachexia.

The development and maintenance of muscle tissue is dependent on the balance between protein synthesis and protein degradation, controlled through various anabolic and catabolic signaling pathways. Dysregulation of these pathways can result in muscle atrophy, which arises in many chronic illnesses. The ubiquitin proteasome system (UPS) has a central role in regulating skeletal muscle physiology. Previous work utilizing USP19 knock out mouse models has demonstrated that USP19 plays an important role in muscle wasting and can protect against denervation-induced muscle atrophy [#2].

We have previously demonstrated that inhibition of USP19 enzymatic activity spares the muscle wasting observed in limb-casted and denervated mouse models of muscle wasting. Here, we report the discovery of a novel, highly potent and selective inhibitor of USP19 (ADC-846) and demonstrate its utility in a cancer-induced muscle atrophy model *in vivo*. Pharmacological inhibition of USP19 by ADC-846 increased lean muscle and fat mass following oral dosing in a Lewis Lung Carcinoma-induced cachexia model and reduced the cachexic index by >60% compared to controls. This data, in combination with our previous work detailing the effect of USP19 inhibition on muscle force and function, provides a much-needed novel pharmacological strategy for therapeutic intervention in muscle wasting conditions.

**LB023**

**Discovery, development and characterization of potent and selective USP11 inhibitors.**

Forum Kayastha, Bandish Kapadia, Noah B. Herrington, Anirban Roychowdhury, Glen E. Kellogg, Ronald B. Gartenhaus. Virginia Commonwealth University, Richmond, VA.

Ubiquitin-mediated events are emerging as the gatekeeper in cell proliferation, and the recognition of deubiquitinating enzymes (DUBs) as the critical regulators of these processes is rapidly growing. Several studies in the last decade confirmed that the USP11 (Ubiquitin-Specific proteases 11) had promoted tumor growth and metastasis. Indeed, pan-cancer data analysis reveals enhanced expression of USP11 in numerous malignancies, including lymphoma. Our lab has established that USP11 deubiquitinates and stabilizes the translation initiation factor eIF4B (Eukaryotic Initiation Factor 4B) to promote eIF4B-dependent oncogenic translation and to facilitate the proliferation of diffuse large B-cell lymphoma (DLBCL). Brody and colleagues screened 2000 FDA- approved compounds and reported that the anti-neoplastic agent mitoxantrone is an effective non-specific inhibitor of USP11, which was later observed to target USP15. Thus, catalytically targeting USP11 provides substantial challenges as it encompasses high homology with the functional orthologs USP4 and USP15. Addressing this major limitation, Spiliotopoulos et al. identified a unique USP11-specific allosteric site without impeding USP15 and USP4 activities. Utilizing this platform, we performed a virtual screening of more than 10 million compounds from publicly available databases and identified 307 potential unique hits. The chemical library of these hits was assessed using an in-house optimized in-cell high throughput screening assay. The primary screening revealed that 16 of the hit compounds with $EC_{50}$ less than 10μM. The two most potent, structurally different, USP11 inhibitors, RBF4 and RBF11, were further evaluated. *In-vitro* deubiquitinase activity assay of USP11 showed minimal inhibition on treatment with compounds (as we are targeting an allosteric site), which indicates that RBF4 and 11 do not bind to the catalytic domain. Thermal shift assays revealed that RBF4 preferentially stabilizes USP11 compared to other functional paralogues USP4 and USP15, which validates the selective USP11-RBF4 interaction. Further, treatment with RBF4 and 11 hampered DLBCL proliferation in a dose-dependent manner. Significantly, treatment with selected compounds depleted USP11-dependent oncogenic expression and, thus, the colony-forming capacity of DLBCL. These results show that these novel compounds can serve as an ideal tool to increase our understanding of USP11 biology and may emerge as an example of potent and selective USP11 inhibitors. Currently, we are evaluating their anti-cancer potency in pre-clinical models.

**LB024**

**Pharmacokinetics of a novel viscoelastic suspension of trastuzumab biosimilar for high-concentration, low-volume subcutaneous injection.**

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Background: Xeris Pharmaceuticals (Chicago, IL, USA) has developed the proprietary XeriJect® technology, which uses a novel viscoelastic suspension for delivery of high-concentration, low-volume subcutaneous (SC) injections of therapeutic antibodies. Trastuzumab (TmAb) is a monoclonal antibody that selectively binds and inhibits human epidermal growth factor receptor 2 protein (HER2). It is widely used for the treatment of certain types of breast, stomach, and esophageal cancer, and is commercially available for both intravenous (IV) infusion (Herceptin®, Genentech Inc., South San Francisco, CA, USA) [Herceptin IV] and SC injection (Herceptin Hylecta®, Genentech Inc., South San Francisco, CA, USA) [Hylecta SC]. XeriJect TmAb is a stable formulation of a trastuzumab biosimilar at a higher concentration (432 mg/mL) than Hylecta (120 mg/mL) for SC administration, thus enabling lower injection volumes. This study evaluated and compared XeriJect TmAb SC (120 mg) to Herceptin IV (10 mg/kg) and Hylecta SC (120 mg) in a minipig pharmacokinetic model.

Results: Among a total of 12 minipigs, Herceptin was administered IV at 10 mg/kg over a 90-minute infusion and all other formulations were administered at a target dose of 120 mg SC. Due to the higher concentration of XeriJect TmAb, the injection volume of Xeriject TmAb (0.28 mL) was considerably lower than Hylecta (1 mL). XeriJect TmAb was rapidly absorbed similar to Hylecta after SC administration with a median $T_{max}$ of 1.0 days with both formulations and a mean $C_{max}$/Dose of 9.7 and 9.5 kg*µg/mL/mg, respectively. In contrast, Herceptin IV produced a median $T_{max}$ of 0.06 days and $C_{max}$/Dose of 18.0 kg*µg/mL/mg. All formulations (Herceptin IV, Hylecta SC, XeriJect TmAb SC) demonstrated similar elimination profiles and exposure as assessed by $AUC_{last}$/Dose. The mean [SD] $AUC_{last}$/Dose of XeriJect TmAb (108 [4.2] day*kg*µg/mL/mg) was similar to Hylecta SC (109 [22.4] day*kg*µg/mL/mg) and Herceptin IV (97 [7.5] day*kg*µg/mL/mg). The mean [SD] elimination half-life of XeriJect® TmAb SC (11.4 [10.1] days) was also similar to Hylecta® SC (11.3 [4.8] days) and longer than with Herceptin® IV (8.8 [2.1] days).
Conclusion: We have demonstrated for the first time that administration of XeriJect TmAb, a stable high-concentration formulation of trastuzumab administered SC, produced rapid absorption and similar pharmacokinetics to commercially available Herceptin IV and Hylecta SC in a preclinical pharmacokinetic model.

**LB025**

**First disclosure of AZD5335, a TOP1i-ADC targeting low and high FRα-expressing ovarian cancer with superior preclinical activity vs FRα-MTI ADC.**

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Folate receptor alpha (FRα) is a cell surface GPI-anchored protein overexpressed in several solid tumors with highest prevalence in ovarian cancer and lung adenocarcinoma but restricted expression in normal tissues. An antibody drug conjugate (ADC) with a microtubule inhibitor (MTI) payload recently received accelerated approval from the FDA for FRα-expressing platinum-resistant ovarian cancer. We describe for the first time the preclinical activity of AZD5335, an FRα-targeting antibody conjugated to AZ's proprietary topoisomerase 1 inhibitor (TOP1i) payload, AZ14170132, with a homogeneous drug-to-antibody ratio of 8 (DAR8) and potential benefits vs an MTI-based ADC. AZD5335's primary mechanism of action is to deliver TOP1i payload into FRα-expressing cancer cells, leading to DNA damage and apoptotic cell death. The TOP1i payload mediates bystander killing, which is important for targeting tumors with less than uniformly positive expression. Here, we report that a single dose of AZD5335 at 2.5 mg/kg was sufficient to provide a robust and durable anti-tumor response in FRα-expressing ovarian cancer cell line xenografts (CDX) with a tumor growth inhibition (TGI) of 75%-94% and median best tumor volume reduction >30% in 14/17 (82%) ovarian cancer patient-derived xenografts (PDX) evaluated. FRα-expression levels (by IHC and deep-learning based image analysis) correlated with efficacy in the tested PDX models, and we observed that AZD5335 was also active in models with low levels of target expression (75% of cells with FRα staining of 2+), expected to be representative of patients who would be ineligible for treatment with the MTI-ADC. Furthermore, AZD5335 demonstrated superior activity vs an FRα-MTI benchmark ADC with respect to anti-tumor activity and duration of response in two PDX models with low-to-medium FRα expression at equal or higher drug doses (e.g., in OV0857-CIS: 96% TGI vs 24% TGI at 5 mg/kg and 95% TGI vs 2% TGI at 2.5 mg/kg of a single IV dose AZD5335 and FRα-MTI, respectively). These data indicate that AZD5335 is a promising therapeutic candidate for the treatment of ovarian cancers across the spectrum of FRα-expression.

**LB026**
**Porcine brain-derived hydrogel carriers for intratumoral administration of cellular immunotherapies enhances anti-tumor potential in post-resection GBM.**
Meghan Logun, Zev Binder, Donald O'Rourke. University of Pennsylvania, Philadelphia, PA.

Glioblastoma (GBM) has shown remarkable resistance to peripherally administered CAR T cell therapy. This is in part because the nuanced requirements for successful CAR-T cell trafficking into brain tumors are not well understood. Intracranial administration has been shown to be both safe and effective in delivery of other anti- cancer drugs in GBM, with one example being Gliadel, involving the placement of carmustine chemotherapy-loaded wafers into the post-resection space for sustained release into local brain tissue. Recurrence of GBM is most often local, suggesting that regional therapies might both serve the immediate and long-term needs of the patients. Here we used extracellular matrix-based scaffolds to encapsulate and locally deliver CAR T cells to GBM for enhancement of CAR T cell infiltration and promotion of effector function against GBM. We decellularized and solubilized commercially procured fresh frozen porcine brain tissues to create temperature-responsive porcine brain matrix solutions that gelled at physiologic body temperatures. We confirmed extent of decellularization using DNA quantification of processed tissue and quantified major protein composition of solubilized product using mass spectrometry. Gelation kinetics and resulting hydrogel structures were quantified using turbidometry and rheology, with an average gelation time of 15$\pm$ 8 min and comparable storage modulus range of 100-200 Pa to Matrigel or Collagen 1-based commercial hydrogel products. Both unmodified healthy donor T cells and donor T cells transduced with EGFRvIII-targeting CAR scFv were >75% viable over 4d *in vitro*, and release of doses ranging from 2M-5M CAR T cells after encapsulation were observed over 4d *in vitro* using Transwell inserts. Released CAR T cells were found to be potently cytotoxic in tumor-killing assays using EGFRvIII+ U87MG target cells at 1:1 E:T within impedance cytotoxicity assays, performing comparably to freshly made non-encapsulated CAR T cells and better than CAR T cells released from Matrigel or Collagen scaffolds at the same timepoints. We have developed a mouse model of GBM resection and recurrence to evaluate the efficacy of hydrogel-delivered CAR T cell treatment after resection surgery to capitalize on a currently underutilized treatment window. This injectable scaffold will support an immediate clinical need by supporting delivery, expansion, and actuation of CAR T function within the solid tumor microenvironment without the challenges of peripheral delivery to a specialized organ system.

**LB027**
**In vivo delivery of novel CD89 fusion receptor to myeloid cells by mRNA activates anti-tumor immunity.**
Thomas E. Prod'homme, Shannon Argueta, Hongyun Zhao, Michael Gorgievski, Neha Diwangi, Edward Cochran, Bruce McCreedy, Yuxiao Wang, Daniel Getts. Myeloid Therapeutics, Cambridge, MA.

Despite the revolution provided by immunotherapy, sustained clinical benefit has yet to be achieved for most of patients with advanced solid tumors. Myeloid cells readily accumulate in tumors, in some cases contributing up to 75% of the tumor mass. While supporting tumor growth and being associated with unfavorable prognosis, myeloid cells can mediate phagocytosis of cancer cells and cytotoxic tumor killing and promote adaptive immune responses through antigen presentation and co-stimulation, therefore representing promising targets in cancer therapy. The ability to engineer circulating and tumor associated myeloid cells to activate their ability to elicit anti-tumor adaptive immunity is an attractive approach to harness systemic anti-tumor immunity. However, it remains challenging to specifically target and activate myeloid cells *in vivo*. To overcome this hurdle, we have developed a novel *in vivo* myeloid cell engineering platform in which a chimeric antigen receptor (CAR) is generated by fusing a tumor

recognition scFv with the alpha chain of human Fc receptors (CD89). The stable expression and function of these receptors requires the endogenously expressed common Fc receptor gamma chain (FcRγ), which expression is mostly restricted to myeloid cells. For *in vivo* engineering, the construct is encapsulated and delivered in lipid nanoparticles (LNP). Trophoblast cell surface antigen 2 (TROP2) is overexpressed in most human solid epithelial cancers, as compared to low expression in corresponding normal tissue. Increased TROP2 expression has been linked to increased tumor growth and has been implicated as a prognostic marker in these cancers, supporting the development of therapies targeting TROP2. Intravenous infusion of LNP encapsulating mRNA encoding the anti-TROP2-CD89 fusion protein results in the uptake of the LNPs and expression of the chimeric receptor fusion protein in myeloid cells. In immunodeficient xenograft models of hepatocellular carcinoma and triple negative breast cancer, delivery of LNP mRNA encoding GPC3-CD89 or TROP2-CD89 fusion proteins resulted in anti-tumor efficacy, confirming the ability of this approach to program myeloid cells. Repeat dosing studies showed significant anti-tumor efficacy following bi-weekly administration of TROP2-CD89. Furthermore, in the B16/10 syngeneic melanoma model, treatment with the melanoma antigen gp75-CD89 fusion protein was also associated with the initiation of broad systemic immune responses, characterized by tumor infiltration by activated $CD8^+$ T cells, reduced tumor-associated Tregs and activation of antigen presenting cells in spleen. When infused in cynomolgus monkeys, TROP2-CD89 LNP led to cell surface expression of anti-TROP2 CAR in myeloid cells. Together these studies highlight the potential of in vivo delivery of CD89 fusion proteins to program myeloid cells to recognize and kill cancer.

**LB028**

**Reprogramming of tumor-infiltrating immune cells using a tumor-associated macrophage-targeted TLR7 agonist.**

John Victor Napoleon, Fenghua Zhang, Rami A. Alfar, Taylor A. Wolt, Philip S. Low. Purdue University, West Lafayette, IN.

Myeloid derived suppressor cells (MDSCs) and tumor associated macrophages (TAMs) are two of the more immunosuppressive cell types in a tumor microenvironment (TME). Although TAMs and MDSCs can be converted from tumor-supporting to tumor-suppressing phenotypes by treatment with proinflammatory immune stimulants (e.g. TLR7 agonists) in vitro, such strategies have proven to be too toxic in vivo because of their systemic activation of immune cells. Therefore, for such immune stimulants to be used to reprogram the immune system in solid tumors, they must be targeted specifically to the immune cells in the TME.

One strategy for targeting TAMs/MDSCs is to exploit a receptor that is expressed solely on these cell types. Because folate receptor beta (FRβ) is expressed exclusively on myeloid cells and since FRβ acquires its ability to bind folic acid (FA) only in inflamed tissues, we have previously exploited FA to target attached TLR7 agonists (TLR7a) to FRβ+ TAMs/MDSCs in solid tumors. While significant repolarization of TAMs and MDSCs was observed in this previous study, premature systemic release of the attached TLR7a caused unwanted toxicity. However, since FRβ was later found to reside in the same endosome as TLR7, we hypothesized that constructing a more stable FA-TLR7a conjugate with a non-cleavable linker should not only prevent premature TLR7a release, but also deposit the FA-TLR7a into an endosome containing a TLR7 receptor, thereby enabling selective activation of TAMs and MDSCs in the TME. The data summarized here confirm this hypothesis. Thus, free TLR7-1A was found to stimulate massive production of IL-6, TNF-α and IFNγ upon addition to human peripheral blood in vitro or injected into live mice, suggesting cells in both species respond aggressively to TLR7-1A. However, performance of the same studies with FA-targeted TLR7-1A stimulated barely or nondetectable levels of the inflammatory cytokines, confirming that FA-TLR7-1A is unable to enter and activate cells that lack a folate receptor (FRβ). More importantly, intravenous administration of FA-TLR7-1A into mice bearing an orthotopic 4T1 breast tumor totally blocked metastases and induced tumor regression without causing detectable toxicity. Flow cytometric analyses of the residual tumor tissue further demonstrated that FA-

TLR7-1A increased M1/M2 macrophage ratios and elevated infiltration of CD8+ T cells into the tumor masses. Taken together, these data demonstrate that a targeted TLR7 agonist can rejuvenate immune cells in a solid tumor without systemically activating the immune system.

**LB029**

**AWT030: a first-in-class bi-functional IL-21 fusion protein selectively activates tumor infiltrated CD8 T cells and suppresses Treg cells.**

Fan Ye, Jianing Huang, Sandra Chen, Ella Li, Jenny Jiang, Hanna Lin, Michael Hua, Danny Huang, Zoey Huang, Joyce Kwan, Lili Cheng, Ziyang Zhong. Anwita Biosciences, San Carlos, CA.

The tumor microenvironment (TME) has been identified as a significant obstacle to the success of immunotherapy, including anti-PD-1 therapy. The TME comprises several factors that limit the response rate to immunotherapy, including immunosuppressive cells such as regulatory T cells (Tregs), which directly or indirectly interact with effector cells and undermine the tumor recognition, activation, proliferation, and survival of the effector cells, thereby promoting tumor growth. To overcome suppressive TME, we designed AWT030, a first-in-class bi-functional IL-21 fusion protein that comprises a stability and potency optimized IL-21 mutein and an engineered functional domain that targets T cells. AWT030 is designed to selectively activate tumor-infiltrating CD8 T cells and suppress Tregs while avoiding the potential suppressive effect of IL-21 on dendritic cells.Compared to wild-type IL-21, mAWT030 greatly enhances the tolerability, half-life, and anti-tumor activity. In anti-PD-1 therapy resistant breast cancer EMT6 model, fibrosarcoma MCA205 model, and anti-PD-1 therapy responsive colon cancer MC38 model, mAWT030 achieved 100% complete response without noticeable toxicity, demonstrating a powerful synergy between tumor specific CD8 T cell activation axis and Treg suppression axis induced simultaneously by mAWT030. In addition, mAWT030 also exhibited a strong synergistic effect with anti-PD-1 antibody. Immunophenotyping revealed that AWT030 had minimal impact on circulating lymphocytes while altering the suppressive TME, with a significantly increased CD8 T cell population and suppressed Treg population. In summary, AWT030 is a novel bi-functional IL-21 fusion protein with high tumoral specificity, the excellent safety and antitumor activity of mAWT030 observed preclinically supports clinical development, focusing on treating PD-1 resistant/relapsed patients or combining with anti-PD-1 therapy.

**LB030**

**SHR-A1921, a novel TROP-2 ADC with an optimized design and well-balanced profile between efficacy and safety.**

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Trop-2 is a promising target for ADC therapy due to its high expression in many solid tumors. The approval of Trodelvy, a Trop-2 directed ADC, for the treatment of refractory or drug-resistant triple negative breast cancer (TNBC) demonstrated the therapeutic value of Trop-2-targeted ADC. However, a Boxed Warning for severe or life-threatening neutropenia and severe diarrhea suggests the safety of Trodelvy needs to be improved. Here, we presented a novel Trop2-directed ADC, SHR-A1921, consisting of a topoisomerase I inhibitor (Proprietary payload, SHR9265) conjugated to a proprietary IgG1 mAb via cleavable linkers. SHR-A1921 demonstrated several advantages over other Trop-2 directed ADCs in the field. SHR9265 is a novel exatecan derivative designed by Hengrui with a better liposolubility and cellular permeability. SHR-A1921 had a drug-to-antibody ratio (DAR) of 4. Compared with other Trop-2-targeted ADCs in the field, such as Trodelvy, TINA-SHR7971[1] (a molecule synthesized using the published structure of DS-1062), and SKB264, SHR-A1921 has considerable advantages as follows: (1) Stronger binding affinity to both human and rhesus macaque TROP-2 than TINA-SHR7971[1]; (2)

Improved plasma stability in plasma of different species presumably due to the proper steric hindrance which was purposely designed on the payload for reducing non-intended cleavage; (3) Stronger bystander cell killing effect presumably due to the increased lipophilicity of the payload vs. that of the payload in TINA-SHR7971[1]; (4) Superior *in vivo e*fficacy in a PSCC[2] CDX Model (FaDu) with high Trop-2 expression (TGI 101% vs 53% [TINA-SHR7971[1]] @ 1 mpk) and in an ovarian cancer CDX Model (SK-OV-3) with moderate Trop-2 expression (TGI 63% vs. 23% [TINA-SHR7971[1]] @ 3 mpk; 87% vs. 16% [TINA-SHR7971[1]] @ 10 mpk); (5) ≥ 2X longer half-life in patients# vs. SKB264 vs. IMMU-132, supporting more flexible dosing frequency; (6) Lower free toxin/ADC ratio# regarding PK exposure in patients compared with SKB264 (< 1% vs. 5-6%); (7) approximately linear pharmacokinetics profile in patients with $T_{1/2}$ ranging from 2.5 to 4.5 days. In summary, SHR-A1921 is a novel anti-TROP2-targeted ADC with a high permeable payload and optimized DAR demonstrating great stability and high potency in both *in vitro* and *in vivo* studies. SHR-A1921 also showed compelling efficacy and good safety profile from 50+ subjects of Phase I clinical trial in China (NCT05154604). Pivotal phase III trial for NSCLC is planned in China. (Notes: 1. TINA-SHR7971 is a molecule that Hengrui synthesized using the published structure of DS-1062. 2. PSCC: pharyngeal squamous cell carcinoma. #. non-head-to-head comparison.)

**LB031**

**SHR-A1811, a novel anti-HER2 ADC with superior bystander effect, optimal DAR and favorable safety profiles.**

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Current clinical HER2-targeting ADCs, especially T-DXd, have shown strong efficacy in HER2-expressing/mutant cancers, however, with adverse events such as hematologic toxicities and interstitial lung disease (ILD)/pneumonitis. Here we presented a potential best-in-class HER2-directed ADC, SHR-A1811, which was composed of trastuzumab, a stable and cleavable linker, and a novel topoisomerase I inhibitor payload (SHR9265). SHR9265 was a delicately selected exatecan derivative with a better liposolubility and cellular permeability: SHR9265 had a higher AlogP value than SHR7971, synthesized using published DXd structure (3.67 *vs*. 2.72). Consistently, SHR9265 showed ~5 times higher membrane permeability (PAMPA model) at pH5 and pH7.4. Cell killing activity of SHR9265 was ~3 times more potent than SHR7971. SHR-A1811 had a drug-to-antibody ratio (DAR) of 5.7, and showed HER2-dependent growth inhibition against various breast cancer and gastric cancer cell lines. In the bystander killing system, SHR-A1811 was able to kill both SK-BR-3 (HER2+) and MDA-MB-468 (HER2-) cells when co-cultured, with the $IC_{50}$ on MDA-MB-468 of 0.28 nM. The *in vitro* efficacy of SHR-A1811 was comparable with anti-Her2-SHR9265 (DAR 7.5) and ADC1 (synthesized using T-DXd structure), and stronger than anti-Her2-SHR9265 (DAR 3.5). In SK-BR-3 (HER2 high), JIMT-1 (HER2 moderate) and capan-1 (HER2 low) xenograft models, SHR-A1811 treatment resulted in a dramatic and sustained inhibition of tumor growth. A significantly stronger antitumor activity was observed for SHR-A1811 than ADC1 under the same dosages. Moreover, SHR-A1811 showed good stability and improved safety profiles, presumably due to the proper steric hindrance which was purposely designed on the payload (SHR9265). Less than 2% of payload release was observed in human plasma after a 21-day incubation. The HNSTD of SHR-A1811 in cynomolgus monkeys was 40mpk with thymus as the main target organ. No death and lung lesions were observed at dose levels up to 70mpk for 42 days, while ADC1 led to one male cynomolgus monkey death on day 10 at 70mpk with multiple lung damages. These findings were consistent with safety profiles observed in a multi-center, dose-escalation phase I clinical trial (NCT0444620). Patients with different solid tumor types received initial intravenous doses of SHR-A1811 from 1 to 8.0 mg/kg. Only trace amount of free toxin was detected. The Cmax of payload was 3.85 ng/ml at 8mg/kg of SHR-A1811. The incidence of G2 ILD was < 2.5% and the treatment discontinuation rate was 5.1% across doses. In summary, with a highly permeable payload, optimized DAR, great potency and better clinical safety profiles, SHR-A1811 has demonstrated the best-in-class potential. Currently

SHR-A1811 has entered phase II and phase III clinical studies for breast cancer, gastric cancer, colorectal cancer, and NSCLC (NCT05424835, NCT05482568, NCT04818333, NCT05349409).

**LB032**

**Kinase GRK3 connects angiogenesis and neuroendocrine differentiation in prostate cancer progression by enhancing epigenetic activity of HDAC2.**

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Treatment-related neuroendocrine prostate cancer (t-NEPC, here as NEPC) is an aggressive subset of castration-resistant prostate cancer (CRPC), found in ~20% of lethal CRPC. The mechanisms underlying the progression of prostate cancer to NEPC are largely unclear, and new drug targets are desperately needed. NEPC is known to be highly vascularized. Elevated expression of NE markers and increased angiogenesis are two prominent phenotypes of NEPC, and thus are expected to be linked. However, direct molecular links between these two phenotypes are still elusive, whose elucidation will substantially expand our knowledge in NEPC and enable the development of effective treatments for NEPC. Through RNAi & cDNA screening and functional validations, we previously discovered that GPCR-kinase 3 (GRK3) is essential preferentially for highly metastatic cancer cells as compared to lowly metastatic cancer cells. The mechanisms of GRK3 in prostate cancer progression were mostly unknown.
Our new data indicate that GRK3 is significantly overexpressed in metastatic prostate tumors from patients, especially in NEPC. GRK3 promotes both angiogenesis and neuroendocrine differentiation in prostate cancer cells, indicating that it is a key missing link for these two phenotypes. Mechanistically, GRK3 enhances the epigenetic repressor activity of histone deacetylase 2 (HDAC2) to suppress key repressors of angiogenesis or NE phenotype. Through compound library screening, we have identified several compounds that block kinase activity of GRK3 much more potently than that of GRK2, the closest-related kinase to GRK3. Of note, our GRK3 inhibitors could substantially reduce angiogenesis and NE marker expression, as well as significantly inhibit NEPC cell growth in culture and in mouse xenografts.
In summary, kinase GRK3 connects angiogenesis and neuroendocrine differentiation in prostate cancer progression. Its mechanism of actions is at least in part through enhancing HDAC2's epigenetic activity. Results based on our novel GRK3 inhibitors suggest that GRK3 is a valuable new drug target for aggressive prostate cancer.

**LB034**

**EWSR1, Ewing sarcoma breakpoint region 1, maintains centromere identity by binding to centromeric R-loops.**

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The centromere DNA-kinetochore complex is the specialized chromatin structure that mediates chromosome attachment to microtubules and is required for high-fidelity chromosome transmission. CENP-A is the centromere-specific histone H3 variant that epigenetically determines centromere position on the chromosome to ensure kinetochore assembly. Despite its importance, the precise mechanism of maintenance of CENP-A, i.e., the maintenance of centromere identity, remains obscure. In this study, we found that EWSR1 (Ewing Sarcoma Breakpoint Region 1) is required for CENP-A deposition at centromeres by binding to centromeric RNA. We determined that the CENP-A binding domain of EWSR1 is the SYGQ2 region within the prion-like domain, which plays an important role in phase separation for chromatin remodeling. Our data suggest that EWSR1 guards CENP-A in centromeric chromatins by binding to centromeric RNA. This is a novel mechanism to maintain the position of the

centromere on the chromosome i.e., centromere identify. As EWSR1-FLI1 is the oncogenic fusion protein in Ewing sarcoma and as it inhibits EWSR1 function as a dominant negative mutant, it contributes to chromosome instability in Ewing sarcoma, which may lead to therapy resistance and metastasis.

**LB035**

**The role of nucleophosmin1 mediated caspase2 activation in acute myeloid leukemia cell death and cell survival.**

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Nucleophosmin 1 (NPM1) mutation is the primary genetic lesion in adult acute myeloid leukemia (AML). Typically, a nucleolar protein, NPM1 mutations cause cytoplasmic delocalization of NPM1(*NPM1c+*), triggering leukemogenesis. Interestingly, *NPM1c+* mutation confers enhanced chemosensitivity. However, the mechanism underlying *NPM1c+* mutation-induced oncogenesis or chemosensitivity remains unclear. We previously reported that, in response to genotoxic stimuli, NPM1 forms a platform for caspase-2 activation in the nucleolus. Caspase-2 is a pro-apoptotic protein and a known tumor suppressor. Our results indicate that nucleolar caspase-2 activation is specific to *NPM1wt* cells. In the *NPM1c+* cells, caspase-2 activation is exclusively cytosolic, suggesting that caspase-2 is activated in the same sub-cellular compartment as NPM1 localization. Loss of caspase-2 rescued the increased sensitivity of *NPM1c+* cells to apoptotic cell death. This is accompanied by reduced cleavage of the apoptotic substrate, caspase-3. Notably, caspase-2 deficient *NPM1wt* cells showed no significant difference in the percentage of apoptotic cell death or endogenous substrate cleavage compared to its caspase-2 wild-type counterpart. This suggests that caspase-2 may contribute to the chemosensitivity of *NPM1c+* cells. Strikingly, in unstimulated cells, loss of caspase-2 leads to prolonged G1 arrest and progressive loss of cell viability. Immunophenotyping of *NPM1c+* cells revealed a remarkable increase in the CD14+population representing monocyte/macrophage-like features, suggesting that loss of caspase-2 results in terminal differentiation. Exogenous expression of *NPM1c+* in caspase-2 deficient *NPM1wt* cells impaired viability, confirming that *NPM1c+* cells require caspase-2 for its growth and proliferation. Transcriptomic analysis revealed that loss of caspase-2 significantly downregulates pathways involved in stem cell pluripotency. In particular, FGF2, IGFR, WNT, LIF, and one of the core members of the self-renewal transcription factor, OCT 4, were downregulated in the absence of caspase-2 in *NPM1c+* cells. This reveals a novel role for caspase-2 in maintaining *NPM1c+* AML stemness. Taken together, our study shows that NPM1c+ mediated caspase-2 activation regulates AML cell death and survival cascades, a key determinant of chemosensitivity and leukemogenesis.

**LB036**

**IRF8 regulates tumor cell sensitivity to intrinsic ferroptosis by repressing p53.**

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Tumor cell resistance to ferroptosis, which is a recently discovered CTL induced cell death pathway, remains incompletely understood. We report here that interferon regulatory factor 8 (IRF8) functions as a regulator of tumor cell intrinsic ferroptosis. Genome-wide gene expression profiling identified ferroptosis pathway as an IRF8-regulated pathway in tumor cells. IRF8.KO tumor cells are resistant to intrinsic ferroptosis induction and also exhibit decreased ferroptosis in response to tumor specific CTL killing. Loss of IRF8 increases p53 express in tumor cells and knocking out Tp53 in IRF8.KO cells restored tumor cell sensitivity to intrinsic ferroptosis induction. We were able to show that treating tumor-bearing mice with IRF8-encoding plasmid NTC9385R-mIRF8 encapsulated in a DOTAP-cholesterol lipid

nanoparticle significantly reduced tumor growth and increased the percentage of dead cells in the tumor. Our data therefore determine that IRF8 represses p53 expression to maintain tumor cell sensitivity to intrinsic ferroptosis.

**LB037**

**Identification of imatinib-induced long noncoding RNAs involved in suppression of tumor growth mediated by bcr-abl oncogene.**

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Chronic myeloid leukemia (CML) is a hematological malignancy mainly caused by the *Bcr-Abl* oncogene that results from the reciprocal translocation between human chromosome 9 and 22, and occurs in more than 90% of CML cases. *Bcr-Abl* oncogene is also involved in tumorigenesis of other leukemia such as acute lymphoid leukemia (ALL). Although progress has been made in the understanding of signal transduction in Abl-mediated transformation, the molecular mechanisms underlying Abl-induced tumorigenesis are still not fully understood. It is well known that imatinib treatment can greatly suppress Bcr-Abl-mediated development of leukemia, but intracellular molecules induced by imatinib and their roles in the tumorigenesis remain largely to be determined. On the other hand, the majority of human transcripts lack protein-coding capacity, which are defined as noncoding RNAs (ncRNAs). Long noncoding RNAs (lncRNAs) play important roles in various vital biological processes. Notably, increasing number of lncRNAs have been linked to human diseases, including cancers. However, the role of lncRNAs in Bcr-Abl-induced leukemia remains largely unexplored. Here, we identified several imatinib-induced lncRNAs as critical regulator of Bcr-Abl-induced tumorigenesis. Most of these lncRNAs expressed in a very low levels in Bcr-Abl-positive cells from chronic myeloid leukemia patients. Interestingly, they could be significantly induced in Abl-positive leukemic cells treated by imatinib. Silencing these lncRNAs promoted survival of Abl-transformed human leukemic cells in experiments *in vitro* and xenografted tumor growth in mice, whereas ectopic expression of particular lncRNAs sensitized the cells to apoptosis and suppressed tumor growth. In concert, knockout of some murine lncRNAs in Abl-transformed cells accelerated cell survival and the development of leukemia in mice. Furthermore, some lncRNA deficient mice were generated, and we observed that knockout of particular murine lncRNAs facilitated Bcr-Abl-mediated primary bone marrow transformation. Moreover, animal leukemia model revealed that lncRNA deficiency promoted Abl-transformed cell survival and development of leukemia in mice. In summary, these findings unveil an inhibitory role of several critical lncRNAs in Abl-mediated cellular transformation, and provide new insights into molecular mechanisms underlying Abl-induced leukemogenesis.

**LB039**

**Ovarian cancer cell-derived exosomal UCA1 reprograms glucose metabolism in stromal fibroblasts.**

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Tumor progression is stringently regulated by the co-ordinated signaling between the tumor cells and the tumor microenvironment (TME) through diverse autocrine, paracrine and exocrine signaling mechanisms. Recent studies have shown that the exosomes that play a crucial role in the transfer of oncogenic macromolecules and oncometabolites to different cellular components of the TME can be targeted for therapy. Therefore, with the focus on identifying the exosomal long non-coding RNA (lncRNA) that can be therapeutically targeted, we investigated the profile of ovarian cancer cell derived lncRNAs and their functional role in ovarian cancer pathophysiology. Analysis of exosomes derived from a panel of high grade serous ovarian cancer cell lines indicated that UCA1 (Urothelial Cancer Associated 1) is the most

abundantly packaged lncRNA in the exosomes. Similarly, exosomes from patient-derived ovarian cancer cells exhibited higher levels of UCA1. Ascites from ovarian cancer patients also indicated high levels of UCA1 in the ascites-derived exosomes. Results using PKH67-labelled ovarian cancer cell-derived exosomes and MRC5 fibroblast cell line indicated the exosomal transfer of UCA1 to the fibroblasts. Since paracrine signaling has been shown to induce metabolic reprogramming of peritumoral fibroblasts, we interrogated whether exosomal transfer of UCA1 could reprogram the glucose metabolism in the fibroblasts. Results from the Agilent Seahorse glycolytic stress assay indicate that the exosomal UCA1 promotes reprogramming of glucose metabolism in fibroblast cells while depleting of UCA1 in the exosomes failed to induce such metabolic reprogramming. Results from the analysis of the key glycolytic enzymes in the stromal fibroblasts, post-exosomal UCA1 uptake also corroborate this conclusion. Thus our results provide primary evidence that the exosomal-UCA1 induces pro-tumorigenic metabolic reprogramming in peri-tumoral fibroblasts. Together with the known oncogenic role of UCA1 in many different cancer cells, our findings indicate that targeting UCA1 could form a productive precision cancer strategy in ovarian cancer, targeting both the tumor cells and tumor stromal cells. This research was supported by the Department of Defense Ovarian Cancer Research Program Award W81XWH-18-1-0066, W81XWH-22-1-0415, the National Institute of General Medical Sciences grant P20 GM103639 and The National Cancer Institute of the National Institutes of Health grant P30 CA225520.

**LB040**

**P62-mediated degradation of TIF-IA drives the senescence associated secretory phenotype.**

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Advanced age is one of the most significant risk factors for malignancy. One mechanism proposed for age-associated cancer risk is the accumulation of senescent cells in ageing tissue. These cells secrete a cocktail of pro-tumorigenic factors known as the senescence associated secretory phenotype (SASP). The SASP is driven by the NF-κB transcription factor. However, the initial triggers of NF-κB in senescence remain unclear. Here we describe a novel mechanism that triggers NF-κB and the SASP. Using multiple *in vitro* and *in vivo* models, we demonstrate accumulation and cytoplasmic to nuclear translocation of the PolI complex component, TIF-IA, upon senescence induction. siRNA knockdown revealed TIF-IA is not required for the cell cycle effects of senescence but is essential for transcription of SASP factors, nucleolar enlargement (a characteristic of senescence and marker of organismal ageing) and full senescence. We make the novel observation that in steady state, TIF-IA is targeted for autophagosomal degradation by the cargo receptor, p62, which is inhibited in senescence and promoted by inactivation of the DNA repair protein, ATM. Based on these data, we propose a model whereby the P62-TIF-IA interaction is lost downstream of DNA damage and ATM activation, which causes TIF-IA accumulation, the SASP and senescence. Using tissue from the well characterized *nfkb1*$^{-/-}$ mouse model of aging, and old and young mice, we show that TIF-IA accumulates in colonic mucosa with age, which is further enhance by *nfkb1* deletion. These exciting new data identify a new role for TIF-IA and have considerable relevance to age-related cancer.

**LB041**

**EZH2 and ATF6 sense metabolic stress to balance MHC class I antigen presentation in melanoma.**

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Unleashing the immune anti-tumor response through immune checkpoint inhibitors (ICIs) is a promising strategy to combat many solid-tumor malignancies, including metastatic melanoma. When successful, the anti-tumor response is potent; however, around half of melanoma patients fail to respond to ICIs. Patient responsiveness to ICIs has been characterized by increases in MHC class I (MHC-I) expression driven by

increases in oxidative metabolism. Though the harsh conditions of the tumor microenvironment (TME) are known to be immunosuppressive, the specific mechanisms connecting metabolic stress and antigen presentation are not well understood. To recapitulate and investigate the ICI-responsive phenotype *in vitro,* a model of metabolic remodeling was developed which forces melanoma cells (A375, A101D, B16F10) to metabolically adapt to the absence of glucose. Proteomic profiling indicates a reversible, adaptive phenotype reminiscent of published ICI-responders, and pathway enrichment shows analogous increases in oxidative metabolism and restoration of MHC-I expression. This phenotype, and its impact on MHC-I expression, significantly increases tumor cell sensitivity to T-cell-mediated killing *in vitro.* Additionally, successful adaptive remodeling of JAK1 and IFNAR1 KO cell lines suggests the metabolism-mediated induction of MHC-I is independent of IFN signaling. Proteomic analysis of three metabolically conditioned melanoma cell lines identified downregulation of the histone methyltransferase EZH2 (Enhancer of Zeste Homolog 2) and the activating transcription factor ATF6 as key determinants of MHC expression. In these studies, we mechanistically explore the control of MHC-I antigen presentation through the transcriptional and post-translational dysregulation of ATF6 and EZH2. Here, we demonstrate that ATF6 overexpression, and thereby activation of the adaptive unfolded protein response (UPR), prevents induction of MHC by EZH2 inhibition. These data suggest EZH2 and ATF6 act to balance MHC-I antigen presentation during metabolic stress whereby loss of EZH2 increases and activation of ATF6 prevents MHC antigen presentation. Additional and ongoing studies coupling EZH2 and ATF6 inhibition will provide crucial insight into this mechanism and have the potential to influence adjuvant therapy development.

**LB042**

**Targeting ATR enhances the antitumor efficacy of patritumab deruxtecan (HER3-DXd)in tamoxifen-resistant ER$^+$ breast cancer cells by inducing DNA damage and apoptosis.**

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**Background:** HER3, a member of the ERBB family of receptor tyrosine kinases that activates multiple oncogenic signaling pathways, is overexpressed in 50-70% of breast cancers (BC). HER3 mRNA expression is highest in luminal (ER$^+$) breast tumors. Approximately 30% of ER$^+$ breast tumors are de novo resistant to tamoxifen. Therefore, therapeutically targeting HER3 with HER3-DXd, an antibody-drug conjugate (ADC) composed of a fully human anti-HER3 IgG1 monoclonal antibody (patritumab) covalently linked to a topoisomerase I (TOP I) inhibitor payload (deruxtecan) via a tetrapeptide-based cleavable linker, can be an effective treatment for tamoxifen-resistant (TMR) ER$^+$ BC. After assessing HER3-DXd's efficacy as a single agent, we sought to identify a synergistic partner to maximize its antitumor activity in HER3$^+$/ER$^+$ TMR BC.

**Methods:** Whole-genome high-throughput siRNA screening (Ambion Silencer Select Human Genome siRNA Library V4) was performed to identify synergistic partners for maximizing HER3-DXd's antitumor efficacy. The synergistic antitumor effects were assessed *in vitro* using a soft agar colony formation assay and a clonogenic assay in TMR HER3$^+$/ER$^+$ MCF7 and T47D BC cells and *in vivo* using xenograft mouse models of these cells. Targeting specificity was determined using siRNA. Treatment effects on cell cycle progression, DNA damage, apoptosis, and expression of proteins of interest were assessed by flow cytometry, comet assay, staining with annexin V-PE and 7-AAD, and Western blotting, respectively.

**Results:** HER3-DXd inhibited the anchorage-independent growth of HER3$^+$/ER$^+$ cells by >50% at 5 nM and their colony formation at 5-25 nM ($P < 0.05$). Among the synergistic targets identified by whole-genome high-throughput siRNA screening, inhibiting ATR with siRNA or BAY1895344 showed the greatest synergistic effect with HER3-DXd in TMR HER3$^+$/ER$^+$ BC cells. In contrast, no synergistic effect was observed with the combination of BAY1895344 plus patritumab or control ADC (IgG-DXd),

suggesting its dependence on HER3-DXd-mediated delivery of DXd. To further confirm the targeting specificity, we knocked down ATR or TOP I expression using siRNA in TMR $HER3^+/ER^+$ BC cells and then treated the cells with HER3-DXd or BAY1895344, respectively. A synergy was also observed, indicating that the drugs achieve the synergy by targeting ATR and TOP I. The combination of HER3-DXd plus BAY1895344 reprogrammed cell cycle progression from G2/M arrest to sub-G1 arrest by inhibiting both ATR/Chk1/cyclin A2/CDK2 and ATR/Chk1/cyclin E/CDK2 signaling. The combination also induced DNA damage, which was further confirmed by the reduced expression of H2AX, an ATR substrate that contributes to DNA repair, and the increased expression of γH2AX (phospho-H2AX at Ser139), an indicator of DNA damage. HER3-DXd and BAY1895344 synergistically inhibited the growth of both TMR $HER3^+/ER^+$ MCF7 ($P < 0.0001$) and T47D ($P < 0.01$) xenografts in mice.
**Conclusion:** The combination of HER3-DXd plus ATR inhibitors has therapeutic potential for overcoming tamoxifen resistance in $HER3^+/ER^+$ BC.

**LB044**
**Modeling the role of ARID1A in colon cancer using patient-derived organoids.**
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Loss-of-function (LOF) mutations in genes encoding subunits of SWI/SNF chromatin remodeling complexes are found in approximately 25% of human cancers. Colorectal cancers (CRC), the second leading cause of cancer related deaths, are particularly vulnerable to SWI/SNF driver mutations, harboring subunit alterations in up to 55% of patient tumors. ARID1A, a BAF subcomplex specific subunit, is the most frequently mutated subunit in human cancers (12%) and CRCs (10-12%). Additionally, ARID1A was recently identified as a leading genetic alteration in inflammatory bowel diseases such as Crohn's and Ulcerative Colitis, conditions which are at higher risk of developing CRC. These data suggest an important role in ARID1A regulation of colonic tissue homeostasis and the potential for ARID1A mutation as an initiating or early driving event in CRC, which has not yet been modeled in human colon tissues. To examine the role of ARID1A in colon cancer development, we took a bottom-up strategy using CRISPR edited patient-derived colon organoids to model ARID1A LOF mutations in combination with classic CRC mutations APC and TP53. We evaluated the functional consequences of ARID1A mutation on cell growth using colony formation and proliferation assays. To systematically investigate the phenotypic contribution of ARID1A loss in these cell contexts, we employed transcriptomic and epigenomic profiling with RNAseq and ATACseq. We found that triple knock-out (TKO) of ARID1A, APC and TP53 confers a growth advantage in colon organoids, and induces dramatic morphological changes such as loss of symmetry and the breakdown of cystic architectures. At the gene expression level, ARID1A mutations led to shared and cell context specific transcriptional changes enriched for pathways such as G2M checkpoint, KRAS Signaling, and STAT5 signaling. Mechanistically, we show that ARID1A dependent chromatin accessibility changes at putative enhancers are linked to promoters of differentially expressed genes. These data suggest that ARID1A can regulate multiple cancer hallmark pathways depending on the cellular context and indicate a pleiotropic tumor suppressive function requiring further investigation in clinically relevant genetic backgrounds.

**LB045**
**Transcriptome-wide association study identifies susceptibility loci and genes for lung cancer risk .**
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Objective: Genome-wide association studies (GWAS) have identified more than 50 loci for lung cancer risk. However, susceptibility genes and the underlying mechanisms for these risk loci remain largely

unknown. We conducted a transcriptome-wide association study (TWAS) to identify susceptibility genes for lung cancer.
Methods: Transcriptome data from normal lung tissue and whole-genome sequencing data from 444 participants of European ancestry in the Genotype-Tissue Expression (GTEx, version 8) were used to build lung-tissue models to predict gene expression levels. Lung-tissue prediction models were successfully established for 10,802 genes with a model performance $r > 0.1$ and $P < 0.05$. We also built joint-tissue models using the joint-tissue imputation (JTI) framework, which leverages transcriptome data from lung tissue and 48 other tissue types from the 444 participants in GTEx. Joint-tissue models were successfully established for 12,629 genes with a model performance $r > 0.1$ and $P < 0.05$. These prediction models were applied to the GWAS data comprised of 29,266 lung cancer cases and 56,450 controls of European ancestry, in order to evaluate genetically predicted gene expression levels in association with lung cancer risk.
Results: We found 44 genes whose genetically predicted expression levels were significantly associated with overall lung cancer risk at the Bonferroni correction significance threshold. Among the 44 genes, four were located at least 500kb away from any of the leading variants identified previously in GWAS. Of these four genes, only the *CCHCR1* gene has been reported in previous TWAS, and the other three genes, *LY6G5B*, *PRSS16*, and *C19orf54*, have never been reported in previous GWAS or TWAS. For each of these three novel genes, consistent associations were observed in both lung-tissue and joint-tissue models. For the 40 genes located in previously identified GWAS loci, after adjusting for GWAS-identified variants, the associations for the majority of them became non-significant. However, the associations did not change materially for *UCKL1* or *PRPF6*. These results suggest that the associations for these two genes were independent from previous GWSA-identified signals. We also identified 10 genes located at least 500kb away from GWAS-identified loci that were associated with lung cancer histological subtypes, e.g., adenocarcinoma (*DCBLD1* and *AQP3*), squamous cell carcinoma (*ZSCAN26*, *BLOC1S2*, *ABCF1*, and *ZSCAN9*), and small cell lung cancer (*BTN2A2*, *TMA16*, *RP11-218F10.3*, and *FRS3*). An additional 7 genes located in GWAS loci were associated with risk of lung cancer histological subtypes but not with overall lung cancer risk, including 4 genes for adenocarcinoma (*TP63*, *STN1*, *FAM227B*, and *TPRG1*) and 3 genes for squamous cell carcinoma (*DDAH2*, *OR2H2*, and *NELFE*).
Conclusion: Our TWAS identified lung cancer susceptibility genes, providing new insight into the genetics of lung cancer etiology.

**LB046**
**Characterizing the role of inflammation-induced epigenetic alterations in modulating the immune microenvironment during lung cancer initiation.**
Na Wang, Ray-Whay Chiu Yen, Hamza Khan, Malcolm V. Brock, Hariharan Easwaran, Stephen B. Baylin, Michelle Vaz. Johns Hopkins University School of Medicine, Baltimore, MD.

One of the most important goals of developing timely detection and effective intervention strategies for lung cancer is to understand the biology and molecular mechanisms involved in early-stage evolution of this disease. Chronic inflammation is a major driver of lung cancer and contributes to its pathogenesis by inducing genetic and epigenetic abnormalities, as well as shifts in key immune cell populations. However, the evolution of these changes and the mechanisms underlying the early stages in development of pre-neoplasias and progression to invasive tumors have not been well delineated. Monolayer cell culture systems, while providing valuable insights into lung cancer development, lack the complexity of an intact organ system. This study aims to overcome these limitations by using a 3D lung organoid model to delineate the role of epigenetic alterations in driving initiation of non-small cell lung cancer. The stem cell enriched nature of the organoids makes it an ideal system to evaluate the implications of epigenetic alterations in these key cell types and their role in cancer initiation. To better identify the epigenetic alterations that play key roles in lung cancer initiation, cigarette smoke condensate (CSC) was applied to normal lung organoids to mimic chronic inflammatory exposure. Epigenomic and transcriptomic

alterations were evaluated by genome-wide DNA methylation analysis, ATAC-seq, and RNA-seq. Flow cytometry and confocal microscopy were used to evaluate changes in composition of cell populations comprising the organoids. Long-term CSC exposure caused distinct morphological changes in organoid structure and cellular composition, accompanied by an increased proliferative potential. Analysis of changes in cell composition revealed increases in Krt14 expression in CSC treated organoids, leading to key shifts in basal stem cell populations from a $TP63^{+}KRT5^{+}$ to a $TP63^{+}KRT5^{+}KRT14^{+}$ population. This was accompanied by reduction in differentiated cell types, as analyzed by qRT-PCR. Analysis of genome wide DNA methylation showed increases in promoter DNA methylation in key genes associated with lung tumorigenesis. Most importantly, co-culture studies involving culturing CSC treated and control organoids with key immune cells revealed that chronic CSC treatment caused modulation of the microenvironment from a pro- to an anti-inflammatory state. Our results suggest that chronic inflammation causes key shifts in populations of lung stem cells with an associated decrease in differentiation potential. These changes are accompanied by DNA methylation and gene expression changes suggestive of an increased tumorigenic potential. Finally, these changes in the CSC treated organoids are associated with the ability to modulate the function of key immune cells associated with lung tumorigenesis from a pro- to an anti-inflammatory phenotype. Results from our study, will aid in developing novel biomarker-based methodologies to distinguish and treat lung cancer in its early stages.

**LB048**

**AR-targeted linked-read sequencing reveals complex AR structural variants in CRPC tumors.**

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Inhibition of the androgen receptor (AR) transcription factor is the mainstay therapeutic strategy for systemic prostate cancer. However, development of a resistance phenotype termed castration resistant prostate cancer (CRPC) enables disease progression that underlies virtually all prostate cancer mortality. In the majority of CRPC, AR is transcriptionally re-activated in part due to accumulation of numerous alterations in the AR gene. These genomic alterations include missense mutations in exons that encode the ligand binding domain of AR , amplification of the AR gene body and/or an upstream transcriptional enhancer, and AR structural variants (SVs) resulting from inaccurate repair of DNA double strand breaks. In CRPC patient samples, these AR genomic alterations co-occur within tumors, which makes elucidating the fitness contribution of each alteration difficult. While short-read DNA sequencing has allowed for characterization of these events, they are underpowered to preserve linkage information. To address this challenge, we performed AR-targeted linked-read DNA-sequencing of CRPC cell line and patient derived xenograft (PDX) models with the goal of resolving sub-clonal and complex AR SVs. This approach confirmed the heterogeneity of AR SVs in these PDXs, including phased AR SVs that can be traced back to a single high molecular weight DNA molecule. These phased AR SVs were consistently found to co-occur in CRPC specimens with amplification of AR. Our results define a new class of AR SVs where multiple rearrangements co-occur on amplified DNA molecules to yield a complex array of AR gene structures in CRPC.

**LB049**

**ALKBH5 emerges as a novel regulator of the BCR pathway.**

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Global epitranscriptomic methylation of mRNA at the N6 position (m6A) is reported to be altered in B-cell and tumor development. However, the mechanistic underpinnings of its role in the pathogenesis of

Diffuse large B cell lymphoma (DLBCL) are unknown. It is well established that the B-cell receptor (BCR) pathway is a primary oncogenic driver in aggressive B-cell lymphomas. We previously reported that the Ataxia Telangiectasia Mutated (ATM)/ Hu Antigen R (HuR)axis, as well as Eukaryotic Translation Initiation Factor 4A (eIF4A), modulate BCR signaling. However, the impact of RNA modifications on BCR induction is still poorly understood. Addressing this burgeoning question, we noted enhanced expression of AlkB Homolog 5 (ALKBH5), an m6A demethylase, in naïve B-cells treated with BCR mimetics. Similarly, mRNA levels of ALKBH5 were reported to be modestly enhanced in IgM-treated human B-cells (GSE156195). Consistently, immunization of C57BL/6 mice with sheep red blood cells (SRBC) significantly enhanced ALKBH5 in splenic B-cells. Next, evaluating the impact of BCR induction in lymphomagenesis, we observed a robust increase in the demethylase expression in BCR mimetic-treated DLBCL cells. Notably, increased expression of ALKBH5 was observed in a murine EμMyc model and primary DLBCL tumors. In coherence, the enhanced transcript levels of ALKBH5 in TCGA-DLBCL have prognostic implications. To gain functional insight, we depleted ALKBH5, which resulted in a robust reduction of BCR pathway proteins. Unexpectedly, the mRNA levels of CD79a and BLK were minimally modified, while the SYK, BTK, and CARD11 messages were reduced. Further studies are ongoing to evaluate the potential mechanism of mRNA transport and/or stability.

**LB052**

**Electronic nicotine delivery system (ENDS) use among LatinX youth in low-resource communities: Implications for policy and regulation.**

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Introduction: Electronic nicotine delivery system (ENDS) use by youth who never previously used tobacco is a major health concern, as ENDS have been linked to DNA damage, inflammation, and cancer development. FDA's regulation of ENDS products includes how the tobacco industry markets ENDS to young people, including youth from marginalized communities. LatinX youth are among the largest and fastest growing populations in the country, yet little is known about their susceptibility to using ENDS, including perceived risks of harm and addiction. The purpose of this study was to characterize ENDS use among LatinX youth in low-resource communities, and perceptions of its harm and addiction.
Methods: N=622 middle and high school students identifying as LatinX and enrolled in a Title I school district in NJ completed an online survey during a class period. Lifetime ENDS use and its correlates were tested in bivariate and logistic regression models.
Results: Based on Spanish-English language preference, 33% of LatinX youth had low acculturation to the US and 35% had high US acculturation: their overall prevalence of lifetime ENDS use was 20%. LatinX ENDS users were 3x more likely to acquire ENDS products from others (e.g., adults) than to purchase or obtain them themselves (16% vs. 5%). LatinX youth were considered further 'susceptible' to using ENDS based on their curiosity (30%), expected use within a year (11%), or uptake if offered by a friend (17%). In multivariable logistic regression models of the odds of ENDS use, LatinX students in Grade 11 were more likely to have ever used ENDS vs. those in Grade 7 (OR=1.23, 95% CI=0.77, 1.95). After adjusting for acculturation, LatinX youth who were more curious (OR=6.85, 95% CI=3.64, 12.87), intended to use ENDS in the next year (OR=4.96, 95% CI=2.24, 10.98), and would use the product if offered by a friend (OR=5.47, 95% CI=2.80, 10.68) had greater lifetime odds of being ENDS users. Also, LatinX youth who perceived no/low risks (OR= 2.02, 95% CI=1.22, 3.34) or health harms (OR=2.19, 95% CI=1.29, 3.73) were more likely to have ever used ENDS. When examined simultaneously, LatinX youth who both perceived no/low risk as well as no/low health harms were the most likely to have used ENDS products in their lifetimes (OR=3.10, 95% CI=1.72, 5.62).
Conclusions: Across the acculturation continuum, LatinX youth from marginalized communities are especially susceptible and using ENDS. Prevention efforts directed towards these communities may

benefit from health education messaging that emphasizes health harms and addiction potential to reduce ENDS experimentation.

**LB054**
**Establishment of an infrastructure for rapid pandemic response at the Frederick National Laboratory.**
Nancy V. Roche, Troy Kemp, Ligia Pinto, Tom Hickey. NCI-Frederick/Leidos Biomed. Research, Inc., Bethesda, MD.

The sudden onset of the 2019 SARS-CoV-2 pandemic required agile development of standards and efficient validation of assays to assess prevalence of infection as well as immune responses to infection and vaccination. Leveraging their experience in HPV serology and standards, the Vaccine, Immunity and Cancer Directorate (VICD) at the Frederick National Laboratory for Cancer Research (FNCLR) pivoted to address this unmet need in SARS-Co-V2 serology clinical testing and research. This standardization effort required the collection and processing of large volumes of blood from SARS-Co-V2 infected and uninfected individuals into serum and peripheral blood mononuclear cells (PBMCs). Collaborations with specimen collection sites across the United States were established. Following qualification for anti-SARS-CoV-2 IgG and IgM levels in independent laboratories, VICD assembled reference evaluation panels, which were used to assist the FDA's performance evaluation of commercial assays submitted for EUA approval. To date, 185 different shipments of the standard or validation panel have been sent to both domestic and international labs. These materials are also available to the SARS-CoV-2 serology community for assay calibration and performance evaluation which greatly facilitates assay data harmonization. In addition, the NCI Serological Sciences Network (SeroNet) was born from this initiative and expertise, resulting in the establishment of Capacity Building Centers (CBCs) for sample collection from different healthy, cancer and immunocompromised cohorts at Mount Sinai, Arizona State University, the University of Minnesota, and Northwell Feinstein. The NCI and FNLCR simultaneously collaborated to develop a network of investigators focused on advancing research on the immune response to SARS-CoV-2 infection and vaccination among diverse and vulnerable populations, including cancer patients. Their research has resulted in over 326 peer-reviewed publications. The CBC's have enrolled patients in longitudinal studies, resulting in a centralized collection of annotated, well characterized serum, PBMCs and clinical data. Numerous cancer cohorts, but predominantly Multiple Myeloma, are included. Furthermore, technology development was supported at the CBC's. Based upon this success, the VICD in collaboration with NCI is pursuing an even more innovative effort in pandemic preparedness to establish a Center for Serology and Data Emergency Preparedness (CESDEP); a global network able to activate and pivot to address pandemic-level threats, while continuing to expand the development of immunological assays that can inform clinical decisions for cancer and other immunocompromised patients.

**LB056**
**Crowdsourcing rare cancer research in the Hack4NF GENIE-NF tumor identification and classification challenge.**
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A key challenge in rare tumor research is the paucity of genomic data that can be used to understand and devise better therapeutic strategies for rare cancers. Furthermore, the "curse of dimensionality," in which

data has many features, such as genetic variants, but few specimens, makes it difficult or impossible to use conventional machine learning techniques to explore these data. To address these challenges in the context of tumors associated with the rare disease neurofibromatosis, we ran a hackathon to stimulate the development of methods to better understand the biology of tumors related to this disease. The hackathon had three challenges centered around variant effect prediction, drug discovery, and genomics. The genomics track of the hackathon leveraged the AACR Project GENIE database (1) and challenged participants to develop new frameworks that accurately use GENIE data to classify neurofibromatosis-related tumors. They were asked to first identify the neurofibromatosis-related tumors in the dataset. They were then asked to use one or more novel classification methods to classify the tumor samples into different groups based on genetic features. To help them do this, we provided access to version 13 of the GENIE database to the hackathon participants, though they were allowed to integrate other relevant datasets. The expected output was a classification method that differentiates different types of NF1, NF2, and schwannomatosis-related tumors using clinical sequencing data, as well as a list of the most important features in the algorithm for differentiating tumor types. Domain expert judges qualitatively scored each team's rationale for defining and including "NF-related tumors" in their project, and scored the feature list based on the presence of known important biomarkers and features in NF tumors as well as potentially novel features that the algorithm identified. A technical judge also scored the code repository based on documentation and clarity of code. Two teams from the GENIE subchallenge were awarded prizes - Team Next GeNLP as the best overall GENIE challenge submission, and team "Artificial Intelligence for neurofibromatosis" for best project documentation. Both winning teams used methods based on natural language processing (NLP) techniques to reduce the dimensionality and complexity of the variant data, and to identify new representations of NF-relevant tumors, and then applied downstream analysis methods such as distance calculations and feature prioritization to better understand the genomic profiles of different tumors. While these methods and tools focused on NF-specific tumor types, we anticipate that they could be re-used by others to better explore the biology and interrelatedness of other rare tumors within the GENIE database.

(1) The AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine Through An International Consortium, Cancer Discov. 2017.

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**LB058**

**A transcriptome-wide gene expression outlier analysis pinpoints therapeutic vulnerabilities in colorectal cancer.**

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The number of effective therapies approved for metastatic colorectal cancer (mCRC) is still limited and multiple strategies are continuously explored to expand the drug target repertoire. Among these, the identification of overexpressed genes has prompted the discovery of actionable oncogenic dependencies in multiple tumour types.

Starting from RNA sequencing data, we identified transcriptome-wide gene expression outliers, defined

as samples showing abnormal expression for a particular gene, across 226 CRC cell lines, considering both overexpression and underexpression events as positive or negative outliers. Then, the distance of each outlier from gene-specific reference points, absolute expression values and differential expression values were considered in a multi-filter strategy to select extreme gene expression outliers, with the hypothesis that they are more likely to be functionally relevant in cancer cells. We also profiled genetic and epigenetic features of CRC cell lines based on whole exome sequencing and DNA methylation microarray data.
Extreme positive and negative gene expression outliers were found for 3,533 and 965 genes, respectively, and only some of them were associated with underlying genetic and epigenetic alterations. Gene expression alterations with known therapeutic or diagnostic value in CRC were pinpointed as extreme positive and negative outliers thus confirming the validity of the approach. Annotation of overexpressed enzyme genes according to the Target Development Level (TDL) classification revealed numerous enzymes for which inhibitors are already available. We next explored underexpression events to identify potential synthetic lethal targets. Intriguingly, we found that CRC models lacking expression of the MTAP gene were sensitive to treatment with an inhibitor of the PRMT5:MTA complex currently under clinical development.
We found that mapping extreme and transcriptome-wide positive and negative gene expression outliers in CRC cell lines is an effective strategy to identify putative drug targets and biomarkers, independently from the underlying genetic or epigenetic alterations. We indeed present a comprehensive atlas of CRC extreme gene expression outliers which includes events with diagnostic or therapeutic relevance. This resource could also serve as a reference for further discoveries in CRC and other tumour types.

**LB059**

**DNA damage and somatic mutations in mammalian cells after irradiation with a nail polish dryer.**

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Ultraviolet A (UVA) rays are long wavelengths that can penetrate the deeper layer of the skin and are responsible for the immediate tanning effect. A multitude of natural and artificial sources of UV exposure exist, including UV-emitting lamps that are commonly used in dentistry, cosmetics, and medical laboratories. Recently, several reports suggested that long-term use of UV-nail polish dryers, used for curating nail polish formulas, known as gel, may increase the risk for developing skin cancer. However, no experimental evaluation has been conducted to reveal the effect of radiation released by UV-nail polish dryers on mammalian cells. Here, we address the continuous exposure of clients and employees to UVA light by employing both murine and human primary cell models for irradiation, in an acute and chronic manner, using a UV-dryer machine. We, then, subject the cells to DNA sequencing either by bulk whole-genome sequencing after clonal expansion or by single-molecule duplex sequencing without clonal expansion. Our findings show that UVA-exposed samples manifest an increase of C:G>A:T transversions compared to control cells. The patterns of mutations found in these cells can be recapitulated by mutational signatures previously attributed to reactive oxygen species (ROS), namely COSMIC signatures SBS18/36. These results are further corroborated by the presence of high levels of ROS in irradiated samples, consistent with 8-oxo-7,8-dihydroguanine damage and mitochondrial dysfunction. Importantly, we show that in contrast to UVB exposure, UVA does not induce an increase of C:G>T:A transitions, indicating that different types of ultraviolet light radiation affect DNA through distinctive molecular mechanisms. Finally, re-examination of somatic mutations from skin cancers reported by the International Cancer Genome Consortium (ICGC) reveals that SBS18/36 are ubiquitously present in melanoma and account for 12% of the previously annotated driver mutations. In summary, this study demonstrates that radiation emitted by UV-nail polish dryers can both damage DNA and permanently

engrave mutations on the genomes of mammalian cells.

**LB060**

**Integrated analysis of transcriptomics and kinase activity data for better characterization of cancer models.**

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Introduction: Quantitative measurements of transcripts and proteins are key to investigate the basal state of a biological system, while functional proteomics inform about the active state of regulatory networks. Here we describe how the integration of transcriptomics and kinase activity data lead to a better characterization of various cancer models.
Methods: We performed RNA sequencing (RNAseq) and kinase activity profiling of 63 Patient Derived Xenograft (PDX) models from six tumor types (Breast, Ovarian, Colon, Melanoma, Lung and Acute Myeloid Leukemia, AML). RNAseq was performed on an Illumina NovaSeq platform. The data was DESeq2-normalized and log2-transformed. Protein Tyrosine Kinase and Serine-Threonine Kinase activities were profiled on PamChip® peptide microarray. To identify the role of kinase signaling related genes we defined a set of signaling-specific genes (n=2932), based on the elements from the reactome signal transduction pathway database (n=2560) and additional kinases (n=372) represented on Pamchip, that was used for further analysis. Integrated analysis of transcriptomics and kinase activity data was performed using Multi Omics Factor Analysis (MOFA).
Results: Principal Component Analysis (PCA) of RNAseq data using all included genes or 2932 kinase signaling-specific genes showed clustering of the data according to cancer type, with ovarian cancer showing most heterogeneity, which indicates the importance of kinase signaling in these malignancies. Interestingly, with integrated RNAseq-Kinase activity data all except ovarian cancer show clustering of cancer types on the MOFA Factor 1 - Factor 3. Pathway analysis on the highest ranking 100 genes from principal component 1 of RNAseq data (capturing variation between AML and the other tumor types) resulted in 60 KEGG pathways. Importantly, highest ranking 50 genes and 47 peptides comprising MOFA Factor1 identified 115 significant KEGG pathways, and the statistical score of pathways identified by RNAseq alone was further improved. Finally, significant correlation between gene expression and kinase activity was found for selected PDX model per malignancy. Furthermore, ranking PDX models based on correlation score provided suitable tool to select PDX models for disease or pathway specific research question.
Conclusion: Integrating transcriptomics with kinase activity data can be used to confirm transcriptomics findings on a functional level and provides deeper biological insights than transcriptomics alone. We show that integrative analysis leads to more significant and a higher number of enriched pathways. High correlation between two datasets allows for selecting animal models addressing specific research questions. Integrated analysis of transcriptomics and kinase activity data has great potential in improving diagnosis, prognosis and prediction of response to treatment.

**LB061**

**Proteomic-based stemness score measure oncogenic dedifferentiation and enable the identification of druggable targets.**

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Progression and therapeutic resistance in cancer have been strongly associated with the acquisition of a stemness phenotype. Here, we provide new stemness indices for assessing the degree of oncogenic dedifferentiation in tumor samples. We used a machine learning model to predict the stemness molecular phenotype based on proteomic data. The prediction model was built from human pluripotent stem cell from the Human Induced Pluripotent Stem Cells Consortium (HipSci) and applied to compute stemness indices on the Clinical Proteomic Tumor Analysis Consortium (CPTAC) tumor samples, consisting in their proteogenomic hallmarks of stemness. The obtained stemness scores based on protein expression are novel and original, and are significantly more robust compared to our previous published work. The obtained proteomic score is able to classify stem cells and non-stem cell classes. The initial analysis of over 2000 tumor samples obtained from twelve types of primary carcinomas of breast, ovary, lung, kidney, uterus, brain (pediatric and adult), head and neck, liver, stomach, colon, and pancreas has confirmed our previously published results. Indexing of CPTAC tumors with proteomic stemness score brought us with previously unappreciated findings. We integrated the stemness scores computed using proteins with gene expression, DNA methylation, microRNA, copy number alteration and protein post-translational modification to identify coherent proteogenomic stemness association. Our initial findings identified proteins and phospho-proteins as active nodes of signaling pathways and transcriptional networks that drive aggressiveness of the primary tumors that cause resistance to existing therapies. The correlation between stemness scores and protein expression resulted in the identification of potential drug targets for anti-cancer therapy both tumor-specific and shared among different tumor types. Our results also revealed stemness-associated proteins predictive of clinical outcome across analyzed tumor types. Finally, we validated some stemness targets by immunohistochemistry in independent samples and confirmed the association with clinical outcome. Targeting the proteins here identified and cellular mechanisms that drive a stemness phenotype with existing or novel drugs may eventually lead for clinical development of effective cures for cancer patients.

**LB062**

**Identifying novel targets for CAR-T therapies from single cell RNA-sequencing data.**

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Chimeric antigen receptor T (CAR-T) cell therapies have revolutionized cancer treatment. While CAR-T has yielded tremendous clinical success for patients with liquid tumors, its potential remains to be unleashed against solid tumors. One key challenge is identifying optimal targets for these therapies: cell surface proteins that are expressed highly and uniformly by a tumor's constituent malignant cells, and minimally so by healthy tissues. Employing a systematic, data-driven analysis, we first charted the landscape of existing CAR-T targets in the clinic, identifying the leading targets in each indication based on tumor selectivity and safety metrics. Next, from patient tumor single cell transcriptomics data, we performed a genome wide search across many different solid tumor types to identify new and candidate CAR-T targets with better selectivity and safety scores than extant ones. Remarkably, in almost all indications, we could not find such better targets, testifying to the near optimality of the current target space, at least in accordance with our measures. However, one striking exception is HPV-negative head and neck squamous cell carcinoma (HNSC), for which there is currently a dearth of existing CAR-T

targets in clinics. Specifically, our investigation has discovered 20 novel CAR-T targets for treating HNSC and one for treating glioblastoma more precisely and safely.

**LB063**

**An integrated transomics approach reveals significant differences between EGFR inhibitors with the potential to identify novel targets to overcome EGFR resistance.**

Christopher J. Nicholson, Arudhir Singh, Caitlin Westberg Brown, Simon P. Fricker, Jon Hu, Samantha Dale Strasser. Pepper Bio, Cambridge, MA.

While first- and second-generation EGFR inhibitors (EGFRi) have shown success against EGFR mutant non-small cell lung cancer (NSCLC), the emergence of resistance mechanisms has prevented long-term treatment for many patients. The third-generation inhibitor osimertinib, the current standard of care, is still prone to resistance. Combined with the failure of several late-stage candidates it is clear a novel approach is required. We have developed a transomic analysis platform, PROSPER (Protein Regulation with Objective Seeking Powerful Evidence Retrieval), employing an integrated analysis of genomics, transcriptomics, proteomics, and phosphoproteomics, termed transomics. This proprietary analysis allows us to interpret complex biological relationships and provide a functional map of the biochemical drivers of disease. We investigated the effects of a cross-section of EGFRi in a panel of EGFR mutant lung cancer cell lines and performed a transomics analysis to identify key differences between different EGFRi and drive novel drug discovery to circumvent resistance to EGFR-targeted drugs. Seven drugs, both approved and unapproved EGFRi, were evaluated against three NSCLC cell lines. The cell lines expressed wild-type EGFR (as a control), Ex19del activating mutation (to model EGFR mutant NSCLC), or L858R activating mutation plus T790M gatekeeper mutation (to model a subtype of EGFRi resistant NSCLC). Approved drugs included erlotinib, afatinib (first- and second-generation EGFRi, respectively), and osimertinib (third-generation drug that can overcome the T790M gatekeeper mutation that confers resistance to earlier drugs). The remaining drugs (maverlertinib, naquotinib, olmutinib, rociletinib) are unapproved third-generation inhibitors. The concentration required to inhibit cell growth ($IC_{50}$) was determined after a 72 h incubation and viability was assessed by CellTiter-Glo. For the transomic analyses, the same drugs were incubated for 24 h with the $IC_{50}$ concentration and cells were harvested for genomic, transcriptomic, proteomic and phosphoproteomic analyses. The subsequent analysis revealed striking differences between the seven drugs. Principal component analysis shows two distinct clusters between approved and unapproved drugs. Furthermore, the transomic signature for the third-generation approved drug osimertinib was significantly different to that for the drug olmutinib which failed clinical development due to toxicity. Transomic analysis of EGFRi has the potential to identify important differences between successful drugs, drugs that failed in clinical development, and to identify non-EGFR targets that may overcome resistance to current drugs. This hypothesis is currently being investigated across various resistant and undruggable cancers to unlock novel therapeutic targets.

**LB064**

**Using a genomic only approach to a radiation-age association for supplementing differentiation amongst lung diseases and cancer using PMBCs.**

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Purpose: Ionizing radiation may serve as a reference variable to estimating biological age since it mirrors cumulative DNA damage similar to aging processes. To do this, we aim to establish a rad-age association using genomics as its foundation and clinical applicability with lung disease as an overlapping health concern.
Methods: Two datasets were combined and used to empirically find the age cutoff between young and old

patients: GSE42488 (Data-A1) and GSE53351 (Data-A2). With age as both a categorical and continuous variable, two other datasets that include radiation exposure are used to test the interaction between radiation and age: GSE21240 (Data-B1) and GSE23515 (Data-B2). A radiation only data was also used in conjunction to evaluate trend analysis with increasing exposure levels: GSE20173 (Data-R1). The gene lists are oriented in preranked lists for both pathway and diseases functional analysis. Finally, these genes are used to evaluate another dataset via GSE42834 (Data-D1) on the clinical relevance in differentiating lung diseases given ethnicity and sex using both pairwise t-tests and linear models including pneumonic, tuberculosis, active and non-active sarcoidosis, lung cancer, and healthy controls.
Results: Using 12 well-known genes associated with aging, a threshold of 29 years old was found to be the difference between young and old patients. The two interaction tests (rad and age as continuous plus rad and age as categorical) yielded 234 unique genes such that pathway analysis flagged IL-1 signaling and PRPP biosynthesis as significant with high cell proliferation diseases and carcinomas being a common trend. From the radiation only t-tests, we see a trend of continued upregulation in gene expression as radiation levels increase in 15 of the 17 common genes. When looking at pairwise comparison of disease, ethnicity, or sex, 10 genes were statistically significant, 3 of which had a combination of pairwise significance and fold-change, while *LAPTM4B* (probe identifier ILMN1680196) was the only gene with significant interaction (p-value=0.004) between lung disease, ethnicity, and sex and fold change greater than two.
Conclusion: The results corroborate an initial association between radiation and age given inflammation and metabolic pathways and multiple genes emphasizing mitochondrial function, oxidation, and histone modification. Being able to tie rad-age genes to lung disease supplements a geroscience approach in future work.

**LB065**
**Interface-guided phenotyping of coding variants.**
Kivilcim Ozturk, Rebecca Panwala, Jeanna Sheen, Prashant Mali, Hannah Carter. UC San Diego, La Jolla, CA.

Understanding the consequences of single amino acid substitutions in cancer driver genes remains an unmet need. High-throughput mutagenesis is emerging as a powerful tool to probe the varying consequences of different amino acid substitutions across the length of a protein or protein domain, however it is currently limited to specific functional readouts such as target protein abundance or functional assays. Studying the effects of genetic perturbations on cellular programs and fitness has been challenging using traditional pooled screens. Over the last few years, there has been a surge of interest in Perturb-seq style assays to measure the transcriptional consequences of genetic perturbations ranging from whole gene knockout to amino acid substitutions in single cells. While providing greater function insight, these sequencing-based methods are not yet scalable to exhaustive mutagenesis, necessitating selection of target mutations. In this work, we hypothesized that examining the consequences of perturbing distinct protein interactions could provide a useful abstraction of the phenotypic space reachable by individual amino acid substitutions. To explore this hypothesis, we employed a Perturb-seq style approach to generate mutations at physical interfaces of the transcription factor RUNX1, with the potential to perturb different interactions, and therefore produce transcriptional readouts implicating different aspects of the RUNX1 regulon. We analyzed these readouts to identify functionally distinct groups of RUNX1 mutations, characterize their effects on cellular programs and study the implications for cancer mutations. Our work demonstrates the potential of targeting protein interaction interfaces to better define the landscape of prospective phenotypes reachable by amino acid substitutions.

**LB066**
**Machine learning algorithm for drug discovery targeting new co-transcription factors of HIF1A.**

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Hypoxia-inducible factor 1 alpha (HIF1A) activation drives cellular adaption to low oxygen stress in malignant and non-malignant cells. HIF1A transcriptionally regulates many genes in key processes like angiogenesis and metastasis, facilitating the cell's survival. Interestingly, HIF1A is able to carry out its regulatory functions by forming protein-protein interactions with its co-transcription factors. Since low oxygen conditions are frequently present in cancerous cells, we predict that these co-transcription factors could serve as new cancer therapeutic targets. Our recent work has thus focused on identifying motifs in HIF1A ChIP-Seq sequences and discovering novel HIF1A co-transcription factors. In this study, we leveraged cutting-edge deep learning methods, including a hybrid convolutional neural network (CNN) and recurrent neural network (RNN) based motif-discovery model, and discovered several novel motifs. We also look to predict potential therapeutic drugs against the identified co-transcription factors using a machine-learning drug discovery model that evaluates therapies based on their half-maximal inhibitory concentration (IC50). We have so far found several small molecules that could adequately modulate the activity of the identified co-transcription factors. Our results could lead to new therapeutic approaches against HIF1A-dependent cancers such as colon, breast, gastric, lung, brain, and prostate.

**LB067**

**A confident and operator-independent deep segmentation model to measure residual tumor volume in the follow-up MRIs for glioblastoma.**

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Introduction: Magnetic resonance imaging (MRI) is the most common tool to examine glioblastoma. Preoperative MRI can be used to initial diagnosis, and surgery planning, while follow-up MRIs can be used to evaluate treatment responses, identify recurrency, and detect side effects. The follow-up MRIs are usually taken after the first-line therapy, such as maximal safe resection. In the past, radiologists manually segment the tumor regions from normal brain tissue on follow-up MRIs, which is time-consuming, error-prone, and challenging. Several deep-learning (DL) models have been developed utilizing preoperative images, but their performance has yet to be evaluated on follow-up MRIs. In this research, we built the largest follow-up MRI cohort (311 patients) to assess these DL models and their generalizability and performance on independent preoperative and follow-up images. We also made the first follow-up-based deep learning models for this specific task.
Methods: All evaluation deep learning models (10 models) were trained by the Brain Tumor Segmentation challenge 2020 (BraTS'20) and evaluated by fifty pairs of preoperative and follow-up scans from our institution. The segmentation form our institution is evaluated by board certified radiologist. MRIs in the BraTS'20 dataset were all preoperative scans. After the evaluation, we randomly assigned 264 patients' scans from our institution to the training dataset and 47 patients' scans to the testing dataset. We compared three types of models in our follow-up deep learning model, including 1) UNet-3D, 2) UNet-3D+transfer-learning, and 3) UNet-3D+transfer-learning+baysian-learning. The benchmark for all models was the Dice similarity coefficient (DSC). DSC can measure the spatial overlap between model prediction and ground truth. The value of DSC is between zero to one. Zero means no overlap and one indicates complete overlap.
Results: Our study demonstrates that the BraTS'20 trained models' performance decreased by 13.05% in independent preoperative MRI scans and 19.04% in follow-up MRI scans. The most significant mismatch regions were FLAIR hyperintense regions (3.68% drop in independent preoperative scans and 10.61% drop in independent follow-up scans ) and Non-enhancing core (5.20% drop in independent preoperative

scans and 11.99% drop in independent follow-up scans ). Our best model can achieve the best DSC among three tumor regions compared to all evaluation models (FLAIR hyperintense regions: DSC 0.77 V.S. DSC 0.58; Enhancing tumor region: DSC 0.87 V.S. DSC 0.68; Non-enhancing tumor region: DSC 0.92 V.S. DSC 0.55 ).
Conclusion: Maximal safe resection induced brain structure change, decreasing the performance of the preoperative-based DL model. Implementing a follow-up MRI-based segmentation model is essential to make accurate and generalizable results to address structural changes after maximal safe resection. Our follow-up DL model demonstrates the DSC score can be recovered. We commit to further developing the tool to assist radiologists in handling follow-up MRIs for glioblastoma patients.

**LB069**
**Predictive modeling of drug sensitivity based on the genetic dependency.**
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Functional genetic screens have been frequently used to repurpose existing drugs or to identify new drug targets. However, the associations between hits on screens and candidate drugs have been unclear, and a manual approach to directly infer drug candidates from “druggable” candidates suffers from its laborious, ad hoc investigation and low accuracy. Here, we describe a systematic approach to explore the associations between genetic dependencies and drug sensitivity of cancer cell lines and to identify potential drug repositioning opportunities. We first developed drug sensitivity predictive models trained on genome-wide CRISPR-Cas9 knockout viability profiles and large-scale drug response screens of 4,518 drugs from DepMap. We obtained the highest predictive power from the decision-tree-based models and observed previously reported associations between gene perturbation and drug response. For example, cancer cells that are more sensitive to *TP53* or *MDM2* perturbation were more sensitive to MDM inhibitors, and *EGFR* or *ERBB2* dependency strongly predicted the sensitivity of cells to EGFR inhibitors. Interestingly, for 3,260 drugs with known targets, only 1.5% of these genes were selected as top features to predict drug sensitivity, indicating that genes that are not the direct targets of drugs should be considered. In addition, we applied our models to the CRISPR-Cas9 knockout screens that target 1,805 “druggable” genes and predicted the sensitivity of cancer cells to known and novel drugs. Furthermore, we were able to validate the candidate drugs using cell viability assays. Finally, a multi-class classification deep learning model, based on the TorchDrug framework, reaches higher recall values than a decision-tree-based method but with much lower precision values. Our study provided the first systematic evaluation between large-scale gene dependency and drug responses. The machine learning predictive model provides an in-silico approach to perform drug screens from functional genetic screens.

**LB070**
**Predicting distant recurrences in invasive breast carcinoma patients using their clinicopathological profiles.**
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Purpose: To determine whether the classification-based machine learning (ML) or artificially intelligent (AI) techniques can predict distant recurrence flags (yes or no) in invasive breast cancer patients using the data comprising several clinicopathological measurements such as pathological staging of tumor and surrounding nodes deemed both pre and post neoadjuvant (i.e. either chemo, radiation or hormone based) therapy including imaging based therapy-response as well and the status of adjuvant therapy (Chemo or

Anti-Her2/Neu antibody) therapy were administered to patients post resection of their tumor to minimize the possibility of diseases-recurrence in them.
Method: The clinicopathological data from a retrospective study of 900 breast cancer patients at Duke university (posted at The Cancer Imaging Archive, i.e. TCIA) was tapped for our study. These patients received neoadjuvant therapies such as Chemo-, Radiation or Endocrine Hormone based therapy, had their responses evaluated through imaging and pathological staging (both tumor and nodal), and received Adjuvant therapies (such as Chemo or Anti-Her2/Neu antibody therapy) post tumor resection . The patient entries pertaining to ungraded tumor responses from therapies or those labelled as 'Not applicable (NA)' for any of the aforementioned clinicopathological parameters were filtered out retaining 161 patient-entries which were further split into train and test sets in 90:10 ratio where 90% were used for training ($n_{train}$=144) and 10% for testing ($n_{test}$=17) of several ML models. Classification based machine learning models such as Random Forest (RF), C-Support Vector Classification (SVC) and Supervised Neural Network (aka Multi-Layer Perceptron, i.e. MLP) were employed to train and test aforementioned clinicopathological parameters to predict distant recurrence flags or labels (i.e. Yes or No). The training of the model was conducted using ImaGene software. Out of the three aforementioned models, RF seemed to have performed best with grid search activated over tree-depth hyperparameter to predict distant recurrence flags in test patients ($n_{test}$=17) at AUC=1.0 ($p<0.002$). Further, the validation of the model was conducted using external clinicopathological dataset of 20 patients from Dartmouth-Hitchcock (DH) Medical center which yielded AUC>0.75.
Conclusion: Random Forest model trained using grid search through tree-depth hyperparameter can predict distant recurrence flags (Yes or No) in invasive breast cancer patients at AUC in the range of 0.75 1.0 across multiple institutions, thereby aiding portability of models and advancing the idea of multi-instutional ML/AI model validations in Invasive Breast Carcinoma subtype of Breast Cancer.

**LB071**

**A phenomics platform combining imaging and artificial intelligence for rapid validation and advancement of novel oncology targets.**

Jenny Rudnick, Kiran Nadella, Chase Neumann, Shane Rowley, Ethan Gardner, Shadi Swaidani, Aimee Iberg, Lu Chen, Daria Beshnova, Aurora Blucher, Rebecca Sarto Basso, Malini Rajan, Kevin Fales, Ashraf Saeed, Christopher Bailey, Weston Judd, Chrissy Egbert, Joel Ellis, John Ansede, Pouya Hadipour, Kevin Jessing, Janet Paulsen, Paul Rearden, Vamshi Manda, Sashi Kasimsetty, Sashi Kasimsetty, Michael Hancock, Harish Shankaran, Bryan Ellis, Meenakshy Iyer, Carl Brooks, Ashish Bhandari, Chris Gibson, Irit Rappley, Laura Schaevitz, Imran Haque, Hayley Donnella, Michael Cuccarese, Marie Evangelista.
Recursion Pharmaceuticals, Salt Lake City, UT.

The emergence of technological innovations has created the opportunity to envision new approaches to discover therapeutics at scale. We combined advances in high content microscopy with arrayed CRISPR genome editing techniques and machine learning (ML) to build a rigorously controlled dataset enabling exploration of biology and chemistry at scale. Phenotypes from millions of perturbations in multiple cell types were embedded in a unified representation space and leveraged to accelerate discovery and reverse translation, ultimately yielding novel biological insights, and optimizing the advancement of lead molecular series through structure-activity relationships (SAR). Here, we demonstrate the capability of our platform to discover potential cancer therapies with distinct mechanisms of action. First, we describe the identification of a novel compound series that potentiates the effects of immunotherapy in syngeneic mouse models, producing complete responses and immunological memory, while also limiting peripheral inflammation. Specific novel chemical entities (NCEs) caused robust CD45+ cell influx into the tumor microenvironment and significantly attenuated exhausted T cells and immunosuppressive macrophages, thereby enhancing anti-tumor immunity. Strikingly, the same NCEs suppressed peripheral inflammation while sustaining elevated levels of intra-tumoral proinflammatory cytokines. Second, we highlight a novel and differentiated strategy to potentiate PARP inhibitor response in homologous repair deficient (HRD) -

negative or HR-proficient ovarian cancers. NCEs altered the expression of genes within the DNA damage repair (DDR) network and cell cycle checkpoints to synergize with PARP inhibition *in vivo* and re-sensitized a PARP-resistant patient-derived xenograft (PDX) model. Collectively, we believe future efforts on the industrialization and integration of various technological innovations across biology, chemistry, automation, data science, and engineering will ultimately modernize drug discovery and radically improve patient lives.

**LB073**

**NCI's broad-use collections: Accelerating discovery process by improving access to individual-level genomics and other -omics data.**

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Genomic data sharing increases our understanding of factors that influence health and diseases by enabling additional research questions from secondary data users, increasing statistical power through combining multiple data sources, facilitating reproducibility and validation of research results, and supporting innovation with the development of research tools and methodologies. Research using large-scale genomic and other-omics data is no longer limited by storage space or slow downloading speed for individual datasets. Innovations like cloud infrastructure enable computing over many datasets at multiple locations at once. These developments have increased the need for faster, more efficient processes for data access and sharing. To support scientific exploration and meet the demand for analysis of biomedical data, the National Cancer Institute (NCI) has created two large collections of the broad-use studies within the database of Genotypes and Phenotypes (dbGaP). These collections comply with the consent of the study (data use limitations) for how secondary access to studies are determined. NCI's Collection of Datasets for General Research Use comprises 284 studies with individual-level data sets, and permits approved users to explore broad research interest, including methods and tool development. NCI's Collection of Datasets for Health, Medical, and Biomedical (HMB) Research Purposes is comprised of 65 studies of individual-level that are permitted for research interests specific to any health, medical, or biomedical research only. The HMB collection could be used for methods and tool development; research interests involving ancestry/populations studies must be dependent on a health/medical condition. Through these collections, investigators will have the potential to add access to 349 datasets to their approved research projects or submit a new project request for access to the broad-use collections. As new genomic studies are registered by NCI for release through dbGaP, they will be automatically added to these collections. NCI anticipates through the implementation of these broad-use collections a requestor could have access to approximately 70% of NCI's studies in controlled-access repositories.The streamlined access to broad-use datasets expedites data sharing and potentially accelerates the discovery process. This approach reduces redundancies for obtaining controlled-access data.

**LB074**

**Towards the next generation cancer dependency map.**

Kirsty Wienand, Samantha Stokes, Philip G. Montgomery, Kelly Sullivan, Joshua M. Dempster, William C. Hahn, Jennifer A. Roth, Francisca Vazquez. Broad Institute of MIT and Harvard, Cambridge, MA.

Cancer genome alterations that cause cancer cells to grow also confer vulnerabilities, which normal cells lack. The challenge is that, for the majority of cancers, we do not understand the relationship between the genetic alterations and the dependencies they cause. To solve this problem, we are creating a Cancer Dependency Map (DepMap) by systematically identifying genetic dependencies and small molecule sensitivities as well as discovering the biomarkers that predict them. Since its inception, DepMap has compiled data for over eighteen hundred cell line models, performed more than a thousand CRISPR

screens, and drug sensitivity profiles using PRISM in over 500 cell lines as well as developed new analytic methods for the identification of cancer vulnerabilities. DepMap is being used by researchers around the world and numerous potential therapeutic targets have been identified using this resource. However, we are still in the early stages of having a complete map and we will discuss our ongoing work towards making the Cancer Dependency Map more powerful. The next generation DepMap will include more complex cellular models including 3D models, representing pediatric and rare cancers, and expanding the perturbation space to include combinations. Additionally, we are performing novel types of omics characterization and building new computational and visualizations tools. These new scientific directions will help make progress towards our identification of the landscape of cancer vulnerabilities and create a roadmap for cancer therapeutics.

**LB075**
**Cancers adapt to their mutational load by buffering protein misfolding stress.**
Susanne Tilk, Judith Frydman, Dmitri Petrov, Christina Curtis. Stanford University, Stanford, CA.

In asexual populations that don't undergo recombination, such as cancer, deleterious mutations are expected to accrue readily due to genome-wide linkage between mutations. Despite this mutational load of often thousands of deleterious mutations, many tumors thrive. How tumors survive the damaging consequences of this mutational load is not well understood. Here, we investigate the functional consequences of mutational load in 10,295 human tumors by quantifying their phenotypic response through changes in gene expression. Using a generalized linear mixed model (GLMM), we find that high mutational load tumors up-regulate proteostasis machinery related to the mitigation and prevention of protein misfolding. We replicate these expression responses in cancer cell lines and show that the viability in high mutational load cancer cells is strongly dependent on complexes that degrade and refold proteins. This indicates that upregulation of proteostasis machinery is causally important for high mutational burden tumors and uncovers new therapeutic vulnerabilities.

**LB076**
**A novel spatial trajectory inference method for detecting regional breast cancer progression from spatial transcriptomics data.**
Duy Pham, Quan Nguyen. University of Queensland, Brisbane, Australia.

Spatial transcriptomics is a rapidly emerging field that allows for the study of gene expression within a tissue sample. This technology has the potential to provide valuable insights into the molecular mechanisms underlying cancer progression and could be particularly useful in identifying specific regional cancer progression. By studying the regional spatial trajectory of cancer progression, we can develop more targeted therapies and better predict the likelihood of metastasis. Additionally, analyzing gene expression patterns in specific regions of the tissue can reveal the molecular mechanisms driving cancer growth in those areas, potentially uncovering new therapeutic targets.
In this study, we created a spatial trajectory analysis method, named as stLearn - PSeudo-Time-Space (PSTS) algorithm, to investigate specific regional cancer progression. Spatial trajectory analysis is a statistical method that allows for the identification of patterns of transitional gene expression over time and space within a tissue sample. In detail, this method incorporates spatial features, such as tissue sub-structure locations and gene expression changes, to model the trajectory from a single snapshot of transcriptomics data. Also, we evaluated the effectiveness of PSTS by utilizing the cancer progression model in human breast cancer samples.
Using this approach, we were able to identify specific regions within the breast tissue that displayed distinct progression branching trajectories patterns of transitional gene expression associated with ductal carcinoma in situ and invasive ductal carcinoma. These regions were found to be characterized by a high

level of cellular diversity and were associated with aggressive forms of breast cancer.
The results of this study suggest that spatial transcriptomics and spatial trajectory analysis could be a powerful tool for identifying specific regional cancer progression and may help to inform the biomarkers for the development of targeted therapies for breast cancer.
Overall, this study highlights the potential of spatial transcriptomics and spatial trajectory analysis to provide valuable insights into the molecular mechanisms underlying cancer progression and could have significant implications for the diagnosis and treatment of cancer.

**LB077**

**Analysis of indel and structural variant error profiles in deep next generation sequencing data.**

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**Background:** Accurate detection of low frequency mutations is of critical importance in the study of genetic heterogeneity, such as on the detection of minimal residual diseases for leukemias. Our prior work has resulted in successful error suppression for substitutions ($10^{-4}$-$10^{-5}$). However, the error profiles of indels and structural variants (SVs) remain elusive.
**Results:** In this work, we generated ultra-deep sequencing data using our previously established dilution models (COLO829) on known somatic indels (n=23) and SVs (n=17). We discovered that the error rate of indels ($10^{-6}$) and SVs ($<2\times10^{-7}$) are 100- to >1000-fold lower than that of SNVs. This finding was fully recapitulated in our analysis of 347 indels and 1248 SVs discovered from a relapsed B-ALL cohort of 103 patients, although homopolymer indels can have high error rates (>1%). We then performed a comprehensive study of homopolymer indels in 361 cancer driver genes by using whole genome data from 1662 healthy donors from the SJLIFE cohort. Our data indicated that the number of repeating units are highly predictive relative to the error rate of homopolymer indels ($R^2$=0.988, $p=4.89\times10^{-8}$). Utilizing these insights, we assayed end-of-induction remission samples from 72 B-cell lymphoblastic leukemia patients that relapsed by selecting ~5 somatic clonal SNV/Indel/SV markers, which confirmed that SVs and indels have >10-fold lower error rates than SNVs. Our next generation sequencing (NGS) approach had 44 positive detections (61%) and outperformed the current standard method of clinical flow cytometry (n=37; 51%) for detecting minimal residual disease. The NGS-based method detected 92% of designed markers for samples with MRD>0.3%, and this detection rate dropped to 27% for MRD between 0.1% and 0.01%, indicating the difficulty in recovering mutant molecules when their frequencies are very low.
**Conclusions:** Overall, we established indel and SV error profiles in deep next generation sequencing data enabling superior tumor detection performance at very low burdens, with lower error rates than what is observed for SNVs. Our work will have a significant impact on the clinical diagnosis and monitoring of human cancers and beyond.

**LB078**

**pbfusion: Detecting gene-fusion and other transcriptional abnormalities using PacBio HiFi data.**

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Sarcomas are a broad group of soft tissue and bone cancers that can be difficult to treat leading to a high mortality rate. Sarcomas comprise two broad genomic classes: (1) simple karyotypes, where a single oncogenic structural variant (SV) clonally expands a subtype that is diagnostic and relevant to tumor burden tracking; and (2) complex karyotypes, genomic instability, where SVs continuously arise throughout tumor evolution resulting in heterogeneous cellular subtypes. Class two sarcomas are harder to characterize using genome sequencing because there may be multiple low-frequency mutations. In both genomic classes, accurate and sensitive detection of fusion transcripts is needed to interpret functional consequences, to understand tumor biology and evolution, and potentially identify new targets for therapy. Many fusions have complex structures that cannot be uniquely resolved using short reads due to a lack of exon connectivity. PacBio full-length RNA isoform sequencing resolves complex fusions, providing more accurate breakpoints, and a complete sequence readout of the associated fusion transcript. To date, long-read fusion detection software was designed for high-error sequencing. PacBio HiFi data provides both full-length transcripts and accurate base calls. Here we present a fusion detection tool, pbfusion, specifically designed for HiFi sequence data, and apply it to sarcoma patients from both classes. pbfusion converts mapped sequences (either HiFi reads or Iso-Seq isoforms) into transcript objects that are annotated with reference gene models. Annotations determine whether transcripts are discordantly mapped, overlap differing genes, strand swap, transcriptional readthrough, or contain novel exons. The discordant exonic boundaries are treated as breakpoints between two genomic locations. All breakpoints are clustered with a multi-directional chaining algorithm and annotated with exonic information, gene names, and quality information.
To test our method, we applied pbfusion to twelve samples from 8 sarcoma patients from both genomic classes. We discovered the known and novel fusions, including validated driver events in the fusion-driven samples (e.g. ASPSCR1-TFE3 in alveolar soft part sarcoma and SS18-SSX2/1 fusion in synovial sarcoma). This approach demonstrates the utility of HiFi sequence data for identification of fusion transcripts in patient samples, and the use of pbfusion in quantifying and annotating these events. pbfusion provides a user-friendly interface, can process a sample in a few minutes, and is freely available to the research community on Bioconda.

**LB079**

**An end-to-end Visium spatial transcriptomics computational pipeline for generating low-code interactive reports of spatial insights.**

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In recent years, methods for analyzing and interpreting expression data have evolved rapidly. Newer and more complex datasets are being developed faster than ever, and the resources needed to perform rigorous data analysis have increased dramatically. In spatial transcriptomics analysis, researchers face many technical challenges: data management, compute power, and programming knowledge. In addition to these technical barriers, a constantly evolving field can make it difficult for scientists to understand if they are performing adequate quality control, proper data integration, choosing situationally correct methods, and showing accurate biological insights. Transcriptomics data comes with many meaningful caveats that make having the proper technical resources just a single piece of the puzzle. These obstacles keep scientists with valuable biological questions from maximizing the power of Spatial Transcriptomics. To

address these challenges, we developed an end-to-end Visium spatial transcriptomics computational pipeline for generating interactive figures and reports for low-code exploration. This pipeline automated data quality control, batch-effects correction, and multiple peer-reviewed analysis techniques to perform a spatially resolved analysis of tissue heterogeneity. To demonstrate the effectiveness of this pipeline, we performed an analysis on a cohort consisting of 3 patients with adenocarcinoma and 1 patient with signet ring cell carcinoma, each with 1 sample of primary colon tumor and its matched adjacent normal tissue. All samples were formalin-fixed paraffin-embedded (FFPE) provided by BioChain Institute. The tissue sections were stained with Hematoxylin and Eosin, and the transcriptome was mapped using 10x Genomics Visium Spatial Gene Expression for FFPE. The raw base call files were then processed into feature-barcode matrices, followed by quality control measures that included data filtering, sample integration, batch-effect removal, and normalization. The pipeline then combined multiple analysis methods for feature extraction from genes and spatial spots, including clustering, neighborhood detection, and gene module detection. Further, we leveraged clinical metadata to perform differential expression and abundance analyses of the extracted features across different tissue and disease types. The pipeline finally generated an interactive report detailing analysis of spatially-resolved tissue biology, pathology, and microenvironment, allowing a scientist to analyze and explore the data further. This pipeline to identified differences in the spatial arrangement between tumor and normal tissue, in addition to demonstrating the spatial arrangements that differentiate adenocarcinoma and signet ring cell carcinoma relating to the invasive front and body of the tumors.

**LB080**

**SAVANA: a computational method to characterize structural variation in human cancer genomes using nanopore sequencing.**

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Whole-genome sequencing (WGS) of human cancers has revealed that structural variation, which refers to the rearrangement of the genome leading to the deletion, amplification of reshuffling of DNA segments ranging from a few hundred bp to entire chromosomes, is a key mutational process in cancer evolution. Notably, pan-cancer analyses have revealed that both simple and complex forms of structural variation are pervasive across diverse human cancers, and often underpin drug resistance and metastasis. To date, the study of cancer genomes has relied on the analysis of short-read WGS on the dominant Illumina platform, which generates short, highly-accurate reads of 100-300bp that allow the study of point mutations at high resolution. However, detection of structural variants (SVs) using short reads is limited, as breakpoints falling in repetitive regions cannot be reliably mapped to the human genome. As a result, our understanding of the patterns and mechanisms underpinning structural variation in cancer genomes remains incomplete.

In contrast to short-read sequencing, long-read sequencing technologies, such as Oxford Nanopore and PacBio, permit continuous reading of individual DNA molecules over 10 kilobases, thus providing unparalleled information to resolve SVs in repetitive regions and complex genome rearrangements. However, novel bioinformatics methods that account for the higher error rate of long-read methods are needed to take advantage of their capabilities for cancer genome analysis.

Here, we present SAVANA, a novel structural variant caller for long-read sequencing data specifically designed for the analysis of cancer genomes. To identify both somatic and germline SVs, SAVANA takes

as input long-read WGS data from a tumor and normal sample pair. SAVANA scans sequencing reads to detect split reads and gapped alignments, which are then clustered to define putative SVs. Next, SAVANA applies a machine learning-informed set of heuristics to remove false positives arising from mapping errors and sequencing artifacts.
Extensively validated against a multi-platform truthset, we show that SAVANA identifies a range of somatic rearrangements with high recall and precision, outperforming existing tools while maintaining a lower execution time than competing methods. In patient samples, SAVANA identifies clinically relevant alterations, such as oncogenic gene fusions, with high accuracy. Additionally, SAVANA permits the reconstruction of double minutes, multi-chromosomal chromothripsis events, and SVs mapping to highly repetitive regions, including centromeres. In sum, SAVANA permits the characterization of complex structural variants and can uncover clinically relevant mutations across diverse cancer types with high accuracy.

**LB081**

**Concurrent characterization of somatic nucleotide mutations, 5mC, and 5hmC methylation in clinical research samples using a single nanopore sequencing assay.**

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Genomic and epigenomic variants accumulate during cancer progression, resulting in a tumor consisting of heterogeneous collections of cells, or clones, with distinct profiles of genomic and/or epigenomic alterations. The ability to characterize a broad set of somatic (epi)genomic variations simultaneously, using a single sequencing assay, facilitates cancer research and can help inform treatment.
Here we describe a comprehensive approach to analyze native tumor DNA with long nanopore reads. First, we demonstrate that shallow (<1x) whole genome nanopore sequencing provides sufficient information to observe large-scale copy number aberrations. This enables rapid and affordable screening of samples with an aim of selecting those that contain variation(s) of potential interest; such samples can then be sequenced to higher coverages for a higher resolution picture of specific structural variants.
We further demonstrate an analysis workflow vimba for high coverage (>30x) nanopore sequencing of tumor/normal datasets that detects clone- and haplotype-resolved structural variations in patient-derived research samples. We provide an example of haplotype- and breakpoint-resolved compounded amplification and loss of heterozygosity (LOH) of a region containing the EGFR gene in an admixed kidney tumor sample.
Finally, we describe the suitability of this assay to gather genome-wide (epi)genomic variations (e.g., SNPs, indels, SVs, microsatellite instability) across tumor samples of different cancer types. For the first time with native long nanopore reads we are able to capture 5mC and 5hmC methylation information and map it over some of the most challenging repetitive regions of the genome. This information can empower a better understanding of cancer-type-specific (epi)genomic alterations, which in turn can help map out the landscape for further research.
Our approach demonstrates how nanopore sequencing can be used as a single comprehensive platform to better understand and resolve the complexity of (epi)genomic somatic alterations in tumor samples.

**LB082**

**The CytoR1: a standardized dielectrophoretic platform for label free sorting.**

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Dielectrophoresis (DEP), a label-free electrokinetic technique, has a long history of being used to characterize and separate target subpopulations from mixed samples. A large body of literature has investigated these parameters with unique setups and limited cell varieties. The technical challenge of the technique coupled with the uncertainty in the breadth of data has limited the commercialization and widespread adoption of DEP for biological applications. The work presented here aims to evaluate a range of prior applications of DEP on a standardized platform. A variety of cell types were characterized and compared to literature values considering variables such as frequency, voltage, and flow rate. Once a cell type's frequency response curve is generated, it is possible to design workflows to enrich that cell type from a bulk mixture, without antibody labeling. Here, we test various cell types and characteristics on a microfluidics-based, benchtop cell sorting technology, the CytoR1. First, the CytoR1 successfully facilitated the separation of ovarian cancer stem cells (CSCs) from peripheral blood mononuclear cells (PBMCs), both from mice. From a 56.2% CSCs and 43.8% PMBCs co-culture, we were able to deplete 50% of the PMBCs while retaining the original CSC population. Further work investigated the ability to sort neuron subpopulations. Live neurons can be isolated from a bulk preparation and further enriched by size and phenotype with varying voltage and frequency settings. By modulating voltage and flow rate in addition to frequency, a distinct population of small neurons can be isolated for further study. Finally, cell state can be investigated. Red blood cells were characterized in both their native and oxidatively stressed states. Curves show a distinct shift towards higher frequencies with increasing oxidative stress suggesting the ability to separate these populations from each other. Together these data suggest the versatility of a robust, DEP-based sorting platform, the CytoR1, for label free cell sorting.

**LB085**

**Antitumor activity of AZD0754, a dnTGFbRII armored STEAP2 targeted CAR-T therapy, in preclinical models of prostate cancer.**

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Prostate cancer is traditionally considered an immunologically "cold" tumor type rendering patients insensitive to immunotherapy. Targeting surface antigens on tumors through cellular therapy can induce a potent anti tumor immune response to "heat up" the tumor microenvironment. However, many antigens expressed on prostate tumors are also found on normal tissues, potentially causing on-target, off-tumor toxicities and a sub-optimal therapeutic index. Our target discovery and validation efforts identified STEAP2 as a superior prostate antigen for therapeutic targeting. Importantly, STEAP2 is a highly prevalent prostate cancer antigen displaying high, homogeneous cell surface expression across all stages of disease. A novel lead generation approach facilitated the development of a potent and specific armored STEAP2 CAR-T therapeutic candidate, AZD0754. This second generation CAR-T product is armored with a dominant-negative TGFβRII, thereby bolstering activity in the TGFβ-rich immunosuppressive environment of prostate cancer. Armored STEAP2 CAR-T cells demonstrate favorable in vitro properties, robust dose dependent in vivo efficacy in STEAP2 expressing cell line- and patient derived- mouse xenograft models and encouraging preclinical safety. Taken together, this data builds confidence in the specificity and potency of this potential first in class STEAP2 targeted CAR-T therapy and supports future clinical development.

**LB087**

**IFN-gamma targets tumor vascular endothelial cells, causing impaired perfusion and tumor growth suppression in adoptive T cell therapy.**

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CD8 T cell-secreted interferon-gamma (IFNγ) is a key factor in controlling adoptive T cell therapy (ACT) outcome. However, IFNγ effects on non-tumor cells (e.g., stroma and blood vessels) that could indirectly impact the therapy are not well characterized. We aim to dissect the spatiotemporal effects of IFNγ in the tumor microenvironment in ACT and the underlying mechanism of the therapy. An ACT using *ex vivo*-activated ovalbumin (OVA)-specific OT-1 T cells suppressed the growth of MCA-205-OVA-GFP fibrosarcoma or MOC2-SIINFEKL oral squamous cell carcinoma in wild-type mice. However, in IFNγR1-deficient hosts, the ACT failed, indicating that non-tumor cells were crucial targets of IFNγ. Furthermore, the efficacy was also abrogated in bone-marrow chimeras generated by transferring the wild-type bone-marrow to lethally irradiated Tie2-Cre-Ifngr1$^{flox/flox}$ mice. As IFNgR1 expression is specifically deficient in endothelial cells in these chimeras, this result indicates that IFNγ action on endothelial cells is indispensable for ACT. Early after T cell transfer (1.5 days), the fraction of OT-1 T cells infiltrating the tumor was small (0.098% of CD45$^+$ cells) but produced high IFNγ (36.3%), resulting in the peak IFNγ concentration (47.3 pg/ml) in the whole tumor tissue in a 7-day observation period. In contrast, the highest accumulation of OT-1 T cells with low IFNγ production (2.9%) was observed on Day 4.5. Intravital imaging revealed that at Day 1.5, IFN-γ-RES-YFP-OT-1 T cells with a high level of IFNγ secretion (strong YFP$^+$ signal) localized mainly along the tumor vessels. While more OT-1 T cells were observed in the MCA-205-OVA-GFP tumor tissue near the blood vessels on Day 4.5 than on Day 1.5, their IFNγ secretion was minimal. Degeneration of endothelial cells was observed via electron microscopy 3-4.5 days after T cells transfer. Consistent with this finding, CD31$^+$ endothelial cell density decreased on Day 4.5 compared to Day 1.5 and significantly reduced blood perfusion in the entire tumor as observed by near-infrared imaging after IR800-albumin infusion. H&E staining of tumor sections showed tumor necrosis on Day 4-7. In summary, our study demonstrates that endothelial cell targeting of CD8 T cell-derived IFNγ is critical for tumor suppression in ACT, impairing blood perfusion. These results provide novel insights into the mechanism of ACT.

**LB089**

**ACE2016: an off-the-shelf EGFR-targeting γδ2 T cell therapy against EGFR-expressing solid tumors.**

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Introduction: CAR-αβT therapies have been shown to improve clinical outcomes in hematological malignancies; however, solid tumors still remain as challenges to CAR-αβT therapies due to tumor microenvironment, limited tumor infiltration or antigen escape. Previous research findings indicate γδ2 T cells are involved in tumor surveillance by responding to phosphoantigen overexpressed by most of cancers. The antibody cell conjugation (ACC) technology has the advantage to link cancer-targeting antibodies on cell surface of immune cells without genetic modification. This study applies ACC technology to generate an off-the-shelf EGFR-targeting γδ2 T cell therapy ACE2016, and presents the promising potency of ACE2016 against EGFR-expressing cancer cells.
Methods: γδ2 T cells were expanded from healthy donor PBMCs and αβ T cell population were depleted. Both γδ2 T cells and αEGFR were covalently conjugated to selected DNA aptamers that enable DNA hybridization to generate EGFR-targeting γδ2 T cells, ACE2016. The characteristics and antibody conjugation of ACE2016 was evaluated by flow cytometry, and *in vitro* cytotoxicity and *in vivo* anti-tumor potency was investigated using luminescence-based cytotoxicity assay and the orthotopical model with EGFR-expressing cancer cells, respectively.
Results: ACE2016 showed nearly 100 % of αEGFR antibody conjugation and exhibited EGFR-specific binding activity. These features conferred ACE2016 with enhanced *in vitro* cytotoxicity against EGFR-expressing cancer cells compared to unconjugated γδ2 T cells, while ACE2016 and γδ2 T cells showed no

significant difference of anti-tumor potency against EGFR-negative cancer cells. Further characterization studies demonstrated that co-cultured with target cells significantly activated ACE2016 along with enhanced degranulation and cytokine production without measurable IL-6 secretion. Moreover, ACE2016 suppressed EGFR-expressing breast cancer cells *in vivo* in the orthotopic xenograft model without weight loss or toxicological observations.
Conclusion: ACE2016, an EGFR-targeting γδ2 T cell product, was successfully generated as an effective off-the-shelf treatment for EGFR-expressing solid tumors. This study provides the evidence for the in vitro and in vivo efficacy of ACE2016 against EGFR-expressing cancer cells to support the clinical application against EGFR-expressing tumors.

**LB090**
**IL-18 secreting chimeric antigen receptor T cells targeting glypican-3show superior persistence and antitumor immunity against hepatocellular carcinoma.**
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Over the last decade, immunotherapy has revolutionized the way we treat cancer primarily by boosting the body's own immune system to help fight cancer. In particular, an engineered T cell therapy, namely chimeric antigen receptor (CAR) T cell therapy, is a more aggressive way to modify T cells to recognize cancer cells and destroy them. Although these CAR-T therapies have shown remarkable clinical responses in patients with hematological malignancies, their efficacy in solid tumor treatment has been disappointing due to many challenges: lack of tumor-specific antigen targets, loss of the CAR T-cell persistence, the inability of CAR-T cells to effectively infiltrate into solid tumors, toxicities of cytokine release syndrome and neurologic toxicity, and the immunosuppressive tumor microenvironment (TME). In this regard, we report here that we have successfully developed a CAR-T cell therapy, referred to as EU307, to treat one type of solid tumor, hepatocellular carcinoma (HCC). EU307 is a fourth-generation CAR-T therapy that targets the HCC-specific tumor antigen of glypican-3 (GPC3), and also secretes IL-18 which results in autocrine co-stimulation of CAR-T cells and reprogramming of the TME into a tumor-killing environment. Through a sophistically optimized manufacturing process in our good manufacturing practices (GMP) facility, we are able to manufacture CAR-T cells with stem cell memory (TSCM) and central memory (TCM) phenotypes. Functionally, EU307, when infused with as little as $0.1 \times 10^6$ total cells per animal, showed superior *in vivo* persistence and antitumor immunity in an HCC tumor-bearing mouse model. Furthermore, a single dose toxicity study in the same disease mouse model determined the no observed adverse effect level (NOAEL) as $5.0 \times 10^5$ total cells per male and $1.0 \times 10^6$ total cells per female. Taken together, our studies demonstrate that we have a developed a novel fourth-generation CAR-T therapy to treat HCC, a solid tumor that expresses a high-level of tumor-specific GPC3, by overcoming previously limiting factors in the development of CAR-T therapies against solid tumors.

**LB091**
**Design, characterization and preclinical validation of a combinatorial CAR-based immunotherapy against colorectal cancer with HER2 amplification.**
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Adoptive immunotherapy based on chimeric antigen receptor (CAR)-T cells has led to successful treatment of some hematological malignancies, but it remains extremely challenging for solid tumors, mostly because of "on-target off-tumor" toxicity, as observed in the case of anti-HER2 CAR-T treatment of colorectal cancer (CRC) with HER2 amplification. To enable adoptive immunotherapy against HER2-amplified CRC, we therefore considered a combinatorial strategy based on the synNotch-based artificial

regulatory network. A synthetic Notch receptor was employed in which the extracellular domain is an anti-HER2 scFv and the intracellular domain contains the GAL4-VP64 artificial transcription factor. Engagement of the anti-HER2 domain by target cells drives GAL4-VP64 cleavage and translocation to the nucleus, where it drives expression of a CAR under a GAL4-responsive promoter. In this way, only cells co-expressing both HER2 and the CAR target are killed. As a CRC-specific CAR target we selected CEA, product of the CEACAM5 gene. CEA expression is restricted to the digestive tract and is increased in cancer. For the generation of HER2-synNotch CEA-CAR effectors, we chose the natural killer cell line NK-92 and transduced it with two lentiviral vectors, encoding respectively the HER2 synNotch and the second-generation anti-CEA CAR with CD28 costimulatory domain. Transduced cells were repeatedly sorted in the ON and OFF state to select those with the best CAR induction after synNotch engagement; cloning of sorted cells led to identification of an optimally responsive clone (clone 5F), showing no basal CEA-CAR expression and massive induction in the presence of HER2-overexpressing cancer cells. In vitro, the 5F clone displayed selective cytotoxicity against HER2++/CEA++ CRC cells, with minimal killing activity against HER2++/CEA- breast cancer cells, or against CRC cells expressing CEA but without HER2 amplification. In vitro 3D models highlighted better recruitment and infiltration by NK-92 clone 5F respect to NK-92 WT cells, only of HER2++ organoids. In vivo, the clone 5F significantly impaired tumor growth in two different HER2++ CRC models. The observed selective efficacy both in vitro and in vivo of the HER2-synNotch/CEA-CAR approach opens a perspective for possible clinical applications in cases of HER2-amplified CRC displaying primary or secondary resistance to HER2/EGFR blockade.

**LB092**

**Identification of target antigens for logic-gated CAR-T therapeutics for the treatment of clear cell renal cell carcinoma: conditional targeting of CA9 with a PSMA PrimeR.**

Nickolas Attanasio, Levi Gray-Rupp, Stanley Zhou, Samuel A. Williams. Arsenal Biosciences, Inc., South San Francisco, CA.

Chimeric antigen receptor T (CAR T) cell therapeutics have produced curative outcomes in hematological malignancies but have yet to be developed into safe and effective therapies for solid tumors. A major obstacle to developing targeted therapies against solid malignancies is the identification of targets that are abundantly and broadly expressed on tumor cells while exhibiting an absence of expression on high toxicity risk normal tissues. Logic-gated CAR T cells that inducibly express CAR upon recognition of a target antigen by a secondary engineered priming receptor (PrimeR) offer a novel approach to permit conditional killing activity in the presence of two antigens. Here, we identify PSMA and CA9 as attractive priming and cytolytic antigen targets, respectively, in clear cell renal cell carcinoma (ccRCC). Using a bioinformatic discovery path, we show PSMA and CA9 mRNA are co-expressed in 94% of ccRCC tumors (n=530, TCGA). Immunohistochemical (IHC) assessments further reveal robust membranous PSMA and CA9 protein coexpression in 80% of ccRCC (n=416). Expression of CA9 and PSMA was evaluated across disease stage and co-expression was confirmed throughout disease progression. Distant metastases similarly showed evidence of common expression. In contrast, IHC assessment of normal tissues revealed limited membranous coexpression of PSMA and CA9 in normal high-risk toxicity tissues. These findings collectively support the utility of PSMA and CA9 as target antigens for AND-logic-gated therapeutics for the treatment of ccRCC.

**LB093**

**Engineering tumor infiltrating lymphocytes from sarcoma and colorectal tumors with membrane bound IL-15 for IL-2 independent expansion and enhanced cytotoxicity against autologous tumor cell lines.**

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Tumor infiltrating lymphocytes (TIL) have shown promising efficacy in immunologically “hot” solid tumors that have a high level of T cell infiltration, such as melanoma. However, current treatment regimens require high dose IL-2 administration to support TIL survival, which limits their clinical applications due to IL-2 related toxicity. Obsidian Therapeutics is engineering TIL with membrane bound IL-15 (mbIL15) to eliminate the dependence of TIL on exogenous IL-2, potentially enhancing the tolerability of TIL therapies. Because there is a high unmet medical need in patients with tumors colder than melanoma, we evaluated mbIL15-engineered TIL expansion and functionality from colorectal cancer (CRC) and sarcoma biopsies. Using an IL2-independent, proprietary rapid expansion process (REP) we successfully expanded mbIL15-engineered TIL from both CRC and sarcoma, despite significantly lower T-cell numbers in the tumor tissues (average of 12-15% CD45+ TIL versus 64% in melanoma). mbIL15-engineered TIL showed an enrichment for CD8+ T cells throughout the REP and high T cell receptor variable beta chain (TCR Vbeta) diversity. mbIL15-engineered TIL also exhibited lower immune checkpoint expression (LAG3/PD-1) and higher activation marker expression (CD25/CD27/CD28) when compared with unengineered TIL expanded from the same tumors using a conventional REP with IL-2. mbIL15-engineered TIL were polyfunctional, as defined by expression of more than one effector molecules (CD107a, perforin, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and granzyme b) in response to CD3/ CD28 stimulation. To examine the cytotoxic function of mbIL15-engineered TIL, we developed autologous cell lines from the same tumors that were used to generate the TIL. Using whole exome and RNA sequencing we found that the autologous tumor cell lines maintained expression of conserved tumor antigens and HLA-expression when compared with the primary tumor. When co-cultured with the autologous tumor cell lines, mbIL15-engineered TIL secreted higher levels of IFN-γ and induced higher cytotoxicity as compared to unengineered TIL cultured with IL-2. Taken together, these data demonstrate that mbIL15-engineered TIL can successfully be expanded from comparatively “cold” tumors with low T-cell infiltration, such as CRC and sarcoma, while maintaining high TCR diversity and polyfunctionality and demonstrating higher cytokine production and cytotoxic activity against autologous tumor lines, compared to conventional TIL with IL2.

**LB094**

**Arginine pre-conditioning improves T-cell potency and metabolic fitness measured by real-time impedance and seahorse assays.**

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Background: Enhanced T cell performance and fitness are imperative for the success of adoptive T cell-based therapies. Beyond the types of genetic modifications to CAR/TCR T cells, there is a growing body of literature demonstrating that relatively simple preconditioning protocols can also be used to improve T cell fitness/function. We studied, the impact that preconditioning in elevated concentrations of leucine, glutamine, and arginine has on the killing efficacy and bioenergetics of MART-1-specific TCR T cells. Methods: Using the Agilent xCELLigence RTCA eSight and Seahorse we assessed the killing efficiency and bioenergetics of engineered T-cells after Arginine, Glutamine, and Leucine pre-conditioning using MART-1 specific TCR T cells. $CD3^+$ T-cells (Hemacare, Seattle, WA) were transduced with retrovirus SAMEN-DMF5 with a CD34 marker gene, against MART-1. The T cells were pre-conditioned in a range of concentrations varying between 0-6mM for 7 days, followed by a killing assay using MART-1 expressing melanoma cell line as target cells (624.38) engineered to express a red-fluorescent nuclear protein. The comparison was made with transduced T-cells grown in RPMI (no added amino acid supplementation denoted as RPMI_TCR), RPMI supplemented with Arginine (Arg_TCR) and non-

transduced T cells. The T cell killing was measured using impedance/imaging-based assays. CD34 assessment was performed using Novocyte and SRC (spare respiratory capacity) and oxygen consumption rate (OCR) were measured using seahorse assays.
Results: Whereas supplementing the growth medium with 6 mM Arginine increased killing efficacy dramatically (up to ~6-fold), elevated leucine and glutamine concentrations were found to have minimal impact on MART-1 TCR T cell killing of melanoma cells. Arginine (6 mM) supplementation increased basal respiration, ATP linked OCR, and maximal respiration compared to the RPMI control. SRC of Arg_TCR T cells was significantly higher than RPMI preconditioned T cells, a parameter previously correlated with T cell persistence. To check the effect of a shortened pre-conditioning period, 2, and 4 days of pre-conditioning were done along with the 7 days method. After a preconditioning step of only 2 days, Arginine preconditioned T cells acquired a killing efficacy that is >2x higher compared to their counterparts RPMI_TCR T cells. Extending the duration of preconditioning from 2 to 4 days has minimal impact on the RPMI control T cells but more than doubles the killing efficacy of the high Arg grown T cells.
Conclusions: In conclusion, Arginine pre-conditioning significantly improved T cell potency and mitochondrial respiration through metabolic rewiring.

**LB095**
**Hybrid TCR-CAR design surpasses conventional CARs and patient-derived TCRs in targeting an ultra-low-density neoantigen.**
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Mutation-associated neoantigens (MANAs) are exquisitely cancer-specific therapeutic targets. However, MANAs are present at ultra-low densities on the cancer cell surface (as few as 1-2 copies per cell), leading to the challenge of eliciting a sufficiently robust therapeutic effect. We combined components of both T cell receptors (TCRs) and chimeric antigen receptors (CARs) to create a new receptor with improved potency against an ultra-low-density MANA. From CARs, we utilized the antibody-based antigen recognition domain (i.e. the single chain variable fragment, scFv) and the integrated co-stimulation that amplifies T cell activation. From TCRs, we utilized the multi-chain signaling platform that facilitates high antigen sensitivity. This new receptor, termed a TCR Embedded ScFv for Long-term Activation (TESLA), showed promising characteristics when tested with the H2-scFv which targets the p53 R175H mutation presented on HLA-A*02:01 (R175H/A2). Using CRISPR-based homology directed repair in primary human T cells, we tested 15 configurations of appending the H2-scFv to subunits of the TCR complex to identify a design that maximized T cell cytotoxicity and interferon gamma release in co-cultures with cancer cells expressing endogenous levels of the R175H/A2 antigen. In this system, we showed that the optimal TCR-embedded configuration of the H2-scFv produced similar levels of cytotoxicity and interferon gamma secretion as patient-derived TCRs targeting the same R175H/A2 MANA, while conventional H2-CARs were unable to produce any T-cell activation. We then used a multiple stimulation co-culture system to identify a co-stimulation domain combination (MyD88 and CD40) that improved serial cytotoxicity and proliferation of H2-TESLAs when incorporated on the intracellular side of the TCRbeta chain. Finally, we compared the H2-TESLA receptor to patient-derived TCRs modified with the same MyD88 and CD40 co-stimulation domains. In vivo, H2-TESLAs cured all mice in a tumor model, while co-stimulation-modified TCRs produced only temporary tumor control. Moreover, in vivo, H2-TESLAs elicited 100-fold greater T cell expansion than co-stimulation-modified TCRs. In conclusion, we demonstrated that by combining aspects of both CARs and TCRs, the TESLA

receptor improved T cell reactivity against an ultra-low-density neoantigen compared to conventional CARs and patient-derived TCRs.

**LB096**

**IL15-engineered tumor infiltrating lymphocytes (cytoTIL15$^{TM}$) exhibit activity against autologous tumor cells from multiple solid tumor indications without IL2.**

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Tumor infiltrating lymphocyte (TIL) therapy has shown promising results in the treatment of metastatic melanoma. However, TIL therapy has conventionally required co-administration of IL2, which is associated with toxicity in patients. We previously showed that melanoma TILs engineered to express membrane-bound IL15 (mbIL15) under the control of the ligand acetazolamide (ACZ) can achieve IL2-independent expansion during manufacturing, antigen-independent persistence *in vitro* and anti-tumor efficacy *in vivo*. In the current study, we extend the cytoTIL15 cell therapy product concept to indications beyond melanoma including non-small cell lung cancers (NSCLC), triple-negative breast cancers (TNBC) and head and neck squamous cell carcinomas (HNSCC), tumor types which represent significant unmet medical needs, particularly in the post-checkpoint inhibitor refractory setting. TILs from primary NSCLC, HNSCC and TNBC were engineered to express mbIL15 in the presence of ACZ and expanded in the absence of IL2 using our proprietary rapid expansion protocol (REP). CytoTIL15 cells were predominantly CD8 positive, enriched for mbIL15 expression and maintained T cell receptor variable beta chain (TCRVβ) diversity throughout expansion. *In vitro* antigen- and cytokine-independent survival and polyfunctionality of cytoTIL15 cells was measured from cultures that included ACZ. To assess anti-tumor activity, cytoTIL15 cells were co-cultured with autologous patient-derived cell lines (PDc) or tumor digests from patient-derived xenografts (PDx), and cytotoxicity and IFNγ release into supernatant was measured. *In vitro,* cytoTIL15 cells + ACZ exhibited similar or increased polyfunctionality compared to unengineered TIL + IL2. Unlike unengineered TILs, cytoTIL15 cells + ACZ persisted in an antigen-free setting without IL2, were cytotoxic to autologous PDc and released IFNγ in response to autologous PDx tumor digest. Taken together, these data show that IL2-independent, fully functional cytoTIL15 cells can successfully be generated from tumors such as NSCLC, HNSCC & TNBC, which afflict large numbers of patients.

**LB097**

**Armored bicistronic CAR T cells with dominant-negative TGF-β receptor II to alleviate antigenic heterogeneity and suppressive immune microenvironment in glioblastoma.**

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Introduction: We have completed two CAR T cell clinical trials for glioblastoma (GBM) and have identified several key challenges to therapeutic efficacy, including the inherently heterogenous genomic landscape and the immunosuppressive tumor microenvironment (TME) found in GBM. Our previous study showed that EGFR variant III (EGFRvIII)-targeting monovalent CAR T cells reduced target-positive tumor cell populations, but tumor recurrence resulted from target-negative tumor cells, highlighting the limitation of single-target approaches in heterogenous tumors. With regards to the highly immunosuppressive TME in GBM, we found that transforming growth factor-β (TGFβ) was present in the GBM TME as a major driver of suppression of the anti-GBM response in clinical samples. TGFb is consistently highly expressed in both GBM tumor cell lines and patient tumor tissues.
Methods: We used two parallel scFv constructs, independently targeting both IL13Rα2 and EGFRvIII, in

combination with a truncated dominant negative (dn) TGFβ receptor II. This trivalent construct was designed to explore possible additive effects in both in vitro and in vivo GBM model systems to limit tumor escape and overcome the immunosuppressive GBM TME. The CART-EGFR-IL13Rα2-dnTGFb construct broadened the targeted tumor cell repertoire, blocked TGFβ signaling, and served as a sink for free TGFβ in the GBM TME to overcome the suppressive function of TGFβ.
Results: The tri-modular CAR T construct had an enhanced proliferative response when compared with the CART-EGFR-IL13Rα2 construct, *in vitro*. In co-culture assays, this construct led to reduced PD-1 expression and increased central memory phenotype, when compared to the bicistronic CAR T construct, which suggested a lower fraction of exhausted T cells. Tri-modular CAR T cells blocked the suppressive pSmad2/3 signaling pathway, leading to the increased tumor killing activity in co-culture experiments with both adherent and suspension GBM cell lines. In an immunodeficient mouse model, tri-modular CAR T cells eradicated tumor cells efficiently and mice had a longer median survival when compared those treated with the bicistronic CART-EGFR-IL13Rα2 cells, lacking the dnTGFb receptor II.
Conclusion: Overcoming the adaptive changes in the local TME and addressing antigen heterogeneity will be required to improve the clinical efficacy of CAR T-directed strategies. Our combination work showed that bicistronic CART constructs cooperate with truncated TGFβ receptor II efficiently. In summary, the dominant-negative TGFβ RII CART-EGFR-IL13Rα2 structure is a promising strategy to address the clinical challenges of antigenic heterogeneity and the immunosuppressive TME in GBM we have observed in our two GBM CART cell trials at UPenn.

**LB098**
**Combination of a PD-1/CTLA-4 bispecific antibody with orally active PD-L1 inhibitor MAX-10181 effectively increased the percentage of tumors inhibited in animal model study.**
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The key players in the current immuno checkpoint inhibition (ICI) treatment are antibodies or bispecific antibodies targeting PD-1. PD-L1 or CTLA-4. While these drugs provided a small number of patients with tumor shrinking (ORR between 10-30%) as a single agent, they did not lead to desirable treatment survival as measured by OS/PFS. Many combination studies are in progress in both preclinical and clinical settings to find an effective way to achieve prolonged OS/PFS in ICI treatment. Herein , we would like to report our most recent findings of combination of a PD-1/CTLA-4 bispecific antibody with our orally active, clinical stage PD-L1 inhibitor MAX-10181 in animal model study. The key observations are as follow:
•The PD-1/CTLA-4 bispecific antibody group (n=10, average starting tumor volume= 68 $mm^3$) demonstrated (a) 2 tumors (20%) in the slow volume shrinking mode with volume between 60-20 $mm^3$, and (b) 4 tumors (40%) in the fast volume growing mode with volume above 500 $mm^3$
•The combination group (n=10, average starting tumor volume= 68 $mm^3$) demonstrated (a) 2 tumors (20%) in the fast volume shrinking mode with volume less than 20 $mm^3$, (b) 1 tumor (10%) in the slow volume shrinking mode with volume between 60-20 $mm^3$, and (c) only 1 tumor (10%) in the fast volume growing mode with volume above 500 $mm^3$
These results indicate the potential of prolonged OS/PFS to combine PD-1/CTLA-4 bispecific antibody with orally active, small molecule MAX-10181 which recently completed Phase I trial. We will present the full study results in the coming AACR meeting.

**LB099**
**Organoid-based drug efficacy evaluation model for immunotherapy.**

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The endogenous immune system of patients are known to be activated through immuno-oncology drug, which activates the patient's own immune system to target and attack tumors. Due to the relatively low occurrence of side effects and sustained success, these therapeutic techniques have gradually replaced the traditional cancer treatment regimen. The creation of an *in-vitro* screening platform for pre-evaluating the efficacy of immunotherapeutic drug candidates was highly essential despite the fact that many of the new emerging therapies had failed clinical trials.An *in-vitro* platform mirroring the interaction between a patient's unique Major Histocompatibility Complex (MHC) and T-cell receptor (TCR) is necessary for predicting the precise efficacy of immunotherapies. The tumor cells' MHC antigen molecule attaches to the T-cell receptor (TCR), allowing T-cells to manifest their tumor-killing activities as a result. The link between immunological checkpoint (ICP) in tumor cells and their receptors in T cells allows tumor cells to evade the effects of T-cell-mediated tumor death.We would like to introduce our "ODISEI" platform, a robust efficacy evaluation tool that enables the recapitulation of a patient's unique immune system, utilizing tumor organoids and PBMC from the same donors. Using PD-1/PD-L1 inhibiting antibodies, the enhanced functionality of our ODISEI platform as an effective evaluation platform for immunotherapy drugs was thoroughly examined. Additionally, we created several ODISEI platforms in order to recreate the precise interactions between tumor organoids and various immune sub-populations (T-cells, macrophages, regulatory T-cells, and dendritic cells). Our ODISEI platforms were validated by utilizing a variety of different drug candidates and evaluating their ability to eradicate tumors upon interaction with various reactive immune cells and tumors. As a result, our highly innovative ODISEI platform can be used to screen a variety of different immunotherapeutic drug candidates and distinguish their precise efficacy.

**LB100**
**Understanding how IL-2 cytokine synergizes with PD-1 therapy during chronic viral infection.**
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In this study we have examined how the cytokine interleukin-2 (IL-2) synergizes with programmed cell death-1 (PD-1) directed immunotherapy during chronic lymphocytic choriomeningitis virus (LCMV) infection. PD-1 blockade in combination with IL-2 is one of the most effective combination therapies in this very stringent LCMV mouse model of life-long chronic infection with irreversible T-cell exhaustion. Our paper makes the following points: First, we show that the more effective viral control seen after PD-1 + IL-2 combination therapy compared to PD-1 monotherapy is mediated by the CD8+ T-cell response. Then we identify the virus-specific CD8+ T cells that proliferate and respond to the combination therapy and show that these are the same lymphoid resident PD-1+ TCF-1+ stem-like CD8+ T cells that act as resource cells to maintain the CD8+ T-cell response during chronic infection and also respond to PD-1 blockade. However, the combination therapy dramatically changes the differentiation program of these chronic resource CD8+ T cells and results in the generation of transcriptionally and epigenetically distinct effector CD8+ T cells that resemble highly functional effector CD8+ T cells seen after an acute viral infection. In contrast, PD-1 monotherapy does not modify the differentiation program and one gets more virus-specific CD8+ T cells but they are transcriptionally and epigenetically similar to what is seen in untreated chronically infected mice. This epigenetic inflexibility of exhausted CD8+ T cells is a potential barrier to PD-1 therapy and the ability of this combination therapy to modify the epigenetic signature of virus-specific CD8+ T cells during chronic infection could be an important determinant of the striking synergy seen between IL-2 therapy and PD-1 blockade. We also highlight the importance of blocking the PD-1/PD-L1 inhibitory pathway at the target site for effective viral control. Expanding the CD8+ T cell population and generating better effector cells is important but it is also critical to block PD-1 inhibitory signals at the target site for optimal immunotherapy. Finally, we show that CD25 engagement with IL-2

plays an important and essential role in the observed synergy between IL-2 cytokine and PD-1 blockade. Either blocking CD25 with an antibody or using a mutated version of IL-2 that does not bind CD25 but still binds CD122/132 almost completely abrogated the synergistic effects seen after PD-1/IL-2 combination therapy. There is currently considerable interest in PD-1 + IL-2 combination therapy for cancer patients and our fundamental studies defining the underlying mechanisms of how IL-2 synergizes with PD-1 blockade should inform these human translational studies.

**LB101**

**Cell-generated IL12 combined with PD-1 inhibition produces local and abscopal immune activation to eradicate metastatic melanoma and pancreatic cancer.**

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Background: Cytokines have been FDA approved for cancer immunotherapy for treatment of metastatic melanoma and renal carcinoma for over 30 years (1, 2). To overcome stability and toxicity limitations seen with high dose cytokine immunotherapy, we developed a delivery platform, called cytokine factories, composed of genetically engineered epithelial cells encapsulated in biocompatible polymers. These clinically translatable cytokine factories are able to safely deliver high local doses of pro-inflammatory cytokines, such as interleukin-12 (IL12), and allow for controlled and predictable dosing *in vivo*.

Results: Tumor-adjacent administration of IL12-based cytokine factories *in vivo* created a high local cytokine concentration (IP space) without substantial leakage into the systemic circulation. In addition, administration of cytokine factories in combination with anti-PD1 checkpoint inhibitors caused reduction of tumor burden by over 60% when delivered as a monotherapeutic to mice with metastatic melanoma (3). Further, when administered in combination with local anti-PD1 checkpoint inhibitors, these cytokine factories led to reduction of intraperitoneal tumor burden by over 80% after only 10 days of treatment. Finally, we evaluated the ability of this therapy to treat pancreatic tumors which are notoriously known to be resistant to immunotherapy. We found that the median survival for control animals was 45 days. However, 8/8 of animals treated with IL12 cytokine factories survived throughout the duration of the study (110 days), demonstrating a significant extension of overall survival time.

Conclusions: Our findings demonstrate efficacy of cytokine factories as single agent and combination therapeutics in preclinical animal models and provide rationale for future clinical testing for the treatment of metastatic peritoneal cancers in humans.

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**LB102**

**Hexyl-(cuban-1-yl-methyl)-biguanide (HCB) suppresses N-glycosylation of immune checkpoint proteins B7-H3 and B7-H4, reverses tumor hypoxia, decreases intratumoral regulatory T cells, and increases intratumoral CD8+ T cells in the ovarian dependent ER+HER2- SSM2$^{ucd}$ mammary cancer allograft model.**

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McCarra,[1] Brenda Koniar,[1] Craig Flory,[1] Beverly Norris,[1] Robert J. Schumacher,[1] Swaathi Jayaraman,[4] John Hawse,[4] Emmanuel S. Antonarakis,[1] Emanuel F. Petricoin,[5] Julia Wulfkuhle,[5] Robert D. Cardiff,[6] Elizabeth A. Ambrose,[1] Gunda I. Georg,[1] Kaylee L. Schwertfeger,[1] Michael A. Farrar,[1] Brad St. Croix,[7] Matthew P. Goetz,[4] David A. Potter[1]. [1]University of Minnesota, Minneapolis, MN; [2]Macalester College, St Paul, MN; [3]Pomona College, Claremont, CA; [4]Mayo Clinic, Rochester, MN; [5]George Mason University, Fairfax, VA; [6]University of California, Davis, CA; [7]National Cancer Institute, Bethesda, MD.

Introduction: Immune checkpoint blockade (ICB) has clinical activity in triple negative breast cancer (TNBC) but is less effective in the ER+HER2- signature, where there is a cold immune microenvironment (IM) and regulatory T cells (Tregs) may suppress effector T cells. Agents that activate the IM by turning cold tumors hot may support ICB. The biguanides hexyl-benzyl-biguanide (HBB) and its bioisostere hexyl-(cuban-1-yl-methyl)-biguanide (HCB) are candidate agents to activate the IM because they potently inhibit biosynthesis of immunosuppressive epoxyeicosatrienoic acids (EETs) and EET-driven oxidative phosphorylation (OXPHOS), while blocking N-glycosylation of immune checkpoint (IC) proteins. We hypothesized that reversal of hypoxia by biguanides in the ovarian dependent ER+HER2- STAT1 KO SSM2$^{ucd}$ mouse mammary carcinoma (MC) model would suppress Tregs and promote effector T cells in the tumor IM. While the SSM2$^{ucd}$ model did not express immune checkpoint protein PD-L1 (B7-H1), it did express related IC proteins B7-H3 and B7-H4. We hypothesized that by inhibiting OXPHOS and reducing N-glycosylation of immune checkpoint proteins, HBB and HCB may promote efficacy of ICB. We chose the SSM2$^{ucd}$ model to test impact of HCB on the ER+ MC IM.
Results: SSM2$^{ucd}$ cells exhibited longer tumor latency (60 days) than the basal 4T1 (10 days) and 67NR (20 days) mouse MC models. SSM2$^{ucd}$ tumor reimplantation shortened latency by more than half, to 20 days. Immunohistochemistry showed that B7-H3 and B7-H4 protein levels were 1.2 (P=0.001) and 1.3-fold (P=0.04) higher in reimplanted tumors vs. control. In SSM2$^{ucd}$ cells, HCB inhibited N-glycosylation of B7-H3 (P=0.01) by 35% and B7-H4 (P=0.02) by 45% and suppressed TGFβ induction of B7-H3 by 21% (P=0.02) and B7-H4 by 79% (P=0.001) at 24 hours, while 14,15-EET promoted N-glycosylation of B7-H3 (1.2-fold; P=0.03) and B7-H4 (1.3-fold; P=0.04) at 4 hours. Effects of HBB and HCB on anti-CD3 and anti-CD28 stimulated mouse splenocytes were assayed. The proliferative effects of HBB on CD4+ and CD8+ cells peaked at 12 uM ($p<0.001$) and for HCB at 6.25 uM ($p<0.001$). Tregs decreased with HCB at a threshold of 12 uM ($p <0.001$) and with HBB at 25 uM ($P < 0.001$) while 14,15-EET increased Tregs (1.2 fold; P=0.02) and suppressed the CD8+:Treg ratio (0.79; P=0.02). In the SSM2$^{ucd}$ mammary allograft model, although HCB 12mg/kg daily did not inhibit tumor growth, it reduced intratumoral hypoxia by 20% (P=0.01), increased CD8+ TIL by 4.4-fold (P=0.04), decreased Treg:CD4+ TIL ratio by 76% (P=0.02), and decreased Treg:CD8+ TIL ratio by 88% (P=0.01) relative to control.
Conclusion: B7-H3 and B7-H4 expression inversely correlated with latency of ER+ MC and may represent targets for immune checkpoint antibodies and their drug conjugates. HCB, an inhibitor of OXPHOS and EET biosynthesis, reduced intratumoral hypoxia, increased CD8+ TIL and reduced the Treg:CD8+ ratio, potentially supporting ICB therapy of ER+ MC by turning cold tumors hot. Supported by CDMRP BCRP Grant BC180596, Award Number W81XWH-19-1-0099

**LB103**
**Inhibition of interleukin-25 ameliorates immune checkpoint inhibitor-induced pneumonitis and exhibits antitumor activity.**

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Background: Immunotherapy has revolutionized the treatment of a variety of malignancies; in particular,

the use of immune checkpoint inhibitors (ICIs) has improved outcomes and extended patient survival in a number of tumor types. However, ICIs oftentimes induce significant immune related adverse events (irAEs) that warrant therapy cessation, thereby limiting the overall effectiveness of this class of therapeutic agents. Some of the currently available therapies that are used to treat ICI-irAEs might also blunt the antitumor activity of the ICIs themselves, therefore there is an urgent need to identify therapies that have the potential to be administered alongside ICIs to optimize their use.
Methods: Using an established translationally relevant murine model of ICI-induced pneumonitis in which anti-PD-1 and anti-CTLA-4 antibodies are co-administered over the course of a month to MC38 tumor-bearing B6/lpr mice, a single dose of putative anti-pneumonitis therapies was administered five days after the onset of ICI therapy. Tumor growth was measured daily, and the presence of immune infiltrates was assessed in various tissues by immunohistochemistry forty days post-ICI therapy initiation.
Results: A single dose of LNR125, a murine anti-IL-25 antibody, was sufficient to prevent the onset of pneumonitis in a dose-dependent fashion. Treatment with LNR125 significantly reduced the numbers of the immune cell infiltration to the lungs. Moreover, a single dose of LNR125 in combination with anti-PD-1 and anti-CTLA-4 antibodies also exerted antitumor activity superior to that of the two ICIs, including a dramatic increase in complete responses (CRs; 75% CRs for dual ICIs therapy plus LNR125 (400ug dose), 0% CRs for dual ICIs therapy). Mechanistically, LNR125 increased not just the numbers, but also the specific cytotoxic activity of the tumor infiltrating lymphocytes.
Conclusions: LNR125 is the first reported agent that is able to abrogate the onset of pneumonitis and at the same time exert a remarkable antitumor activity in a translationally relevant model of ICI-induced immune related adverse events. These findings suggest that Interleukin-25 inhibition may serve a dual role in the therapy of solid tumors that are treated with ICIs, both enhancing the antitumor activity of the ICIs and allowing for extended ICI therapy by suppressing autoimmune and immune related side effects.

**LB104**

**Preclinical evaluation of the role of Galectin-1 in tumor immune evasion associated mechanisms using a highly selective Galectin-1 small molecule inhibitor.**

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Galectin-1 (Gal-1) is a β-galactoside-binding lectin that is highly expressed within the tumor microenvironment (TME) of some aggressive cancers and whose high expression correlates strongly with poor survival. Following a data mining exercise of external publication data and additional clinical biomarker datasets, we found strong evidence concluding the potential role of Gal-1 in the establishment of a fibrotic tumor stroma and mechanisms associated with tumor invasion, metastasis and angiogenesis. However, there was limited data regarding the role of Gal-1 in immune escape by tumors to make any strong conclusions/pathway reconstruction around this specific TME mechanism of action (MOA). To further strengthen the potential role of Gal-1 in tumor immune evasion we completed a series of preclinical in vivo and in vitro activities, using a highly selective Gal-1 small molecule inhibitor developed at Galecto Biotech. To examine the role of Gal-1 inhibition in vivo, we investigated the effects on tumor volume of the highly selective Gal-1 inhibitor, in a syngeneic mouse model of lung adenocarcinoma. RNA profiling of vehicle and compound treated tumors was also implemented following completion of the in-life phase. Oral administration of the Gal-1 inhibitor reduced mouse lung adenocarcinoma growth and increased the immune gene expression within the tumors, showing a strong association to that of exacerbated NK cell activity. No additional cytotoxic T cell gene changes were uncovered in the Gal-1 inhibitor treated tumors. We further evaluated the overall T cell compartment, relevant to the clinical TME setting, by stimulating human peripheral blood mononuclear cells (hPBMC's) incubated with and without the Gal-1 inhibitor to observe the potential blockade of any known immune checkpoint inhibitor (ICI) resistance mechanisms. Interestingly, we found a variety of

immune suppressive proteins to be inhibited, including IL-17, when hPBMC's were incubated with the Gal-1 inhibitor. In summary, the data presented suggests highly selective Gal-1 inhibition could provide an effective, monotherapy (or be used in combination with ICI's) to boost immune infiltration and/or activation in the TME of lung adenocarcinoma and potentially other aggressive cancers.

**LB107**

**A precision DNA methylation test to triage HPV positive women before referral to colposcopy-driven biopsies or ablative treatment in cervical cancer screening clinics worldwide.**

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Cervical cancer is one of the most common cancers in women. Despite progress in prevention through Human Papilloma Virus (HPV) vaccination and success in early detection of cervical cancer through cytologic screening and HPV detection, there remains an unequal cervical cancer burden in low-resource settings, both in developed and developing countries. We have previously shown that the *CervicalMethDx* test can provide a Cervical Intraepithelial Neoplasia (CIN) grade 2-3 risk score, by assessing DNA methylation in a panel of three human genes (*ZNF516, FKBP6* and *INTS1*) in samples from the United States (US), Puerto Rico, and Chile. We now tested the performance of the *CervicalMethDx* test on HPV-positive CIN2 and CIN3 cases (n=113) from Honduras collected from participants in the ESTAMPA clinical trial (NCT01881659). ESTAMPA (EStudio multicéntrico de TAMizaje y triaje de cáncer de cuello uterino con pruebas del virus del PApiloma humano; Spanish acronym) is a multicentric study of cervical cancer screening with HPV testing and assessment of triage methods in Latin America, which has accrued close to 50,000 participants from twelve recruitment centers in nine Latin American countries since 2013. We hypothesized that the *CervicalMethDx* test can identify HPV positive women most likely to be diagnosed with CIN grades 2 and 3 by anatomic pathologists, before they are referred to colposcopy-driven biopsies. We assessed DNA methylation by quantitative Real Time Methylation Specific PCR (qMSP) analysis of sodium bisulfite-modified genomic DNA. Primers and probes were previously designed to specifically amplify the promoters of the 3 genes of interest and the promoter of a reference gene, *β-actin*, to assess DNA input. We performed blinded retrospective studies on well-characterized clinical samples in PreservCyt sample transport media (ThinPrep, Hologic), comparing DNA methylation levels in samples from Honduras and 88 HPV-positive previously tested US samples with No Intraepithelial Lesions or Malignancy (NILM), as controls. Our results showed that the *CervicalMethDx* test can correctly classify 96% of CIN2 (n=62) samples with 92% Sensitivity, 98% Specificity, and an AUC of 0.96 as well as 97% of CIN3 (n=51) samples with 96% Sensitivity, 98% Specificity, and an AUC of 0.97. Moreover, the assay correctly classified 96% of CIN2-CIN3 samples combined (n=113) with 95% Sensitivity, 98% Specificity, and AUC of 0.97, when compared to samples with NILM (n=88). Our results suggest that the *CervicalMethDx* test is a new and valuable tool to stratify HPV positive women prior to colposcopy-driven biopsies in developed countries and ablative treatment in developing countries, most of which are unnecessary. These results warrant further evaluation of the *CervicalMethDX* test in prospective, population-based studies, assessing the use of precision DNA methylation algorithms to triage HPV positive women before referral to colposcopy-driven biopsies or ablative treatments in cervical cancer screening clinics world-wide.

**LB109**

**Network analysis of gut microbiome throughout a whole foods based high fiber dietary intervention reveals complex community dynamics in melanoma survivors.**

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Recent evidence has demonstrated that the gut microbiome modulates response to immune checkpoint blockade (ICB) treatment in melanoma patients. Microbiome modulation via a habitual high-fiber diet was associated with significantly improved progression-free survival (PFS) in melanoma patients on ICB. Previous findings have suggested that this pro-response is associated with known fiber-responsive taxa and Short Chain Fatty Acid (SCFA) producing taxa. However, little is known about the communications responsible for stimulating the aforementioned taxa. To explore community dynamics and identify potential keystone communicating taxa, we conducted microbial association network analysis throughout a high-fiber dietary intervention (HFDI) in melanoma survivors. Ten patients were enrolled to the HFDI study and were provided with whole-food-based fiber-enriched meals for the duration of six weeks. Fecal samples were collected longitudinally, and whole genome sequencing (WGS) of the fecal microbiome was used to calculate microbiome composition profiles. OTU abundance data was then used to construct, analyze, and compare association networks across timepoints via the R package NetCoMi. Overall changes in community dynamics were assessed via changes in global network properties, and significant taxa were identified quantitatively via differences in calculated centrality measures as well as visually by NetCoMi's selection of hubs, or specific keystone taxa. Network analysis across timepoints demonstrated a general increase in connectivity by both quantitative and visual comparison throughout the HFDI. Analysis via multiple association statistics revealed a general rise in several measures of centrality of many known fiber- responsive and SCFA-producing taxa, with many consistently becoming hub taxa. Consensus networks generated by overlapping several networks generated from different association statistics revealed a consistent increase in centrality in two particular species: Ruminococcus bromii and Dorea longicatena. Analysis of networks constructed from only differentially associated taxa revealed similar results, with R. bromii and D. longicatena having numerous changes in associations with fiber-responsive taxa. Increases in general connectivity measures indicate that a HFDI prompts the gut microbiome to become a more interconnected and dynamic community over time. This, in conjunction with an overall increase in centrality in known fiber-responsive and SCFA-producing taxa, reflects an overall pro-response to the HFDI. Networks constructed via different association statistics yield varied results, with many identifying different taxa as hubs. Despite this heterogeneity, network analysis consistently identified D. longicatena and R. bromii as potential keystone species with an important role in communicating with fiber-responsive taxa. Further analyses are needed to characterize communications between keystone species and SCFA-producing taxa.

**LB110**

**Racial and ethnic disparities associated with the intratumor microbiome in female cancers.**

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Background: The intratumor microbiome is implicated in tumor initiation, progression, and altered immune response to cancer therapies. Furthermore, recent studies have revealed correlations between microbial abundance and racial disparities in cancer. While these investigations provide novel insights into cancer disparities research, few have investigated which racial and ethnic disparities exist for patients with female malignancies. In this study, we characterized the intratumor microbiome according to racial and ethnic disparities in common female malignancies including breast, cervical, uterine, and ovarian cancers.
Methods: We examined the intra-tumoral microbiome in the breasts, cervix, uterus, and ovaries (n = 2630). Raw tumor RNA sequencing data were downloaded from The Cancer Genome Atlas (TCGA) and

aligned to bacterial genomes using the PathoScope 2.0 framework and references genomes provided by the NCBI nucleotide database. Potential contaminants were identified and removed from downstream analyses by associating individual microbe abundance with total microbe abundance in each sample. Microbial abundance was correlated to race, ethnicity, and prognostic variables (Kruskal-Wallis test or Cox regression, $p < 0.05$). Finally, we validated our results using transcriptomic sequencing data downloaded from the GEO NCBI data portal.
Results: Significant dysregulation of bacterial microbes according to race and ethnicity was observed in these cancers, but most notably in breast cancer in which 6 species correlated strongly with survival. Of particular significance were *Veillonella Parvula* and *Mycobacteroides chelonae*, which were both significantly dysregulated in Black breast cancer patients, with low abundance of both species correlating to poor survival. *Cupriavidus Taiwanensis* and *Delftia Acidovorans* were more abundant in Black breast cancer patients, with high abundance correlating to poorer prognosis. Black patients were also diagnosed at significantly later cancer stages in cervical cancer. We also observed significant correlations of bacterial microbe abundance with prognostic and treatment variables in these cancers, including pathologic TNM staging, neoplasm presence, therapy outcome, and more.
Conclusion: Our study is the most comprehensive to date investigating racial differences in the intra-tumoral microbiome in common female cancers. We found that differences in intratumoral microbial abundance may account in part for observed racial and ethnic disparities in cancer prevalence and progression in these cancers. Further studies are needed to investigate the specific mechanisms by which these microbes contribute to these observed cancer disparities within the tumor microenvironment.

**LB111**
**Epigenetic mechanism underlying pathogenesis of skin photo-damage and actinic keratoses.**
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Chronic skin exposure to solar ultraviolet B (UVB) radiation causes cutaneous inflammation, sun-burns and pre-malignant lesions known as Actinic Keratosis (AK). About 20% of AKs may progress to highly invasive malignant lesions known as Squamous Cell Carcinoma (SCC). These lesions carry multiple mutations in oncogenes and/or tumor suppressor genes. However, the molecular mechanism underlying AK is not incompletely defined. Here, we demonstrate that cutaneous UVB exposure leads to activation of bromodomain-4 (BRD4), an epigenetic modifier. We observed enhanced expression of BRD4 and its hyper-acetylation marks, H3K18ac and H3K27ac in AK, accompanied by elevated transcription of BRD4-target genes: MMP13, CXCL9, IL33, Apol19b, IL19 and Oasl2. Using confocal microscopy, we observed enhanced nuclear accumulation of BRD4 and H3K27ac in the sun-exposed lesional skin, AK and in SCC tissues from patients. $Ptch^{+/-}$/SKH1 mice irradiated with to chronic UVB manifest similar molecular alterations in highly photo-damaged skin. By manipulating BRD4 expression in human skin SCC A431 cells, we identified two important BRD4-regulated signaling pathways, which may be involved in the molecular pathogenesis of AKs and SCCs. BRD4-mediated inflammatory cytokine signaling is regulated by EZH2-SOCS3-NFkB/STAT3 axis while Myc-Survivin-CDK4-E2F1 axis drives tumor cell hyper-proliferation. Co-localization of BRD4/H3K27ac and Survivin-E2F1 was contiguous from sun-burn skin to AK and SCC. We observed enhanced copy number of Myc in human AK lesional skin and associated SCCs. We also show that Myc-Survivin-CDK4-E2F1 axis is critical in driving the progression of tumor keratinocytes to highly invasive and fast growing SCCs. This signaling acts by enhancing the expression epithelial-mesenchymal transition transcription factors and other proteins involved in UVB-induced SCC development. 9cUAB30, a RXR agonist developed by us blocked BRD4 activation and its downstream signaling in A431 and SCC13 cells. Oral administration of 9cUAB30 abrogates UVB-induced inflammation and keratinocyte hyper-proliferation in murine skin in BRD4 dependent manner. 9cUAB30 treatment attenuates growth of human SCC xenograft tumors in highly immune-suppressed mice, a response which was significantly more profound when the drug was

administered with a survivin inhibitor, LQZ-7I. These translational studies demonstrate that UVB-induced cutaneous BRD4 drives AK pathogenesis and their progression to invasive SCCs.

**LB112**

**Increasing clinical trials diversity through Community Research Registry (CoRR): The Community Champions for clinical trials program.**

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Introduction: The lack of representation of minoritized and marginalized (MaM) populations in Clinical Trials (CTs) has been noted as one of the key factors that contribute to cancer health disparities. The challenges of CT diversity are multifactorial, including barriers at the personal, provider, health systems and protocol levels. Guided by a vision of developing and implementing inclusive CTs that address the priorities identified by our communities, we created a Community Research Registry (CoRR) with three primary aims: 1. Maintaining a CoRR database of participants who may be eligible for participation in future CTs; 2. Using the database, facilitate matching of MaM populations to Clinical Trials; and 3. Facilitating access of MaM populations to CTs.
Methodology: The Mayo Clinic CoRR is IRB-approved and was implemented in 2022 through bidirectional engagement of communities, partnership with community leaders to foster community input, dissemination of CT information to high-risk communities, and use of evidence-based education materials for community outreach. Participants are recruited in MaM communities (including clinics, churches, health events) and through effective use of social media. Organized events for recruitment include: 1. CT education; 2. provision of culturally tailored CT educational materials and T-shirts; and 3. data collection from those who consent to be part of the registry. The inclusion criteria for the registry are adults and ability to read and write in English or Spanish. Informed consent is obtained prior to completion of the registry form and participants receive $10 incentive. The data is collected using REDCap, and includes name and contact information of participants, demographic information, chronic disease status and explicit consent to be contacted for future research studies (with option to state type of study).
New Unpublished Data: Within 7 months of CoRR implementation, 184 MaM participants signed up with 92% Black (including 10% African immigrants), 7% Hispanic, 69% female, median age of 64 years, 24% rural residents, and most of the participants had high school level education. Over 30% of participants reported medical history of hypertension, high cholesterol, arthritis and/or diabetes. 8% reported cancer. Approved investigators (with appropriate community engagement training) have direct access to de-identified CoRR information though Tableau for the purpose of estimating participants for their study. Tableau has been found to be effective for investigators to explore the database for CT/research match. All requests for participants matching are approved by a CoRR Board that includes community representatives, prior to matching. CoRR CT matching is now in its pilot phase, with Mayo Clinic studies identified for matching.
Conclusion: The feasibility of the CT CoRR has been established, and has led to over 20 CoRR Champion organizations, including churches, sororities, fraternities, and the American Legion Post.

**LB113**

**Bio-printed 3D cell models and high-content imaging for testing anti-cancer compounds.**

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The discovery and screening of anti-cancer drugs is a vast field that is constantly evolving. More

advanced cell-based models are needed to have an efficient way of studying cellular and subcellular effects of compound treatments, especially in early drug discovery. 3D cellular models that more accurately represent various microenvironments are very important for accurate drug screening and disease modeling. However, the complexity of those models is a limiting factor for the wider adoption of 3D models for research and screening. To address this, we have developed an automated workflow that includes a multitool 3D printing/automation robot enabling printing cells in a hydrogel-based matrix and high-content imaging for the characterization of phenotypic effects of compounds. We demonstrate examples of several bio-printed 3D cell models that can be used for testing anti-cancer drugs. For the development of 3D cellular models, we used VitroGel / VitroInk, xeno-free (animal origin-free) bio-functional hydrogel matrices. Hydrogel matrices with cells were used for printing 3D cellular patterns in 96 well plates, or dispensing cells in hydrogel “domes”. We have utilized a variety of widely used cancer lines HCT-116, HELA, HepG2, and MCF-7, as well as patient-derived triple-negative breast cancer cell line 4IC. Cells mixed with hydrogels/inks were dispensed/printed into a 96-well format using the multi-tool robotic platform, BioAssemblyBot. The platform enabled efficient dispensing/printing of cells into domes, lines, or other patterns. This assay was used for compound testing and evaluation of the anti-cancer effects of various drugs. The integrated system included a high-content confocal imager (IXM-C) and enabled automated seeding, bioprinting, liquid handling, plate transferring, as well as high-content imaging. The 3D bioprinted cells were monitored daily using imaging in transmitted light. In 2-3 days, cells formed spheroids in the matrix. Those were treated with a panel of anti-cancer drugs including doxorubicin, cytarabine, taxol, mitomycin, romidepsin, cisplatin, trametinib, and other compounds with various modes of action. Cells were treated with compounds for 72h, then stained and imaged for the endpoint measurements. 3D models were stained with viability dyes including Calcein AM, EthD, and Hoechst stain to evaluate numbers of live, dead, and total cells, then imaged using the IXM-C confocal imaging system. Image analysis allowed the characterization of cytostatic and cytotoxic effects of various compounds measuring the impact of compounds on cell proliferation, number of spheroids, spheroid size, cell death, and apoptosis. Different measurements, including spheroid size, and numbers of live and dead cells were used to determine EC50s for different compounds and cell types. The results showed the feasibility of 3D cellular models bioprinted with ECM matrices for anti-cancer drug screening workflows. An increase in throughput and ease of operation was achieved through automation. Also, imaging and data analysis methods provided valuable information about complex compound effects in 3D printed and cell-tissue-engineered cancer models.

**LB114**

**Colon cancer preventive efficacy of ONC201 and naproxen alone or in combination in FAP relevant PIRC rat model.**

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Colorectal Cancer (CRC) is the third most common cancer and leading cause of mortality in the US. While the rate of CRC incidence has steadily dropped in the last decades, recent rising trends among young adults are a major concern. Hence, preventing CRC in the general population would aid in reducing cancer mortality, especially in high-risk FAP patients for which there are no FDA approved preventive agents. Here, we evaluated TRAIL-inducing agent ONC201, alone and in combination with NSAID naproxen (NAP) for CRC prevention in PIRC rat model of FAP. PIRC rats (20 female rats/group) are bred and randomized by age into control and intervention groups. Starting at 6 weeks of age, PIRC rats were treated with ONC201 by gavage [25mg/kg; 2X/week (LD) or 50mg/kg; 1X/week (HD)]; NAP 200 ppm in AIN-76A diet [continuously or 1 week ON/OFF]; or the combination of ONC201 and NAP. Control group rats received vehicle only. Colonic polyps’ (CP) development (occurrence, number, and

size) was monitored by periodic colonoscopies and upon termination at 32 weeks of age. Colonoscopy data indicated significantly fewer CPs in the HD ONC201, NAP alone dosing regimens, and combination groups. At termination, colonic tumors were harvested and subjected to histopathological evaluation. Colonic tumors were histologically classified as hyperplastic polyps, adenomas, and adenocarcinomas (ADCA). In vehicle treated rats, histological assessment showed multiplicity of 2.4±0.4 (Mean±SEM) hyperplastic polyps; 9.14±0.92 adenomas, and 2.4±0.27 ADCA. A significant decrease in the number of hyperplastic polyps ($p<0.05$) was observed in rats treated with combination of ONC201 and NAP continuous dosing regimens. LD and HD ONC201 regimens resulted in modest (11.3%, $p=0.45$) and significant (48.8%, $p<0.0001$) inhibition of colonic adenoma multiplicity, respectively. As anticipated, NAP dosing regimens showed 78-85% inhibition ($p<0.0001$) of adenoma multiplicity. Combination treatment showed >90% inhibition of adenomas. Importantly, LD ONC201 inhibited colonic ADAC multiplicity by 78.8% ($p<0.0001$) and remaining treatment groups showed no colonic ADACs. This histological data clearly showed that ONC201 (25 mg 2X/week or 50 mg 1x/week) significantly suppresses colonic tumor progression to colon adenoma and adenocarcinomas. NAP, either continuously or intermittent dosing, resulted in 85% and 78% inhibition of adenoma multiplicities, respectively, suggesting that intermittent dosing may be similarly effective for CRC prevention. Biomarker analysis suggested increased apoptosis (TRAIL, Caspase-3) and decrease in proliferation markers (PCNA, Cyclin D1). Our data warrants investigating ONC201 with NAP combination for CRC prevention in high risk FAP patients. (Supported by NCI-PREVENT 75N91019D00020-75N91020F00004)

**LB115**

**Simultaneous whole-body ultrasound contrast imaging: a novel high throughput preclinical platform for targeted molecular imaging studies.**

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Ultrasound contrast agent (UCA) based molecular imaging is a rapidly growing field in biomedical research. Ultrasound (US) is a non-radiative imaging modality that is frequently used to investigate various human diseases in the clinic. US molecular imaging is a novel imaging modality that utilizes targeted contrast agents to add molecular information about the imaging target, including vascular biomarkers of cancer. Pre-clinical animal models are commonly used to evaluate and validate targeted contrast agents before extending them for FDA-approval. Key impediments in the preclinical evaluations are the short *in vivo* half-life of microbubbles (MBs) (less than 30 minutes), limited transducer scanning area, and focusing one target at a time, which all make this strategy low throughput. Moreover, whole-body imaging strategy is not available for US imaging compared to other imaging modalities, such as PET, CT, and MRI. To overcome these imitations, we have developed a multi-mouse US imaging platform using an Automated Breast Volume Scanner (ABVS) system. In addition, we have synthesized a novel breast cancer vascular-specific B7-H3 targeted microbubbles (TMBs) using a microfluidic device. We tested these TMBs in a genetically engineered transgenic mice model [FVB/N-Tg (MMTV-PyMT)634Mul/J] (n=32) that develops breast cancer in the mammary fat pad. The US imaging results of conventional Vevo 2100 system indicated that B7-H3-TMBs could provide target specific contrast signals in the tumor vasculature compared to control MBs. We used this MBs for whole-body imaging using an ABVS system with two mice simultaneously in a stage. We imaged multiple tumors (10 tumors/mouse, 2 mice per scan, n=20 tumors) with a single bolus injection of TMBs per mouse. The results successfully showed tumor specific contrast signals from tumors independent of tumor size. Introduction of this whole-body scanner, and its ability to scan multiple mice simultaneously allows rapid imaging of multiple tumors with 3D quantification of tumor size by administering a single TMB injection enabling without significant contrast loss. Our approach makes this US-MI as high throughput and can be used to test multiple contrast agents for investigating different aspects of biological problems including screening of vascular biomarkers of human diseases.

**LB118**

**Evidence of tumor response in orbital lesions treated with tebentafusp in metastatic uveal melanoma patients.**

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**Background:** Tebentafusp, a bispecific (gp100 x CD3) ImmTAC that can redirect T cells to target gp100+ melanoma cells, has shown a superior overall survival (OS) benefit compared to investigator choice for HLA-A*02:01+ patients (pts) with untreated metastatic uveal melanoma (mUM). Although local control rates for primary UM are high, a minority of patients with mUM have recurrent or untreated orbital disease. Here we present safety and efficacy from tebentafusp treated mUM pts with orbital lesions including intraocular lesions.
**Methods:** Tumor response according to RECIST v1.1 and ocular Adverse Events (AEs) were assessed in a pooled analysis of mUM pts with orbital lesions from 3 tebentafusp trials: IMCgp100-01 (Phase (Ph) 1/2, 19 2L+ mUM pts), IMCgp100-102 (Ph 1/2 2L+ mUM, 146 pts) and IMCgp100-202 (Ph3 1L mUM, 245 tebentafusp pts). Pre-treatment biopsies, taken prior to the IMCgp100-202 trial, were analyzed by immunohistochemistry for gp100 and CD3+ or CD8+ T cell infiltration prior to tebentafusp treatment.
**Results:** In the 3 clinical trials, 12 mUM pts with radiologically detectable orbital lesions received tebentafusp, the majority of which were intra-ocular. All lesions were stable or had achieved shrinkage. 5/12 pts had orbital target lesions (TL), of which 4/5 had achieved tumor shrinkage (best % change -3 to -40) and 1/5 had no change in tumor size. All non-target orbital lesions were stable or achieved a complete response. 9 eye disorder AEs occurred in 5 pts, the majority of which were G1. The most common (7/9) AEs were extra-ocular local edema. One G3 eye pain AE, in a post eviscerated eye, did not resolve and occurred in the setting of disease progression resulting in treatment discontinuation. All other eye disorder AEs recovered and none led to tebentafusp discontinuation. 37 primary UM and 195 liver metastasis baseline biopsies were evaluated. Expression of gp100 in primary UM and mUM was high (95% and 80%, respectively). CD3+ and CD8+ T cell numbers were generally lower in primary UM compared to liver metastasis (median CD3: 231 and 504 cells/mm$^2$, p=0.035; CD8: 121 and 238 cells/mm$^2$, p=0.53, respectively).
**Conclusions:** Following tebentafusp treatment, tumor reduction or stabilization of all orbital lesions including intra-ocular lesions were observed. Most AEs were extra-ocular mild local edema. Primary UM showed a high level of gp100 expression and also T cell infiltration. These preliminary signals of tebentafusp activity in orbital disease, including intra-ocular lesions, suggest a role for tebentafusp as a neoadjuvant therapy in UM and warrant further investigation.

**LB119**

**Recurrence score for recurrence over survival outcome in the landmark TAILORx trial.**

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Overall survival is the most relevant endpoint in clinical research and patient care. Nonetheless, cancer recurrence has been frequently utilized as a surrogate endpoint, particularly in the context of multi-gene assays and molecular signatures. The 21-gene recurrence score (RS) was originally established against distant recurrence for prognosis in breast cancer. However, it is unclear whether RS has a weight for the recurrence over survival prognosis. We investigated RS on the choice of clinical endpoints in the Trial

Assigning Individualized Options for Treatment (TAILORx), which had OS, invasive disease-free survival (DFS), and recurrence-free interval (RFI) in addition to distant recurrence-free interval as the clinical endpoints. Both midrange (11-25) and high-range (26-100) scores were associated with RFI, high-range only with DFS, but not significantly associated with OS in the multivariable Cox proportional-hazards regression models. Of 462 death events, 33.1% (153 events) were ascribed to breast cancer and 91.8% (424) were due to DFS-related events that included 24.5% other cancers, 34.2% unknown and 33.1% breast cancer. It is DFS that was wound up as the most representative surrogate endpoint for survival. The lack of survival significance was probably due to the nature of 21-gene assay weighted for recurrence rather than not having enough event numbers in the TAILORx clinical trial. The data call for establishing biomarkers directly towards OS or DFS to increase survival with more precision.

**LB121**

**BrainChild-03: Intraventricular B7-H3 CAR T cells for recurrent/refractory central nervous system tumors and non-pontine diffuse midline glioma in children and young adults.**

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Background: B7-H3 expression on central nervous system (CNS) tumors offers a potential therapeutic target for adoptive cellular therapy.
Methods: A 3+3 statistical design was employed to assess the safety of repeated B7-H3 chimeric antigen receptor (CAR) T cells administered into the intracranial ventricular system (ICV) using an intrapatient dose escalation without lymphodepleting chemotherapy in pts 1-26 years of age with recurrent/refractory CNS tumors including (non-pontine) diffuse midline glioma (DMG) (NCT04185038, Arm B). Primary endpoints were feasibility to manufacture sufficient CARs and safety of weekly infusions for 3 weeks of 4-week cycles for 2 cycles. Secondary endpoints were best overall response (BOR) and correlatives of CAR activity.
Results: CAR manufacturing was achieved in 20/22 (91%) enrolled pts. Eight pts were inevaluable (rapidly progressive exam precluding initial infusion (n=5), removal from protocol therapy due to progressive disease (n=2), and lack of evaluable disease prior to initial infusion (n=1)). No dose limiting toxicities were observed, identifying the maximally tolerated dose regimen (MTDR) as DR3 (maximum dose: $10x10^7$ B7-H3CARs). Subjects received 98 ICV doses (median: 6 doses/patient, range: 3-13). For evaluable patients, the most common adverse events were headache (12/12, 100%), fatigue (9/12, 75%), nausea/vomiting (7/12, 58%), and fever (6/12, 50%). There was no cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS). Imaging analysis is ongoing with current BOR being stable disease. CAR T cells were detected in the CSF in 9/12 evaluable pts (75%). Serial CSF/serum cytokine analysis and mass spectrometry are in process.
Conclusion: Repeated ICV administration of B7-H3 CAR T cells is tolerable with circulating CAR T cells detectable in the CSF post infusion. Further investigation is warranted, along with future studies interrogating iterative enhancements such as modifications for enhanced potency and multi-antigen targeting.

**LB122**

**Arima-HiC sequencing accurately detects clinically-relevant structural variants in pediatric leukemia samples.**

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Genetic structural variants (SVs), especially those leading to gene fusions, are well-known oncogenic drivers. These SVs can produce overexpression or loss-of-function of certain genes, or generate chimeric fusion proteins. Thus, they serve as important disease biomarkers across several cancers and can also represent therapeutically targetable alterations. Detecting these SVs at a gene-level resolution can be challenging with lower resolution karyotyping approaches, or even RNA sequencing approaches, due to difficulties with culture, sample stability, low transcript abundance, or low-quality RNA. In addition, the ability of fluorescence in situ hybridization (FISH) techniques to detect gene fusions is limited by the targeted nature of these assays as they depend heavily on the design and selection of each probe. Here we use a novel method, Arima-HiC sequencing, that utilizes DNA to evaluate 12 pediatric leukemia samples and determine this assay's effectiveness in detecting clinically-relevant SVs. We first selected 5 archived (cryopreserved) pediatric acute myeloid leukemia (AML) samples (archival period range: 1-4 years) known to be either fusion-positive (n=3) or fusion-negative (n=2) via prior clinical genetic testing (i.e., chromosomes, FISH, and/or microarray). All samples underwent Arima-HiC sequencing. Briefly, chromatin digestion, end-labeling, and proximity ligation were performed prior to DNA purification per the Arima-HiC protocol. Purified DNA was next prepared as a short-read sequencing library and sequenced on a HiSeq X. The raw reads were aligned and deduplicated, and SVs were called using HiC-Breakfinder software. For a discovery set, we then additionally selected 7 pediatric leukemia samples—6 precursor B-cell acute lymphoblastic leukemias (ALL) and 1 AML—for Arima-HiC sequencing (as above). These cases had undergone standard-of-care cytogenetic (karyotyping, FISH, microarray) and/or molecular (targeted cancer NGS sequencing panel) testing clinically, and a genetic driver / known gene fusion had not been identified. Using Arima-HiC sequencing, we identified the clinically-relevant SV in each of our 3 fusion-positive AML cases, consistent with the original diagnostic cytogenetic finding (n= 1 *RUNX1-RUNX1T1* fusion, 1 *CBFB-MYH11* fusion, and 1 *CBFA2T3-GLIS2* fusion). The 2 fusion-negative AML cases were also negative for structural gene fusions by HiC data. In our discovery sample set, Arima-HiC sequencing was able to find clinically-relevant SVs that were not previously detected in 3 of 6 samples: a *KMT2A-MLLT10* fusion was found in an AML case, and a *ZNF384-EP300* fusion was found in each of 2 ALL cases. A *ABHD17B-PTK2B* fusion was found in another ALL case, although *ABHD17B* may be a novel partner *PTK2B* and is undergoing validation. Rearrangements involving *KRAS* and *EGFR* were detected in the final two ALL cases, and are also undergoing validation. Overall, this study demonstrates how Arima-HiC sequencing can provide diagnostic value in pediatric leukemia specimens via the identification of clinically relevant SVs.

**LB123**

**Poorer outcomes in EGFR L858R-driven NSCLC treated with osimertinib may be addressed with novel combination of BLU-945 and osimertinib.**

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Background: Osimertinib, a 3rd-generation (gen) EGFR tyrosine kinase inhibitor (TKI), is standard of care in front-line (1L) patients with advanced EGFR mutant NSCLC; however, not all subgroups may benefit equally. In the phase 3 FLAURA study, patients with exon 19 deletions (ex19del) had a median progression-free survival (mPFS) of 21.4 months (mo); patients with L858R had a shorter mPFS of 14.4 mo. Poorer outcomes with L858R have also been reported with other 3rd-gen TKIs aumolertinib and lazertinib. These patients are a potential poor-risk subgroup for these therapies.
Here, we explored outcomes of patients with L858R-driven NSCLC using real-world datasets (RWDs), analyzed potential contributors to poorer outcomes, including co-mutation incidence and osimertinib potency for each mutation, and report preclinical proof of concept of combination treatment BLU-945, an investigational next-gen L858R inhibitor, with osimertinib.

Methods: Two large RWDs were analyzed for survival outcomes in 1L osimertinib-treated patients with ex19del or L858R from MD Anderson Cancer Center (MDACC; n=105) and the clinical-genomic Guardant INFORM database (EGFR ctDNA baseline positive; n=1386). $IC_{50}$s of osimertinib on *EGFR* mutations and wildtype were determined in BaF3 cells. Preclinical studies of combination BLU-945 and osimertinib were performed in L858R-driven BaF3 xenograft models.
Results: Both RWDs confirmed poorer prognosis for 1L osimertinib-treated patients with L858R *vs* ex19del. MDACC cohort showed a 12-mo PFS rate of 63% for L858R (n=45) *vs* 82% for ex19del (n=60); mPFS was immature. Guardant INFORM cohort (ctDNA baseline positive) had a median time-to-treatment discontinuation of 8 mo for L858R (n=517) and 11.4 mo for ex19del (n=869), *P*=0.003. Poor prognosis factors (including TP53 mutations and co-mutation number) were not significantly different between L858R and ex19del; both had a similar number of off-target mutations in post-osimertinib samples.
Strong association was found between osimertinib cellular $IC_{50}$ and osimertinib clinical trial outcomes (mPFS). Osimertinib exhibited most clinical and cellular activity on ex19del, followed by L858R, then G719X, and then exon 20 insertions.
BLU-945 in combination with osimertinib in a BaF3 L858R xenograft model demonstrated a longer duration of response *vs* osimertinib monotherapy.
Conclusions: In both RWDs, 1L osimertinib-treated patients with L858R-driven NSCLC had poorer outcomes *vs* ex19del, consistent with osimertinib's weaker activity on L858R. Preclinically, BLU-945 in combination with osimertinib increased L858R inhibition, resulting in more durable antitumor activity in L858R xenografts vs osimertinib alone, supporting rationale for combination treatment in patients with L858R mutations. This combination is being evaluated in 1L patients with L858R in the SYMPHONY study (NCT04862780).

**LB126**
**RELA fusion-positive ependymoma and diffuse midline glioma treated with VAL-083 under expanded access - case reports.**
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Ependymoma can occur anywhere in the central nervous system (CNS), but often occurs near the ventricle of the brain and central canal of the spinal cord. Ependymoma accounts for 5.7% of all childhood and 1.9% of all adult CNS tumors. RELA fusion-positive ependymoma is a subgroup associated with supratentorial location, higher WHO grade and worse prognosis. Diffuse Midline Glioma (DMG) is another relatively rare CNS tumor, originating in the midline locations of the brain (including thalamus, pons and spinal cord), accounting for 10% of all childhood and less than 4% of adult CNS tumors. For ependymoma standard treatment includes surgery and radiation therapy, with limited systemic options other than clinical trials for recurrent disease. For DMG, surgical intervention is restricted to biopsy, with radiation as standard therapy. Systemic options for both ependymoma and DMG are limited.VAL-083 is a bi-functional DNA-targeting agent which rapidly induces inter-strand DNA cross-links at N7-guanine inducing double-strand breaks causing cell death and acts independent of MGMT DNA repair and H3F3 K27M mutation status in high-grade gliomas. We report on 2 patients: one with ependymoma and one with DMG, treated with VAL-083 under an expanded access program. Both patients had recent disease progression and limited therapeutic options. Case #1: a 47-year-old male who was diagnosed with a left parietal high grade (3) anaplastic ependymoma and with IDHwt and unmethylated MGMT promoter status. Inter- and intragenic fusion analysis of tumor tissue revealed RELA fusion-positive ependymoma. He had undergone 2 resections and had received radiation and multiple systemic treatment regimens. Case #2: a 21-year-old male who was diagnosed with DMG of the brain stem, with IDHwt and unmethylated MGMT promoter status. He had undergone radiation therapy,

and multiple treatment regimens. Both patients were not eligible to participate in any clinical trial and received VAL-083 under an expanded access program. They initiated treatment with VAL-083 (30 $mg/m^2$ for 3 consecutive days every 21 days) and have completed 3 cycles. Both are neurological and radiological stable, they continue to receive VAL-083. No adverse events have been reported and no dose reductions have been required. These cases highlight that VAL-83 may be a treatment option for recurrent RELA fusion-positive ependymoma and DMG refractory to other treatment regimens. Additional safety and efficacy outcomes related to patient status will be presented at the meeting.Clinicaltrials.gov Identifier: NCT03138629. The treatment plans for this EAP patient were approved by MD Anderson Cancer Center IRB.

**LB127**

**VAL-083 in patients with recurrent glioblastoma treated under expanded access program.**

Carlos Kamiya-Matsuoka,[1] Shiao-Pei Weathers,[1] Rebecca A. Harrison,[2] Nazanin K. Majd,[1] Ashley E. Aaroe,[1] Stephanie Knight,[1] Teresa Hanna,[1] Timothy A. Gregory,[1] John Langlands,[3] Dennis Brown,[3] Vinay K. Puduvalli[1]. [1]MD Anderson Cancer Center, Houston, TX; [2]BC Cancer, University of British Columbia, Vancouver, British Columbia, Canada; [3]Kintara Therapeutics Inc, Menlo Park, CA.

Current standard-of-care for glioblastoma (GBM) includes surgery followed by concurrent therapy with radiation and temozolomide (TMZ) and adjuvant TMZ. Almost all GBM patients experience recurrent/progressive disease despite upfront standard of care treatment, with a median overall survival of 3-9 mo. after recurrence. There are limited treatment options available upon progression of disease which may include potential participation in clinical trials. However, patients may not meet the strictly defined entry criteria to participate in these clinical trials. VAL-083 is a first-in-class bifunctional alkylating agent that acts independent of O6-methylguanine-DNA-methyltransferase (MGMT) methylation status. Under an Expanded Access (EA) program, we have treated 24 patients with recurrent GBM, who were not eligible to participate in clinical trials with VAL-083. Four (4/24; 17%) patients had leptomeningeal disease (LMD) at time of enrolment. While safety data was assessed for all patients, those without LMD (20 patients) were evaluated for efficacy. All patients evaluated for efficacy received chemoradiation with TMZ, and the mean number of adjuvant TMZ cycles was 5 (± 6.2). The median time from last progression to start of VAL-083 was 0.65 mo. (95%CI: 0.32-1.55) and median KPS was 80 (25-75P: 70-90). Eight (8/20; 40%) patients had 2 or more prior recurrences, 9/20 (45%) patients had multifocal disease, and 5/20 (25%) had prior lomustine. Eighteen (18/20; 90%) patients had unmethylated promoter status for MGMT and 18/20 (90%) were IDH wild type. All patients had at least 1 mutation, with 11/20 (55%) having 5 or more mutations, and one patient had hypermutator phenotype with MSH6 mutation. The most common mutations were, TERT 11/20 (55%), PTEN 9/20 (45%), and TP53 6/20 (30%). All patients started treatment with VAL-083 at 30 $mg/m^2$ administered on 3 consecutive days every 21 days. Seven patients received bevacizumab concurrently with VAL-083. VAL-083 was well tolerated and the main adverse events were consistent with prior experience, i.e., thrombocytopenia and neutropenia. Eight (8/24; 33%) patients had a dose reduction, 7 of which were due to thrombocytopenia, and 1 due to neutropenia. Five patients with thrombocytopenia had prior lomustine. As of cut-off date (05 Jan, 2023), median progression free survival (mPFS) and median overall survival (mOS) from last disease progression was 5.9 mo (95%CI: 3.9-7.9) and 9.4 mo (95%CI: 3.0-14.3), respectively. In patients without multifocal disease mOS was even longer, 14.3 mo (95%CI: 3.9-14.3). Use of VAL-083 in this expanded access study showed benefit in the treatment of recurrent GBM patients even those who have had multiple recurrences and were not candidates for the treatment through clinical trial. Additional safety and efficacy measures will be presented at the meeting.Clincialtrials.gov Identifier: NCT03138629. All EA treatment requests and plans were approved by MD Anderson Cancer Center IRB.

**LB128**

**Cardiovascular disease mortality among cancer survivors by race and ethnicity in the United States.**

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**Background** Cancer survivors have higher cardiovascular disease (CVD) mortality than the general population but comprehensive data by race and ethnicity are limited.
**Methods** Data from 17 Surveillance, Epidemiology, and End Results (SEER) registries were used to identify adults diagnosed with invasive cancer at ages ≥20 years from 2000 to 2018. CVD mortality was calculated for five mutually exclusive race/ethnicity categories (non-Hispanic American Indian/Alaska Native [AIAN], non-Hispanic Asian or Pacific Islander [API], Hispanic, non-Hispanic Black [Black], and non-Hispanic White [White]). Standardized mortality ratios (SMR) were estimated to compare mortality rates in cancer survivors to their counterparts in the general population. Analyses were stratified by demographic factors, CVD subtype, and cancer site.
**Results** During a mean follow up of 5.3 person-years among 5,786,876 survivors (20-64 years, 49.3%; men, 51.2%; White, 71.7%; Black, 10.1%; Hispanic, 10.9%; API, 7%, AIAN, 0.3%), 358,970 CVD deaths (cardiac death, 76.9%; cerebrovascular death, 15.9%) occurred. CVD mortality per 10,000 person-years was highest among survivors who were Black (122.9), followed by White (114.7), AIAN (86.7), API (78.6) and Hispanic (75.3). In contrast, SMRs were greatest among AIAN survivors (SMR=1.42, 95% CI=1.32-1.51), followed by API (SMR=1.39, 1.37-1.41), Hispanic (SMR=1.29, 1.27-1.30), Black (SMR=1.19, 1.17-1.20) and White (SMR=1.08, 1.07-1.08) survivors. Among survivors ages 20-64 years, Black survivors were approximately three times as likely to die from cardiac death as Hispanic or API survivors (46.7 vs. 14.5-17.6 per 10,000). SMR, however, was 1.47 among Hispanic survivors (95% CI=1.42-1.51) and 1.80 among API survivors (95% CI=1.42-1.51), greater than 1.25 among Black survivors (95% CI=1.22-1.27). Across cancer types, the highest SMR was among API survivors of pancreatic cancer (SMR=5.27, 3.83-7.08) and brain cancer (SMR=4.56, 3.10-6.48) in ages 20-64 years and among Hispanic (SMR=3.77, 3.09-4.56) and API survivors of brain cancer (SMR=3.24, 2.40-4.28) in ages ≥65 years.
**Conclusion** Black survivors had the greatest CVD mortality of all groups, demonstrating that CVD health disparities in the general population extend to cancer survivors. Despite the lower absolute mortalities, relative risks compared to their counterparts in the general population were greatest among API and Hispanic survivors, implying a greater impact of cancer diagnosis on the risk of CVD death among survivors of these populations. Targeted primary care and cardio-oncologic intervention strategies are needed to improve CVD outcomes and achieve cardiovascular health equity among cancer survivors.

**LB130**

**Single-cell multiomics analysis of myelodysplastic syndrome predicts clinical response to DNA methylation inhibitor therapy.**

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Epigenetic alterations, such as DNA methylation aberrations, are a hallmark of human cancer. This observation has led to the assessment of hypomethylating agents (HMAs) as potential drugs to treat oncological patients, being considered the clinical approval of these pharmacological compounds in the therapy of myeloid malignancies as one of the main achievements of this scientific field. A good example is myelodysplastic syndrome (MDS), a pre-leukemia disorder that can evolve into acute myeloid leukemia (AML), where very few therapeutic options were available until the introduction of HMAs.

However, it remains unsolved how we can predict if an MDS patient will response or not to the epigenetic drug and for how long. Using the great analytical power of the newly developed single-cell technologies, we have herein tackled this issue. To study the evolution of the clonal molecular and cellular architecture MDS upon the treatment with HMAs, we performed single-cell DNA sequencing (scDNA-seq) of an amplicon panel for 53 genes commonly mutated in myeloid malignancies and single-cell protein sequencing (scProt-seq) of 45 cell-surface proteins to provide a simultaneous landscape of the genetic setting and immunophenotype. We sequenced hundreds of thousands of cells from these MDS patients where paired bone marrow samples were obtained at the time of diagnosis and at the end of at least six cycles of azacitidine treatment. Our study has unveiled that the co-occurrence of particular truncating or stop codon mutations in the same myeloid progenitor cell; the multibranched dynamics of the mutant clones; and the persistence of particular cell lineages with distinct mutational profiles (such as non-classical monocytes and CD8+ effector T-cells) can predict the clinical response of MDS patients to HMA therapy.

**LB131**

**Association between epigenetic heterogeneity of esophageal adenocarcinoma and response to first-line immunochemotherapy in LUD2015-005 Trial.**

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Immune checkpoint inhibitors (ICI) were recently approved as a first-line treatment for inoperable esophageal adenocarcinomas (EAC) in combination with chemotherapy (CTX). Unfortunately, even though EAC has one of the highest tumor mutation burdens among all cancer types, response to immunochemotherapy (ICI+CTX) is highly variable, and the underlying molecular basis is incompletely understood. While genomic features such as mutations and copy number alterations in EAC are highly variable across samples, DNA methylation array data from numerous studies suggested that EAC can be clustered into a few consistent subtypes. We thus hypothesize that epigenetic heterogeneity of EAC may contribute to or associate with patients' heterogeneous response to ICI+CTX, possibly through modulation of key genes or neoantigens. To test this hypothesis, we took advantage of a unique ICI+CTX LUD2015-005 trial in which inoperable EAC patients received first-line ICI for four weeks (ICI-4W), followed by ICI+CTX. Instead of methylation array, we also used a new DNA methylation sequencing technology, TET-Assisted Pyridine-Borane Sequencing (TAPS), on 64 tumor and 15 adjacent normal tissue samples collected from 23 EAC patients before and throughout treatment. Unlike prior studies that used methylation arrays which only cover ~2.5% of all CpG sites in the genome, TAPS detects genome-wide, base-resolution DNA methylation information. Furthermore, many previous studies did not account for variability in tumor content between samples, which could impact downstream DNA methylation analyses. In view of this, we proposed an analytical framework that includes tumor content and local copy number as key parameters, which estimates the tumor and stromal methylation and tests for differentially methylated regions (DMR). We also performed unsupervised clustering based on large scale genome-wide methylation pattern and revealed 2 major tumor clusters. Using the whole genome data, we identified a large set of shared tumor-specific DMRs, revealing that hypomethylation across wide regions of the genome and hypermethylation of certain gene bodies are common features in EAC. We also identified a set of shared outcome-associated DMRs in pre-treatment samples, which predicts better progression-free survival at 12 months. We further performed subgroup analysis of the 2 tumor clusters. Interestingly, we found higher numbers of cluster-specific prognostic DMRs with stronger effect sizes. This suggests that tumor subtypes may respond differently to treatment, and should be considered

separately in statistical analyses. Altogether, these results indicate that a detailed understanding of tumor epigenetic heterogeneity will improve patient stratification in immunochemotherapy.

**LB132**

***In silico* mutant neoantigen prediction - a promising avenue for target identification in human adrenocortical carcinoma.**

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**Introduction:** Adrenocortical carcinoma (ACC) is one of the most aggressive endocrine malignancies and confers a poor prognosis in advanced stages. Effective treatments are lacking. The results of immune checkpoint inhibition were disappointing with few responders only deriving clinical benefit. For the development of novel immunotherapies such as tumor vaccines and T cell-based treatments, target identification is essential. Tumor-specific mutant neoantigens that may be recognized by T cells in the context of major histocompatibility complex (MHC) I are promising candidates.
**Methods:** We performed whole exome-sequencing in 10 ACC samples with matched blood controls. Somatic mutations were identified using an in house bioinformatics pipeline. By coupling POLYSOLVER for HLA typing with netMHCpan, *in silico* binding affinity of tumor-specific neoantigens to MHC was calculated taking into account both peptide and HLA sequence information. Strong binding (SB) was defined as <0.5% rank, weak binding (WB) as 0.5-1.9% rank and no binding (NB) as >2.0% rank of all peptides to MHC.
**Results:** Across 10 ACC patients, we identified 1067 unique somatic mutations (median 49.5, ranging from 15-590), affecting 989 different genes. Binding affinity changed for 414 predicted neoantigens from NB to WB, 80 from NB to SB and 82 from WB to SB. The mutant neoantigen load per patient ranged from 10 to 235 (mean 66.2) and was positively correlated to the total number of non-synonymous single nucleotide variants ($R^2$ 0.8977, 95% CI 0.79-0.99; $p<0.0001$).
**Discussion:** This is the first study that demonstrates successful *in silico* neoantigen profiling in ACC. Mutant neoantigens were predicted to be present both in ACC with high and low tumor mutational burden. These data will pave the way for *in vitro* validation and hold potential to develop therapeutic cancer vaccines and T cell-based cancer immunotherapy in ACC.

**LB133**

**An orally bioavailable degrader targeting androgen receptor and the splice variant in castration resistant prostate cancer.**

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The development and progression of prostate cancer depends on androgen receptor (AR) whose sustained activity albeit the use of anti-androgen drugs accounts for one of the major mechanisms leading to drug resistance. As such, the clinical benefits of current therapeutic agents remain rather limited for the advanced prostate cancer. Given that expression of constitutively active AR variants (AR-Vs) that lack the ligand-binding domain (LBD) clinically associates with the drug resistance and poor survival, targeting both AR and the LBD-truncated AR-Vs is anticipated an effective approach to overcome therapy resistance. Here, we present Proteolysis Targeting Chimeras (PROTAC) compound ITRI-148, capable of effectively degrading both full-length and LBD-truncated AR by targeting the proteins via the N-terminal

domain to Cereblon-mediated ubiquitination and degradation. Degradation of AR and AR-V7 suppresses target gene expression, and inhibits cell proliferation with accompanied apoptosis activation in AR-V7-expressing castration-resistant prostate cancer (CRPC) cell models. *In vivo*, this compound displays good pharmacokinetics profiles and oral bioavailability with strong antitumor efficacy towards castration-, enzalutamide-resistant CWR22Rv1 and VCaP xenograft models. These data provide compelling evidence that by targeting AR NTD for induced degradation of active AR protein variants, ITRI-148 is a promising agent for the intervention of advanced therapy-resistant prostate cancers.

**LB134**

**Targeting coactivators to inhibit ESR1 fusion-driven breast cancer growth.**

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Background: In-frame chimeric proteins that are encoded by *ESR1* exons 1 to 6 (ESR1-e6) fused to C-terminal gene partners can induce hormone-independent growth and endocrine therapy (ET) resistance of estrogen receptor-α (ERα) positive breast cancer by driving a unique transcriptional signature (PMID: 34711608). However, the mechanism how active ESR1 fusions reprogram transcription is not understood. Moreover, the lack of targeted treatment due to the loss of ligand binding domain (LBD) in *ESR1* gene fusions necessitates the need to develop new therapies targeting active ESR1 fusions that promote tumorigenesis.
Methods: An estrogen response element (ERE) DNA pull down assay was performed to identify coactivators recruited by active ESR1 fusions. An alamar blue assay was utilized to assess cell growth after treatment with pharmacological inhibitors to the coactivators. Single cells isolated from ERα+ patient-derived xenografts (PDX) were used to form organoids (PDxOs). A CellTiter-Glo 3D assay was conducted to measure the growth of PDxOs after treatment with coactivator inhibitors.
Results: Using ERE DNA pull down assays, we identified bromodomain-containing proteins (BRDs) and steroid receptor coactivators (SRCs/p160 family members) as key regulators recruited by active ESR1 fusions from nuclear extracts of breast cancer cells. A pan-BET family inhibitor (BETi), birabresib, and pan-SRC inhibitors (SRCi), SI-2 and SI-12, inhibited the growth of ERα positive breast cancer cells and PDxOs expressing an active ESR1 fusion or LBD point mutation. Importantly, the combination of birabresib and a CDK4/6 kinase inhibitor (palbociclib) conferred the largest growth reduction of PDxOs expressing an ESR1-YAP1 fusion. RNA sequencing showed BETi and SRCi led to downregulation of ERα target gene expression and cell cycle pathways, with upregulation of apoptosis pathways. Here, we propose a model whereby BRD and SRC protein families are major regulators of active ESR1 fusion-driven gene transcription and growth and positioned the potential therapeutic significance of BETi and SRCi to be tailored to treat active ESR1-translocated breast tumors.
Conclusions: Active ESR1 fusions cannot be treated with standard-of-care ET due to the lack of the LBD of ERα. Therefore, it is critical to investigate how active, in-frame ESR1 fusion proteins induce gene transcription and to develop molecular mechanism-based targeted therapies. Here, we revealed two groups of coactivators (BRDs and SRCs) as critical transcriptional regulators of active ESR1 fusion-driven tumor biology. Our study demonstrated the efficacy of a BETi (birabresib) and SRCi (SI-2/SI-12) in inhibiting the viability of active ESR1 fusion/LBD mutation expressing breast cancer cells and 3D grown PDxOs. Importantly, birabresib combined with a standard-of-care CDK4/6 inhibitor conferred the best PDxO growth reduction.

**LB137**

**Ovulatory years prior to menopause and postmenopausal endogenous hormone levels.**

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Background: Estimated lifetime ovulatory years (LOY) is the difference between ages at menopause and menarche subtracting time for events interrupting ovulation. We tested whether LOY influence sex-hormone levels in postmenopausal women not using hormone therapy.
Methods: Hormones, including estradiol and testosterone, were measured in 1976 postmenopausal women from the Nurses' Health Study in plasma collected in 1990. Effects of age, years since menopause, body mass index (BMI), smoking status, and hysterectomy on hormone levels were assessed by t-tests and ANOVA. Multivariable linear regression was used to assess associations between LOY and hormones adjusted for potential confounders and the trend in hormone levels per 5-year increases in LOY estimated.
Results: Women in the sample averaged: 61.4 years old at blood draw, 11.0 years since menopause, with a BMI of 25.8 kg/m$^2$. 17.5% had a hysterectomy and 13.8% were current smokers. These characteristics were associated with one or more hormone levels and included as adjustment variables. Each 5-year increase in LOY was associated with a 6.0% increase in testosterone (95% CI 3.3%, 8.7%) in all women. In women with above-average BMI, each 5-year increase in LOY was associated with a 7.3% (3.5%, 11.2%) increase in testosterone, a 6.9% (2.7%,11.3%) increase in estradiol, and a 6.9% (2.9%,10.9%) increase in estrone.
Conclusion: Greater LOY is associated with higher testosterone in postmenopausal women as well as greater estradiol in those with above-average BMI. This may be due to an accumulation of functioning stromal and thecal cells from repeated ovulation and peripheral conversion of testosterone.

**LB138**

**The conception of an Oncology Neurodiversity Work Group to address cancer disparity for individuals with autism and intellectual or developmental disability (IDD) at the Sidney Kimmel Cancer Center.**

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Studies have suggested that autistic individuals are as much as 30% more likely to receive a cancer diagnosis which may be associated with co-occurring IDD or birth defects. Following a diagnosis of cancer, outcomes have been documented to be significantly worse for autistic individuals. Autistic individuals experience numerous barriers to care across healthcare settings, including a lack of appropriate communication with providers, a lack of accommodating healthcare environments, lack of mental health support, and too few providers who are trained in working with individuals with autism or IDD. This leads to numerous negative health outcomes, including lower likelihood of having a regular source of care, lower satisfaction with healthcare, reduced screening utilization, delays in recognizing early symptoms of disease, higher ED utilization, and anxiety and stress associated with healthcare visits. We report on the results of a recent Medical Oncology provider survey conducted from 10/31/22 - 12/16/22 to evaluate for opportunities to address barriers to care facing individuals with autism and/or IDD. Of the 49 respondents comprised of physicians and nurse practitioners, 93.9% (46) noted receiving five or fewer hours of specific training or education regarding caring for populations with IDD. Additionally, providers cited the following barriers most commonly related to delivery care to patient with IDD: Lack of strategies to enhance communication on site (32, 65.3%), Inadequate time allotted for visits (28, 57.1%), Inadequate staff training and education related to this group (27, 55.1%), Limited resources to support vulnerable or lower socio-economic status populations (22, 44.9%), and Fragmentation of care (19, 38.8%).

The Sidney Kimmel Cancer Center and the Jefferson Center for Autism & Neurodiversity have collaborated to develop a culturally competent care design aimed at understanding the needs of our neurodiverse population through a joint research effort. The results of our Medical Oncology provider survey identified the need for training of providers and staff to optimize the patient experience, creation of accessible cancer screenings, and the adoption of an environment that supports the sensory and communication abilities and needs of neurodivergent patients. Providers that adopt best practices in the care of autistic and/or IDD individuals have noted that patients and families report marked improvement in the rating of their patient experience in the visit. The desired product is care plans, appropriate medical settings, and trained providers that will meet the needs of this population and improve overall health outcomes.

**LB139**

**Factors associated with clinical trial participation among female cancer survivors in Maryland.**

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Background: There are known disparities in cancer-related clinical trial participation within the oncology community, including disparities by race/ethnicity, age at cancer diagnosis, education, and obesity status. Our study examines the associations between these factors and cancer clinical trial participation among a diverse population of female cancer survivors living in Maryland- a racially and socioeconomically diverse state that is a significant resource to research cancer health disparities.
Methods: The Maryland Behavioral Risk Factor Surveillance System (BRFSS) is an ongoing telephone-based disease surveillance program that collects data on lifestyle factors, health status, and healthcare access. The Maryland BRFSS takes a sample of ~15,000 non-institutionalized Maryland residents (≥18 years) per year and includes state-specific modules, including a cancer survivorship module to elicit responses among respondents who indicated they had a past or current cancer diagnosis. This study restricted data to female cancer survivors who participated in the Maryland BRFSS in 2011, 2013, 2015, 2017, 2019, and 2020. Maryland BRFSS respondents who reported having more than one type of cancer, did not report the type of cancer diagnosed, or had skin cancer except for melanoma were not eligible for the study. Weighted multivariable logistic regression models were constructed to examine the associations between risk factors (race/ethnicity, education, age at diagnosis, obesity, and cancer type) and odds of clinical trial participation, determined by the yes/no response to the question, “Did you participate in a clinical trial as a part of your cancer treatment?”
Results: A total of 1,633 female cancer survivors were included in the analysis (1,353 non-Hispanic White (NHW); 280 non-Hispanic Black (NHB)). The average age at cancer diagnosis among the respondents was 55 years (SD=16 years), with breast cancer as the most common cancer diagnosis (41.1%). A total of 489 women reported being obese. Only 5.8% of the respondents reported participating in a clinical trial for their cancer treatment; this response differed by race/ethnicity (5.1% of NHW vs 8.9% of NHB; $\chi 2$ $p$= 0.01). NHB women were 2.57 times more likely to participate in a clinical trial (95% confidence intervals (CI): 1.19, 5.53) compared to NHW women. Women with breast cancer were 3.19 times more likely (95% CI: 1.55, 6.56) to participate compared to women with other cancer types. Lastly, women who reported obesity at survey were less likely (adjusted odds ratio: 0.41; 95% CI: 0.19, 0.88) to participate compared to non-obese women. Associations between age at cancer diagnosis (≥50 years vs <50 years), education (college graduate vs. not), and clinical trial participation were not statistically significant.
Conclusions: Our results demonstrate that among Maryland female residents with a history of cancer, NHB women were more likely to participate in clinical trials for their cancer treatment, compared with NHW women. We also found significant associations with cancer type, obesity, and odds of clinical trial participation. Our study findings should be interpreted with consideration of the study limitations, including not having the ability to ascertain the respondents’ eligibility and/or their invitation to participate in clinical trials during their cancer treatment.

**LB140**

**Inequity in cancer crowdfunding among LGTBQ+ cancer survivors.**

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Background: Lesbian, gay, bisexual, transgender, queer, and other sexual and gender minority (LGBTQ+) individuals are twice as likely to live in poverty and carry a greater cancer burden than non-LGBTQ+ individuals. Crowdfunding, a type of online fundraising, is increasingly used for cancer-related financial support, but the extent to which LGBTQ+ inequities exist in crowdfunding success has not previously been studied.
Methods: In December 2022, we extracted 494,242 publicly available crowdfunding campaigns from GoFundMe using web-scraping. We then applied two textual dictionaries to categorize the sample for analysis. The first dictionary identified health campaigns that were in English and contained cancer terms (n=196,038). The second dictionary used a list of terms from prior research that were supplemented by an LGBTQ+ study advisory board to stratify the cancer campaigns by LGBTQ+ identity (yes vs. no) of the campaign creator and/or beneficiary. Outliers in the fifth and ninety-fifth percentile for fundraising goal amount were dropped resulting in a final sample of N=179,793 campaigns for analysis. Summary statistics and regression models were calculated using Stata 17 to describe differences in funding goals, amount raised, and number of donors by LGBTQ+ identity, adjusting for year of the campaign and geographic location.
Results: In total, the average campaign goal was $15,891 (Standard Deviation (SD): $13,816), average amount raised was $6,281 (SD: $5,409), and average number of donors was 61 (SD: 47). There were n=1,280 LGBTQ+ cancer campaigns (0.65%). In multivariable models, LGBTQ+ cancer campaigns had goals that were on average $2,009 lower than non-LGBTQ+ cancer campaigns (95% Confidence Interval (CI): -$2,798 - -$1,220, p<0.001), when controlling for year of campaign and geographic location. Similarly, LGBTQ+ cancer campaigns raised $500 less than non-LGBTQ+ cancer campaigns (95% CI -$821 - -$178; p=0.002) when controlling for year of campaign and geographic location. LGBTQ+ campaigns had on average 2.81 more donors than non-LGBTQ+ campaigns (95% CI: 0.05 - 5.56, p=0.046), adjusting for year of campaign and geographic location.
Conclusions: When controlling for year of campaign and geographic location we observed significant disparities in goal amount and funds raised between LGBTQ+ and non-LGBTQ+ cancer campaigns. However, LGBTQ+ campaigns on average had more donors. Our findings suggest that while there may be stronger community among LGBTQ+ populations (i.e., higher number of donors), LGBTQ+ cancer survivors may face substantial financial burden inequities. LGBTQ+ specific supportive services and interventions may help improve economic equity among cancer patients.

**LB142**

**Metabolically defined body size phenotypes in relation to subsequent colorectal cancer risk.**

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Overweight and obesity have been linked to increased risk of several diseases, including colorectal cancer, but the underlying mechanisms are not fully known. An earlier study analyzed metabolically defined body size phenotypes in relation to colorectal cancer risk, using combinations of C-peptide (a marker of insulin resistance) and body mass index (BMI). Higher serum C-peptide concentrations were associated with higher colorectal cancer risk in both overweight and normal weight individuals compared to individuals with low levels of C-peptide and normal weight. The aim of the present study was to see if these results could be replicated and to further explore the role of metabolism and body size phenotypes

in colorectal cancer subtypes. We conducted a nested case-control study of 1010 individuals with colorectal cancer and 1010 individually matched (age, sex, cohort, year of blood sampling and data collection, number of freeze-thaw cycles of plasma samples and fasting status at blood sampling) control participants from the population-based Northern Sweden Health and Disease Study. The blood samples and data used in our analyses were collected at a mean of 9.7 years prior to case diagnosis. Participants were categorized as (1) metabolically healthy/normal weight (BMI < 25 kg/m$^2$), (2) metabolically healthy/overweight (BMI ≥ 25 kg/m$^2$), (3) metabolically unhealthy/normal weight (BMI < 25 kg/m$^2$), or (4) metabolically unhealthy/overweight (BMI ≥ 25 kg/m$^2$), where metabolically healthy was defined as C-peptide in the lowest tertile, as in the previous study. We used multivariable conditional logistic regression to calculate odds ratios and confidence intervals and adjusted for tobacco smoking, recreational physical activity and alcohol consumption as potential confounders. In our preliminary results, metabolically unhealthy/overweight individuals had a significantly higher risk of developing colorectal cancer compared to metabolically healthy/normal weight individuals (adjusted odds ratio =1.44, 95% CI 1.13-1.85). The odds ratio for metabolically unhealthy/normal weight individuals was 1.23 (95% CI 0.92-1.65) and for metabolically healthy/overweight individuals 1.23 (95% CI 0.89-1.70). These results support the potential of a more nuanced analysis of metabolism and body size to better understand their etiological contribution to colorectal cancer development. To gain more knowledge about how the role of metabolism and body size might differ between tumor subtypes, we will also analyze body size phenotypes in relation to subtypes of colorectal cancer based on anatomical tumor site, *KRAS* and *BRAF* mutations and microsatellite instability status of the tumor.

**LB143**

**Improving treatment outcomes: a digital solution for remote patient monitoring of stomatitis for patients receiving Dato-DXd.**

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Instances of stomatitis are very painful and compromise nutrition and oral hygiene as well as induce an increased risk for local and systemic infection. Stomatitis is a well-known toxicity of radiotherapy, chemotherapy and other targeted therapies and is associated with dose interruptions, reductions, and treatment discontinuation. For upcoming Dato DXd phase III trials, the mitigation strategies included patient education delivered at the site and supported by printed material to foster continued adherence to an oral care protocol (OCP).

The study team thought to leverage digital technology, namely patient-provisioned smartphones to bring the OCP right “to the patients’ fingertips”, while enabling health care providers at the site to monitor onset or worsening of stomatitis for each patient.

The digital solution consists of a patient interface for data acquisition and personalized education/behavioral reinforcement, and a clinician interactive data dashboard to access, in real time, patients’ records of stomatitis-related symptoms and their impact on nutrition and hydration, respectively. For a few days post infusion, the solution surfaces personalized illustrated vignettes to provide the participant with simple recommendations for self-care and probe to contact the clinical site team when symptom(s) reach a given morbidity threshold. The clinical team can visualize data on demand or upon being prompted, to quickly assess trends towards stomatitis worsening and decide on the next course of action.

This solution for remote patient monitoring of stomatitis risk is deployed in four studies and is planned for an additional three, reaching approximately a total of 7,000 patients across 49 sites in 16 countries. Early feedback from patients confirmed the engaging design, the low burden daily usage, and the perceived utility of the solution. Patients who had experienced stomatitis previously indicated that such a solution would have given them confidence to engage with their site sooner to receive care. Further structured feedback from clinical study teams and analyses of clinical trial data are planned to inform

patients' sustained compliance using the solution and its effectiveness in mitigating high grade of stomatitis and helping patients to better manage their treatment and outcomes.

**LB144**

**The nationwide population-based cohort study on the risk of immune-mediated diseases and risk of cancer in KOREA.**

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Background: Immune-mediated diseases (IMDs) constitute a clinically heterogeneous group of disorders, affecting up to 5%-7% of the population worldwide. Disorders caused by IMDs are a group of diseases caused by abnormal activities of immune cells, which overreact or attack the body to show extreme inflammatory reactions or lose the ability to recognize and counter tumor cells. There is increasing evidence that IMDs play an important role in carcinogenesis and associated with the higher risk of cancers. To provide nation-wide evidence on the link between IMDs and cancer, this study evaluated the association between IMD and risk of cancer using the National Health Insurance Service-National Sample Cohort (NHIS-NSC) in Korea.
Method: Among a total of 1,137,861 cohorts registered between 2002 and 2019 from the NHIS-NSC , the cohorts with first cancer diagnosis at the time of death were excluded and 1,056,170 cohorts were included in the analysis. IMD and cancer cases were identified as at least one hospitalization or two outpatient visits using the Korean Standard Classification of Diseases-8 (KCD-8) codes. We required the IMD diagnosis to be present at least 12 months before the cancer diagnosis. The associations of overall and 47 specific IMDs with overall and 75 specific cancer risk were estimated by relative risks (RRs) and 95% confidence interval (95% CI) using a log-binomial regression model adjusted for sex and age.
Results: Total 44,329 (4.20%) IMD cases were identified. A total of 3,094 (6.97%) cancer cases were documented in IMDs and 51,056 (5.09%) cancer cases in non-IMDs. Overall IMD was significantly associated with the 1.38-fold higher risk of cancer (95% CI=1.33-1.43). Specifically, 32 out of 47 (68.09%) IMDs showed the statistically significant association with the increased risk including relatively strong or moderate associations; psoriatic (RR=9.75; 95% CI=2.43-38.99), rheumatic heart disease (RR=6.71; 95% CI=4.07-11.10), primary biliary cholangitis (RR=5.00; 95% CI=4.35-5.75), systemic scleroderma (RR=4.18; 95% CI=2.34-7.45), giant cell arteritis (RR=4.17; 95% CI,1.53-11.39), sarcoidosis (RR=3.61; 95% CI=1.63-7.96), aplastic anemia (RR=3.54; 95% CI=1.01-12.43), Wegener's granulomatosis (RR=3.07; 95% CI=1.09-8.69), polymyositis (RR=2.92; 95% CI=1.91-4.48), polymyalgia rheumatica (RR=2.84; 95% CI=2.18-3.72), systemic lupus erythematosus (RR=2.84; 95% CI=1.77-4.53). When cancers were classified by body system, the more prominent associations between overall IMD and cancers in respiratory-intrathoracic organs (RR=3.73; 95% CI=2.85-4.88), urinary tract (RR=1.62; 95% CI=1.23-2.14), thyroid and other endocrine glands (RR=1.58; 95% CI=1.46-1.71), and lip-oral-cavity-pharynx (RR=1.55; 95% CI=1.12-2.15) were observed.
Conclusion: This retrospective cohort study provides the nation-wide population-based evidence that IMDs are associated with the increased risk of cancers supporting the immunomodulation in carcinogenesis.

**LB145**

**The relationship of socio-behavioral factors to the symptom experience of Asian American breast cancer survivors: a structural equation model.**

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Background: Despite an increasing number of studies among breast cancer survivors, little is known

about how socio-behavioral factors influence the symptom experience of racial/ethnic minority breast cancer survivors, especially Asian American breast cancer survivors. The identified socio-behavioral factors could give directions for development of unique intervention components that are tailored to a specific population in needs.
Purpose: This study examined the relationship of multiple socio-behavioral factors including attitudes, perceived barriers, social influences, and self-efficacy to the symptom experiences of Asian American breast cancer survivors.
Methods: This secondary analysis was conducted using data collected from 195 Asian American female breast cancer survivors. Subjects were recruited from January 2017 to June 2020 through online and offline communities and groups. The study variables were measured using validated instruments such as the Questions on Attitudes, Self-Efficacy, Perceived Barriers, and Social Influences (QASPS); the Cancer Behavior Inventory (CBI); and the Memorial Symptom Assessment Scale-Short Form (MSAS-SF). The hypothetical model was built based on Bandura's self-efficacy theory. Mediation analysis was conducted using structural equation modeling with SPSS version 26.0 and Amos 28.
Results: The overall fitness of the hypothetical model to the data was acceptable ($\chi 2$=51.36, p<.001, the goodness of fit index [GFI] = .95, adjusted GFI [AGFI] = .89, comparative fit index [CFI] = .96, Tucker-Lewis index [TLI] = .94, normed fit index [NFI] = .94, and root mean square error of approximation [RMSEA] = .08 Attitude and perceived barrier and social influence had a significant direct effect on self-efficacy (direct effect, β = 0.247, p =.003; β = -0.552, p =.003; β = 0.241, p =.002, respectively). Attitudes indirectly influenced symptom distress scores through self-efficacy (β =-0.054, p = .019). Perceived barriers had a significant direct effect (β = 0.605, p=.003), indirect effect (indirect effect, β = 0.121, p =.024), and a total effect on symptom distress scores (total effect, β = 0.726, p =.004). In addition, social influence had an indirect effect on symptom distress scores through self-efficacy (β = -0.053, p = .017)
Conclusions: The findings supported that the self-efficacy for coping had a mediating effect on their symptom experience among Asian American breast cancer survivors as well as
identified meaningful associations with attitudes, perceived barriers and social influences and symptom experience. Future research and practice should consider socio-behavioral factors, especially self-efficacy, in symptom management of Asian American breast cancer survivors.
Acknowledgments: The parent study was funded by the National Institutes of Health (NCI/NINR; 1R01CA203719).

**LB146**
**Imaging surveillance practice patterns among older adults with stage I/II non-small cell lung cancer.**
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Background: Following completion of curative-intent treatment, patients with non-small cell lung cancer (NSCLC) are recommended to undergo routine imaging surveillance to detect potential cancer recurrence. We aimed to evaluate adherence to guideline recommended imaging surveillance and factors associated with either adherence or non-adherence.
Methods: We utilized data from the Surveillance, Epidemiology, and End Results (SEER)-Medicare Program for the years 2003-2016. Participants were included if they were diagnosed with stage I/II NSCLC, survived for more than 2 years following completion of treatment, between the ages of 65-85, and received either surgical resection or stereotactic body radiotherapy (SBRT) as their primary treatment. Participants were considered to have received guideline-recommended imaging surveillance if there was record of receiving at least one computed tomography (CT) or chest x-ray (CXR) during the following four time windows following completion of curative intent treatment: 90-270 days, 360-450 days, 540-630 days, and 720-810 days. Logistic regression analysis was used to evaluate whether race/ethnicity, sex, age group, treatment type, stage of disease, histology, insurance status, marital status, and comorbidity

burden were associated with adherence to guideline-recommended imaging surveillance. All analyses were performed using SAS 9.4.
Results: A total of 14,296 NSCLC patients were included in the present analysis. Most participants were male (57.9%) and Non-Hispanic (NH) white (85.8%). Notably, only 21.9% of patients received guideline-recommended imaging surveillance during the entire 2-year follow-up period. When evaluating adherence within the first year of follow up only, 51.1% of participants met adherence criteria. Factors associated with receipt of guideline-recommended surveillance during the 2-year surveillance period include being married (OR: 1.17; 95% CI:1.06-1.29), receipt of neoadjuvant systemic therapy (OR: 1.76, 95% CI: 1.12-2.76), and a Charlson comorbidity score of >=3 (OR: 1.17, 95% CI: 1.03-1.33). Factors associated with not receiving guideline-recommended imaging surveillance included NH Black race (OR: 0.73, 95% CI: 0.59-0.91) and receipt of adjuvant systemic therapy (OR: 0.68, 95% CI:0.57-0.81), adjuvant radiotherapy (OR: 0.50, 95% CI: 0.40-0.61), or SBRT only (OR: 0.74, 95% CI: 0.63-0.87).
Conclusion: Adherence to guideline-recommended imaging surveillance is low in this population. Strategies to increase compliance to imaging surveillance, particularly among NH black populations, are needed.

**LB147**
**The mediating effects of socio-behavioral factors on the needs for help among Asian American breast cancer survivors.**
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Background: Breast cancer survivors often report their high needs for help during the transition to cancer survivorship. During the COVID-19 pandemic, technology-based programs are increasingly popular because of their high flexibility and accessibility in delivering information and coaching/support to address the current needs for help among cancer survivors. Yet, little is known about how socio-behavioral factors influence the effects of a technology-based intervention on the needs for help of racial/ethnic minority breast cancer survivors, especially Asian American breast cancer survivors.
Purpose: The purpose of this secondary analysis was to examine the multiple socio-behavioral factors (including attitudes, self-efficacy, perceived barriers, and social influences related to breast cancer survivorship) mediated the effects of a technology-based intervention on the needs for help among Asian American breast cancer survivors.
Methods: This analysis was conducted with the data from 199 Asian American breast cancer survivor women who were recruited from January 2017 to June 2020 through online and offline communities/groups. The needs for help were measured using the Support Care Needs Survey-34 Short Form (SCNS) with five domains on psychological, information, physical, support, and communication needs. Mediation analysis was conducted using the PROCESS macro within SPSS. The analysis determined the mediating effects of four socio-behavioral mediators on the needs for help at pre-test [$T_0$], post 1-month [$T_1$], and post 3-months [$T_2$] of a technology-based intervention.
Results: Overall, all the mediators had statistically significant mediation effects on all types of needs for help ($p < .05$) at different points. Attitudes and social influence presented statistically significant mediation effects on the total needs for help score over 3 months ($T_0$, $T_1$, and $T_2$). Perceived Barriers had mediation effects on all types of needs for help over 1 month ($T_0$, $T_1$). Self-efficacy mediated the effects on all types of needs for help only at post 1 month ($T_1$).
Conclusions: The findings supported that all the socio-behavioral factors (attitudes, self-efficacy, perceived barriers, and social influences) mediated the effects of a technology-based intervention on the needs for help of Asian American breast cancer survivors. Future research and practice should consider socio-behavioral factors to reduce their needs for help during their
survivorship process
Acknowledgments: The parent study was funded by the National Institutes of Health (NCI/NINR; 1R01CA203719).

**LB150**

**Endothelial protein kinase D1 signaling induces development of aggressive cancer stem-like cells for breast cancer progression and lung metastasis.**

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Breast cancers demonstrate robust angiogenesis known as vascular niches despite an abundance of endogenous antiangiogenic factors in the tumor microenvironment. Aberrant angiogenesis may contribute to therapeutic resistance in antiangiogenic treatment via interactions between vascular endothelial cells (ECs) and cancer stem-like cells (CSCs, a subset of malignant cells critical for tumor initiation, metastasis and therapeutic resistance). Emerging evidence suggests that ECs in the tumor vasculature may confer CSC features to cancer cells by developing abnormal vascular niches. Protein kinase D1 (PKD1), a serine threonine kinase upstream of PI3Kinase/Akt and MAPK/Erk1/2, regulates angiogenesis and tumor progression. By analyzing RNA-sequencing data in the Cancer Genome Atlas (TCGA), we also found a poorer overall survival of the patients in the *PKD1* high cohort compared with the patients with *PKD1* low in BC tissues. Interestingly, our recent studies suggest that enhanced EC PKD1 signaling facilitates arteriolar niche formation for CSC self-renewal in breast cancers. However, the role and mechanisms of EC PKD-1 signaling in arteriolar niche establishment for CSC expansion in tumor progression is a critical yet underexplored and underappreciated field. We propose that EC PKD1 signaling in the vascular niches is essential for the expansion of CSCs to promote malignant progression of BCs. To test this hypothesis, we established a BC model in EC-specific *PKD1* knockout mice and the control mice and developed a co-culture model of ECs and BC cells. By combining with molecular and cellular approaches and immunofluorescence microscopy, we observed that EC PKD1 signaling was critical for BC growth and survival of tumor-bearing mice, and particularly important in metastasis of BCs to the lungs. Analysis of the BC models indicated that there was an obvious enrichment of CSCs in the tumor vasculature of EC PKD1-positive mice. Moreover, there was a significant increase in expression of CD44 and ALDH1, the key CSC-related genes in BCs, in the primary tumors of mice with lung metastases. Co-culture of human microvascular ECs with human BC cells further confirmed that ECs promoted the expression of these two genes in tumor cells, along with the expression of other genes related to CSC self-renewal and epithelial mesenchymal transition. Co-culture with ECs also led to an increase in cell migration and colony formation in BCs. Importantly, the PKD1 signaling dictated the changes in CSC-related gene expression and aggressive behaviors in BC cells. In conclusion, EC PKD1 signaling in the vascular niches is critical for phenotype switching of BC cells to aggressive CSC features, thereby promoting BC progression and lung metastasis. Targeting PKD1-signaling-induced arteriolar niche establishment and this niche interaction with CSCs could provide potential novel and effective therapeutic strategies against tumor angiogenesis and CSC expansion.

**LB151**

**Exploring the tumor microenvironment in a spatial context with SpaCET.**

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Uncovering the composition and structure of the tumor microenvironment is critical to a mechanistic understanding of tumorigenesis and therapeutic resistance. Spatial transcriptomics (ST) technology has enabled profiling the molecular features of tumor tissue with position information. However, the spatial probe of various ST strategies with a 10~100 μm diameter might capture a mixture of transcripts from multiple cells or cell lineages. Cell type deconvolution in ST data of tumor tissues remains challenging for existing methods, which are designed to decompose general ST or bulk data. We develop the Spatial

Cellular Estimator for Tumors (SpaCET) to infer cell identities from tumor ST data. Without the need of inputting cell references, SpaCET estimates malignant and non-malignant cell abundance by using a gene pattern dictionary of copy number alterations in common malignancies and a hierarchical atlas of immune/stromal cells, respectively. SpaCET provides higher accuracy than existing methods based on eight ST datasets on seven cancer types with matched double-blind histopathology annotations as ground truth. Furthermore, SpaCET can reveal the potential intercellular interactions at the tumor-immune interface by integrating inferred cell fractions with the ligand-receptor interaction network. We expect that SpaCET will be a valuable tool for spatial cancer biology.

**LB153**

**Inhibiting BRD4 as a proposed alternative therapeutic strategy for HPV16-associated head and neck squamous cell carcinoma.**

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Integration of Human Papilloma Virus (HPV) 16 into human genomes is an integral event for cancer progression in Head and Neck Squamous Cell Carcinoma (HNSCC). There is a need to differentiate treatment for HPV-16 integrated HNSCC from other subtypes. We hypothesize that by inhibiting Bromodomain Extra Terminal (BET) protein BRD4, a transcriptional co-regulator of host and viral genes can advance treatment in patients with the integrated subtype of HPV16 associated HNSCC. Through small molecule inhibition and knockdown of BRD4 in seven model HPV-16 integrated cell lines, we demonstrated that BRD4 down-regulates the expression of viral genes E6, E7, independent of viral E2 type of integration. Given that E6 expression is mediated by BRD4, E6-p53 interactions was abrogated as revealed through increased expression of p53. Furthermore, BRD4 inhibition caused downregulation of c-Myc. This result also suggests the function of BRD4 in the regulation of proteins in cell cycle regulation, including E2F1 and p21. BRD4 inhibition was also shown to delay DNA damage response (DDR) by reducing the expression of RAD51 in 6 out of 7 cell lines. RNA-sequencing results give further insight regarding the downregulation of genes involved in DDR – RAD51, BRCA1 and BRCA2 in all cell lines. RNA-sequencing data also gives us an understanding of the implication of BRD4-inhibition in other genes involved in cell cycle regulation. Knockdown of BRD4 done through small hairpin RNA (shRNA) constructs phenocopied the effects shown through inhibition of BRD4. Additionally, treating the above-mentioned cell lines with BRD4 inhibitor (JQ1) followed by radiation showed further downregulation of viral oncogenes E6, and E7. This suggests that combining radiation and JQ1 treatment can be a viable combination treatment. De-escalating radiation treatment with BRD4 inhibition has potential to be an effective combination treatment for HPV16 integrated HNSCC.

**LB154**

**Circ0003039 interacts with calreticulin to inhibit the progression of hepatocellular carcinoma by suppressing wnt/beta-catenin signaling pathway.**

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**Background**: Circular RNAs (circRNAs) are covalently closed and single-stranded RNAs which play critical roles in various biological processes and diseases including cancers. The functions and mechanisms of circRNAs in hepatocellular carcinoma (HCC) are still need to be clarified.
**Methods**: Custom circRNA microarray was performed to investigate the profile of circRNAs in HCC. Expression of circ0003039 and its clinical significance in 60 pairs of HCC and matched non-cancer liver tissues were identified and confirmed by microarray and quantitative RT-PCR. The biological functions of circ0003039 were investigated by gain-and loss-of-function experiments in vitro and in vivo. RNA pull-

down, mass spectrometry, RNA-immunoprecipitation, Co-immunoprecipitation, fluorescent in situ hybridization were used to identify circ0003039-protein interaction. The downstream pathway regulated by circ0003039 was explored using RNA-seq and verified using western blot and immunofluorescence.
**Results**: Expression of circ0003039 was reduced in HCC tissues and positively associated with the overall survival of HCC patients. Circ0003039 was located in cytoplasm of HCC cells. Ectopic overexpression of circ0003039 inhibited the proliferation, colony formation, migration and invasion of HCC cells and the growth of HCC xenograft in mice, while knockdown of circ0003039 had opposite effects in HCC cells. Mechanistically, circ0003039 bound to the N-terminal domain of CALR protein and acted as a scaffold to enhance the interaction of CALR with CAPN2, which promoted the degradation of CALR protein by the enzymatic activity of CAPN2. Rescue experiments showed that circ0003039 inhibited HCC proliferation via suppressing CALR protein. Finally, we found that circ0003039 inhibited HCC cells by suppressing CALR-mediated wnt-βcatenin signaling pathway.
**Conclusions**: Our study demonstrates that circ0003039 bind to N-terminal domain of CALR and facilitated CAPN2 to degrade CALR protein, which inhibits the development and progression of HCC via suppressing CALR-mediated wnt-βcatenin signaling pathway, and circ0003039 is a potential prognostic biomarker and therapeutic target for HCC.

**LB155**
**In vivo imaging of vascular pathology in mouse orthotopic glioma model using functional ultrasound.**
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Neovascularization together with aberrant vasculature is a hallmark of glioblastomas. Understanding tumor vasculature can have a pivotal role in planning therapeutics approaches. The objective of this study was to investigate morphological, vasculature and blood flow changes induced by aggressive tumor growth, using in vivo imaging - magnetic resonance imaging (MRI) and functional ultrasound (fUS) imaging in a mouse model of orthotopic glioma. Female NMRI nude mice were xenografted orthotopically with U-87 MG, a human glioblastoma, cells ($5 \times 10^4$) at the age of 8 weeks. Four weeks after implantation the mice we anesthetized with isoflurane and scanned with 11.7 T small animal MRI (Bruker) for tumor volumetry. Thereafter, the mice were imaged with Iconeus One imaging system (Iconeus, Paris, France) for structural information of brain vasculature and relative cerebral blood volume (rCBV) changes. High resolution vascular imaging was performed after intravenous injection of microbubble contrast agent (SonoVue, sulphur hexafluoride microbubbles). The average tumor size was $58.8 \pm 27.6$ mm$^3$ (mean ± SD, n=7) four weeks after implantation. Aberrant vasculature could be visualized with structural fUS imaging as and the necrotic core of the tumor could be observed. A reduced rCBF was observed in ipsilateral hemisphere on the relative power doppler signal timeseries as a lower amplitude and faster decay compared to contralateral side. As a summary, in vivo fUS imaging is a noninvasive tool to visualize vascular changes induced by aggressive brain tumor growth which are not detectable with conventional imaging methods (CT, MRI, PET, SPECT) and can provide a novel readout for efficacy studies.

**LB156**
**Identified the ISG15 mediated extracellular vesicles drives ovarian cancer progression and metastasis: extracellular vesicular ISG15 is a potential biomarker and therapeutic target.**
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Background & Objective: High-grade serous ovarian cancer (HGSOC) accounts for over 80% of all epithelial ovarian cancer (OC) diagnoses, and the majority of patients with HGSOC are diagnosed with advanced metastatic disease. Aberrant expression of interferon stimulated gene 15 (ISG15) has been demonstrated in human malignancies, and we have identified that ISG15 is highly expressed in HGSOC tumors and peritoneal ascites. This study seeks to determine whether ISG15 contributes to HGSOC progression and metastasis through extracellular vesicle (EVs) secretion.
Method: ISG15 expression was analyzed in ascites samples and primary ovarian cancer cells (POCC) from different patients by ELISA and WB. Cell surface biotinylation assay was done to show the modulation of endo and exocytosis by ISG15 and STAT3. Immunoprecipitation pull down assay was done to demonstrate the interaction of ISG15 with activated STAT3. Confocal microscopy showed the co-localization of STAT3 with the endosome marker TSG101. In-vivo studies were done using bio-luminescence imaging in orthotopic ovarian tumor mouse models to measure tumor progression and metastasis.
Results: ISG15 was found to be significantly elevated in HGSOC metastases (pelvic, mesenteric and diaphragmatic samples) as compared to primary ovarian tumors or benign samples. We observed that ISGylation was increased in POCC cells derived from ascites with increased USP18 expression. Our results confirmed a significant decrease in EV's secretion in ISG15 KD POCC cells. Mice injected with ISG15 OE -POCC cells showed increased tumor burden when compared to the ISG15Kd cells in mice. Furthermore, we observed a significant reduction of ovarian tumor growth and metastasis in an orthotopic mouse model treated with a small molecule inhibitor-DAP5 that targeted ISG15 or exosome blocker (Amiloride) compared to untreated mice. In addition, we found the expression of ISG15 within the EVs represents a promising development in elucidating prognostic markers for HGSOC patients.
Conclusion: Based on our results, ISG15 expression is elevated in HGSOC patient ascites and metastatic disease sites. The aberrant expression of ISG15 in patient ascites plays a key role in the secretion of EVs carrying ISG15, which contributes to HGSOC progression and metastases. Our study provides the pre-clinical evidence regarding new molecular targets, novel prognostic markers, and innovative therapeutic strategies for HGSOC, aiming to ultimately improve the survival of patients suffering from this disease.

**LB157**

**Cereblon inhibits prostate cancer progression and metastasis by negatively regulating 6PGD.**

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As the third enzyme of the pentose phosphate pathway (PPP), abnormally elevated levels of 6-phosphogluconate dehydrogenase (6PGD) have been documented in various human cancers. We demonstrate that reduced cereblon (CRBN) protein expression is the underlying mechanism of elevated 6PGD expression in metastatic prostate cancer cells. We establish 6PGD as a new endogenous substrate for CRBN by demonstrating that it interacts directly with CRBN and is ubiquitinated by CRL4CRBN. In

addition, CRBN negatively regulates prostate cancer cell progression and metastasis, as abnormally high 6PGD, in the absence of sufficient CRBN, enhances the metastatic potential of prostate cancer in vitro and in vivo. Our findings show convincingly that carbohydrate metabolism regulated by 6PGD is linked to prostate cancer metastasis via CRBN. Based on these data, we propose that the 6PGD-CRBN axis may be a suitable target for further research into new therapeutics for mitigating prostate cancer metastasis.

**LB158**

**Rapid cancer cell perineural invasion utilizes amoeboid migration.**

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The invasion of nerves by cancer cells, or perineural invasion (PNI), is potentiated by the nerve microenvironment and is associated with adverse clinical outcomes. However, the cancer cell characteristics that enable PNI are poorly defined. Here, we generated cell lines enriched for a rapid neural invasive phenotype by serially passaging pancreatic cancer cells in a murine sciatic nerve model of PNI. Cancer cells isolated from the leading edge of nerve invasion showed a progressively increasing nerve invasion velocity with higher passage number. Transcriptome analysis revealed an upregulation of proteins involving the plasma membrane, cell leading edge, and cell movement in the leading neural-invasive cells. Leading cells progressively became round and blebbed, lost focal adhesions and filipodia, and transitioned from mesenchymal to amoeboid phenotype. Leading cells acquired an increased ability to migrate through microchannel constrictions and associate with dorsal root ganglia than non-leading cells. ROCK inhibition reverted leading cells from an amoeboid to mesenchymal phenotype, reduced migration through microchannel constrictions, reduced neurite association, and reduced PNI in a murine sciatic nerve model. Cancer cells with rapid PNI exhibit an amoeboid phenotype, highlighting the plasticity of cancer migration mode in enabling rapid nerve invasion.

**LB159**

**Deregulation of CTC-associated RPL/RPS protein expression inhibits melanoma brain metastasis.**

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Melanoma brain metastasis (MBM) is associated with poor prognosis and low survival rates. MBM is diagnosed clinically in up to 60% of melanoma patients and in up to 80% of patients at autopsy. Circulating Tumor Cells (CTCs) are “seeds” of fatal metastasis and the smallest functional units of cancer. Our previous studies have employed a multilevel approach to identify a common CTC signature for ribosomal protein large/small subunits (RPL/RPS) which is linked to MBM development and progression[1]. Here, we hypothesized that targeting ribosomal biogenesis prevents MBM onset in CTC-derived xenografts. First, we treated CTC-injected MBM mice with RPL15 inhibitor omacetaxine to monitor metastatic progression. Second, parallel cohorts of mice were treated with CDK4/CDK6 inhibitor palbociclib with or without omacetaxine to assess effects of cell proliferation. Total flux of MBM signal was measured by IVIS and found to be higher in untreated mice. Mouse necropsies revealed decreased levels of MBM, along with metastatic dissemination to other organs. Third, collected mouse blood was analyzed by the FDA-approved Parsortix™ platform to enumerate CTC/CTC clusters (IHC), and evaluate their gene expression (RNA-seq). Lastly, metabolic profiling of inhibitor-treated versus untreated MBM CTC-derived clones was performed in vitro. Collectively, our findings indicate that the deregulation of ribosomal proteins inhibits MBM progression. This implicates a specific vulnerability of aggressive CTC subsets with high RPL/RPS content in MBM patients. RPL/RPS genetic screening could be a prognostic indicator of MBM severity and clinical outcomes. Our work promotes the concept that therapies targeting

RPL/RPS proteins merit exploration as potential suppressors of metastatic progression in general, MBM in particular. 1. Bowley T, Lagutina I, Francis C, Sivakumar S, Selwyn R, Taylor E, et al. The RPL/RPS gene signature of melanoma updates CTCs associates with brain metastasis. *Cancer Research Communications* 2022;2: 1445-57.

**LB160**

**Mdm2 regulates metastasis and associated cellular processes through modulation of Sprouty4 in a p53-independent manner.**

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Mdm2 and its homologue MdmX form an E3-ligase complex that is best understood as the major regulator of p53. Yet Mdm2 and MdmX have functions in cells that are independent of their ability to degrade p53. Amongst the functions regulated by Mdm2 is cell migration, although the molecular mechanism(s) involved have not been well characterized. We show, in a p53 *null* model, that either siRNA knockdown of Mdm2 or MdmX as well as pharmacological inhibition of the Mdm2/X complex E3-ligase can reduce migration of cells grown as monolayer or invasion of cells from pre-formed spheroids into collagen-based matrices. This is consistent with our observation that Mdm2 ablation or inhibition leads to decreased cell spreading and attachment of cells to the extracellular matrix. In line with these findings, we found that modulation of Mdm2, MdmX or the Mdm2/X complex impacts focal adhesion (FA) formation, a main step in cell attachment, spreading and migration. Physiologically, Mdm2 silencing leads to decreased metastatic burden in mouse models. Mechanistically, we have discovered that Mdm2 modulates the RNA levels of Sprouty4 and that Sprouty4 is needed for the effects of Mdm2 knockdown on cell migration, FA formation and metastasis. Taken together, we have discovered a pathway by which Mdm2, through the activity of the Mdm2/X complex, mitigates FA formation, migration and ultimately metastasis by regulation of Sprouty4 independently of p53. Our findings suggest that blocking Mdm2 or the Mdm2/X complex might be a potential target to prevent metastasis.

**LB161**

**Single cell bioprinted cell circuits for the systematic assessment of cell-cell communication in the early tumor microenvironment.**

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The specific communication of multiple cell types in the tumor microenvironment plays a critical role in cancer progression. Current engineering methods have failed to adequately replicate the complexities of the tumor microenvironment (TME). In particular, generating engineered tissue-like environments with multiple TME cell types has remained challenging. Here we demonstrate the capability to pattern complex single cell circuit configurations, using a novel microfluidic bioprinting method, to study cell-cell communication in the early TME. A microfluidic dispenser (Biopixlar, Fluicell AB) was optimized to determine the delivery pressure (5 – 80 mbar), internal vacuum (0 – 80 -mbar), and external vacuum (0 – 80 -mbar) to enable highly controllable deposition of single cells suspended in complete media supplemented with polyethylene glycol (15 mg/mL, 1:1) at $1x10^6$ cells/mL. Flow conditions were optimized for human cells: MDA-MB-231, MCF7, PC3, breast epithelial cells (MCF10a), fibroblasts, cancer associated fibroblasts, THP-1 derived macrophages, CD4+ T cells, CD8+ T cells, human umbilical vein endothelial cells (HUVECs), and mesenchymal stem cells. As proof of concept, the optimized settings were used to replicate a 2D tumor biopsy region of interest with high spatial precision. Next, cell-cell communication circuits were fabricated with cancer cells (PC3 or MDA-MB-231) and HUVECs.

Communication circuits were bioprinted as 4 by 4 cell arrays, with 100 μm spacing between each cell, equal number of HUVECs and cancer cells, and three different cellular arrangements: alternating cell types, like cell types grouped, and groups of four like cell types. The circuits were live cell imaged for up to 30 hours to observe cell migration patterns, proliferation, and morphological changes as a function of cell-cell communication circuit arrangements. Optimal printing parameters were identified as 80 mbar delivery pressure, -25 mbar internal vacuum, and -55 mbar external vacuum. These parameters maintained >99% cell viability and ±10 μm spatial precision of printed cells. Live cell imaging of circuits containing PC3s or MDA-MB-231s with HUVECs on collagen substrates revealed changes in migration patterns, proliferation, and morphology depending on the surrounding cellular arrangement. HUVECs were highly migratory throughout the duration of the experiment, frequently extended protrusions towards nearby HUVECs, but did not display the same level of interaction with PC3s as they did with MDA-MB-231s. In MDA-MB-231 circuits, irrespective of patterning, we identified clear tendencies of HUVECs to herd MDA-MB-231s, travel overtop of MDA-MB-231s, collect and carry visible particles released from MDA-MB-231s, and maintain dendritic morphology instead of undergoing the expected vascular tubulogenesis. We found that HUVECs had the best morphology when clustered in groups of four and proliferated most when surrounded by MDA-MB-231s (alternating pattern). We found that MDA-MB-231s only proliferated when surrounded by HUVECs and had the least displacement when surrounded by like cells. These results demonstrate a method to precisely bioprint single cell circuits, enabling the investigation of cellular spatial organization and composition within the tumor microenvironment as it relates to tumor initiation and progression.

**LB162**

**A multiplex organoid avatar drug testing platform for precision medicine.**

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The promise of precision medicine is to improve patient outcomes by making better therapeutic choices based on cancer causing somatic mutations. This strategy has shown limited success in colorectal cancer, in part because the genetic rules governing resistance and sensitivity are not fully elucidated. Direct drug sensitivity testing of individual patient-derived organoids is an attractive addition to this decision process because it does not require understanding of all drug-somatic mutation interactions. Individual patients avatars can be challenged with a variety of drugs and response used to inform patient care. We have developed a panel of colon cancer organoid avatars and uniquely tagged each one with a lentiviral vector possessing identifying DNA sequence bar codes flanked by common PCR primers. Each bar-code is uniquely identifiable and quantifiable from a mixture of organoids by nanopore sequencing of a common PCR product. Treatment of the mixture of organoid avatars over time under multiple drug conditions and sampling at different time points allowed the real-time monitoring of relative Darwinian fitness of each organoid in the mixture. We demonstrated the utility of this approach at identifying both expected and novel drug responses for individual organoids. Resistance to both nutlin and irinotecan were correctly predicted by TP53 somatic mutations while sensitivity to both lapatinib and ibrutinib were best predicted by mutations in EGFR signaling pathway. Patterns of drug response revealed unexpected common mechanisms. Future work will focus on informing patient care decisions with these results and determining if improvements in patient outcomes are realized.

**LB163**

**A microfluidic platform for developing circulating tumor cells (CTCs) organoids for precision medicine in pancreatic cancer.**

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Introduction
Pancreatic ductal adenocarcinoma (PDAC) is predicted to be the second leading cause of cancer death in the US by 2030. Besides the late detection and lack of effective treatments, the greatest treatment hurdle to PDAC is the ineffectiveness of patient-derived tumor models. Precision medicine-based treatment decisions require the generation of consistent and trackable complex organoid models that is not achievable by tissue biopsies. Liquid biopsies such as blood, instead, contain circulating biomarkers released by the tumor, among which especially interested in for this study are circulating tumor cells (CTCs). This work develops microfluidic 3D organoids from CTCs to be used as a precision medicine tool to predict and guide the drug response of early stage I-IIB (PDAC) patients.
Experimental Methods
CTCs are isolated from PDAC patient blood samples using microfluidic Labyrinth following removal of red blood cells with 6% dextran. Collected cells post-isolation are immunofluorescence (IF) stained with DAPI (nuclei), CD45 (leukocyte), pancytokeratin (type I/II cytokeratin 1-8, 10, 14- 16 and 19), Vimentin, and Epithelial cell adhesion molecule (EpCAM) to identify CTCs and characterize their phenotypes. The mold of the microfluidic platform for CTC organoids is fabricated with the standard SU-8 photolithography. The PDMS-based device is tested with a low seeding number of a pancreatic cancer CTC cell line labeled fluorescently. Decellularization of cancer associated fibroblasts (CAFs) generates the in vitro matrix that supplies amino-acid precursors for PDAC metabolism, promoting CTC organoid formation. Serial images of cell growth are obtained throughout the course using a Ti-20 microscope. After each 7-day culture, organoids are dissociated from the device and IF stained using a similar panel as above (with Ki67 marker instead of CD45 for cell proliferation). Preliminary gemcitabine dosage-performance analysis is performed on-chip.
Results
CTC count from the tested patient panel (n=16) averages 17.0±8.4/mL of blood. CAFs are successfully decellularized on-chip and the embedded pancreatic CTC cell line growth curve is generated. IF staining of the dissociated cells from the organoids confirms the proliferation of the loaded pseudo-CTCs.
Conclusion
The work above demonstrates the successful isolation of CTCs from PDAC patient blood samples and an engineered microfluidic platform to expand low abundance of cells into organoids. It has a potential to be tested and employed to expand CTCs from PDAC into organoids for drug screening.

**LB164**
**Entinostat restores sensitivity to olaparib in two in vitro models of PARPi resistant ovarian cancer cells.**
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The use of Poly ADP ribose polymerase inhibitors (PARPi) has revolutionized the treatment of homologous recombination (HR) deficient ovarian cancer tumors. A subset of these tumors exhibit genetic and acquired resistance to PARPi treatment. We have previously shown histone deacetylase inhibitors (HDACi) resensitizes ovarian cancer cells to PARPi. The objective of this study is to examine the effectiveness of combination HDACi and PARPi treatment in two in vitro models of mouse ovarian cancer cells. To investigate these effects, we used the following three ID8 murine ovarian cancer epithelial cell lines: *TP53*$^{-/-}$ (HR deficient), *TP53*$^{-/-}$*/BRCA2*$^{-/-}$ (HR proficient), and an olaparib resistant line *TP53*$^{-/-}$*/BRCA2*$^{-/-}$-OR (ID8_OR). We also created ovarian cancer organoids from these three lines. In short, 8-week old female C57BL/6 mice were injected with 7 million untreated cells in PBS. Following the formation of ascites, mice were sacrificed and organoids were derived from both ascites and tumors. For viability assays, cells were pretreated with entinostat (Ent, 0.25uM) for 24h, followed by 72h (2D

cells) or 7 days (organoids) of Ent (0-2uM), olaparib (Ola, 0-40uM), or in combination. Cell proliferation and viability was assessed using MTS and ATP-based assays. For immunofluorescence and western blotting analysis, cells were treated with 0.5uM Ent, 10uM Ola or the combination.*TP53* adherent cells and organoids treated with 0.5uM Ent + 10uM of Ola significantly reduced cell proliferation when compared to Ola alone (p= 0.0028, 0.0021 Two-Way ANOVA). When compared to Ola and Ent alone, *TP53/BRCA2* adherent cells treated with 0.125uM of Ent + 2.5uM of Ola also reduced cell proliferation (p=0.0011 and p=0.0072, 0.0845). Lastly, ID8_OR adherent cells and organoids treated with 0.125uM of Ent + 2.5μM of Ola significantly reduced cell proliferation when compared to Ola alone (p=0.0018, 0.0264).Immunofluorescences analysis revealed, when treated with Ola and Ent combination, adherent *TP53* (p= 0.002, n.s), *TP53/BRCA2* (p=0.0033, 0.0315), and ID8_OR (n.s) cells displayed increased expression of DNA damage marker gH2AX when compared to Control and Ola alone. We also observed decreased expression of RAD51 in cells treated with combination when compared to control and Ola alone, *TP53* (p= <0.0001, 0.0023) and ID8_OR (n.s). In our organoids, we found an increase in gH2AX across all three cell lines when treated with Ola+Ent compared to control and Ola alone: *TP53* (p= <0.0001, 0.0057), *TP53/BRCA2* (n.s), and ID8_OR (p= 0.0116, 0.0151). Similarly, we found a decrease in RAD51 when cells were treated with Ola+Ent compared to control and Ola alone: *TP53* (p= n.s, 0.0418) and ID8_OR (n.s). Lastly, preliminary western blot analysis of the organoids, revealed a decreases in proliferation marker PCNA in both adherent cells and organoids when comparing the control to combination treatment. In conclusion, the combination of Ola and Ent treatment restores responsiveness of two in vitro models of genetic and acquired PARPi resistance through increasing DNA damage and reducing DNA repair and proliferation.

**LB165**

**A novel PDX-based 2-stage screening platform can identify active drug combinations against KRAS-mutated colorectal cancer.**

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Introduction: Targeting $RAS^{mut}$ colorectal cancers (CRC) remains challenging and targeting has focused on the RAS-MAPK cascade. MEK inhibitors for $KRAS^{mut}$ tumors were not successful *in vivo* or in clinic as they slowed tumor growth, but did not cause regression at clinically achievable doses, however, potential for synergy has been shown. *In vivo* modeling commonly guides decisions to advance to the clinic but these studies are heterogeneously conducted, with rare use of statistically justified sample sizes or prespecified alternate hypotheses. The purpose of this study was to develop a robust PDX screening platform for pre-clinical evaluation of combination strategies, and pilot it with MEK inhibitor combinations.

Methods: The screening model was based on Simon's two-stage design, with alternate hypothesis of 30% response rate (null hypothesis of 5% response rate). Stage 1 involved testing drug combinations against 5 $KRAS^{mut}$ PDX models. Drug combinations that demonstrated efficacy in Stage 1 (≥10% decrease in tumor volume at Day 22 post-treatment) were tested in Stage 2 against an additional 13 PDX models. The null hypothesis was rejected if ≥3/18 PDX models showed response. This design yields a type I error rate of 0.05 and power of 0.8. Twenty RAS-MAPK cascade inhibitor-based combinations were tested. RP2D drug doses were used.

Results: In Stage 1, MEKi plus CDK4/6i combination demonstrated efficacy in 3/5 models. This effect was sustained in Stage 2 (9/13 models with ≥10% decrease in tumor volume) for a total of 12/18. MEKi/BETi was next best (total 4/18 models). MEKi/PARPi and MEKi/ERKi - 3/18. Two combinations (MEKi/TKi and MEKi/AKTi) passed Stage 1, but failed in Stage 2 (2/18 total). Other combinations did not meet efficacy threshold in Stage 1 and were deemed inactive. In an expansion study of various $RAS^{mut}$ PDX models (6 codon 12/13 $KRAS^{mut}$, 5 codon 61/146 $RAS^{mut}$ and 4 $RAS^{wt}$), trametinib/palbociclib differentially attenuated tumor growth relative to untreated controls and caused

tumor regression in all tested PDX models. Protein expression analysis (RPPA) demonstrated increases in cell cycle regulators (Cyclin-B1, FOXM1, PLK1) in non-responders, and higher pMEK and FOXO3 protein levels in responders. Combination treatment resulted in decreased expression of MAPK/ERK and CDK4/6 pathway-related proteins. mRNA expression analysis (GSEA) revealed that MEKi-resistant PDX models were enriched in cell cycle, ERK, mTORC1, ER stress and DDR signaling pathways. Primary drug combination toxicity (weight loss) was most pronounced with trametinib/palbociclib compared to tram or palbo alone. Using *in vivo* dose/response matrix screening, we maintained >90% efficacy while significantly reducing toxicity (decreasing weight loss from >15% to <10%) by decreasing palbociclib dose and maintaining tram dose intensity.
Conclusions: The two-stage screening design demonstrated efficacy, time and cost-efficiency in drug combination screening. Efficacy of MEKi/CDK4/6i, MEKi/PARPi and MEKi/BETi combinations in $RAS^{mut}$ CRCs was confirmed. These combinations are now being explored in prospective clinical trials.

**LB168**
**Efficacy analysis of the DeepNeo neoantigen prediction tool for antitumor vaccine development.**
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Antitumor peptide vaccine is an alternative and attractive way to eradicate tumors by the aid of host immune system. The current peptide vaccine development is hampered by several hurdles. These include heterogeneity of tumor cells, applicable in vivo stability of peptides, and others. One of the major obstacles is the precise prediction of cancer antigen, which can be loaded efficiently onto antigen presenting cells. We present here a neoantigen prediction algorithm DeepNeo comprised of DeepNeo-MHC and DeepNeo-TCR, which calculates the antigenicity of neoantigen based on the MHC binding and TCR activation potential. We examined the efficacy of DeepNeo using ELISPOT and two allogenic mouse tumor models. By treating the combination of long peptides targeting MC38 colon or B16F10 melanoma cell lines, we have shown the DeepNeo effectively determines neoantigens for the development of antitumor peptide vaccine. Analysis of tumor-infiltrated lymphocytes (TIL) supported our results. Undergoing study using patient-derived xenograft model treated with autologous, trained PBMC with personalized peptide vaccine will be discussed further.

**LB169**
**Machine learning-based approach for glioblastoma drug repurposing on real-world patient data.**
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Glioblastoma (GBM) is the most aggressive and deadly type of brain cancer. The poor survival rate of GBM is often attributed to the low therapeutic efficiency of current front-line therapy, treatment resistance, and high recurrence rate. Sizeable clinical data of cancer patients preserve a great potential for drug discovery and drug repurposing research, enabling comprehensive screening of treatment effects among frequently-prescribed medications. To efficiently characterize potent drug candidates and develop novel treatment strategies for GBM, we designed a machine-learning-based pipeline that predicts repurposable drug combinations from large-scale patient claims data. Our pipeline first identified pseudo-randomized drug-user and non-user cohorts from heterogeneous clinical records and balanced the confounding effects using the Inverse Probability of Treatment Weighting (IPTW) method. Next, our model computed the averaged treatment effect on the treated (ATT) values to determine the treatment

effects of our candidates. Our methodology identified several promising drug combinations that have been found to be effective in inhibiting GBM in cell line experiments. This research was conducted by integrating computational and experimental approaches to identify drug combinations, providing a promising strategy for the development of new treatments for GBM.

**LB170**

**Artificial intelligence-assisted morphology-based detection and enrichment of malignant effusion tumor cells as a method for molecular profiling.**

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Patients with metastatic breast cancer (MBC) can develop malignant effusions (MEs) when excess fluid and tumor cells accumulate in the pleural or peritoneal space. MEs are associated with significant morbidity and mortality. Tropism of tumor cells in these spaces is poorly understood and tumor cells residing in these fluids are difficult to study with traditional methods due to low abundance. To overcome this limitation and understand the pathobiology of MEs in MBCs, we applied a label-free method to isolate tumor cells based on morphology using an artificial intelligence (AI) model This method enabled isolation of live cells for downstream molecular analysis and characterization of morphological heterogeneity.

We collected ME samples from 9 MBC patients with cytology-confirmed MEs, including 3 triple negative and 6 hormone receptor-positive/HER2-negative breast cancers. ME cells were imaged and isolated using a Deepcell AI model trained on a separate cohort of MEs to isolate and enrich tumor cells based on deep learning representations of morphological features. Quantitative morphological features were then extracted from cell images to perform cluster analysis and visualized by dimensionality reduction projections. In parallel, flow cytometry (FC) was used to quantify % EpCAM+/Claudin4+ tumor cells in ME samples. Copy number (CN) analysis was performed before and after enrichment with as few as 200 cells from each sample. Tumor cell fraction (TCF) estimates were calculated from the AI classifier, FC, and CN analyses.

CN profiling of malignant cells sorted by the AI classifier revealed genomic alterations common in breast cancer in 7 of 9 patients. We observed increased amplitude of genomic alterations in tumor-enriched vs. unenriched samples, indicating successful tumor cell enrichment in 14 out of 16 samples. We observed a significant correlation in TCF between the AI classifier and FC with a Spearman test (rho=0.568, p0.008). In some cases, the AI classifier reported higher TCF than FC. This could indicate that the AI model detected tumor cells missed by antibody labeling or false positives from morphologically similar cell types, such as macrophages and mesothelial cells. Remarkably, analysis of serial ME samples from the same patient revealed morphological shifts in cell populations which were consistent with changes in TCF by CN analysis.

We demonstrate the feasibility of label-free identification and isolation of tumor cells in MEs using an AI classifier. CN profiling of isolated tumor cells verified enrichment from low initial frequencies. Future work will include expression analysis of ME tumor and immune cells, and analysis of organoids derived from MEs. Combining molecular and morphological profiling of MEs will enable new insights into their pathobiology and inform targeted treatment strategies for patients with this condition.

**LB171**

**WSI based prediction of *TP53* mutations identifies aggressive disease phenotype in prostate cancer.**

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In prostate cancer, there is an urgent need for objective prognostic biomarkers that identify a tumor's metastatic potential at an early stage. While recent analyses indicated *TP53* mutations as candidate biomarker, molecular profiling in a clinical setting is complicated by tumor heterogeneity. Deep learning models that predict the spatial presence of *TP53* mutations in Whole Slide Images (WSIs) offer the potential to mitigate this issue. To assess the potential of WSIs as proxy for spatially resolved profiling or as biomarker for aggressive disease, we developed *TiDo*, a deep learning model that achieves state-of-the-art performance in predicting *TP53* mutations from WSIs of primary prostate tumors. On an independent multi-focal cohort, we could show successful generalization of the model, both at patient and lesion level. Hence, the model offers insight into which lesions on a WSI most likely contain a *TP53* mutation. Analysis of model predictions revealed that false positive (FP) predictions could at least partially be explained by *TP53* deletions. This suggests that some FP carry another alteration of which the effect converges in the same histological phenotype. Comparative expression analysis and histological cell type analysis identified such common phenotype (related to stromal composition) in both TP and FP predictions. This indicates that WSI-based models might not be able to perfectly predict the spatial presence of individual *TP53* mutations. However, we show they have the potential of capturing a tumor's aggressive potential by observing a downstream phenotype of the tumor cells and TME associated with a biomarker of aggressive disease (*TP53*).

**LB172**

**Improved T-cell and B-cell receptor repertoire profiling and immunophenotyping for biomarker discovery.**

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T-cell receptor (TCR) and B-cell receptor (BCR) repertoire profiling, also referred to as adaptive immune receptor repertoire (AIRR) profiling, holds great potential for the understanding of disease mechanisms and for the development of new treatments in infectious disease, autoimmunity, and immuno-oncology. This potential could be greatly improved by combining information about receptor clonotypes with immunophenotypes of T- and B- cells. A new technology we developed that combines profiling of all human TCR and BCR variable regions with phenotypic characterization of immune cells using the same workflow could be particularly useful. The TCR and BCR immunophenotyping method proposed involves RT-PCR amplification and sequencing of the CDR3 regions of the TCR and BCR genes, and subsequently determining the expression levels of the most informative T- and B-cell phenotyping genes. Preliminary results show that this method allows for comprehensive profiling of all seven TCR and BCR chains from a single sample, in a highly reproducible manner, directly from micro-samples including cancer tissue, whole blood, sorted cells and more. Bioinformatic analysis of the next-generation sequencing (NGS) data from the TCR and BCR clonotypes profile combined with RNA expression profiling of the same samples results in a richer data set that includes the identification of major immune cell subtypes and their activation status. Data from human cancer tissue and whole blood samples will be presented.

**LB173**

**A bioinformatics approach to determine the potential benefit of blocking both Galectin-1 and Galectin-3 in cancer.**

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Galectins are a family of Beta-galactoside-binding lectins that control numerous cellular processes in health and disease, including fibrosis, carcinogenesis and tumour immune evasion. Evidence is starting to emerge that blockade of more than one galectin may provide additional benefit within the oncology arena due to the overlapping biological functions associated with these lectins. Due to the rich and robust external datasets associated with the two most previously studied galectins, we initially investigated the potential therapeutic benefit of a dual blockade approach of Galectin-1 (Gal-1) and Galectin-3 (Gal-3). A systematic assessment, consisting of three steps, was implemented to conclude the potential clinical effects of dual Gal-1 and Gal-3 inhibition and the specific cancers which may benefit the most from this approach. Step 1 consisted of pathway map reconstructions, depicting the role of Gal-1 and Gal-3 in tumor invasion, metastasis and angiogenesis, to confirm overlapping and independent cancer pathways associated with both galectins. These maps were constructed based on evidence from published literature and additional external databases. Step 2 aligned and ranked the associated expression of Gal-1 and Gal-3 at both the mRNA and protein level across different cancer types. This profiling included data from publicly available TCGA and CPTAC databases and additional external sources. Step 3 tested the highest ranked Gal-1 and Gal-3 mRNA and protein expressing cancers for association of survival by stratifying patients into four cohorts: Gal-1 low/Gal-3 low, Gal-1 high/Gal-3 low, Gal-1 low/Gal-3 high, Gal-1 high/Gal-3 high. Survival profiles quite clearly show that high expression of both galectins leads to poor survival in certain cancer types but not all. To summarize, using a bioinformatics approach, we have provided the initial evidence that Gal-1 and Gal-3 are highly expressed at both the gene and protein level in several cancers with their combined expression strongly correlating with poor survival rates. This data suggests, in certain cancer types, that targeting dual inhibition of Gal-1 and Gal-3 will have the potential to improve patient survival to a greater extent than a single Galectin blockade approach.

**LB174**

**Diabetes-associated breast cancer is molecularly distinct and shows DNA repair deficiency.**

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Diabetes commonly affects cancer patients. In breast cancer, it decreases patient survival. Diabetes also impacts African American women more so than European American women, potentially contributing to the breast cancer health disparity. In this study, we investigated the influence of diabetes on breast cancer biology in a diverse patient cohort using a three-pronged approach that included analysis of orthotopic human tumor xenografts, patient tumors, and breast cancer cells exposed to diabetes/hyperglycemia-like conditions. We aimed to identify shared phenotypes and molecular signatures by investigating the metabolome, transcriptome, and tumor mutational burden. Diabetes does not enhance cell proliferation but induces mesenchymal and stem cell-like phenotypes linked to increased mobility and odds of metastasis. It also promotes oxyradical formation and both transcriptome and mutational signatures of

DNA repair deficiency. Moreover, food- and microbiome-derived metabolites tend to accumulate in breast tumors in presence of diabetes, potentially affecting tumor biology. Breast cancer cells cultured under hyperglycemia-like conditions acquire increased DNA damage and sensitivity to DNA repair inhibitors. Based on these observations, diabetes-associated breast tumors may show an increased drug response to DNA damage repair inhibitors that are cancer therapeutics.
Key words: Breast cancer, diabetes, metabolome, transcriptome, metastasis, DNA repair, mutational signature, health disparity

**LB175**
**Ploidy as a predictive biomarker for gemcitabine sensitivity in triple-negative breast cancers.**
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Polyploid giant cancer (PGCC) state is a common response of cancer cells to various stressors including chemotherapy, irradiation, hypoxia and viral infection. Upon stress, PGCCs adopt an endoreplication in which the genome replicates, mitosis is omitted, and cells grow in size, leading to resistance. How accessible endoreplication is to a cell, therefore, directly translates to its resistance to therapy. In this study, we hypothesized that endoreplication and PGCC state are more plausible to cancer cells that already have a higher ploidy content prior to therapy. To test this hypothesis, we developed a comprehensive three-tier framework consisting of i) computational, ii) *in vitro* and iii) *in silico* components.
We designed a set of ordinary differential equations (ODEs) to model how cells enter and exit the PGCC state in response to a given stressor. We engineered how this *in silico* approach interacts with *in vitro* experiments into a broadly applicable software solution called CLONEID. The software uses computer vision to monitor phenotypic changes in cell and nuclear size from standard bright-field microscopy and classify cells into PGCC and non-PGCC states. We used CLONEID to test various therapeutic agents for their ability to select for a stable near-tetraploid population in a set of near-diploid cell lines. Through spontaneous cell fusions, we also obtained tetraploid breast cancer cells matched with their parental lines. Altogether, this framework, now, enables us i) to monitor the PGCC state experimentally ii) in matched cell lines with differential DNA content iii) to model the successful entrance and exit rates to and from the endoreplication state, respectively.
As the first application of this framework, we tested the ability of our matched triple-negative breast cancer (TNBC) lines (SUM-159 and MDA-MB-231) to access the PGCC state upon treatment with 18 commonly-used chemotherapy agents. We observed that gemcitabine caused continued cell growth without cell division in both tetraploid SUM-159 and MDA-MB-231 cells whereas near-diploid parental cells were hypersensitive to the treatment. Consequently, tetraploid cancer cells continued to safely grow in the presence of gemcitabine. Furthermore, these PGCCs re-entered the proliferative cell cycle and grew in cell number when treatment is terminated.
Gemcitabine (GEM)-based chemotherapy is a standard treatment for patients with TNBC although its efficacy is limited mainly due to drug resistance. Moreover, such poor response rate is coupled with severe side effects that frequently leads to the failure of vital organs, serious secondary diseases, and co-morbidities. Our findings suggest that ploidy is a predictive biomarker for gemcitabine sensitivity. Thus, we expect our findings and three-component framework strategy to help stratify the TNBC patient population by their response to gemcitabine. In addition, our mathematical modelling approach has the promising potential to inform personalized dose optimization and to effectively decrease administered gemcitabine dose for a subset of patients, which would alleviate therapy-associated side effects and lethalities.

**LB177**

**Tumor sideomics: spatial characteristics and functional deregulation in colorectal cancer.**

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Clinical evidence suggests that right-sided colorectal cancer (CRC) tends to have a worse prognosis compared to left-sided CRC. The distinct functional mechanisms underlying this "sidedness" remain cryptic and poorly understood. Thus, molecular classification of CRC is of paramount in targeting specific subtypes and personalizing treatment for patients. To identify targetable molecular features to improve treatment regimens, we performed multi-faceted network analyses of hundreds of CRC patients. In our network model, epigenetic features, differential expression of transcript abundance, and mutational frequency were used to identify widespread gene dysregulation and perturbed pathways to reveal disrupted networks distinct to right or left sided colorectal cancer. We discovered histone related genes and distinct epigenetic signals are at play in right-sided CRC. Additionally, our studies reveal "at risk" patient characteristics suggestive of survival differences, to improve diagnostic predictors based on CRC sidedness. Overall, our integrative approach serves to link molecular profiles distinct between left and right sided colorectal cancer, while improving upon previous models of CRC and paving the way towards identifying more effective treatment alternatives from a previously overlooked, readily characterized subtype of colorectal cancer.

**LB178**

**Cell free RNA for prediction of PDAC in de novo symptomatic patients presenting for EUS-FNA.**

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Endoscopic ultrasound (EUS), the primary procedure for the diagnosis of pancreatic ductal adenocarcinoma (PDAC), the most lethal cancer, is invasive. We aim to develop a liquid biopsy approach using plasma cell-free messenger RNA to stratify patients prior to EUS and assist in the precision diagnosis of PDAC. We found a set of plasma cell-free messenger RNA biomarkers and a set of robust reference genes to classify PDAC patients from intermediary pancreatic pathologies such as: benign cases, pancreatitis, and intraductal papillary mucinous neoplasm (IPMN) with reproducible results validated in separate cohorts. Our results demonstrate a non-invasive liquid biopsy approach for stratifying patient groups before invasive and definitive EUS screening.

**LB179**

**Automated viable circulating tumor cell (CTC) isolation enables efficient single-cell multi-omics analysis in a clinical setting.**

Janet Dickerson, Dylan Dufek, Robbie Huff, Stephenie Jones, Jennifer Barber-Singh, Christopher Brandt, Judy Muller-Cohn, Rolf Muller, Yipeng Wang. BioFluidica, San Diego, CA.

Circulating tumor cells (CTCs) are rare cells found in peripheral blood or other body fluids of cancer patients. Single-cell multi-omics analysis of CTCs can provide critical information and insights for tumor heterogeneity, early detection, residual disease, recurrence, and response and resistance to therapies. However, the adoption of single cell analysis in a clinical setting has been challenging due to complicated and lengthy workflows, lack of automation, low throughput, cell loss, inefficient cell picking, and nucleic acid degradation. To overcome these challenges, we have developed the fully automated LiquidScan liquid biopsy platform as a solution for CTC enrichment and single cell isolation. Whole blood samples

were collected from breast, prostate, and lung cancer patients using BioFluidica blood collection tubes. CTCs were enriched with LiquidScan platform coupled with microfluidic affinity selection of rare cells with antibodies against cancer specific surface markers. Cell eluates released from microfluidic chips were further sorted and individual cells were placed into individual wells of PCR plates. The isolated single cells were processed with either whole genome amplification for DNA analysis or pre-amplification of RNA for transcriptome profiling. Cell viability and RNA integrity were assessed for enriched cells post LiquidScan processing. Whole blood samples were directly loaded to microfluidic chips and processed (capture, wash, release and elute) using Hamilton robot for automated processing with no require to red blood cell lysis. The sample processing time was approximately 3 hours with up to 8 samples processed simultaneously. Enriched cells were 70% viable on average post LiquidScan sample processing. The RNA RIN score for the enriched cells was 6 on average. Success rate of DNA amplifications and RNA pre-Amplifications were both over 90% across all samples processed.
The LiquidScan platform provides a solution for fast, automated single viable cell isolation making downstream molecular analysis possible in a clinical setting. Protocols for RNA pre-amplification and DNA sequencing including copy number analysis and targeted resequencing have been developed. Single cell DNA methylation and proteomics assay development is in progress.

**LB180**
**SPOC, A highly multiplexed platform to study the kinetics of hundreds of biomolecular interactions: applications for early cancer detection.**
Rebecca Cook, Chidozie Victor Agu, William Martelly, Peter A. Bell, Mukilan Mohan, Bharath Takulapalli. INanoBio Inc, Scottsdale, AZ.

Tissue specific tumor neo-antigens and tumor associated antigens (TAAs) play a pivotal role in immune system interaction with cancer cells. Mutated, alternately spliced, over-expressed, mis-folded and abnormally post-translationally modified proteins may elicit immune responses that suppress tumor development. As these cancer specific proteins are distinct from normal self-proteins they offer the possibility of serving as highly-specific cancer biomarkers for early detection. TAAs are increasingly being targeted for priming the immune response to immunotherapies and cancer vaccines. Consequently, there has been an exponential increase in research on neo-antigen profiling using sequencing and multi-omic technologies. However, proteomic tools capable of studying and profiling the cancer proteome are limited. To contribute to this, we are developing a novel proteomic screening platform termed Sensor-integrated Proteome on Chip (SPOC) that enables high-throughput (HTP) screening of cancer proteins for loss or gain of function analysis, and humoral immune response profiling. SPOC platform enables use of surface plasmon resonance (SPR) biosensing for kinetic interaction profiling of 100s to 1000s of proteins at once, which can be applied to discovery and characterization of autoantibodies and neoantigens. SPOC uses human cell-free in vitro transcription and translation to express fusion capture-tagged proteins of interest from DNA plasmids printed in nanowells etched into a silicon slide. As proteins are expressed, they are captured covalently onto an overlaid gold-coated surface in individual spots via the capture ligand. The capture ligand requires the tag to be properly folded for binding, thus proteins on the surface are presumed to be fully folded when captured. When SPOC chips are analyzed using SPR, kinetic measurements of protein interactions can be obtained for each protein spot over time (RMax, Kon, Koff, etc.). SPOC arrays may be probed with a variety of biological molecules including small molecule drugs, antibodies, and other proteins. Data can be analyzed using bioinformatics and machine learning for neo-antigen discovery and to generate distinct signatures of disease. To demonstrate SPOC utility for neo-antigen discovery and characterization, a serum sample with known anti-p53 autoantibodies was analyzed on a SPOC SPR chip containing expressed p53 protein spots distributed throughout the array. Increases in SPR signals were observed for each p53 spot on the array as anticipated.

**LB181**

**Infer cancer cell gene dependency in multiple myeloma using causal AI *in-silico* patient model.**

Brandon Nathasingh, Derek Walkama, Laurel Mayhew, Kendall Loh, Jeanne Latourelle, Bruce W. Church, Yaoyu E. Wang. Aitia, Somerville, MA.

Recent advances in artificial intelligence (AI) and availability of multimodal patient datasets have enabled the construction of complex network models to derive disease molecular mechanisms and predict the impact of therapeutic intervention. However, observational datasets are commonly affected by confounding factors making causal interpretation challenging. Causal inference network methods are particularly suited to facilitate therapeutic intervention studies by inferring the causal structure from sufficiently detailed multi-omic molecular data. The learned models enable *in-silico* loss-of-function screening experiments on patient data by using counterfactual simulation to reveal the impact of a gene loss in a disease model. These models enhance gene dependency characterization and the design of advanced therapeutic interventions.

In this study, we developed an *in-silico* multiple myeloma (MM) patient causal model of overall survival (OS) based on transcriptomic expression, clinical, and genomic alteration data from Multiple Myeloma Research Foundation (MMRF) CoMMpass dataset (IA19). After filtering for data quality and availability, we included 516 patients, with 60% being hyperdiploid. We sampled Bayesian networks using Markov Chain Monte Carlo simulation to infer probabilistic causal relationships between network components that influence overall survival. We then simulated synthetic knock downs of those genes where a path exists to overall survival with posterior probability of at least 0.25. Next, we compared CRISPR-SpCas9 cancer dependency data from DepMap (version 22Q2) for multiple myeloma cell lines against genes predicted to be causal (causal genes) for overall survival. Last, we examined the causal genes that are non-dependent in MM cell lines for upstream genomic alterations to determine if specific patient genomic contexts are affecting the results.

We identified 102 causal genes, including non-coding RNA genes (n=23), driving overall survival. Among them, 70% (56/79, p=2.2e-16, OR=9.5) of the coding genes were found to be MM-dependent in DepMap, with 44 common essential, 7 strongly selective and 5 weakly selective genes. From 23 genes identified as causal and not known to be MM-dependent, 20 (87%) were selective in other cancer lineages and all of them (23/23) had consistent upstream genomic alterations driving their expression.

Causal genes identified from AI-driven *in-silico* experiments to predict overall survival were strongly enriched for known dependent genes from DepMap. Furthermore, we identified causal genes that may be dependent in unique patient genomic contexts. This demonstrates *in-silico* AI causal modelling is a powerful tool for exploring cancer cell vulnerability directly from patient data to advance target discovery.

**LB182**

**Targeting arginine methylation for improved therapy in triple negative breast cancer.**

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Since the discovery of DNA repair deficiency as risk factors for breast cancer, our knowledge of their function in maintaining human genomic integrity and a normal biological function has greatly advanced, ultimately culminating in development of PARP inhibitors that can specifically target patients who harbor these mutations. However, to date PARP inhibitors have demonstrated only modest clinical success as monotherapy in triple-negative breast cancer. In addition, conventional therapies are often coupled with severe toxicity. Therefore, there is a dire need to develop more effective combination therapies. Although there are many existing tools to predict possible actionable targets, it remains challenging to effectively and systematically identify drug combinations for better breast cancer therapeutics. This is because most tumors carry multiple genetic alterations that cannot be suppressed with mono-therapies; and diverse

confounding factors such as intra-tumor and inter-tumor heterogeneity exist across patient populations. Our preliminary proteomics study has revealed a new protein mark called arginine methylation as a novel target for breast cancer. We found that inhibition of arginine methylation can sensitize cancer cells for PARP inhibition treatment. We further assessed and validated this actionable and synergistic drug combination in patient-derived xenograft models. Together, our novel target combinations discovered here may serve as important biomarkers to help develop more effective combination therapy. Furthermore, our results help uncover patient-specific signaling mechanisms, a critical step towards precision medicine in breast cancer therapy, which is a benefit and potential long-term outcome.

**LB183**

**Network biology approach reveals multiple non-essential helper genes to target cancer cell lethality.**

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Understanding the determinants of human gene essentiality is critical for developing anticancer therapeutics. Here, we systematically analyzed the human essentialome from a network perspective. We found that essential genes are mostly context-specific and predicted synthetic lethal interactions based on analysis of context-specific essentiality in distinct genetic backgrounds. The predicted gene pairs are significantly overlapped with known synthetic lethality and further validated across more than 300 cancer cell lines. Moreover, analysis of synthetic lethal interactions among actionable genes helps prioritizing rational drug combinations. Surprisingly, genes in human essentialome (especially spliceosome, RNA transport and cell cycle genes) are enriched for cancer mutations with high functional impact and disease risks. Our network analyses suggest that protein interactome topology and neighborhood community integrated with gene expression, are highly predictive of gene essentiality. Thus, we devised network-based method (SYE-NET) to systematically prioritize the essentialome, which is further validated by functional screens in independent cell lines. Taken together, our study provides a holistic insight into molecular determinants for cell fate and implicates potential biomarkers for synthetic lethality and drug combinations in cancer, a critical step towards precision medicine.

**LB187**

**Case report: APN401, a novel cancer therapy using Cbl-b silenced autologous PBMCs, induced stable disease in two patients with advanced solid tumors.**

Mario Kuttke,[1] Alexander Dohnal,[1] Andreas Tanzmann,[2] Beate Pribitzer,[3] Felix Batrina,[3] Stefan Bunka,[3] Sophia Spagl,[2] Manuela Branka,[2] Sarah Bischof,[1] Kathrin Thell,[1] Maria Urban,[1] Hannes Muehleisen,[1] Bernhard Peball,[1] Bianca Gapp,[1] Angela Halfmann,[1] Markus Raderer,[2] Gerald Prager,[2] Thorsten Fuereder,[2] Romana Gugenberger,[1] Nina Worel[2]. [1]invIOs, Vienna, Austria; [2]Medical University of Vienna, Vienna, Austria; [3]University Hospital Vienna, Vienna, Austria.

Immune checkpoint control is one of several mechanisms that negatively influence the immune system and contribute to the development and progression of cancer. To enhance anti-tumor immune responses in patients with advanced solid tumors, we employ a novel autologous cancer cell therapy, APN401, which allows transient and highly specific silencing of Casitas B-lineage lymphoma-b (Cbl-b) in patients' peripheral mononuclear cells (PBMCs). Cbl-b is an E3 ubiquitin protein ligase that plays a central role in both innate and adaptive immune responses. Here we present two patients with advanced, metastatic solid tumors: one patient diagnosed with appendix carcinoma, and one patient diagnosed with head and neck squamous cell carcinoma (HNSCC). Both showed stable disease (SD) during treatment with APN401.
In an open-label, multicenter Phase Ib trial, two increasing dose levels of APN401 were evaluated in patients with advanced solid tumors. Using invIOs's closed cell-processing platform - Enhancement Platform for immune Cells (EPiC) - patients' PBMCs were purified from leukapheresis products and

subsequently transfected with a small interfering ribonucleic acid (siRNA) to specifically block Cbl-b expression. The entire manufacturing process required less than six hours, and the drug product could be reinfused on the same day. The trial evaluated safety and clinical outcomes, as well as the activity and potency of the drug product. Additionally, various biomarkers were analyzed in patients' peripheral blood, which was collected prior to each treatment cycle. Two patients, one with appendix carcinoma and one with HNSCC, showed stable disease after repeated APN401 treatments at the lowest and/or intermediate dose levels. Subsequent drug product analyses revealed increased IL-2 levels and elevated CD8/CD4 ratios, suggesting potential cytotoxic efficacy. In stimulation assays with HLA class I-restricted viral or tumor antigens, increased IFN-γ levels were detected and served as surrogate markers for improved immunity and tumor reactivity. 10X genomics and TCR clonotyping revealed clonal expansion of T cell subsets throughout the course of the treatment. Taken together, these initial results indicate that APN401, a novel personalized cancer cell therapy based on targeted silencing of Cbl-b in PBMCs, may be a safe and effective immunotherapy for solid tumors. This cell therapy will be further evaluated as a monotherapy along with potential combinations in clinical trials in various solid tumors.
Ethics approval: The clinical trial was approved by the Medical University of Vienna institution's independent Ethics Committee, approval number 1778/2020.

**LB188**

**Discovery and development of fully human TACI/BCMA bispecific armored CAR for the treatment of relapsed/refractory multiple myeloma.**

Garima Agrahari, Wenle Liang, Katie O'Callaghan Katie O'Callaghan, Keming Zhang, Ning Jiang, Xiaoyi Jin, Ryan Feng, Jenna Nguyen, Sam Hassan, Annie Luu, Tony Ruan, Kehao Zhao, Yan Chen. Elpis Biopharmaceuticals, Lexington, MA.

Background: The introduction of novel targeted therapies and immunotherapies has dramatically improved responses and overall survival in multiple myeloma (MM) patients. However, greater than 50% MM achieve refractory or relapse (r/r) within five-year due to MM target heterogeneity and bone marrow tumor microenvironment (TME). The approved B-cell maturation antigen (BCMA) -directed CAR T therapies have shown unprecedented efficacy in advanced r/r MM, yet relapse remains to occur. The shedded soluble BCMA, target escape and bone marrow TME may contribute to the r/r MM mechanism through compromising clinical efficacy and durability. TACI and BCMA are co-expressed on the tumors of ~78% MM patients, and therefore, dual targeted TACI/BCMA CAR therapy could be an attractive strategy in addressing challenges of BCMA mono-CAR therapy. We have developed EPC-004, a fully human anti-TACI and BCMA bispecific armored tandem CAR, aiming to overcome BCMA target escape mechanism, reduce immunosuppression within the bone marrow tumor microenvironment, and be more persistent with engineered armor and the improved cell manufacturing process.
Methods: Fully human anti-TACI, anti-BCMA and anti-PDL1 scFv antibodies with a broad affinity range and epitope coverage have been discovered by proprietary mRNADis$^{TM}$ mRNA display and live cell selection platform. The T cell modulatory activity fine-tuned IL2 was discovered by the mSCAFold$^{TM}$ technology through the structure guided mutagenic library design and high throughput systemic screening. The anti-PDL1 scFv was then fused to the engineered IL2 to generate an armor molecule to enhance CAR activities and persistence. Finally, EPC-004, an optimal bispecific tandem TACI/BCMA armored CAR candidate, was developed by precision engineering of multiple pairs of anti-TACI and BCMA scFvs with monospecific and bispecific target binding properties, spacers and scFv orientation.
Results: EPC-004 demonstrated both mono-specific and bispecific TACI and BCMA target cell engagement and effective cytotoxic cancer cell killing activities. EPC-004 showed potent anti-tumor activity in MM1R, BCMA knock out or TACI knock out MM1R -luciferase orthotopic animal models, which validated the mechanism of EPC-004 in overcoming BCMA or TACI target escape resistance. Furthermore, EPC-004 showed strong persistent CAR-T activity against tumor cells in re-challenging

models in mice. Importantly, the secreted armor molecule selectively activates central memory T cells and contributed to the enhanced anti-tumor activity and persistence *in vivo*.
Conclusion: EPC-004, engineered with multi-modules and multi-mechanisms is a promising candidate which enhances anti-tumor activity, prevents target escape, reduces immunosuppression within the bone marrow tumor microenvironment. These novel mechanisms could potentially translate EPC-004 into durable clinical efficacy in treating R/R multiple myeloma.

**LB192**

**MTL-CEBPA in combination with pembrolizumab converts an immune desert to an inflamed TME in solid tumors resistant to checkpoint blockade.**

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Despite significant advances in outcomes with immunotherapy, most cancer patients do not benefit from currently approved immune checkpoint inhibitors (ICI). The reasons for ICI resistance are multi-faceted and suggest that additional immunomodulation is required to improve outcomes. MTL-CEBPA is a novel immunotherapy based on RNA activation that upregulates expression of a master myeloid transcription factor, CEBPA. The small activating RNA for CEBPA is encapsulated within a NOV340 liposome that targets the myeloid cell lineage. MTL-CEBPA has shown favorable safety and promising clinical activity in combination with tyrosine kinase inhibitors (Sorafenib) in hepatocellular carcinoma (NCT-02716012) [Hashimoto et al, CCR 2021; Sarker et al, CCR 2020]. We recently reported preliminary clinical data from the ongoing multi-center phase 1 TIMEPOINT study (NCT-04105335) evaluating the safety, pharmacokinetics, immunomodulation, and clinical activity of MTL-CEBPA in combination with pembrolizumab in patients with solid tumors who have exhausted standard therapy. This demonstrated a favorable safety profile and initial clinical activity [Plummer et al, JITC 2021]. Here we report the findings from a biomarker pharmacodynamic analysis of paired baseline and cycle 2 tumor sample biopsies in 23 patients from the TIMEPOINT trial. Brightplex® IHC and digital pathology analyses of the samples for myeloid and T cell panels were undertaken, alongside gene expression (Nanostring I/O 360). Prior to study treatment, nine patients out of 23 had an immune cold tumor microenvironment (TME) at baseline as measured by the Immunosign®21 score. Following the combination of MTL-CEBPA with pembrolizumab, seven of these patients converted to an inflamed TME by Immunosign®21 ($P$=0.008). This change in the TME was associated with infiltration of CD8 and cytotoxic T cells (CD8+, GrzB+, Ki-67+) ($P$=0.1). GSEA analysis indicated that a Tstem-like signature was enriched post-treatment. A Brightplex® IHC analysis of myeloid cells in these patients indicated that, post treatment, there was a significant influx of HLA-DR+ myeloid cells into the TME ($P$=0.04). We also observed a significant

increase in the expression of CXCL9, 10, and 11.The remaining 14 patients had an inflamed TME at baseline. Here, we also observed an increase in HLA-DR+ cells, T cells, and chemokines, though to a lesser extent. Further, however, in these inflamed tumors—which have significantly greater infiltration of myeloid-derived suppressor cells (MDSCs) than desert tumors—we observed a reduction in 8/10 patients with detectable PMN-MDSCs ($P$=0.1) post treatment, consistent with the mechanism of action of CEBPA. An expression signature based on 18 genes significantly enriched for clinical response across all patients. Collectively, these data suggest a positive immunomodulatory TME effect of the combination of MTL-CEBPA with pembrolizumab. In both hot and cold TME tumors, the combination drives directed differentiation of progenitor monocytes into HLA-DR+ myeloid cells secreting chemokines that stimulate the ingress of T cells into the TME. We observe a significant positive correlation between the change in cytotoxic T cells and HLA-DR+ myeloid cells post treatment ($P$=0.004). These effects are most pronounced in cold tumors.

**LB194**

**A small molecular-weight anti-lag-3 peptide inhibits colon tumor growth.**

Mohammed Alahmari, Umar-Farouk Mamani, Yuhan Guo, Chien-Yu Lin, Mohammed Nurudeen Ibrahim, Yongren Li, Kun Cheng. University of Missouri- Kansas City, Kansas City, MO.

Lymphocyte activation gene-3 (LAG-3) has emerged as a promising target for cancer immunotherapy. It is expressed on activated $CD4^+$ and $CD8^+$ T cells, and on the surface of B cells, natural killer cells, and dendritic cells (DC). It has also been found to be highly expressed on activated T cells upon treatment with monoclonal antibodies targeting the PD-1/PD-L1 pathway, which may explain the resistance mechanism of monotherapy. Several anti-LAG-3 antibodies are being examined in clinical trials to treat different types of cancers. Despite their specificity and affinity, antibody-based checkpoint inhibitors are hampered by poor tumor permeability and high production costs. In this study, we aimed to discover a small peptide-based anti-LAG-3 inhibitor using a novel biopanning technique developed in our laboratory. We discovered several anti-LAG-3 inhibitor peptides, and the CMA16 peptide showed the highest stability and blocking efficacy. The peptides were synthesized by the solid-phase peptide synthesis using PurePep$^{TM}$ Chorus synthesizer (Gyros Protein Technologies, Tucson, AZ). The molecular weights and purity were confirmed by mass spectrometry and HPLC, respectively. To evaluate the function of the CMA16 peptide, a series of *in vitro* functional assays including binding ELISA, serum stability and cell viability assays were performed. *In vivo* anti-tumor activity of the peptides was examined in five-week-old C57BL/6 mice bearing MC38 cells. CMA16 significantly inhibited the growth of MC38 tumors. Quantification of $CD8^+$ tumor-infiltrating cells revealed a significant increase in this type of immune cells in the group of mice treated with CMA16. These findings support the further development of the CMA16 peptide as potential anti-LAG-3 inhibitor for cancer immunotherapy.

**LB195**

**Immunotherapies that repolarize macrophages and CD4 T cells enhance the effect of chemotherapy in high-grade serous ovarian cancer.**

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We asked if single-cell RNA sequencing (scRNA-seq) analysis of tumor immune cells in high-grade serous ovarian cancer (HGSOC) would reveal ways to enhance effects of neo-adjuvant chemotherapy (NACT). Analysis of 64,097 immune cells from seven HGSOC omental metastases showed that positive effects of NACT on immune responses were counterbalanced by induction of immunosuppressive mechanisms, especially related to antigen presentation and Treg activity. We identified stabilin-1 (Clever-

1) on macrophages and FOXP3 in Tregs as targets and validated actions of their inhibitors in vitro. ScRNA-seq analysis of 69,781 cells from chemotherapy-treated syngeneic mouse HGSOC tumors showed significant agreement with the patient results. Combinations of chemotherapy with anti-stabilin1 antibody and/or FOXP3 inhibitor (AZD8701), FOXP3i, significantly increased median survival of mice with established peritoneal disease. Long-term survivors (300 days+) were resistant to tumor rechallenge. Analysis of bulk RNA sequencing data from treated murine tumors confirmed our hypothesis and suggested potential mechanisms to explain the anti-tumor response seen in the murine models. Tumors treated with chemotherapy plus anti-stabilin1 antibody showed significant enrichment of anti-tumor macrophage signature and antigen presentation pathway compared to chemotherapy alone. Tumors treated with chemotherapy plus FOXP3i in combination with anti-stabilin1 antibody showed significant upregulation of antigen presentation, T cell activation and cytotoxicity and T helper 1 differentiation pathways. There was significant reduction in FOXP3 positive cells and significant increase of Tbet positive cells in FOXP3i treated murine tumors suggesting repolarization of naive T cells in the tumor microenvironment towards a more anti-tumor T helper phenotype. As there are early phase clinical trials of FOXP3 inhibitor and anti-stabilin1 (Clever-1) antibody in patients with advanced cancer, these results suggest ways of improving response to chemotherapy not only in HGSOC but in other cancers that are treated by NACT.

**LB196**

**NOUS-PEV, a personalized cancer immunotherapy targeting neoantigens, induces long lasting, tumor infiltrating memory T cells.**

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Personalized vaccines hold great promise to exert meaningful clinical efficacy, with durable tumor control maintained by a vaccine–induced memory response. NOUS–PEV is a personalized viral prime–boost cancer vaccine that expresses 60 patient–specific neoantigens identified by next generation sequencing (NGS) and selected with a proprietary algorithm VENUS *(Leoni & D'Alise et al, Vaccines, 9, 2021*). Administration is intramuscular, with a priming Great Ape Adenovirus (GAd) vaccination, followed by Modified Vaccinia Ankara (MVA) "boosts", administered in combination with the PD–1 blocking antibody pembrolizumab in patients with metastatic malignant melanoma and non–small cell lung cancer. Data from the Part 1 dose–confirmation cohort of 3 patients demonstrated the combination of NOUS–PEV and pembrolizumab to be safe and well–tolerated, with early indications of efficacy and immunogenicity (Bechter, et al SITC 2022 Poster number: 706). Now in Part 2 extension–expansion cohorts, we present extended safety, immunogenicity and clinical data at 11 months median follow–up for 6 vaccinated melanoma patients. Tolerability remains good with no grade 3 or 4 vaccine related adverse events and activity encouraging, with 4 PRs, 1 SD and only 1 PD as best response. Immune responses were evaluated by *ex–vivo* interferon–gamma ELISpot on PBMC collected at baseline, post pembrolizumab, and post vaccination. Vaccine immunogenicity was demonstrated in all evaluable patients receiving the prime/boost regimen (n=4), with a mean of T cell response of ~ 650 IFN–γ spot forming cells (SFC) per million of PBMC (range 380–1,250 SFC/$10^6$) and with observed induction of both CD4 and CD8 T cell responses which lasted for at least 6 months. By analyzing the intratumoral TCR repertoire, we found increase of T cells by ~3 fold on average post treatment with NOUS–PEV in all evaluable patients (n=3), with expansion and diversification of intratumoral T cell clones. Vaccine–induced TCR clonotypes were found in on–treatment tumor biopsies of 2 vaccinated patients, providing

the proof–of–concept for neoantigen induced T cells homing and infiltrating into the tumor. Overall, these data show that NOUS–PEV continues to be safe, and elicits a robust long lasting immune response and clinical activity.

**LB197**

**A pooled mutant KRAS peptide vaccine activates polyfunctional T cell responses in patients with resected pancreatic cancer.**

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Background: Oncogenic mutations in KRAS are expressed in up to 90% of pancreatic ductal adenocarcinomas (PDAC). Vaccination against mutant KRAS (mKRAS) is thus a promising approach as an off-the-shelf immunotherapeutic treatment for PDAC. We developed a mKRAS peptide vaccine targeting 6 common KRAS mutations (G12V, G12A, G12C, G12R, G12D, or G13D (NCT04117087). We evaluated the mKRAS specific T cell response induced by vaccination in combination with immune checkpoint inhibitors (ICIs) in patients with resected PDAC.
Materials and Methods: This is an ongoing pilot study of a pooled mKRAS long peptide vaccine in combination with ICIs in patients with resected PDAC and mutations in one of 6 KRAS mutations in our vaccine. Patients with no evidence of disease on imaging within 6 months of completion of adjuvant chemotherapy were vaccinated with the mKRAS vaccine (0.3mg/peptide and 0.5mg poly-ICLC (Hiltonol: Oncovir)) weekly for 4 doses followed by booster vaccines every 8 weeks. Patients also received ipilimumab (1mg/kg, every 6 weeks for 2 doses) and nivolumab (3mg/kg, every 3 weeks in the priming phase) followed by nivolumab (480mg, flat dose in boost phase). To evaluate the expansion of mKRAS-specific T cells in the periphery, pre- and post-vaccination peripheral blood mononuclear cells (PBMCs) were restimulated with control or individual mKRAS peptides and assessed for IFNγ release by ELISPOT. To further phenotype the responding CD4 and CD8 T cell compartments, peptide-restimulated PBMCs were evaluated for T cell activation, proliferation, memory and exhaustion marker expression by CyTOF. Paired single-cell RNA and TCR sequencing are being performed to isolate mKRAS-specific TCRs and their corresponding transcriptional profiles. These TCRs are being functionally validated in vitro using CRISPR-Cas12a-based genome knock-in of human T cells.
Results: At the time of data cut off (1/12/2023) 8/11 patients enrolled had a positive mKRAS-specific T cell response in post-vaccine sample timepoints defined by a >5 fold increase in IFNγ producing T cells after peptide restimulation. CyTOF analysis of peptide-restimulated PBMCs demonstrated expansion of polyfunctional (IFNγ+IL2+TNFα+) mKRAS-specific CD4 and CD8 T cells with both central and effector memory phenotypes after vaccination. mKRAS-specific CD4 T cells were induced at a greater proportion. Our single-cell analysis has identified and validated a novel CD4+ TCR that recognizes KRAS G12V in the context of HLA-DRB1*07:01.
Conclusions: This study thus far indicates the induction of *de novo*, high quality mKRAS-specific T cells in the periphery post-vaccine. Ongoing studies will define TCR diversity and clonality of mKRAS-specific T cells to each mKRAS epitope. Overall, our data will be used to identify peripheral T cell-based biomarkers that may be able to predict response to mKRAS-targeted immunotherapy.

**LB198**

**A first-in-human, open-label, multicenter study of intratumoral SAR441000 (mixture of cytokine encoding mRNAs), as monotherapy and in combination with cemiplimab in patients with advanced solid tumors.**

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**Background:** SAR441000 is an intratumoral (IT) therapy consisting of a mixture of mRNAs encoding the cytokines IL-12, IFN-α 2b, GM-CSF, and IL-15sushi. SAR441000 is hypothesized to induce a local and systemic immune response with reduced toxicity, and potentially enhance anti-tumor response when combined with checkpoint inhibitors.
**Methods:** We conducted a phase 1 dose escalation and expansion study (NCT03871348) in patients with advanced/metastatic solid tumors to investigate SAR441000 as monotherapy (QW) and in combination with cemiplimab (350mg Q3W). The recommended dose (RD) of SAR44100 in combination with cemiplimab was further investigated to confirm the anti-tumor activity in patients with metastatic melanoma who had previously exhausted all standard of care options and failed treatment with anti-PD-(L)1 therapies. Serum samples and tumor biopsies were collected at baseline and post-treatment to characterize the PK/PD profile, and immune cell tumor infiltration. Tumor assessment was performed per RECIST 1.1 and iRECIST, where ≥1 non-injected lesion was considered as target lesion. Analysis was triggered after the last treated patient underwent their 2$^{nd}$ tumor assessment.
**Results:** 77 patients were treated, 36 in escalation phase (21 in monotherapy, 15 in combination therapy) across eight dose levels (8µg-4000µg) and 41 in expansion phase. No dose limiting toxicities were observed. During escalation phase, 1 patient (6%) achieved confirmed complete response (iCR), 1 (6%) partial response (iPR), and 3 (20%) stable disease (iSD) as per iRECIST in combination therapy. Paired biopsies from the iPR patient showed significant increases in CD3+ and CD8+ immune cells in both injected and non-injected lesions compared to baseline. Increase of peripheral PK and PD (IFN-γ and IP-10) cytokines were observed post-treatment but no clear dose response relationship was established. Based on the overall data, 4000µg was chosen as the RD. During expansion phase, 2 (5%) melanoma patients achieved confirmed iPR and 12 (29%) achieved iSD as best overall response. Although clinically meaningful responses of injected and/or non-injected lesions were observed in 13 (32 %) patients, responses were mostly limited to loco-regional disease and no significant distant non-injected lesion response was seen. Post-treatment biopsies showed increased CD3+ and CD8+ immune cells in approximately 50% and 40% of injected and non-injected tumors, respectively. Most frequent (>10%) related TEAEs observed in expansion phase included injection site pain (26.8%), fatigue, diarrhea, pruritus, and asthenia (each 12.2%).
**Conclusion:** IT with SAR441000 in combination with cemiplimab was generally well tolerated and demonstrated anti-tumor activity and immune cell infiltration in both injected and non-injected tumors.

**LB199**

**A personalized neoantigen vaccine is well tolerated and induces specific T-cell immune response in patients with resected melanoma.**

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Tumor mutations giving rise to neoantigens have recently emerged as promising targets for cancer immunotherapy. Vaccines delivering tumor-specific neoantigens have demonstrated favorable efficacy and safety results in numerous preclinical and early clinical studies. However, selecting therapeutically relevant neoantigens amongst the myriad of cancer mutations has proven challenging. To overcome this, we have developed a proprietary AI-based target discovery platform, PIONEER, with enhanced predictive performance and increased precision. The PIONEER top-ranked neoantigens were selected and included in the personalized DNA vaccine candidate EVX-02. To assess the ability of the PIONEER designed neoantigen vaccine candidate to induce neoantigen immunogenicity and anti-tumor effect in preclinical models, mice were immunized with a mouse surrogate EVX-02 molecule, mEVX-02. mEVX-02 vaccination completely prevented establishment of tumors and induced neoantigen-specific, polyfunctional CD4+ and CD8+ T cells in the blood and spleen of immunized mice. An enhanced antitumor effect was obtained when combining mEVX-02 with anti-PD-1 mAb treatment. Encouraged by the preclinical results, we conducted a first-in-human multicenter Phase I clinical study of EVX-02 in combination with nivolumab in patients with resected malignant melanoma. The objectives of the trial were to investigate safety and tolerability, operational and clinical feasibility, and immunogenicity of the applied personal neoantigens. Each patient received a fully personalized drug that was produced in a complex process, from biopsy, through genome sequencing, AI-profiling, vaccine design, manufacturing, quality testing, and drug product release. This was succeeded with every single step for each patient. In all patients, EVX-02 treatment was well tolerated, and only very mild adverse events (AEs) have been observed in relation to immunization with EVX-02. Interim data demonstrated neoantigen-specific T-cell immune responses upon EVX-02 treatment and that the responses were mediated by activated CD4+ and CD8+ T cells. The measured T-cell responses were robust and long lasting. Together, these findings validate the precision and predictive power of our proprietary AI platform, PIONEER, and provide proof of mechanism for the DNA-delivery technology in that the encoded neoantigens gave rise to significant immune reactions.

**LB200**

**Indoximod or ibrutinib/indoximod based clinical chemo-immunotherapy drives conversion of extra-tumoral circulating stem-like precursor CD8+ T cells into clonally expanded, rejuvenated effector cells.**

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GA.

Increasing evidence suggests that many of the effector CD8+ T cells reactivated ('rejuvenated") by immunotherapy come from outside the tumor, derived from a circulating pool of "stem-like" memory or "precursor-exhausted" (TPEX) cells. These cells have been characterized in mice, but, despite their importance, circulating counterparts in humans have not yet been identified for study. We hypothesized that immunotherapy designed to enhance immunogenic antigen-presentation during chemotherapy might produce extensive reactivation of these precursor T cells. While the antigen-presentation step occurs in tissues, homing of the rejuvenated T cells to the tumor is via the circulation; thus, we hypothesized that they would be visible in blood. Informative patients were selected from two ongoing clinical trials of children with brain tumors treated with the IDO-inhibitor drug indoximod: a Phase 2 trial (NCT04049669) of indoximod plus chemotherapy; and a Phase 1 trial (NCT05106296) of indoximod, chemotherapy and the BTK-inhibitor ibrutinib, which synergistically destabilizes IDO (*Immunity* 54:2354-2371, 2021). Patient selection criteria included either (i) massive clonal expansion of activated CD8+ T cells on therapy (expanded clones reaching 16-25% of total CD8+ T cells); or (ii) complete radiographic tumor response (CR) on treatment; or (iii) both. Longitudinal blood samples (4-10 samples per patient) were obtained over a period of 6-24 months and analyzed by single-cell RNA-sequencing (scRNA-seq) with paired single-cell T cell receptor sequencing (scTCR-seq). TCR clonotypes of interest were identified based on robust clonal expansion on-treatment; then each clonotype was traced back through earlier samples to the pre-treatment baseline, or the earliest sample in which that clone could be detected. Clones were pooled, subjected to UMAP clustering, and differential gene-expression and trajectory analysis performed. At earliest appearance, each clonotype was enriched for a phenotype dominated by early transcription factors *TCF7* and *IKZF2* (Helios). These cells expressed little *PDCD1* (PD-1) but showed a "hybrid" combination of genes associated with immaturity/arrest (*BACH2, DUSP2, LTB, IL7R, CD160*) and effector/memory (*NKG7, GZMK, GZMA*). Within each responding clone, this "precursor" phenotype could be observed to progressively transition into a mature cytotoxic/effector phenotype (*PRF1, GZMB, GZMH, FGFBP2, KLRB1, IFNG*). Trajectory analysis of this maturation sequence allowed us to analyze key gene-regulatory networks and transcription factor profiles at each stage. To our knowledge, this is the first study in humans to identify this key stem-like precursor population in circulation, allowing us to sequentially follow the molecular changes in these cells during rejuvenation.

**LB201**

**A novel combination of *Listeria*-based immunotherapy and repurposed drug combats colorectal cancer.**

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Background: Colorectal cancer (CRC) has been a difficult-to-treat cancer with a significant proportion of microsatellite stable (MSS-CRC) patients who do not respond to immunotherapy. Currently, various strategies are being investigated to sensitize MSS-CRC tumors by converting them to immunologically "hot" tumors and improve responsiveness to immunotherapy. In this study, we demonstrate a novel strategy of combining a repurposed FDA-approved compound, Pimavanserin tartrate (PVT), with a *Listeria monocytogenes* (Lm)-based vaccine targeting Interferon-Stimulated Gene 15(ISG15), Lm-LLO-ISG15, against MSS-CRC tumors. Our findings demonstrate that both agents synergize to produce $CD8^+$ T cell-dependent therapeutic efficacy in immunotherapy-resistant MSS-CRC tumors.
Methods: The direct killing effect of PVT *in vitro* was confirmed by cytotoxic SRB assay. BALB/c mice bearing CT26, a murine model of MSS-CRC, were randomly assigned to receive either PBS, PVT, Lm-LLO-ISG15, or PVT+Lm-LLO-ISG15. Mice were monitored for tumor growth kinetics to study the anti-cancer effect of each monotherapy and the combination therapy. Changes in immune-related gene and

protein expression were analyzed by qPCR and Western blot, respectively. Various immune cell subsets in the tumors and spleens were evaluated by multi-color flow cytometry and immunohistochemistry. In addition, organs such as the liver, kidney, heart, lungs, spleen, brain, and pancreas were collected for safety analysis.
Results: PVT efficiently induced apoptosis and directly killed both human and mouse MSS-CRC cell lines *in vitro*. Further, PVT treatment enhanced T-cell activation and induced ISG15 expression, thus, sensitizing the tumor cells to targeting by the CTL-mediated immunotherapy, Lm-LLO-ISG15. While each monotherapy failed to demonstrate anti-tumor efficacy, treatment with the combination therapy (PVT + Lm-LLO-ISG15) significantly controlled tumor burden and extended the median survival. The therapeutic efficacy of the combination approach was associated with a higher infiltration of $CD8^+$ T cells in the TME and an increased population of effector memory T cells ($CD4^+CD44^{hi}CD62L^{lo}$) in the spleen. The anti-tumor efficacy of combination therapy on subcutaneous CT26 tumor growth was completely abrogated after the depletion of $CD8^+$ T cells. Moreover, the safety profile of dual therapy was comparable to that of each monotherapy and PBS.
Conclusions: MSS-CRCs are considered "immunologically cold" tumors that represent great challenges for any standalone therapies. In this study, we evaluated, for the first time, the therapeutic efficacy of a novel combination of PVT and Lm-LLO-ISG15 in an aggressive MSS-CRC mouse model. Combination therapy demonstrated a synergistic effect that led to a significant reduction of tumor burden and an extension of median survival. While the underlying mechanism for synergism requires further examination, our findings strongly suggest that combining PVT and Lm-LLO-ISG15 could be a promising approach for MSS-CRC patients.

**LB202**
**Obesity-trained $C1q^+$ macrophages compromiseT cell anti-tumor immunity.**
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Adipose tissue in the mammary gland undergoes dramatic remodeling during obesity and is involved in numerous metabolic diseases. However, how obesity-driven remodeling in adipose tissue regulates anti-tumor immunity remains unclear. Here we provide the detailed cellular atlases of mouse mammary fat pad and tumor at single-cell resolution in lean and obese mice. By leveraging scRNA-seq, we found that high-fat feeding causes a marked increase of macrophage in adipose tissue. Interestingly, a subset of macrophages expressing high levels of C1q complex genes is accumulated in obese subjects. We found that glucocorticoid may as a key regulator of $C1q^+$ macrophage identity. Synthetic glucocorticoid, Dexamethasone, triggers C1q expression in macrophages. We found that $C1q^+$ macrophage derived C1q complex could suppress $CD8^+$ T cell response, thus fueling breast cancer progression. In mouse TNBC tumor, we uncovered a specific inflammatory responsive tumor subset in obese tumors with high CCR2 ligand expression. Obesity-induced TNFα exposure triggers of breast cancer cells reprogramming and CCR2 ligand production. Consequently, CCR2 ligands promote macrophage into tumor. Moreover, depletion of macrophages by anti-CSF1R suppresses tumor growth in obese mice. Our findings define a novel fundamental mechanism of C1q+ macrophage involved in anti-tumor response in obesity.

**LB203**
**Targeted inhibition of IRAK-4 with CA-4948 (emavusertib) sensitizes metastatic brain melanoma to anti-PD-1 immune checkpoint blockade.**
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Introduction: The unique microenvironment within the brain alongside restrictions imparted by the blood brain barrier (BBB) create a sanctuary site for tumors to thrive. Melanoma is often responsive to therapy when present in peripheral tissue, but becomes recalcitrant when sequestered beyond the BBB. An estimated 40-60% of metastatic melanoma patients will develop a brain lesion, even while receiving treatment. Stereotactic radiosurgery (SRS) is the gold standard in intervention for metastatic brain melanoma (MBM), often given with anti-PD1 immune checkpoint blockade (ICB). While this therapy is initially effective, resistance occurs rapidly and ~50% of patients will develop new brain lesions within a year. Treatments at this stage are limited, necessitating the discovery of new therapies. Recent studies have shown that myeloid differentiation primary response protein 88 (MyD88) is activated in melanoma in tumor-associated myeloid cells where chronic myddosome signaling results in defective immunological responses. We have found that myddosome signaling is also prevalent in MBM, likely protecting brain metastases against effector immune detection. We recently identified that CA-4948, a small molecule inhibitor of interleukin (IL)-1 receptor-associated kinase (IRAK-4) and rate-limiting step in the myddosome cascade, can reach therapeutic levels in the brain and demonstrates on-target inhibition in preclinical models of MBM. These data highlight a unique therapeutic opportunity where selective inhibition of pro-tumorigenic cells in the MBM tumor microenvironment (TME) via targeted IRAK-4 inhibition may recondition the immunological landscape of these tumors, sensitizing these difficult-to-treat tumors to immunotherapy.
Hypothesis: Selective inhibition of pro-tumorigenic cells via targeted inhibition of IRAK-4 with CA-4948 will recondition the immunological landscape of MBM, improving anti-tumor response to anti-PD-1 ICB.
Methods: Patient MBM samples were assessed by IHC to define the distribution of myddosome signaling. Preclinical syngeneic MBM tumor models were treated with CA-4948 alone and in combination with anti-PD-1 ICB. Tumors were examined by flow cytometry and IHC to characterize the composition of the immune TME and evaluate the distribution of T lymphocytes. Overall survival was compared between control, single agent, and combination treated animals.
Results: Tumor-associated immune cells and astrocytes comprise the majority of active myddosome signaling in patient MBM. Preclinical models corroborate these findings, with targeted inhibition of IRAK-4 through CA-4948 treatment effective at reducing activation and validating anti-tumor activity in the CNS. Analysis of treated tumors reveal decreased expression of Programmed Death Ligand 1 (PD-L1), and increased numbers of tumor-infiltrating T lymphocytes. Combination CA-4948 with anti-PD-1 ICB resulted in reduced tumor growth, and improved survival over single agent therapy.
Conclusions: Therapeutic inhibition of IRAK-4 in MBM mitigates immune suppression mediated by tumor-associated cells, allowing for improved immune surveillance and tumor infiltration by effector T lymphocytes, resulting in enhanced anti-tumor activity. Thus, CA-4948 may be an effective treatment alongside anti-PD-1 ICB for patients with MBM.

**LB205**

**Feasibility and immunogenicity of adjuvant TG4050, a patient tailored cancer vaccine in head and neck and ovarian cancer.**

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Background: Head and Neck (HNSCC) and Ovarian cancer (OC) are two indications for which

immunotherapy had limited impact so far. Current treatments achieve high rates of initial success through surgery and adjuvant chemo/radiotherapy, but patients (pts) remain at high risk of relapse in both indications. Immune stimulation using a vaccine is a promising strategy to a clinically meaningful improvement. Herein we report phase I data of TG4050, a vaccine engineered to carry a pt tailored antigen payload, in pts with HNSCC (NCT04183166) or OC (NCT03839524).
Methods: Tumor specific variants are identified using next generation sequencing of tumor and normal samples whereby immune relevant mutations are predicted using a machine learning algorithm factoring in parameters known to affect immunogenicity including MHC binding, level of expression, prevalence across clones, and antigen processing. DNA sequences of the mutations of interest, typically 30 per pt, are cloned in a viral vector (Modified Vaccinia Virus Ankara). Following curative intent treatment, HNSCC pts in complete remission were randomized to an immediate vaccination arm to receive weekly doses of TG4050 for 6 weeks followed by a maintenance period of one dose every 3 weeks for up to 20 doses or to a delayed vaccination arm where the same vaccination regimen is initiated at relapse. OC pts received the vaccine upon onset of signs of relapse. PBMC were collected at Baseline and after 6 doses of vaccine. Primary endpoint was vaccine safety and secondary endpoints included feasibility, immunogenicity.
Results: 18 pts received TG4050 (5 OC and 13 HNSCC) treatment with no related grade $\geq 3$ adverse events. Tumor mutational burden (TMB) was on average 3.00±0.98 and 2.43±0.69 mutations per megabase for HNSCC and OC respectively. Despite this low TMB, all pts had sufficient cancer specific neoantigens to design a vaccine. Ex-vivo ELISPOT was completed for 7 pts at the time of submission. A mean of 44% of selected mutations were associated with a reactive T cell responses either spontaneously or after TG4050 stimulation ranging from 6 to 22 mutations per pt. Vaccination induced a stimulation of T cell response in all tested pts on an average of 10 targeted mutations, induction of *de novo* responses was seen in all 7 tested pts and amplification of pre-existing response in 6/7 pts. None of the evaluable HNSCC pts receiving TG4050 immediately after first line treatment had relapse after a median follow-up of 7 months while 2 pts had relapse in the delayed vaccination arm. Furthermore, in OC pts treated at early relapse, TG4050 has normalized disease markers (ctDNA and/or CA-125) for 2/4 pts with stable disease for 9 and 11 months.
Conclusion: TG4050 treatment is feasible and immunogenic in pts with low to moderate TMB. It is effective in inducing T cell responses and first signs of antitumor activity are encouraging for the continuation of development.

**LB208**
**A potent macrophage switching drug D-4559 reduces tumor GROWTH in a hepatocellular carcinoma mouse model.**
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Tumor-associated macrophages (TAMs) play a role in cancer progression and are associated with Sorafenib resistance in hepatocellular carcinoma (HCC)[1]. D-4559 is a new potent macrophage switching nanomedicine technology that selectively inhibits VEGF receptor tyrosine kinases (VEGFR1, 2, 3) in TAMs leading to a functional reprogramming of TAMs toward a pro-inflammatory activated phenotype. Here, we evaluate the effect of D-4559 on the M1 and M2 polarization of TAMs and its anti-tumor efficacy in a murine HCC tumor model. In vivo efficacy of D-4559 was examined in the subcutaneous Hepa 1-6 liver tumor model in C57BL/6 mice. Animals (n=15/group) were treated with D-4559 (*i.p.*, 200 mg/kg daily) and free drug Sorafenib (*p.o.*, 40 mg/kg daily) as a positive control for 4 weeks. The treatment started when the mean tumor size reached approximately 100 $mm^3$, then the animals were randomly allocated into study groups. The day of randomization and treatment was denoted as day 0. Tumor sizes were measured using a caliper for 27 days, M1/M2 macrophage polarization was examined

by flow cytometry at day 16, and cytokine biomarkers were evaluated with MSD Cytokine Multiplex Assay at day 16 and 27.D-4559 significantly reduced Hepa 1-6 tumor growth (**$p$<0.01 vs. vehicle). D-4559 and Sorafenib efficacy were similar with 40% of mice with a tumor volume less than 500 $mm^3$ after 27 days of treatment. At day 16, D-4559 significantly increased M1/M2 ratio by inducing M1 macrophage infiltration and reprogramming of TAMs into M1 macrophages compared to vehicle treatment groups (* $p$<0.05). In contrast, Sorafenib did not switch TAM polarization from M2 to M1 phenotype. Moreover, D-4559 significantly increased TNF-a and IL-8 cytokines in the tumor microenvironment at days 16 and 27, indicative of a M1 signature. Increased levels of TNF-a and IL-8 have also been correlated with better survival in HCC patients[2,3]. D-4559 significantly increased M1 infiltration, shifted TAM polarization from M2 to M1 phenotype and favored pro-inflammatory cytokines suggesting that D-4559 creates an immunopermissive tumor microenvironment leading to a reduction in tumor growth. This preclinical study supports the development of the potent macrophage switching drug D-4559 as a safe and effective agent that can be used systemically for the treatment of HCC or in combination therapies to improve anti-tumor immune response.
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**LB209**

**An ADC composed of daratumumab and lenalidomide is extremely powerful in killing multiple myeloma cells.**

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**Introduction:** Multiple myeloma (MM) is a neoplastic malignancy characterized by the abnormal proliferation of plasma cells with excessive antibody production in the bone marrow. It accounts for approximately 1.8% of all new cancer cases and 2.1% of cancer deaths in the United States and occurs most frequently among older adults (age > 75). While there are a number of treatments with high response rates, MM relapses frequently within a few years and is considered incurable. Herein, experimental studies and results of a new therapeutic agent under development, TE-1146, are reported. TE-1146 is an antibody drug conjugate (ADC) composed of two therapeutic agents already in use in MM, namely, an anti-CD38 antibody, daratumumab (Dara), and lenalidomide (Lena).
**Method:** TE-1146 is designed by employing "HIDAR" technology platform, which enables the preparation of homogeneous ADCs with high DAR (drug to antibody ratio). In the TE-1146 molecules, the Fab is replaced by scFv and a cysteine-containing $Zn^{2+}$-binding motif is engineered at the C-termini of the H chains. Lena molecules are assembled into drug bundles containing 3 Lena molecules and a maleimide group. Two drug bundles are conjugated site-specifically to the $Zn^{2+}$-activated SH groups of the reconfigured antibody molecule, creating an ADC with DAR of 6. TE-1146 was investigated for cytolytic effects against human MM cell lines H929 in cell cultures *in vitro* and transplanted H929 tumor in NOD-SCID mice *in vivo*, in comparison with Dara, Lena, and their combination.
**Results:** In human plasma, TE-1146 has stability comparable with Dara, and the Lena molecules in the drug bundles remain conjugated. In cultures of H929 cells, TE-1146 caused the lysis of H929 cells at least 100 times more effectively than Dara, Lena, and Dara/Lena combination, based on $IC_{50}$ comparison. It was shown that H929 cells internalized and degraded TE-1146 and freed Lena. In NOD-SCID mice, the subcutaneously transplanted H929 cells were allowed to grow into solid tumors in 14 days, and TE-1146 and other agents for comparison were administered intraperitoneally. It was found that one single dose of TE-1146 at 20 nmol/kg (conjugated with Lena at 120 nmol/kg) could retard the growth of the transplanted tumor and ultimately eliminate the tumor over 28-42 days, while the combination of one dose Dara at 20-80 nmol/kg and Lena of 46 μmol/kg given daily could not eliminate the tumor. The amount of Lena used in the combination treatment over a 28-day course is 10,700 times that of Lena in TE-1146.
**Conclusion:** Lenalidomide is extremely powerful in killing multiple myeloma cells if brought into the

MM cells. It is estimated that a very minute amount, probably less than 0.01%, of intraperitoneally injected lenalidomide gets into the transplanted MM tumor in the mouse model. TE-1146 may be a more effective and less toxic drug than Daratumumab/Lenalidomide combination in treating patients with hard-to-treat MM.

**LB210**

**RenNano® mice: A heavy-chain-only antibody platform for the generation of nanobody therapeutics.**

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Monoclonal antibodies have been used to successfully treat various diseases, including tumors, autoimmune diseases, and infectious diseases. Traditional antibodies are comprised of a tetramer of two heavy chains and two light chains, totaling 150 kDa in molecular weight. However, the large size of antibodies can limit the therapeutic application; in particular, the penetration of tumors and the blood brain barrier (BBB) is not always feasible. In contrast to traditional antibodies, heavy-chain-only antibodies (HCAbs) are significantly smaller (~75 kDa), as they contain only two heavy chains. Since the heavy chain variable domain of HCAbs (i.e., VHH or single domain antibody, sdAb, or nanobody) is solely responsible for antigen recognition, nanobodies can function independently as a therapeutic molecule, which may be advantageous for penetrating tumors or the BBB. Previously, we generated a fully human antibody mouse platform, RenMab™, in which the murine heavy chain and kappa light chain variable domains were replaced by the full human heavy chain and kappa light chain V(D)J loci *in situ*. Here, we have further modified the RenMab™ model to generate a fully human heavy-chain-only antibody mouse model, termed RenNano®. The modified heavy chain constant regions of RenNano® mice allow them to spontaneously produce HCAbs. Flow cytometry and biolayer interferometry confirmed that RenNano®-derived HCAbs can bind antigens without light chains. Despite this reliance on the heavy chain only variable regions for antigen specificity, RenNano® mice can generate antigen-specific antibodies with high affinity ($10^{-8}$ -$10^{-9}$ $K_D$) upon immunization with various antigens. In addition, many RenNano®-derived HCAbs exhibited a longer CDR3 length, which could promote the recognition of difficult-to-reach epitopes. Furthermore, RenNano®-derived HCAbs have favorable diversity, and excellent developability properties such as a higher degree of hydrophilicity. Anti-4-1BB HCAbs can also activate 4-1BB-NF-κB signaling in a dose-dependent manner, as demonstrated in reporter assays.
In summary, the full human heavy-chain-only antibody mice, RenNano®, can produce human HCAb with high affinity and good efficacy. Thus, RenNano® is a powerful platform to discover HCAb/nanobodies for various therapeutic applications.

**LB211**

**Discovery of RenNano®-derived human heavy-chain-only antibodies that cross the blood-brain barrier.**

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Therapeutic antibodies have been successfully used to treat several diseases, such as cancers and autoimmune diseases. However, the utility of conventional antibodies for neurological conditions is limited by the blood-brain barrier (BBB). Several strategies to address this issue have been reported, including receptor-mediated transcytosis (RMT) of antibodies using transferrin receptors. We hypothesize

that this strategy could be further improved by the use of single-domain antibodies (sdAbs), such as the variable domain of heavy-chain-only antibodies (HCAbs), or variable new antigen receptors (VNARs), which are significantly smaller, and therefore could be used to more efficiently transport drugs of interest across the BBB. To this end, we developed anti-transferrin receptor 1 (TFR1) HCAbs utilizing our fully human heavy-chain-only antibody mice (RenNano®). We immunized RenNano® mice with recombinant TFR1 proteins, isolated the B cells from spleen and lymph nodes, and performed single B cell antibody screening using the Beacon® Optofluidic system. Most of the antibodies tested were cross-reactive to human and monkey TFR1. Furthermore, even though the antigen specificity relies on the VHH domain instead of a conventional antibody variable domain, the affinity of these HCAbs can reach $10^{-8}$-$10^{-9}$ ($K_D$). Of the 7 HCAbs tested, 6 were internalized into the human brain microvascular endothelial cell line, hCMEC/D3. To assess brain penetration of these antibodies *in vivo*, mice expressing human TFR1 (hTFR1 mice) received a tail vein injection with either isotype control, positive control pabinafusp alfa (a BBB penetrating anti-TFR1 monoclonal antibody enzyme conjugate) analog or RenNano®-derived HCAbs. After 0.5, 6, 24, and 72 h of exposure, mice brains were dissected for the quantification of HCAbs and for immunohistochemical analyses. The levels of anti-TFR1 HCAbs in the parenchyma was significantly higher than isotype controls and pabinafusp alfa analog. In brain sections, HCAbs were clearly observed in the parenchyma, and were colocalized with TFR1-expressing cells. These results demonstrate that HCAbs developed from RenNano® mice are able to penetrate the BBB. Taken together, these data highlight the tremendous potential for HCAbs and its variable domain sdAbs for transporting cargo across the BBB. Due to their smaller size and simpler structure, sdAbs could ultimately provide therapeutic benefit for neurodegenerative diseases, and offer promising potential for tumor penetration.

**LB212**

**BCG022: A novel bispecific antibody-drug conjugate targeting HER3 and MET.**

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HER3 is a unique EGFR family member that plays a role in both tumor progression and drug resistance. Its expression can act as a bypass mechanism for EGFR and HER2-targeted therapies, resulting in therapeutic resistance. MET has also been reported as a bypass resistance mechanism to EGFR-TKI treatment. HER3 and MET are co-expressed at high prevalence in multiple tumor types, including gastric, colorectal, breast, and non-small-cell lung cancer (NSCLC). In addition, HER3 and MET are frequently overexpressed in liver metastases from patients with colorectal cancer, indicating that targeting both proteins may provide clinical benefit. We generated fully human bispecific antibodies (bsAbs) targeting HER3 and MET with cross-species reactivity, using RenLite® mice, which contain the full human heavy chain variable domain with a common human kappa light chain to facilitate future bispecific antibody assembly. These 1+1 bsAbs have demonstrated enhanced internalization compared to the parental monoclonal antibodies in multiple cancer cell lines. These bsAbs were then conjugated with Monomethyl auristatin E (MMAE) to generate HER3 and MET-targeting bispecific ADC (BCG022) candidates. *In vivo* drug efficacies are being screened using cell-derived hepatocellular carcinoma (HCC) and gastric carcinoma xenografts, as well as patient-derived gastric and pancreatic xenograft models. Collectively, these results suggest that BCG022 has the potential to be a novel therapeutic option for HER3 and MET co-expressing tumors.

**LB213**

**Identification of DM004, a first-in-class anti-5T4/MET bispecific antibody-drug conjugate.**

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Co., Ltd., Suzhou, China.

Aberrant MET signaling is frequently found in various types of solid tumors, and is correlated with oncogenic transformation, treatment resistance and poor prognosis. While MET remains an attractive therapeutic target, it is widely expressed on the surface of epithelial and endothelial cells, including normal tissues and tumors. To date, MET-targeting agents are associated with adverse clinical effects, including hypoalbuminemia, peripheral edema and pneumonitis, indicating that alternate treatments and/or modalities are needed. Intriguingly, MET antigen is commonly co-expressed with the oncofetal antigen 5T4 in various cancer types, including head and neck, lung and pancreatic cancer. While 5T4 is highly expressed on primary and metastatic cancers and is associated with adverse clinical outcomes in solid tumors, expression on normal adult tissues is very limited. Although several therapeutic agents targeting 5T4 antigen are currently being evaluated in human clinical studies, none have yet entered the market. To address these challenges, we hypothesized that targeting both MET and 5T4 with a bispecific antibody-drug conjugate (BsADC) could provide a more targeted therapeutic strategy to effectively eliminate tumor cells and reduce systemic toxicity. Here, we report that we have successfully generated two bispecific antibody candidates targeting both 5T4 and MET. The candidates were conjugated with monomethyl auristatin E (MMAE) via a protease-cleavable linker to generate DM004 BsADCs, i.e., Top1-MMAE and Top2-MMAE. In vitro, DM004 BsAbs demonstrated enhanced internalization in the NCI-H226 cell line compared to its parental monoclonal and monovalent anti-5T4 and anti-MET antibodies. In vivo, DM004 BsADCs exhibited robust anti-tumor activity in cell line-derived and patient-derived xenografts of gastric cancer and lung cancer, respectively. In particular, DM004 Top2-MMAE outperformed benchmark ADCs in lung BP0508 PDX models. In summary, we have identified a novel BsADC which may be a promising future treatment for cancers co-expressing 5T4 and MET.

**LB214**

**A first-in-class bispecific antibody-drug conjugate (DM002) targeting HER3 and the juxtamembrane domain of MUC1.**

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Despite the entry of new anti-cancer drugs into the market, there were more than 10 million deaths from cancer globally in 2021, with cancers of the lung, stomach, breast, and pancreas contributing to the most cancer-related deaths in China and the USA. Accordingly, there is an urgent need for improved therapeutic interventions. Antibody-drug conjugates (ADC) are novel drugs that exploit the specificity of a monoclonal antibody for target antigens expressed on cancer cells in order to achieve targeted delivery of a potent cytotoxic payload. More recently, bispecific ADCs (BsADC) targeting two tumor-associated antigens (TAA) have been developed to further amplify tumor cell specificity while minimizing toxicity to normal tissue, thus allowing for broader therapeutic windows. HER3 and MUC1 are two TAAs that are commonly expressed/co-expressed on multiple tumor types, including lung, gastric, breast and pancreatic cancer; however, neither antigen has been successfully targeted by effective drugs: of the candidates targeting HER3 antigen being evaluated in human clinical studies, most are well-tolerated, but with limited efficacy. On the other hand, high levels of MUC1 in diseased tissues can undergo auto-proteolysis, so that drugs targeting the MUC1-N region will be neutralized before reaching the tumor tissues, thereby limiting the recognition of tumor cells. To overcome these challenges, we generated fully human bispecific antibody candidates with human-monkey cross-species reactivity that target HER3 and the juxtamembrane domain of MUC1. These bsAbs exhibit higher endocytic activity in HER3-low tumor cell lines compared with other currently available HER3 mAb. These bsAbs were subsequently conjugated with monomethyl auristatin E (MMAE) via a protease-cleavable linker to obtain first-in-class BsADC candidates, DM002. DM002 candidates showed robust anti-tumor activity in multiple CDX and

PDX models of lung, breast, gastric and pancreatic cancer; most notably, DM002 candidates outperformed benchmark ADCs in BP0508 lung PDX models. Together, these data indicate that DM002 will be a promising therapeutic drug for patients with HER3 and MUC1 co-expressing tumors.

**LB215**

**A first-in-class anti-TROP2/EGFR bispecific antibody-drug conjugate, DM001, exhibits potent anti-tumor efficacy.**

Zhuolin Li,[1] Chengzhang Shang,[1] Xuewa Guan,[2] Gao An,[1] Yuming Guo,[1] Ellen Zhang,[1] Qingcong Lin,[1] Yi Yang[1]. [1]Biocytogen Pharmaceuticals (Beijing) Co., Ltd., Beijing, China; [2]Doma Pharmaceutical (Suzhou) Co., Ltd., Suzhou, China.

EGFR is a well-established target for the treatment of many cancers. However, limitations encountered with current therapies, such as drug resistance and low cytotoxicity, indicate a need for alternative treatments. In particular, antibody-drug conjugates (ADCs) are a promising new therapeutic strategy, due to their potent killing effects and high target specificity. However, the toxicity of the payload can often cause safety concerns with ADCs, so their efficacy and safety must be carefully evaluated. With these challenges in mind, we hypothesized that development of a bispecific ADC (BsADC) targeting EGFR and a second tumor-associated antigen could help to improve the tumor selectivity of the ADC, thereby limiting the occurrence of on-target off-tumor effects. TROP2 and EGFR are co-expressed in multiple types of solid tumors, including head and neck, esophageal, lung, and pancreatic cancers, indicating that this target combination could provide therapeutic benefit for a wide range of tumors. Herein, we developed a novel bispecific ADC, DM001, targeting TROP2 and EGFR, conjugated with monomethyl auristatin E (MMAE) via a protease-cleavable linker. *In vitro*, internalization of DM001 bsAb into a $TROP2^{+}EGFR^{+}$ cell line is comparable with that of its parental monoclonal anti-TROP2 or anti-EGFR antibodies. Tumor killing of double positive cell lines is also comparable between DM001 and its parental ADCs. Compared with single positive cells, DM001 can selectively bind and better kill double positive cells. Mechanistically, DM001 delays progression of the cell cycle and increases the frequency of apoptosis *in vitro* in an antigen-dependent manner. Pharmacokinetic analyses in mice with humanized FcRn (B-hFcRn) demonstrated a similar half-life of DM001 to isotype controls. Importantly, DM001 demonstrated strong anti-tumor activity in several cell line-derived and patient-derived xenografts, including lung and pancreatic tumors. Notably, the efficacy of DM001 was superior to benchmark ADCs in A431 and Panc.02.03 xenografts. Interestingly, the efficacy of DM001 was superior to its parental ADCs in BP0508 lung cancer and BP0209 pancreatic cancer PDX models, but not obvious in Panc.02.03 CDX models, indicating that DM001 may effectively target heterogeneous tumors, which better mimic the tumor microenvironment in patients. In summary, DM001 is a novel bispecific ADC with promising therapeutic potential that can be further exploited to treat TROP2 and EGFR co-expressing tumors.

**LB216**

**Discovery of BCG033, a novel anti-PTK7 x TROP2 bispecific antibody-drug conjugate with promising efficacy against triple-negative breast cancer.**

Sufei Yao, Chengzhang Shang, Gao An, Ellen Zhang, Qingcong Lin, Yi Yang. Biocytogen Pharmaceuticals (Beijing) Co., Ltd., Beijing, China.

Triple-negative breast cancer (TNBC) accounts for 15-20% of metastatic breast cancer incidence, and remains an area of unmet clinical need due to the low rates of overall survival. Recently, the TROP2-targeting ADC sacituzumab govitecan has received an accelerated approval from the FDA for adult patients with metastatic TNBC, as more than 85% of TNBC is marked by TROP2 overexpression. However, the clinical efficacy of ADC therapies targeting TROP2 alone is limited by its on-target toxicity. In an effort to offer therapeutic alternatives that limit this toxicity, we sought to identify other targets to

combat metastatic TNBC in combination with TROP2. PTK7 is highly expressed in breast cancer; notably, PTK7 expression is higher in TNBC than non-TNBC, and is correlated with worse prognosis, tumor metastasis and TNBC progression. PTK7 has also been demonstrated to be enriched in tumor-initiating cells (TICs) in low-passage TNBC, OVCA, and NSCLC patient-derived xenografts (PDXs). We generated fully human anti-human PTK7 x TROP2 bispecific antibodies (bsAbs) from RenLite® mice, which harbor the complete human heavy chain immunoglobulin variable domain with a common human kappa light chain for subsequent bispecific antibody assembly. These bsAbs demonstrated reactivity to human, monkey, and dog antigens, and showed enhanced internalization *in vitro* compared with parental PTK7 antibodies. In addition, these bsAbs showed favorable tumor cell selectivity, as there was minimal internalization of the monovalent antibodies. These bsAbs were than conjugated with Monomethyl auristatin E (MMAE) to generate anti-PTK7 x TROP2 bispecific ADC (BCG033) candidates. BCG033 candidates showed potent anti-tumor activity in several cell line-derived xenografts including TNBC xenografts, indicating that BCG033 has strong therapeutic potential in TNBC and other PTK7/TROP2 co-expressing cancers. Patient-derived TNBC xenografts with co-expression of PTK7 and TROP2 have been screened for future *in vivo* drug efficacy screening. In summary, BCG033 has the potential to exert anti-tumor efficacy in TNBC and other solid tumors co-expressing PTK7 and TROP2.

**LB217**

**A novel bispecific LILRB4/CD3 antibody with potent killing of monocytic acute myeloid leukemia cells.**

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Background: Acute myeloid leukemia (AML) remains one of the highest unmet needs among human cancers. LILRB4 is specifically expressed on M4 myelomonocytic and M5 monocytic AML cells with a lack of expression on normal hematopoietic stem cells and progenitors, making LILRB4 an attractive target for a T-cell redirecting bispecific antibody to treat AML. Using a proprietary bispecific antibody platform, we engineered and optimized a LILRB4/CD3 bispecific antibody demonstrating potent and specific killing of monocytic AML cells in vitro and in vivo. The strong chemistry, manufacturing and control (CMC) attributes and cross-reactivity to non-human primate LILRB4 and CD3 favor this bispecific for rapid advancement to the clinic.

Experimental Procedures: Binding EC50 values for LILRB4/CD3 bispecific antibodies were determined by flow cytometry analysis. The selectivity of the anti-LILRB4 modality to other LILRA/B proteins was assessed by ELISA. The potency of T-cell mediated killing of LILRB4-expressing AML cells was determined by co-culturing human peripheral T cells and monocytic AML THP-1 cells. A human peripheral mononuclear cell (PBMC) assay evaluated potency against LILRB4-positive primary cells (CD14+ monocytes), using CD19+ B cells as a negative control. Tumor bioluminescence kinetics was performed in NSG mice administrated intravenously with human T cells and THP-1-luciferase cells to measure T cell-directed killing of AML cells in vivo. Single intravenous dose of 5 mg/kg was used for PK evaluation in human FcRn transgenic mice.

Results: Two top candidates were selected using biophysical criteria: 1) A monovalent anti-LILRB4 arm together with a monovalent anti-CD3 (1+1); 2) A bivalent anti-LILRB4 arm together with a monovalent anti-CD3 (2+1). The binding EC50 values of the 2+1 or the 1+1 variants to LILRB4 in the THP-1 cells were 0.7 nM and 1.3 nM, respectively. ELISA showed the anti-LILRB4 arm to be highly selective for LILRB4. Both 2+1 and 1+1 variants demonstrated potent killing of the THP-1 cells with EC50 values of 0.5 pM and 4.3 pM, respectively. In the PBMC assay, both variants induced killing of monocytes while sparing B cells, supporting the specificity in inducing T-cell directed killing of the LILRB4-expressing primary cells. The anti-CD3 arm was designed to have a low affinity for CD3 to mitigate the risk of cytokine release while ensuring sufficient T cell activation. Both variants showed potent tumor-growth

inhibition at doses as low as 0.2 mg/kg in the NSG mice with the 2+1 molecule showing superiority in vivo. The PK profile of the 2+1 bispecific in human FcRn-transgenic mice was similar to what is expected of human IgG1 with linear clearance.
Conclusions: A novel bispecific LILRB4/CD3 (2+1) has been identified for development to treat AML based on its potency and specificity in killing monocytic AML cells in both in vitro and in vivo preclinical studies.

**LB218**
**Developing a bispecific anti-ROR1 antibody drug conjugate for hematological and solid tumor treatment.**
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Developing a bispecific anti-ROR1 Antibody Drug Conjugate for hematological and solid tumor treatment
Receptor tyrosine kinase ROR1 is a type I transmembrane protein belongs to the ROR family members. ROR1 is a receptor for Wnt family signaling molecules Wnt5a and is a key regulator of normal cellular process, including cell proliferation, survival, and migration. It is also involved in the development and progression of many types of cancer. Although being an oncofetal protein with limited expression in most of the normal tissues, ROR1 is expressed abnormally in various hematological and solid cancers, making it a highly attractive target for antibody-drug conjugate (ADC) therapy. The current clinical results of ROR1 ADC have been promising in treating patients with relapsed and/or refractory (R/R) hematologic malignancies.
Utilizing our unique and innovated linker platform, we screened many anti-ROR1 ADCs, with defined DAR=4. Those unique ADCs consist of a humanized monoclonal antibody (mAb against single epitope) or a bispecific antibody (BsAb against two epitopes), stably conjugated to an antimitotic agent. The bispecific mAbs that target to two different epitopes of ROR1, are superior to those antibodies that target to single epitope in the binding to ROR1-expressing tumor cells, the induction of tumor cell death and anti-tumor immunity. Our novel linker structure prevents payloads from coming off of the antibody during the circulation, significantly reduced the off-target toxicity. *In vitro* and *In vivo* studies demonstrated the antitumor activity of anti-ROR1 ADCs outperformed the lead anti-ROR1 ADC currently in phase II/Ill trial, providing a promising treatment for hematological and solid cancers with a better safety profile and a larger therapeutic window. The lead candidate molecule, BR111A will start the preclinical studies soon.

**LB219**
**Inducing significant and efficient tumor growth inhibition vs trastuzumab deruxtecan with low drug-load topoisomerase 1 inhibitor ADC using novel peptide linkers for payload conjugation.**
Isabella Attinger-Toller, Rachael Fay, Romain Bertrand, Philipp Probst, Ramona Stark, Roger Santimaria, Dragan Grabulovski, Bernd Schlereth, Philipp Rene Spycher. Araris Biotech AG, Au, Switzerland.

The Araris' site-specific and one-step linker conjugation technology aims at generating stable, safe and highly potent ADCs without the need for antibody engineering prior to payload conjugation. Here, we generated an anti-HER2 ADC using a Topoisomerase 1 (Topo1) inhibitor as payload with highly favorable biophysical properties and superior anti-tumor efficacy compared to Trastuzumab deruxtecan in head-to-head in vitro and in vivo studies. Based on trastuzumab as the targeting antibody and a Topoisimerase 1 inhibitor as payload, we generated highly homogeneous and pure ADCs with a drug-to antibody-ratio (DAR) of 2. In in-vitro assays on target positive cell-lines, the Araris Topo 1 ADC demonstrated potent cell-cytotoxicity in the low nM-range similar to the approved Trastuzumab

deruxtecan which has a DAR of 8. Moreover, the ADC showed excellent stability in mouse, cynomolgus and human sera exemplified by the absence of payload deconjugation or linker cleavage while Trastuzumab deruxtecan showed significant payload loss during the 14d incubation period. Interestingly, despite the improved stability, the kinetics for payload release was highly efficient in human Cathepsin B or human liver-lysosome (HLL) enzyme cleavage assays. Most importantly, the ADC was extremely stable in circulation as shown in pharmacokinetic studies in rodents, demonstrating an exposure profile similar to the unmodified trastuzumab parent antibody. In efficacy studies using an established NCI-N87 colon cancer model (therapeutic setting), a single injection of the Araris Topo 1 ADC at DAR2 at a dose of 52ug/kg (adjusted payload dose) induced superior anti-tumor activity compared to Trastuzumab deruxtecan at DAR of 8, injected at the same payload dose. Complete tumor regression of all tumors (7/7) was obtained at 104ug/kg payload dose and lasted throughout the whole study duration (total 80 days) and was very well tolerated. The data show that Araris Topo 1 ADCs assembled using novel peptide linkers, even at a DAR of as low as 2 have a very efficient anti-tumor activity suggesting optimal drug exposure, targeting and release of the payload. In summary, we show that the Araris Topo1 linker-payloads result in highly potent ADCs with very favorable biophysical properties and extremely efficient payload release as well as an antibody-like exposure profile making them ideal linker-payloads for solid tumor targeting. We anticipate the low-drug load to be favorable in avoiding excessive toxicities in non-targeted tissues. Finally, the Araris bioconjugation technology allows for the generation of tailor-made ADC candidates with improved therapeutic indices.

**LB220**

**S095029: a novel clinical-stage Fc-silenced NKG2A-blocking antibody with best-in-class potential.**

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NKG2A and its ligand HLA-E are increasingly recognized as an important immune checkpoint restraining multiple lymphocyte populations involved in cancer immunity. Initially described as an NK cell inhibitory receptor, NKG2A's role in regulating T-cell functions has recently come to the fore and is increasingly viewed as a key component of its immune suppressive functions. In addition, clinical studies have also provided proof-of-concept evidence for the superiority of dual NKG2A/PD-L1 blockade over anti-PD-L1 monotherapy. As of now clinical safety and efficacy results are only available for a single NKG2A-blocking antibody. This is in contrast with other immune checkpoint receptors (e.g., PD-1) where multiple blocking agents with different pharmacological properties have been assessed in the clinic, thereby providing a broad assessment of the target therapeutic potential and benefit to patients. Here, we report the generation and preclinical characterization of a novel monoclonal antibody (mAb) disrupting the NKG2A/HLA-E interaction. The Fc-attenuated IgG1 mAb S095029 binds NKG2A with a nM-range affinity and reverses the inhibitory effects of the NKG2A/HLA-E interaction in several experimental models, both *in vitro* and *in vivo*. As a single agent S095029 enhanced the killing activity and cytokine secretion of NK and γδ T-cells in co-culture with cancer cell lines of multiple tissue origins. We also show that S095029 increased the antibody-dependent cellular cytotoxicity (ADCC) mediated by Fc-competent mAbs in different antigen systems with high HLA-E expression. Of note, these immune-activating properties of S095029 compared favorably in benchmarking experiments with other clinical-stage anti-NKG2A mAbs. Finally, S095029 combination with PD-1 blocking agents, with or without an additional ADCC mAb component, conferred superior anti-tumor activity compared to treatments without NKG2A blockade.

Overall, our *in vitro* and *in vivo* data support the clinical development of S095029 in tumor settings with adequate HLA-E expression and suggest potential differentiating characteristics compared to other anti-NKG2A mAbs. S095029 is currently being evaluated in phase 1 dose escalation studies as a single agent or in combination with anti-PD-1 therapy (NCT05162755).

**LB221**

**Novel peptide linker-based nectin-4 targeting ADC shows improved tolerability with long-lasting anti-tumor efficacy at low doses.**

Isabella Attinger-Toller, Philipp Probst, Romain Bertrand, Emma Renard, Ramona Stark, Roger Santimaria, Dragan Grabulovski, Bernd Schlereth, Philipp Rene Spycher. Araris Biotech AG, Au, Switzerland.

The Araris site-specific and one-step peptide linker conjugation technology generates stable, safe and highly potent ADCs without the need for antibody engineering prior to payload conjugation. We generated an anti-Nectin-4 ADC that shows superior anti-tumor activity and tolerability compared to enfortumab-vedotin (EV) in head-to-head in vitro and in vivo studies. The Araris ADC is based on enfortumab as the targeting antibody and monomethyl auristatin E (MMAE) as payload. Using a peptide linker and site-specific enzymatic conjugation approach, we generated a pure ADC with a drug-to-antibody-ratio (DAR) of approximately 2 and above 98 percent monomeric content. The Araris ADC demonstrated potent cell cytotoxicity similar to the approved enfortumab-vedotin which has a DAR of 4, excellent stability in mouse, cynomolgus and human sera exemplified by the absence of payload deconjugation or linker cleavage while EV showed significant payload deconjugation. Despite high stability, the Araris ADC releases the free active MMAE metabolite at comparable rate to EV in human Cathepsin B or human liver-lysosome (HLL) enzyme cleavage assays. The ADC was also shown to be extremely stable in circulation in pharmacokinetic studies in rodents, leading to an intact ADC exposure profile comparable to the unmodified enfortumab parent antibody. No free payload was detectable in circulation during the 3 week study by LCMS-MRM. In efficacy studies using a SUM-190PT established breast cancer model, a single injection at a dose of 10 ug/kg normalized by payload induced a complete tumor regression lasting for more than 100 days (i.e. a very durable response or tumor eradication). EV administered at the same payload dose showed only a short and transient (until day 20 only) tumor regression with no animal (0/6) reaching a complete response. Despite the higher in vivo exposure and extremely efficient anti-tumor response at low payload doses, there was no increased toxicity but in contrast, overall tolerability was improved, i.e., less neutropenia, skin involvement and signs of toxicity - the skin toxicity being the dose-limiting toxicity of Enfortumab vedotin in humans and rats. Overall, the highest non-severely toxic dose (HNSTD) in 4-week repeat dose rat toxicity studies for the Araris ADC (25 mg/kg) was 5-fold higher compared to the HNSTD (5mg/kg) reported for Enfortumab vedotin. Our data impressively show that the Araris ADC has superior efficacy and durable anti-tumor response even at 3-fold lower payload dose compared to EV. The improved efficacy in mice and tolerability in rates resulted in a 8-fold better TI for the Araris ADC and offers the opportunity to develop a highly efficacious ADC having potentially lower dose-limiting toxicities such as peripheral neuropathy, rashes or neutropenia.

**LB222**

**An mRNA-based cancer vaccine multi-targeting KRAS mutations inhibits tumor growth by increasing immune response in KRAS mutant LL/2 mouse model.**

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Kirsten rat sarcoma viral oncogene (KRAS) mutations are present in 26% of non-small cell lung cancer (NSCLC) patients (The Cancer Genome Atlas data). Studies have reported the presence of spatial or intra-tumoral heterogeneity of KRAS mutations. Though resistance to mutant KRAS inhibitors targeting a specific form of major KRAS mutations (e.g, G12C or G12D) is multifaceted, intra-tumoral heterogeneity of KRAS mutation is one of the crucial factors for the intrinsic resistance to the inhibitor. Multi-targeting of different KRAS mutations can prevent resistance due to the heterogeneity of KRAS mutations within a

tumor. To achieve this, we incorporated an mRNA-based cancer vaccine containing transcripts for multiple KRAS mutant antigens. Our KRAS cancer vaccine (CV) candidate showed significantly attenuated tumor growth by 37% in the syngeneic KRAS G12C-expressing LL/2 tumor-bearing mice. CV treatment group showed significantly decreased tumor size by 45.4% after biopsy. Moreover, the mice treated with KRAS CV showed spleen enlarged by 42.6% indicating increased immune responses. To confirm, the expansion of T cells in the cancer vaccine-treated group, we collected tumor tissues and analyzed proportions of CD4+ and CD8+ T lymphocytes in the tumor. We found a 14.5-fold increase in infiltration of CD8+ T cells and a 0.5-fold increase in CD8+ CD44+ memory/effector T cells in the tumors from mice treated with KRAS CV. Whereas CD4+ Foxp3+ Treg cells were decreased by 3.9-fold in the tumor. HLA-A*02 is the most common MHC class I allele. To investigate the changes in MHC expression, we conducted FACS analysis using HLA-A*02 antibodies after ex vivo treatment of KRAS CV on the human peripheral blood mononuclear cells (hPBMCs). KRAS CV treatment facilitated higher proportions of HLA-A*02+ monocytes (CD14+) and B cells (CD19+). Though LL/2 tumor model has been considered an immunosuppressive model, our results suggest that the KRAS CV can significantly enhance immune responses, thereby suppressing tumor growth.

**LB223**

**Intratumoral administration of modified vaccinia Ankara expressing a tumor associated antigen and the costimulatory molecules IL-12 and 4-1BBL induces potent systemic long-term immune responses against murine tumors.**

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In the last decade, immunotherapy has revolutionized cancer treatment. However, the lack of potent therapy-induced immune responses against solid tumors due to the immune-suppressive tumor microenvironment (TME) is still a major drawback. One approach to reprogram TME is virotherapy, which can target tumors via destruction of infected tumor cells as well as activating immune cell populations in the TME. A versatile and safe tool for virotherapy is modified vaccinia virus Ankara (MVA), which has already been approved by the FDA as a non-replicating vaccine against smallpox and monkeypox and is also in clinical trials against other infectious diseases and cancer. In the current study, we deciphered the anti-tumorigenic effect of a novel MVA vector encoding a tumor associated antigen (TAA) and the costimulatory molecules IL-12 and 4-1BBL, called MVA-TAA-4-1BBL-IL12. We could show that repetitive intratumoral (IT) injection of MVA-TAA-4-1BBL-IL12 resulted in strong tumor growth control, shrinkage and/or complete elimination of tumors and induction of tumor-specific CD8 T cell response. Furthermore, MVA-TAA-4-1BBL-IL12 treatment promoted a systemic anti-tumor immune response not only against the treated tumor but also untreated, distant tumors. Importantly, local virotherapy also resulted in the generation of tumor-specific memory response, which protected mice against local recurrence after rechallenge. As a conclusion, our findings show that MVA-TAA-4-1BBL-IL12 IT treatment induces an effective anti-tumorigenic immune response in different solid tumor models, which gives rise to long-term protection against cancer recurrence.

**LB224**

**STC-1010 a new therapeutic vaccine promotes tumor cell death.**

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**BACKGROUND** Colorectal cancer (CRC) is the third most commonly diagnosed cancer (10% of new

cases in 2020) and the second leading cause of cancer-related death (9.4%) worldwide. CRC is often detected at late stages, with 38% of metastasis cases at diagnosis. This makes the development of efficient treatments crucial. Recent tremendous progress sets immunotherapy as a treatment option and opens multiple ways to address therapeutic challenges in CRC. Accordingly, we have previously developed therapeutic cancer vaccine based on stimulated tumor cells (STC) technology, that have already shown efficacy in immunocompetent mouse models. Same strategy was used in this study to generate the STC-1010 therapeutic vaccine, a novel human therapeutic CRC vaccine derivate from 3 human CRC cell lines, HCT116, HT29 and LOVO exposed to irradiation, heat shock, chemotherapy agents and haptenized.
**METHODS** We evaluate STC-1010 for its potential to favor an immune stimulatory response on human monocytes-derived dendritic cells (moDCs) from 2 different donors through i) a derived Mixed Lymphocyte Reaction (dMLR) assay, ii) the assessment of their cytokine profile, iii) the evaluation of their capacity to activate autologous CD8+ T cells and finally iv) to promote apoptosis of three CRC cell lines (HCT116, HT29 and LS-174T ).
**RESULTS** We showed that STC-1010 induced IL-8 and IL-12 production, and reduced IL-10 during moDCs maturation. In addition, dendritic cells (DCs) exposed to STC-1010 during the maturation enhanced Interferon gamma (IFNg) production by CD8+ T cells (p=0,06 for donor 1 and p=0,0004 for donor 2) and amplified their anti-tumor activity, in combination with LPS/IFNg and CD40L. CD8+ T cells primed with STC-1010-treated DCs promote massive apoptosis of HCT116 and HT29 cells compared to the condition without STC-1010 (p<0,001 for all donors). The benefit of STC-1010 was not detectable with LS174T cells characterized by a low expression of HLA-ABC and used as negative control in this study.
**CONCLUSION** Taken together, our results showed that Brenus STC-1010 human vaccine is an efficient strategy to educate immune system and promote the CRC cells death *in vitro* lending further support in favor of STC technology based on physical or chemical stimulation and haptenization to generate human cancer therapeutic vaccine.

**LB226**
**FGFR2 modulates ALK inhibition response in high-risk neuroblastoma.**
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High-risk neuroblastoma (NB) has a poor prognosis despite multimodal treatment. To improve survival and minimise treatment side-effects, research has focused on developing more effective therapeutic strategies. Anaplastic lymphoma kinase (ALK) is a promising druggable target as its expression rapidly decreases in healthy tissues postnatally, and it is expressed as a hyperactivated mutant form in ~14% of high-risk NB. The ALK tyrosine kinase inhibitor lorlatinib is a promising treatment, but resistance has been reported, hindering long-term benefits. With a trimodal approach, consisting of genome-wide CRISPR-Cas9-overexpression (CRISPRa) screens, RNA sequencing and high-throughput drug screens, we have identified genes whose expression is associated with decreased sensitivity to lorlatinib creating novel therapeutic vulnerabilities. In particular, we validate the CRISPRa screen hit *FGFR2* as a bypass signalling mechanism desensitising mutant ALK-expressing NB cells to lorlatinib; overexpression of *FGFR2* increased, while silencing decreased resistance of NB cells to lorlatinib. Furthermore, RNA sequencing of lorlatinib resistant (LR) NB cells developed in our lab, compared to parental cell lines, showed FGFR2 to be expressed to a higher level in the LR cells. High-throughput drug screens exposing LR and parental NB cell lines to an FDA-approved drug library of 1430 compounds showed that drugs targeting receptor tyrosine kinases, including FGFR2, were amongst the compounds most significantly effective in reducing the viability of LR NB cells compared to parental cells. The multi-kinase inhibitor ponatinib and the selective FGFR inhibitor erdafitinib acted synergistically with lorlatinib in treating both

parental and LR NB cells although both inhibitors were more effective in inhibiting the growth of LR compared to parental cells when given alone, suggesting that FGFR2 may represent a novel vulnerability to treat lorlatinib resistant NB. *In vivo* studies using patient-derived xenograft (PDX) models of high-risk NB (*MYCN*-amplified and $ALK^{F1174L}$ mutant) showed that combinations of either ponatinib or erdafitinib with lorlatinib decreased tumour growth and increased survival compared to PDXs treated with vehicle or either agent alone. In conclusion, these findings suggest that FGFR2 alters NB sensitivity to lorlatinib and modulation of this pathway in combination with ALK inhibition is a promising approach to improve NB treatment response and ultimately patient survival.

**LB227**

**Leveraging novel Dato-DXd resistance models to inform biomarker discovery and rational combinations to combat drug resistance.**

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Datopotamab deruxtecan (Dato-DXd) is an antibody-drug conjugate (ADC) consisting of a humanized anti-TROP2 IgG1 monoclonal antibody covalently linked to a highly potent topoisomerase I (TOP1) inhibitor payload via a stable, tumor-selective, tetrapeptide-based cleavable linker. Despite its promising early clinical signals, drug resistance is a challenge that may emerge with time. The mechanisms of Dato-DXd resistance are currently unknown. Herein, we created and investigated novel models of Dato-DXd resistance to characterize resistance mechanisms and identify novel combinations to overcome Dato-DXd resistance. We used a cyclical dosing method to develop two Dato-DXd acquired resistant NCI-N87 gastric cancer cell line models from *in vitro* cells (N87-IVR) and *in vivo* tumors (N87-EVR). The N87-IVR and N87-EVR cells were found to be more than 40-fold resistant to Dato-DXd compared to the parental N87 cells. First, we assessed TROP2 levels, Dato-DXd binding and expression of drug efflux pumps known to have TOP1-inhibitor substrates between the parental and resistant cell populations. Minor changes in each of these were not sufficient to drive Dato-DXd resistance in the drug-tolerant models. Subsequently, we performed a proteogenomic analysis to identify which proteins and pathways were modulated in the resistant phenotype. Both the N87-IVR and N87-EVR cells were divergent from the parental N87 cells and, interestingly, they showed similar yet distinct profiles. Among other changes, our analysis revealed a dramatic loss of SLFN11, a putative DNA/RNA helicase, in both resistant models. Previously published data has shown that a combination with an ATR inhibitor (ATRi) can re-sensitize TOP1 inhibitor-resistant cells in SLFN11-low settings. Consistent with these data, we observed that the ATRi ceralasertib (AZD6738) in combination with Dato-DXd re-sensitizes the resistant NCI-N87 cells *in vitro* and *in vivo*. In addition to SLFN11 loss, we also identified a number of other modulations with the potential to drive resistance, e.g. downregulation of the caveolin internalization machinery and interferon response genes. These findings help us refine the mechanism of action of Dato-DXd and shed light on the potential mechanisms of Dato-DXd resistance that may emerge clinically. These data have been used to uncover new biomarkers of Dato-DXd sensitivity and can lead to the development of new clinical strategies to combat Dato-DXd resistance, e.g. the combination with ATRi.

**LB228**

**Replication stress activates the DNA damage response and contributes to lapatinib resistance in HER2-positive SK-BR-3 cells.**

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**Background:** Lapatinib is a small molecular inhibitor of HER2 and EGFR tyrosine kinases, which is approved for HER2-positive metastatic breast cancer as second line treatment. Significant proportion of patients experience disease progression due to acquired resistance. Activation of DNA damage repair (DDR) is one of the drug-resistance mechanism, however, the impact of DDR on sensitivity to lapatinib is unclear. Thus, lapatinib resistance mechanism was explored from the standpoint of DDR activation.
**Methods:** Acquired lapatinib-resistant (LR) SK-BR-3 cell lines were generated by continuously exposing to lapatinib, starting with 30 nmol/L and incrementally increasing to 10 μmol/L over 7 months. MTT assay was used to confirm lapatinib sensitivity. Cell cycle progression was analyzed using BrdU assay. Replication fork speed and stalled fork were analyzed by DNA fiber assay. Expression of the molecules was examined using Western blotting, immunofluorescence assay and transcriptome data analysis. DNA strand breaks and repair efficacy were evaluated through alkaline comet assay.
**Results:** G1/S phase transition was increased and early/late S phase population was increased in SK-BR-3 LR cells. Replication fork speed was accelerated and replication stress are elevated in SK-BR-3 LR cells. p-Chk1, Rad51, Rad51B, Rad51C and XRCC3 were upregulated in SK-BR-3 LR cells. After irradiation or treatment with ATR inhibitor, γ-H2AX was continuously increased in parental cells. In contrast, γ-H2AX did not change significantly in SK-BR-3 LR cells, and Chk1 was activated after irradiation or treatment with ATR inhibitor. Tail of comet disappeared at early time point in in SK-BR-3 LR cells after induction of DNA damage compared with parental cells. These results demonstrated that DDR was activated and DNA damage repair capacity was enhanced in LR cells.
**Conclusion:** Replication stress is elevated in SK-BR-3 LR cells. Up-regulation of molecules involved in the homologous recombination repair pathway was observed in SK-BR-3 LR cells. Moreover, DNA repair capacity was increased in SK-BR-3 LR cells. These data suggested that activation of DNA damage repair pathway caused by replication stress contributes to lapatinib resistance**.**

**LB229**

## Tinengotinib, a novel fibroblast growth factor receptor (FGFR) inhibitor, is potent against resistance mutations in FGFR1/2/3.

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Dysregulated FGFR alterations (FGFR*alt)*, including gene amplification, hot spot mutations, and gene fusion or rearrangement, have been reported in 7% of solid tumors, functioning as oncogenic drivers independent of tumor types. First generation of FGFR inhibitors, such as erdafitinib and pemigatinib, have already demonstrated promising efficacy on multiple cancers with prespecified FGFR*alt*. However, certain kinase mutations identified in the clinics, including gatekeeper mutations and some molecular brake mutations, may cause resistance to first generation FGFR inhibitors and remain challenging for current FGFR inhibitor development. Tinengotinib potently inhibited multiple FGFR1/2/3 resistant mutations both *in vitro* and *in vivo*. In the biochemical and cellular assays, tinengotinib exhibited potent inhibitory activities against FGFR2 gatekeeper mutation V564F, and molecular brake mutation N549K, which were known to cause resistance to the first generation FGFR inhibitors in cholangiocarcinoma patients with FGFR2 fusion or rearrangement. Binding kinetics was measured using surface plasmon resonance (SPR) on FGFR2, and tinengotinib demonstrated very tight binding to the target. To understand the underlying mechanism, we conducted co-crystallization of tinengotinib and wild type kinase domain of FGFR2. The resulting high-resolution X-ray diffraction structure revealed a very unique binding mode of tinengotinib in the ATP-binding site of FGFR2 comparing to other FGFR inhibitors. In addition, homology model demonstrated similar binding mode of tinengotinib to FGFR 1 and 3. This is consistent with the finding of tinengotinib showing excellent potency to FGFR 1/3 gatekeeper mutations. Tinengotinib is well tolerated in the clinical trials. Retrospective analysis showed early promising efficacy

in treating patients with various solid tumors bearing FGFR*alt* . It provides an opportunity to investigate tinengotinib as a tumor agnostic therapy in patients with prespecified FGFR 1/2/3 alterations (pan-FGFR*alt*).

**LB230**
**Critical role for DAXX in triple negative breast cancer.**
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Breast cancer is the most common diagnosed cancer and second cause of mortality in women. In particular, triple negative breast cancer, a subtype with few targeted therapies, frequently presents with metastatic disease and drug resistance. Nearly half of women with triple negative breast cancer will have metastatic disease. New targets and therapies are needed to prevent drug resistance and metastatic disease. We identified a novel protein, DAXX, in a biomarker window trial as a potent inhibitor of breast cancer stem cells (BCSC). BCSC are responsible for drug resistance, metastases, and tumor relapse. In this current study, the role of DAXX in sensitivity to cytotoxic chemotherapy was investigated in the setting of triple negative breast cancer. DAXX depletion using RNAi increased the proliferative capacity of three triple negative breast cancer cell lines (MDA-MB-231, BT549, and MDA-MB-468). This corresponded with increased PARylation of cellular proteins and PARP-1. DAXX was required to limit cell cycle progression through S-phase. Knockdown of DAXX modulated sensitivity to carboplatin, doxorubicin, and paclitaxel *in vitro*. Survival of female nude mice-bearing MDA-MB-231 DAXX-depleted tumors was 100% upon paclitaxel treatment compared to vehicle control-treated mice where the survival rate was 0% after 30 days. This survival advantage was not due to differences in tumor burden or tumor growth rates. These results suggest that DAXX is a critical growth regulator of triple negative breast cancer and possible predictor of response to paclitaxel.

**LB231**
**Overcoming KRAS G12C inhibitor resistance with a chaperone-mediated protein degrader (CHAMP).**
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KRAS is the most frequently mutated oncogene in cancer and is particularly common in lung, pancreatic and colorectal cancers. While KRAS has been undruggable, covalent KRAS G12C inhibitors, sotorasib and adagrasib, that bind preferentially to GDP-bound KRAS and prevent exchange for GTP and interaction with downstream effectors, have been approved for use in NSCLC. While these reagents show promising efficacy and are well-tolerated by patients, acquired drug resistance rapidly develops. The drug resistance landscape of G12C inhibitors is complex, with several mechanisms of resistance already described, ranging from reactivation of mutated RAS or other RAS isoforms, acquisition of new KRAS mutations, and genetic alterations of pro-growth signaling pathways. Thus, an ideal approach to overcoming drug resistance will need to target multiple different mechanisms. We exposed NSCLC and pancreatic cell lines (NCI-H358, NCI-H1373 and MIA PaCa-2, respectively) to increasing doses of sotorasib or adagrasib until the resulting cells grow normally in the presence of 10 μM of each inhibitor. Sotorasib-resistant cells, designated as S-R, remarkably remain sensitive to adagrasib, suggesting that the resistant cells continue to be dependent on mutant KRAS for survival. In contrast, adagrasib-resistant cells, designated as A-R, are resistant to both inhibitors. Treatment of innate and acquired adagrasib-resistant cells with 500 nM adagrasib attenuated RAS activity; however, this is compensated for by

increased KRAS, HRAS and NRAS mRNA and restored p-ERK levels, a surrogate marker of RAS activity. Moreover, analysis of RTK activation in drug-resistant cells reveals that adagrasib treatment induces the activity of specific RTKs in a cell-line specific manner. Interestingly, during the acquisition of adagrasib resistance, several RTKs are strongly induced to escape drug inhibition. These results indicate the need to simultaneously reduce both KRAS G12C and compensating RTK activity to effectively treat KRAS G12C NSCLC. The majority of oncogenic RTKs are known to be dependent on the molecular chaperone HSP90 for their proper folding and stability. Therefore, a heterobifunctional chaperone-mediated protein degrader (CHAMP), RNK07421, was designed to specifically target both KRAS G12C and HSP90. RNK07421 induces an artificial ternary complex with KRAS G12C and HSP90, while also inhibiting the activity of both proteins and promoting proteasomal degradation of KRAS G12C via HSP90-associated E3-ligases. Treatment of adagrasib-resistant NSCLC cells and PDOs with RNK07421 resulted in decreased levels of KRAS G12C, p-ERK and RTKs. Furthermore, *in vivo* treatment with RNK07421 demonstrated dramatic tumor growth inhibition as compared to adagrasib treatment alone. Together, these observations indicate that the novel mechanisms of action of RNK07421 may provide several advantages over G12C inhibitors and possibly other targeted protein degradation agents to effectively treat KRAS G12C-dependent NSCLC.

**LB232**

**Targeting MEK activity in pancreatic tumors resistant to the combination of 5FU, irinotecan, and oxaliplatin promotes response to immunotherapy.**

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FOLFIRINOX, a chemotherapy regimen consisting of 5FU, Leucovorin, Irinotecan, and Oxaliplatin, has been a first-line standard of care for patients with pancreatic adenocarcinoma (PDAC) for the last decade. However, low patient survival rates following treatment highlight frequent occurrence of resistance. Hence there is increasing interest in developing appropriate models of FOLFIRINOX resistance to identify subsequent line therapies. We have generated PDAC cell lines that exhibit acquired resistance to a combination of 5FU (F), Irinotecan (I), and Oxaliplatin (O) (FIO) in vitro. In mice, they form tumors that resemble FOLFIRINOX neoadjuvant-treated PDAC patients in regard to immune cell infiltration and a lack of response to single-agent anti-PD-1 therapy. We also show that similar to human PDAC tumors treated with neoadjuvant FOLFIRINOX, FIO-resistant tumors exhibit increased ERK1/2 phosphorylation. We demonstrate that the MEK inhibitor Trametinib reprograms the FIO-resistant tumors, but not the parental tumors, towards a less immunosuppressive tumor immune microenvironment. Importantly, Trametinib sensitizes FIO-resistant tumors to anti-PD-1 therapy, with the combination treatment significantly enhancing the cytolytic activity of the infiltrating $CD8^+$ T cells and increasing tumor cell death. Taken together, our findings identify enhanced MEK/ERK signaling as a therapeutic target in FOLFIRINOX-resistant PDAC tumors, and suggest evaluating the combination of Trametinib and anti-PD-1 therapy in patients who progress on FOLFIRINOX.

**LB233**

**Preclinical analysis identifies predictive biomarker and potential pathways of resistance to lurbinectedin treatment in small cell lung cancer.**

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**Background:** Small cell lung cancer (SCLC) is an exceptionally aggressive disease with limited treatment options that typically result in transient responses. SCLC is responsible for approximately 250,000 deaths globally per year. Major hurdles to improving SCLC treatment include the development of rapid chemo-resistance and limited second-line therapies. Lurbinectedin is FDA approved as a second-line treatment for SCLC but shows a response in a subset of patients. Therefore, improved mechanistic understanding and identifying predictive biomarkers of lurbinectedin treatment is a major unmet clinical need.
**Methods:** We treated SCLC cell lines and patient-derived xenograft (PDX) models from all major SCLC subtypes with lurbinectedin. Lurbinectedin-mediated changes in signaling pathways were studied by bulk RNA sequencing, western blot, and flow cytometry. Anti-tumor efficacy and toxicity studies were performed in vivo.
**Result:** All human and PDX-derived SCLC cell lines showed sensitivity to lurbinectedin at a nano-molar concentration ranging from 1.905 to 30 nM. Bulk RNA-seq analysis showed lurbinectedin induced changes in neuroendocrine phenotype, DNA damage response and tumor progression markers in vitro. Single agent treatment of lurbinectedin showed remarkable anti-tumor efficacy in an ASCL1-driven PDX model. RNA sequencing analysis identified modulation of genes in multiple signaling pathways including PI3K-AKT, apoptosis and EMT to be significantly associated with the lurbinectedin response in PDX models.
**Conclusion:** There is an immediate need to understand the subsets of SCLC that would be most sensitive to lurbinectedin and identify predictive biomarkers. We demonstrate MYC as a predictive biomarker for lurbinectedin response. We are the first to show that single agent lurbinectedin shows remarkable anti-tumor efficacy in a ASCL1-driven PDX models of SCLC. Furthermore, our pre and post-lurbinectedin treatment transcriptomic analysis identify the pathways that may contribute to primary or acquired resistance to lurbinectedin in SCLC. Finally, we identity candidate targets that would guide the design of future combination clinical trials with lurbinectedin in SCLC.

**LB234**

**Activation of the integrated stress response by the developmental HSF1 pathway inhibitor NXP800.**

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HSF1 is a stress-inducible transcription factor that regulates the eukaryotic heat shock response (HSR). HSF1 activation induces the expression of multiple proteins needed for cellular recovery from stress. HSF1 also plays a key role in tumorigenesis and regulates the expression of a cancer-specific gene signature which is unique to malignant cells and distinct from that activated during the HSR.
We discovered NXP800, the first-in-class orally active HSF1 pathway inhibitor which is now undergoing Phase 1 clinical trial, based on a phenotypic pathway screen. Here we employed various techniques to investigate the mechanism of action of NXP800. Using RNAseq in a panel of human carcinoma cell lines, we identified overlapping gene expression changes in response to NXP800. These included genes regulated by HSF1 and interestingly also genes associated with activation of the integrated stress response (ISR). In contrast, we found no evidence for activation of the unfolded protein response. Consistent with the observed NXP800-induced phosphorylation of eIF2α which is a critical regulator of the ISR, NXP800 increased the protein expression of downstream ISR markers ATF4, CHOP and CHAC1, both in human tumor cells in vitro and in human tumor xenograft models in vivo.
Induction of the ISR is controlled by four stress-activated protein kinases (PKs) that phosphorylate eIF2α. To further explore these kinases in the mechanism of action of NXP800 we used genetic knockdown by siRNA and inhibition by small-molecule tool compounds. Silencing each of the ISR-regulatory PKs revealed that GCN2 was required for ISR activation by NXP800. This was confirmed using two GCN2

inhibitors from different chemical series. Global phospho-proteome analysis showed that altered protein phosphorylation following NXP800 exposure was reversed upon co-treatment with a GCN2 inhibitor. We also demonstrated that activation of the ISR caused inhibition of HSF1 activation when stimulated with an HSF1 activator, thus confirming the link between ISR induction and inhibition of HSF1 activation. Activation of GCN2 and the ISR can occur in response to a variety of stimuli including amino acid deprivation. However, we did not detect a difference in the uptake of amino acids following exposure to NXP800, indicating that NXP800 does not directly impair amino acid uptake. Using an siRNA approach to determine if activation of the ISR components was contributing to growth inhibition following NXP800 exposure, we found that blocking the induction of ATF4 reduced the response of NXP800-sensitive SK-OV-3 human ovarian carcinoma cells to NXP800 treatment.
In summary, NXP800 acts on cancer cells to induce activation of the ISR pathway via GCN2, which then leads to inhibition of HSF1 activation. Further studies are underway to determine the precise molecular target of NXP800 and the mechanism of HSF1 pathway inhibition.

**LB235**
**Mechanistic studies on VNLG-152R-mediated tumor inhibition of triple negative breast cancer.**
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Introduction: Breast cancer remains a major concern as the second leading cause of cancer-related death in women in the United States and the most frequently diagnosed cancer in women globally. Among all the subtypes, triple negative breast cancer (TNBC) is highly resilient and eludes currently available treatment modalities. This study describes the mechanism of action of VNLG-152R in inhibiting TNBC tumor growth.
Methods: TNBC cell lines MDA-MB-231 and MDA-MB-468 originated from TNBC patients of Caucasian American and African American descend, respectively, were challenged with VNLG-152R *in vitro*. The mechanism of action was studied by immunoblotting key proteins, proteasomal degradation assay of Mnk1/2 and transcriptome analysis by RNA-seq. The therapeutic potential of VNLG-152R is demonstrated in NRG mice bearing either MDA-MB-231 or MDA-MB-468 tumor xenografts.
Results: We report for the first time, that, VNLG-152R significantly upregulates Synoviolin (SYVN1) in TNBC cell lines MDA-MB-231 and MDA-MB-468 with concomitant degradation of Mnk1/2. SYVN1 is a known E3-ubiquitin ligase and it ubiquitinates its target proteins, rendering them to proteasomal degradation. However, there was no significant change in Mnk1/2 levels upon treatment with VNLG-152R in presence of SYVN1 inhibitor LS102. A similar result is obtained upon siRNA knockdown of SYVN1. Subsequently, the decreased level of Mnk1/2 diminished the phosphorylation of eIF4E with a concurrent decrease in levels of eIF4E-validated downstream targets, Bcl-2 and Cyclin D1, resulting in tumor growth inhibition. Inhibition of proteasomal degradation by MG-132 prior to treating cells with VNLG-152R further supports that VNLG-152R promotes SYVN1-mediated ubiquitin-proteasomal degradation of Mnk1/2. Further, RNA-seq and Gene Set Enrichment Analysis (GSEA) of differentially expressed genes demonstrate inhibition of mTORC1 (mammalian target of rapamycin complex 1) and NUP153 (Nucleoporin 153) pathways and activation of p53 pathway thereby inhibiting tumor progression. Finally, oral administration of VNLG-152R to mice bearing tumor xenografts of either MDA-MB-231 or MDA-MB-468 significantly inhibited tumor growth and resulted in 87% and 80% tumor growth inhibition, respectively, without apparent host toxicity. Immunoblots of tumor tissue further demonstrate elevated levels of SYVN1 and decreased Mnk1/2 and p-eIF4E, which further confirms the role of SYVN1-mediated proteasomal degradation of Mnk1/2 in inhibiting TNBC tumor growth by VNLG-152R.
Conclusion: As VNLG-152R potently inhibits development and progression of TNBC of different origins by upregulating E3 ubiquitin ligase SYVN1 leading to ubiquitination and proteasomal degradation of

Mnk1/2 thereby decreased oncogenic phosphorylation of eIF4E by Mnk1/2, VNLG-152R could potentially be developed for the treatment of TNBC, irrespective of racial origin.

**LB236**

**ST101, a peptide antagonist of novel I/O target CEBPβ,reprograms MDSCs and promotes an immunoactive tumor microenvironment.**

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CCAAT/Enhancer Binding Protein β (C/EBPβ) is a basic leucine zipper (bZIP) transcription factor that causes aberrant gene expression in many cancers. Upregulated or overactivated C/EBPβ drives oncogenesis by promoting tumor survival and proliferation and is a critical regulator of the immunosuppressive tumor microenvironment (TME). Specifically, C/EBPβ regulates macrophage differentiation, activating a transcriptional program driving macrophage polarization toward immunosuppressive M2-type myeloid-derived suppressor cells (MDSCs). Consistently, activation of C/EBPβ correlates with poor prognosis in several types of human cancer. Thus, targeting C/EBPβ to reprogram tumor-associated macrophages (TAMs) from the M2 toward the immune-promoting M1 phenotype represents an attractive strategy to enhance antitumor immunity. ST101 is a novel peptide antagonist of C/EBPβ dimerization that inhibits C/EBPβ-dependent gene expression. Here we evaluated the impact of ST101 on macrophage differentiation, cytotoxic T-cell activation, and in vivo anti-tumor activity. Primary human macrophages cultured from Peripheral Blood Mononuclear Cells (hPBMCs) were activated toward the M1 or M2 phenotype by LPS and IFNγ or IL-4, respectively. ST101 exposure dose-dependently suppressed expression of M2 markers (CD163, CD206) while inducing M1 markers (CD80, CD86) by flow cytometry and quantitative PCR, resulting in a 40-fold increase in the M1/M2 ratio without substantial impact on cell viability. Next, in co-cultures of T cells with M2 macrophage, ST101 exposure resulted in a 4-fold increase in T-cell activation compared to control M2/T cell co-cultures, as measured by intracellular IFN-γ staining. Importantly, ST101 did not suppress proliferation or activity of T cells cultured alone. Finally, in an orthotopic TNBC model in vivo, ST101 in combination with anti-PD-1 treatment enhanced anti-tumor activity compared to either single agent alone. The observed increase in tumor growth inhibition was accompanied by a reduced TAM fraction and increased intratumoral and peripheral M1/M2 ratios. ST101 is being evaluated in a Phase 1-2 clinical study in patients with advanced unresectable and metastatic solid tumors (NCT04478279). Initial gene expression analysis performed on 8 paired patient biopsies (prior to ST101 exposure and within 24 hrs of ST101 administration during cycle 2 of therapy) collected during dose escalation (4 mg/kg ST101 or greater) indicates a significant decrease in expression of multiple factors involved in M2 polarization, including CD209, SIGLEC5 and IL-24, and T cell suppression, including FoxP3 and inhibitory KIR proteins. The result is a decrease in intratumoral regulatory T cell (Treg) vs. TIL ratio, indicating a shift towards a more immunoactive TME. Overall, these results support a novel, macrophage-driven mechanism of action for ST101 as anticancer agent and suggest the exploration of ST101 in immune-oncology therapeutic strategies.

**LB237**

**Functional characterisation of TRACERx reveals mechanisms of NSCLC evolution.**

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Chromosomal instability (CIN), the increase in the rate of whole/partial chromosome gains and losses, is a driving feature of cancer identified in ~90% of solid tumours. CIN generates intratumor heterogeneity (ITH), drives evolutionary adaptation, and is associated with highly aggressive, drug-resistant tumours and poor prognosis. Aberrations in numerous pathways have been suggested to increase CIN. However, how CIN is initiated and maintained in non-small cell lung cancer (NSCLC) is largely unknown. Here we have analysed multi-region WES sequencing data from patients of the TRACERx study, and identified novel genes involved in the DNA damage response (DDR) that may contribute to chromosomal instability. Using orthogonal methods, we have identified that genetic alteration in 6 genes, particularly FAT1, culminates in deficiencies in homologous recombination repair (HRR). This is manifested by a reduction of RAD51 and BRCA1 foci formation, reduced end-resection rate and increased 53BP1 bodies. We find that these deficiencies in the HRR pathway can lead to an increase in structural CIN and deviation from the modal cjromosome number. Furthermore, we observed an early selection of FAT1 mutations in the TRACERx421 LUSC cohort, which resulted in more genome-doubling. We reprot mirrored sub-clonal allelic imbalance (MSAI) events at the FAT1 gene locus, indicating parallel evolution. We have proceeded to validate these observations using several cell lines, showing that FAT1 loss promotes whole genome doubling (WGD) through the generation of actively replicating binucleated cells through an elevated mitotic error rate. Importantly, the elevated CIN induced by FAT1 loss could be partially ameliorated by co-depletion of the oncogene YAP1. We hypothesize that elevated CIN could synergize with WGD to generate increased intratumor heterogeneity. To investigate this, we modelled the relationship between FAT1 loss and genome doubling in an isogenic cell culture system using PC9 cells which are sensitive to the EGFR inhibitor, Osimertinib. Indeed, we observed an elevated rate of acquired resistance in FAT1 KO genome-doubled PC9 cells, which we attribute to the high CIN level generated by FAT1 loss. In line with our hypothesis, we also observed a significant increase in genome content in FAT1 KO cells, suggesting FAT1 loss induces a second WGD event to escape targeted therapy.
In conclusion, FAT1 is one of the most frequently mutated genes both in the TRACERx421 cohort and in somatic tissues (Martincorena et al, Science 2015). We postulate that FAT1 loss attenuates DDR and exacerbates CIN to enhance tumour heterogeneity, early in lung cancer evolution. Our observation that FAT1 loss leads to elevated EGFRi resistance also provides a unique opportunity to understand how EGFRi resistance arises through elevated CIN.

**LB238**
**Image-based phenotypic profiling of a chemogenomic screening library identifies nobel targets of known inhibitors.**
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The gene encoding epidermal growth factor receptor (EGFR) is a major driver gene in cancer. Many drugs targeting EGFR-associated molecules have been developed, yet many have failed in clinical trials due to a lack of efficacy and/or unexpected side effects. In this study, I used image-based phenotypic profiling to screen a pharmacologically active compound library with the aim of identifying new druggable targets in the EGFR pathway. As anticipated, the phenotypic screen identified compounds that produce phenotypes resulting from targeting a known specific molecule or pathway. The assay also showed that compounds with diverse known mechanisms of action produced similar, EGFR-related cellular phenotypes. Biochemical assays revealed that those compounds share a previously unappreciated common target/pathway, showing that the image-based assay can identify new target molecules that are independent of the compound’s known target.

**LB239**
**Downregulation of spliceosomal proteins hnRNPH1 and H2 in WM266-4 melanoma cells induces**

**immune signaling.**
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This study aims to determine the effect of hnRNPH1/H2 (H1/H2) downregulation on immune-related genes *in vitro*. Due to the molecular heterogeneity of melanoma, it is difficult to treat it, which necessitates new approaches. We discovered compounds that selectively kill melanoma cells by binding to spliceosomal proteins hnRNPH1/H2. RNAseq of melanoma cells treated with H1/H2 siRNA showed upregulation of immune pathways. To validate this effect of genomic modulation of H1/H2 on immune gene expression, we used NanoString method. We treated both WM266-4 cell line and melanocytes with H1/H2 siRNA and scrambled siRNA to knockdown our target gene. Then, RNA was extracted and analyzed by NanoString. Analysis data of melanoma cell line showed a significant difference between RNA samples treated with scrambled and the one treated with siRNA in upregulating immune-related genes. In contrast, data of melanocytes revealed no significant difference between scrambled and siRNA samples and did not show upregulation of these genes. This implies the specificity of this response to melanoma cells. In conclusion, we hypothesize that downregulation of spliceosomal proteins H1/H2 can upregulate immune-related genes, which in turn can improve melanoma patients' survival. Consequently, H1/H2 can be targeted as a novel therapeutic approach.

**LB242**
**Characterizing the fate<tissue distribution and excretion route> of cancer vaccine lipoplex-RNA following intravenous injection of $^{14}$C-DOTMA-lipoplex-mRNA in mice.**
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Messenger RNA (mRNA) has emerged as a new class of therapeutic agent delivered by a carrier lipoplex (LPX) to elicit an immune-response for treating various diseases, including cancers. However, the PK and tissue distribution of the components is not well characterized given its therapeutic novelty and structural complexity. As such, this study aimed to characterize the distribution and metabolic fate of LPX-mRNA using a radiolabeled DOTMA, a component of the LPX-mRNA therapeutic, to aid the development of this anti-cancer agent. To track the fate of LPX-mRNA, DOTMA was first radiolabeled with [$^{14}$C] through chemical synthesis ($^{14}$C-DOTMA, and then mixed with DOPE and mRNA at a specific ratio to form $^{14}$C-DOTMA-LPX-mRNA ($^{14}$C-LPX-mRNA)). The plasma protein binding and blood cell partitioning for both $^{14}$C-LPX-mRNA and $^{14}$C-DOTMA alone were assessed in vitro. Moreover, the in vivo biodistribution and elimination of both $^{14}$C-molecules were characterized following a single intravenous (IV) injection in mice up to 12 weeks.The in-vitro assays showed the $^{14}$C-DOTMA and the $^{14}$C-LPX-mRNA were highly associated with plasma proteins and blood cell as the complexes were spun down at a much higher level as compared to in PBS solution (~5% and ~60% plasma proteins bound and ~60% and ~80% blood cell partitioned in $^{14}$C-DOTMA and the $^{14}$C-LPX-mRNA, respectively). Following in-vivo dosing in mice, plasma radioactivity of both test molecules showed a biphasic elimination profile with a rapid phase of decrease during the first 3 days followed by a prolonged exposure phase. The radioactivity in the whole blood and plasma displayed similar profiles but had minimal partitioning to blood cells, differing from the in-vitro data. We hypothesized that, both drug substances began to associate with plasma proteins/blood cells, then quickly distributed to the tissues and eliminated from the systemic circulation. Among all tissues analyzed, the liver and spleen showed the highest radioactivity levels where the $^{14}$C-LPX-mRNA peaked within the day while $^{14}$C-DOTMA peaked at 3-weeks-after-dose, followed by a long persistency. Lower levels of distribution and persistence of

radioactivity were also observed in other tissues. The route of elimination is mainly through the biliary-fecal route with minimal contribution from the renal route for $^{14}$C-LPX-mRNA. $^{14}$C-LPX-mRNA achieved mass balance at 8-weeks after dose where ~70% was eliminated through feces, 3% through urine and ~20% remaining in tissues. $^{14}$C-DOTMA animals have an under-recovery of about 40-50% in radioactivity due to animals' low intake of food and water which caused a severe reduction in animals' weight. In summary, this study fully characterized the fate of the $^{14}$C-DOTMA and the $^{14}$C-LPX-mRNA in vitro and in vivo in mice. Our data demonstrated that the LPX-mRNA (by tracking DOTMA) mainly distributed to the spleen and liver with a long persistency, consistent with previous study showing the spleen as the major tissue for mRNA distribution. However, the LPX's elimination profile (duration/persistency) is likely very different from that of mRNA. The ongoing work is to track the fate of mRNA component in LPX-mRNA, which could provide more insight on the correlation of DOTMA and mRNA, and help the development of this novel therapeutic modality.

**LB245**

**ONM-501, a dual-activating polyvalent STING agonist, enhances tumor retention and demonstrates favorable preclinical safety profile.**

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Background: The Stimulator of Interferon Genes (STING) plays a crucial role in the innate immune response. Several previous STING agonist development compounds have shown limited therapeutic efficacy in oncology clinical trials. ONM-501 is a novel STING agonist: the endogenous STING agonist 2',3'-cyclic GMP-AMP (cGAMP) is encapsulated within PC7A micelles. PC7A induces polyvalent STING condensation and prolongs immune activation. cGAMP-PC7A nanoparticles offer a dual 'burst' and 'sustained' STING activation. The anti-tumor efficacy and pharmacodynamic analysis of ONM-501 in multiple tumor models have been demonstrated previously. Here we report the pharmacokinetic (PK) and biodistribution (BD) analysis of ONM-501 in mice and safety evaluation in mice, rats and primates.
Methods: PC7A polymers conjugated with LiCOR 800CW were mixed with unlabeled PC7A in 1:9 ratio and cGAMP was encapsulated into micelles to generate an "always-on" fluorescently labelled ONM-501-CW800. Naïve or tumor-bearing mice were injected subcutaneously (SC) or intratumorally (IT) with ONM-501-CW800, respectively, and plasma and multiple organ samples were collected; the whole tissue specimens were first imaged ex vivo using LiCOR Pearl Imaging system, and then homogenized and the fluorescence quantified against standard curves prepared by spiking ONM-501-CW800 into a homogenate of the relevant matrix. PK parameters were calculated using non-compartmental methods. Safety and tolerability were evaluated by single- and multiple-dose SC injections in naïve animals up to the highest feasible doses.
Results: The BD pattern of ONM-501-CW800 was similar after IT and SC injections. The highest concentrations were observed at the injection sites and draining lymph nodes at all timepoints for both routes of administration. The concentrations in the injection site were much higher in tumors following IT than in dermal tissue following SC injection. After a 50 µg dose, systemic exposure to ONM-501-CW800 was ~1.8- and 2.4-fold lower after IT than SC injection based on Cmax and AUC(inf), respectively. The plasma t½ after IT injection, 17.4 hours, was ~1.3-fold longer than after SC injection, 12.9 hours. The Cmax and AUC (inf) in tumors were ~144- and 120-fold higher than in plasma after IT injection, with a t½ of 25.2 hours in tumors. In single-dose toxicology studies, ONM-501 was well tolerated in mice, rats and monkeys without severe or irreversible systemic toxicities up to the maximum feasible SC doses at 74, 45 and 30 mg/kg, respectively. In the 4-week repeat-dose GLP toxicology studies, the highest non-severely toxic SC dose (HNSTD) was 30 and 7.5 mg/kg in rats and monkeys, respectively.
Conclusions: Systemic exposure to ONM-501 was lower after IT than SC administration, which is consistent with increased ONM-501 retention in tumors. Combined with preclinical toxicology studies,

ONM-501 showed a favorable pharmacokinetic, tolerability and safety profile that support its continued development in cancer patients.

**LB246**

**Enfortumab vedotin, a nectin-4-directed antibody-drug conjugate, demonstrates compelling antitumor activity in non-muscle invasive bladder cancer models which predicts minimal systemic exposure when administered by intravesical instillation in patients.**

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Enfortumab vedotin (EV) is a monomethyl auristatin E (MMAE)-containing antibody-drug conjugate directed to Nectin-4, which is highly expressed in bladder cancers. Preclinically, EV has demonstrated tumor cell killing by direct cytotoxicity and bystander effect and can induce the hallmarks of immunogenic cell death. EV improves survival in adults with previously treated locally advanced or metastatic urothelial carcinoma (la/mUC) and is approved in the US, Europe, Japan, and others. Most newly diagnosed bladder cancer cases are non-muscle invasive (NMIBC). Standard treatment of high-risk NMIBC involves transurethral resection followed by intravesical Bacillus Calmette-Guerin (BCG) or chemotherapy. Although response to BCG is high, recurrence is common, and treatment options for patients with BCG-unresponsive tumors are limited, underscoring the significant unmet need. Previously, we demonstrated compelling preclinical antitumor activity of EV in NMIBC models with a favorable safety profile and minimal systemic exposure. EV-mediated antitumor activity was confirmed in a mouse model of NMIBC by both bioluminescence imaging and IHC for hNectin-4-expressing cancer cells. Following intravesical administration of EV, tumor growth inhibition ranged 46-96% across the dose range tested. Colocalization of EV to Nectin-4-positive tumor tissues was confirmed by IHC in the engrafted tumor cells. Systemic EV exposure in tumor-bearing mice was low, consistent with previous nonclinical studies, supporting that the antitumor activity is driven by local exposure within the bladder. In a repeat-dose GLP toxicology study in rats, no systemic toxicities were observed at intravesical doses up to 6-fold higher than the maximum tolerated IV dose. This lack of systemic toxicities that can occur with IV administration in rats was likely due to minimal systemic exposure of both EV and unconjugated MMAE. Currently, the safety, tolerability, and antitumor activity of intravesical EV are being evaluated in a Phase 1 study in adults with high-risk, BCG-unresponsive NMIBC (EV-104, NCT05014139). The initial dose level for EV-104 was selected to be active and predicted to have minimal systemic absorption based on preclinical and known clinical IV data. Here, we present confirmatory clinical data demonstrating that EV and unconjugated MMAE are undetectable in the bloodstream at the starting dose. These findings confirm the translatability of our nonclinical models and provide evidence that intravesical administration of EV in NMIBC is a promising approach that limits systemic exposure. These data support the potential for a favorable safety and activity profile and warrant continued investigation of intravesical EV in patients with NMIBC.

**LB249**

**A genome-scale CROP-seq screen reveals mediators of T cell signaling.**

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CROP-seq/ Perturb-seq screens combine CRISPR perturbation with single-cell RNA sequencing. In brief, cells are perturbed with a pooled sgRNA library and transcriptomic profiles of each cell are recorded

using conventional single-cell RNA sequencing platforms. Currently, the scale of these screens is limited to the perturbation of a couple of hundred genes, possibly up to 1.000 genes, because costs for single-cell library preparation and next generation sequencing are high. Here, we aimed to conduct a genome-scale CRISPR screen within a reasonable budget. We chose Jurkat T cells for this experiment and focused on T cell activation because signaling pathways in these cells are well understood and we could utilize the published knowledge to benchmark the performance of our platform.
To accomplish this, we utilized a CRISPR interference setup in which multiple sgRNAs can be delivered to the same cell, thus "squeezing" more perturbations into one cell. We targeted 18.595 human genes with four sgRNAs per gene and delivered this genome-scale library to Jurkat cells harboring dCas9-KRAB. Cells were stimulated with anti-TCR and anti-CD28 antibodies for 24 hours to activate T cell signaling. Following that, we processed 1.000.000 cells in one go, using the Chromium X platform that has recently been launched by 10X Genomics. Following single-cell library preparation, we amplified a selected set of 374 transcripts and submitted the corresponding library for NGS on one NovaSeq S4 flowcell.
First, we confirmed that the perturbation of 374 marker genes by CRISPR interference led to the downregulation of the cognate targets, suggesting that the CRISPR perturbation workflow is functional. Then, we assessed whether T cell activation could be recapitulated from the chosen markers and found that activated Jurkat T cells can be distinguished from their unactivated counterparts using the signature in question. Finally, we assessed the phenotypes of gene knockouts introduced at genome-scale. Of the 18.595 genes perturbed, a set of 70 genes affected T cell activation, partitioning to 55 activators (whose knockout led to diminished signaling) and 15 inhibitors (whose knockout led to enhanced signaling). Of note, our screen recovered key signaling nodes that are proximal to the TCR and have been well described in the literature, including LAT, LCK, ZAP70, CD3E, ITK, RASGRP1 and VAV1.
Overall, the screen presented here will catalyse a paradigm shift for CROP-seq/ Perturb-seq type CRISPR screens towards genome scale.

**LB250**
**Multiplex screening of PBMCs with DNA barcoded peptide exchanged MHC trimers to identify peptide-specific paired VDJ TCRs at single cell level.**
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Elucidation of the paired TCR receptor-antigen synapse is important both for vaccine development and for targeted therapy. Linkage of T cell responses with specific antigenic peptides will not only guide the development of immunotherapies but also enable treatment response monitoring. Currently, single cell sequencing technology is well established, but the identification of specific pathogenic peptides and associated TCR in single cell level is still challenging. In most cases, ascertaining T cell responses to pathogenic peptides is based on the screening of pooled peptides that do not give the knowledge of corresponding responsive TCR to a particular peptide. We therefore developed a technique that uses DNA barcoded peptide exchanged MHC trimers to stain PBMCs. With this technique, multiple peptide-loaded trimers can be used to stain a PBMC sample in one test tube. Single cells positive for trimers are sorted, amplified, and sequenced together with the DNA barcode. Specific peptides can be identified with the DNA barcodes which are co-amplified with TCRs. Applying this technique, we screened one PBMC sample with several peptide-loaded trimers in one reaction. Utilizing the CDR3 identity of known clones against each of these peptides, we were able to demonstrate and validate that a multi-peptide screen using DNA barcodes is feasible. Further optimization is in progress to reduce background and to increase the multiplexing of barcoded peptide-trimers.

**LB251**
**Blocking genomic instability delays acquired resistance to MAPK inhibitor therapy in melanoma.**

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**Background**: In cutaneous melanoma, the burden of chromothripsis is high prior to targeted therapy, and additional chromothripsis appears to be a key evolutionary mechanism by which cancer rapidly generates and accumulates highly dynamic structural variants (**SV**s). Blocking cancer genomic instability may prevent tumor escape from targeted therapies.
**Methods**: We assembled three cohorts of tissues for WGS-based analysis of SVs. The first cohort consisted of patient-matched normal tissues, *BRAF*$^{V600MUT}$ melanoma tumors before MAPKi therapy and at disease progression (n=10 normal tissues; n=10 pretreatment tumors; n=17 acquired-resistant tumors; n=10 patients). The second cohort consisted of rapid autopsy melanoma tissues (n=3 normal tissues; n=12 acquired-resistant tumors; n=6 metastatic organ sites). The third cohort consisted of cutaneous PDX tumors. To study acquired MAPKi-resistance at the whole-genome level, we subjected PDXs (n=6 models; 1 *BRAF*$^{MUT}$ and 5 *NRAS*$^{MUT}$ models) to MAPKi therapy in NSG mice at doses sufficient to elicit tumor regression, and then generated acquired MAPKi-resistant tumors. In total, we used vehicle-treated tumors (n=6), acquired-resistant tumors (n=12), and patient-matched normal tissues (n=6) to generate WGS data.
**Results**: Analysis of genomic amplicons due to intrachromosomal complex genomic rearrangements (CGRs) and extrachromosomal circular DNAs (ecDNAs) uncovered a significant (unpaired Student's *t*-test, p=0.0002) association between acquired-resistant tumors and CGRs and/or ecDNAs harboring *bona fide* MAPKi-resistance genes and revealed copy number amplification of *BRAF* (range 4.5-27), *NRAS* (range 5-13), *HRAS* (range 13-16), *MYC* (range 12-15) and *EGFR* (CN 4.6-5), known to drive acquired MAPKi-resistance. Moreover, we validated a recurrent ecDNA by direct isolation and high-depth sequencing using a new approach referred to as CRISPR-CATCH. This alternative technique confirmed the circularized junctions of a 890 kb, driver ecDNA within this acquired-resistant clinical tumor sample. Additionally, resistance-specific (versus sensitivity-specific) chromothriptic single-base substitutions (SBSs) enriched for signatures of defects in base excision repair (BER) and in DNA mismatch repair (MMR) (Wilcoxon rank sum test, p=0.04 and p=0.005 respectively) in 14 of 31 resistant tumors (10 of 16 patients). Moreover, breakpoint-sequence analysis inferred non-homologous end-joining (NHEJ) as critical, and homologous recombination repair (HRR) as adjunctive, to DNA double-stranded break repair underlying CGR and ecDNA formation harboring MAPK-reactivation or MAPKi resistance-driver genes. Inhibition of DNA-PKc or PARP1/2, even only initially during MAPKi treatment, suppressed acquired MAPKi-resistance in melanoma cell lines (*BRAF*$^{V600MUT}$ n=3 and *NRAS*$^{Q61MUT}$ n=3) and blunted the expansion of ecDNA + CGR genomic spans. *In vivo*, DNA-PKi in combination with MAPKi forestalled resistance, reduced ecDNA and CGRs size, and suppressed the contribution of NHEJ in 5 out of 5 cutaneous melanoma PDXs analyzed.
**Conclusions**: Our findings advance the concepts that preventing—instead of reversing—acquired resistant phenotypes may be more clinically impactful and that targeting DNA-PK$_{CS}$ and NHEJ lies at the center of this approach in stabilizing cancer genomes during oncogene-targeted therapies.

**LB252**

**HPV integration events are heterogenous, clonally selected and associated with spatially distinct transcriptomic profiles in aggressive HPV positive oropharyngeal squamous cell carcinoma.**

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HPV positive Oropharyngeal Squamous Cell Carcinoma (HPV positive OPSCC) is rising in incidence and, for patients that recur, survival is extremely poor. Recent technological advances have led to a better

understanding of patterns of HPV integration in primary tumors but have not yet tested the evolution of these events in deadly forms of the disease. Therefore, we performed multi-region sequencing analysis on a cohort of 34 recurrent and metastatic patients with HPV positive OPSCC (29% survival rate) plus one recurrent HPV positive model pair, UPCI-90 and UPCI-152. We then used our recently developed capture-based sequencing HPV integration caller and assembler pipeline, *SearcHPV*, to identify patterns of viral integration into the host genome and Oxford Nanopore long-read whole genome sequencing to validate the integration events in selected cases. In total, 1591 HPV integrations were called and assembled. Unexpectedly, our analysis revealed that HPV integration events are heterogenous as multiple independent integration events were observed in different spatial regions of the same tumor. Further, when comparing independent and anatomically distinct regions of recurrence from the same patient, independent integration events were also observed. Heterogeneity of integration structures was found to include variation of local HPV copy number, distribution of the specific HPV integration sites within the structures as well as distinct characteristics of rearrangement breakpoints (human-HPV, HPV-HPV, human-human). Given this observation, we then evaluated the hypothesis that HPV integration events could be clonally selected by comparing the integration profiles from a cohort of matched primary and recurrent tumors. In this cohort, 64 integration events (11 exact same; 53 clustered in the same genes) were maintained throughout tumor progression to recurrence, while other integration events were lost, supporting a model of clonal evolution with driver and passenger integrations. We then explored this further using one recurrent HPV positive model pair, UPCI-90 and UPCI-152, using long-read whole genome sequencing which validated the heterogeneity and clonal selection of HPV integration events. Finally, to test if differences in HPV integration content in spatially distinct regions could drive spatially distinct phenotypic differences, we performed multi-region RNA-seq analysis on 8 HPV16 positive OPSCCs, which demonstrated spatial differences in HPV16 E7 expression, that were validated with RNAscope assays, as well as statistically significant spatial enrichment of independent oncogenic gene sets. Collectively, we report evidence showing that HPV integration events are heterogenous, clonally selected during progression and associated with distinct transcriptomic profiles. Based on this data, we now propose a new working model of disease pathogenesis in which heterogeneity and clonal evolution of HPV integration events serve as a critical driver of the disease process.

**LB253**

**Long-read single-cell sequencing of liver cancer.**

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The protein diversity of mammalian cells is determined by arrays of isoforms from genes. Protein mutation is essential in species evolution and cancer development. Accurate Long-read transcriptome sequencing at single-cell level is required to decipher the spectrum of protein expressions in mammalian organisms. In this report, we developed a synthetic long-read single-cell sequencing technology based on LOOPseq technique. We applied this technology to analyze 447 transcriptomes of hepatocellular carcinoma (HCC) and benign liver from an individual. Through Uniform Manifold Approximation and Projection (UMAP) analysis, we identified a panel of mutation mRNA isoforms highly specific to HCC cells. The evolution pathways that led to the hyper-mutation clusters in single human leukocyte antigen (HLA) molecules were identified. Novel fusion transcripts were detected. The combination of gene expressions, fusion gene transcripts, and mutation gene expressions significantly improved the classification of liver cancer cells versus benign hepatocytes. In conclusion, LOOPseq single-cell technology may hold promise to provide a new level of precision analysis on the mammalian transcriptome.

**LB254**

**Accurate detection of low frequency AML-associated mutations *in vitro* using Duplex Sequencing with enzymatic fragmentation.**

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Sensitive and specific detection of low frequency mutations in acute myeloid leukemia (AML) is critical in research of minimal residual disease (MRD). At variant allele frequencies (VAF) below approximately 1%, PCR and sequencing errors result in prohibitive signal-to-noise ratios with next generation sequencing (NGS). Duplex Sequencing (DS) relates the original top and bottom DNA strands to make double stranded consensus sequences to greatly reduce errors. In two experiments we show further improvement of DS of AML-related genes by the use of enzymatic fragmentation (EF) and an updated gene panel that incorporates 2022 European LeukemiaNet (ELN) recommendations. In the first DS study, 29 AML-related genes (59 kb) were targeted in hybrid capture. Mutant cell line DNA was mixed into DNA from a healthy young donor to simulate MRD. Expected VAFs in mixtures were 1.0-0.003%. Samples comprised a mix of 4 insertions and deletions (indel), a mix of 15 single nucleotide variants (SNV), and 4 serial dilutions of a *FLT3* ITD plus an *NPM1* insertion. Pure diluent DNA was used as a negative control. Each sample was prepared in quadruplicate with mechanical fragmentation (MF) vs EF. DNA input mass was 1,500 ng per replicate, except for 50-250 ng for the *FLT3*/*NPM1* mixes with expected VAF of 1% and 0.1%, respectively. Input masses were set to ensure >95% probability of detection of all mutations in the combined data. All targeted variants down to 0.003% VAF were detected in spike-in mixtures, with expected vs observed VAF highly correlated whether using MF or EF ($R^2$ >0.98 for all mixtures). Duplex molecular depth was 1.2-2.0x higher with EF vs MF across input masses. Panel-wide mean duplex depth per 1,500 ng replicate was 30,376x for MF and 48,036x for EF, for combined mean depths of 121,508x for MF and 192,144x for EF. In the negative control, background mutational calls at spike-in positions were 4/2,993,429 duplex bases using MF and 0/4,780,491 duplex bases with EF, reflecting increased specificity with EF. Next, a revised panel targeting 36 AML-related genes (80 kb) was used for DS with EF. Here differing mutant cell line DNA was mixed into the same diluent from above (the negative control). This mixture harbors 27 variants with expected VAFs of 0.125-0.006% (4 indels, 21 SNVs, a *FLT3* ITD and an *NPM1* insertion), 17 of which (2 indels, 14 SNVs and the *NPM1* insertion) span VAFs of 0.011-0.009% to establish a limit of detection of 0.01%.With 2,000 ng (+/- 10%) input, for 14 samples, panel-wide mean duplex depth was 56,528x. For variants with expected VAF ≥ 0.009%, the assay had >98% sensitivity, >96% specificity, and >98% accuracy at genomic positions of known true positive variants. In summary, DS with EF yields more data per nanogram of DNA than MF, maximizing the use of precious samples. This method exhibits extremely low background mutation signal and a low limit of detection across targets that are valuable in AML MRD research.

**LB255**

**Benchmarking NGS integration site analysis methods in support of long-term safety monitoring of gene therapy products.**

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The FDA Guidance to Industry on Long Term Follow-Up (LTFU) After Administration of Human Gene Therapy Products states the importance of longitudinal testing of gene products introduced into human subjects. Depending on the delivery mechanism, the therapeutic gene product may or may not integrate into the genome. Of particular interest are gene-product integrations near proto-oncogenes which might lead to malignancies. The FDA LTFU guidance states that recipients of an integrating gene therapy modality should be tracked for 15 years, while those receiving a non-integrating therapy modality should

be tracked for 5 years. Therefore, advanced analytical methods are needed to identify, quantify, and track integration events across the genome. Here, we provide a comprehensive evaluation of methods leveraging next-generation sequencing approaches for genome-wide analysis of lentiviral integration events. Our analysis employed well-characterized standards consisting of varying copy number and known integration sites.
The approaches we characterized can be bucketed into two major groups: PCR amplification approaches and target capture-based approaches. All methods detected true positives with strong correlation to theoretical integration site dosage levels down to 1% allele frequency. Comparatively, PCR amplification-based approaches have lower data requirement per sample suggesting higher sensitivity, greater molecular capture, and lower limit of detection compared with target enrichment-based approaches. Target enrichment-based approaches can afford the flexibility to capture the integrated vector, which is of interest for characterizing partial integration events. While all methodologies performed well in our study, the choice of assay (or assays) for testing will depend on numerous factors including but not limited to the viral vector system and construct and starting material availability.

**LB256**
**Type 1 calreticulin mutations in myeloproliferative neoplasms promote glycolysis via intracellular calcium mediated upregulation of GLUT1.**
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Myeloproliferative neoplasms (MPNs) comprise a group of cancers of the bone marrow that can transform into a post-MPN acute myeloid leukemia (AML), at which point the overall survival rate is less than 6 months. JAK2 inhibitors in MPN failed to alter disease progression, emphasizing the need for new treatments. Calreticulin (*CALR*) is one of the MPN driver mutations and encodes an endoplasmic reticulum (ER) calcium (Ca2+) binding protein. *CALR* mutations are classified as either type 1 or type 2. Only type 1 proteins exhibit loss of the C-terminal Ca2+ binding sites. Additionally, type 1 and 2 *CALR* mutations engender significant prognostic differences. Previously published work from our lab showed that type 1 CALR exhibits a loss of Ca2+ binding sites, rendering them unable to bind ER Ca2+ and leading to depletion of ER Ca2+ stores. We next wanted to evaluate where this Ca2+ is localized. Through imaging studies, we found that CALR type 1 expressing cells have significantly increased cytosolic Ca2+ and significantly lower mitochondrial Ca2+ compared to both CALR wildtype and CALR type 2 expressing cells. It has been well established in various cancer types that increased glycolytic activity is prompted in part by elevated cytosolic Ca2+. To test if this was the case in our model, we performed a vast array of metabolic assays in UT-7-MPL cells, a human megakaryocytic cell line, including but not limited to lactate secretion, Seahorse XF Glycolysis Stress Test assay, and metabolite tracing with 13-C labelled glucose. These all confirmed a highly glycolytic profile in UT-7-MPL cells expressing the CALR type 1 mutation. In addition, through evaluation of mitochondrial mass, membrane potential and several other assays, mitochondrial metabolism in CALR type 1 mutant cells appears severely dysfunctional. All the glycolytic and mitochondrial phenotypes are almost fully rescued upon the reintroduction of the Ca2+ binding sites of the CALRwt protein (P and C domains; P+C), indicating that perturbed Ca2+ homeostasis is the driver of these changes. Pharmacological treatment with cytosolic Ca2+ chelator BAPTA-AM or with kaempferol, an activator of the mitochondrial Ca2+ uniporter mimic the P+C rescue effect for both the glycolytic and the mitochondrial phenotypes. In our *in vitro* system CALR type 1 mutant cells express along with other glycolytic factors significantly higher cell surface levels of the glucose transporter GLUT1, and demonstrate enhanced sensitivity to pharmacological

GLUT1 inhibition with BAY-876. Preliminary data in a transgenic mouse model as well as in primary patient samples show that GLUT1 expression is higher in CALR type 1 mutant mice and patient cells, and that the mitochondrial mass in this subset of patients is lower compared to CALR type 2 mutant patients. Finally, we validated that all these phenotypes are JAK/STAT pathway independent and that they are worsened by ruxolitinib treatment, providing a strong rationale for a combination treatment. Together, these data both expand the current knowledge of the role that Ca2+ dynamics play in metabolic reprogramming and can lead to improved targeted therapies for MPN patients.

**LB258**

**Therapeutic advantage of targeting lysosome as signaling hub for metabolic conditions in malignant gliomas.**

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Lysosome, as the digestive system of the cell, participated in numerous cell biological processes, such as macromolecular degradation, cell adhesion/migration, and apoptosis by regulating metabolic status and pro-growth signaling. Therefore, lysosome could be a promising therapeutic target for cancer therapy, but it remains unclear how lysosome is involved in cancer malignancy. In this study, to investigate the roles of function lysosomes in the regulation of malignant status, we evaluated the proteolytic activity of lysosomes using BODIPY-dye conjugated BSA (DQ-BSA) in malignant gliomas characterized by high aggressivity, destructivity, and invasiveness, glioblastoma multiforme (GBM). While most GBM patient-derived cells showed higher lysosomal proteolytic activity than an immortalized human neural progenitor cell line derived from the embryonic brain, levels of lysosome activity positively co-related with sphere formation. Multi-omics analysis demonstrated that the level of lysosomal proteolytic activity was co-related with metabolic status, including activated mitochondria biogenesis and amino acid transport. The orthotopic xenograft model revealed that lysosome activity *in vitro* is a critical biomarker representing malignant phenotypes of GBM. We found that the MiT/TFE family, a master regulator of lysosomal biogenesis, controlled the malignant progression of GBM, including therapy resistance, indicating that quality control of lysosomes is a critical determinant for the malignant properties of gliomas. To develop a novel therapeutic approach for targeting lysosomal function, we performed screening of FDA-approved compounds and found that lysosomotropic agents, which induced galectin-3 punctation as an indicator of lysosomal membrane damage, efficiently suppressed tumor growth. Ifenprodil, a clinically available drug that acts as a lysosomotropic agent, increased intracellular $Ca^{2+}$ levels, resulting in mitochondrial reactive oxygen species-mediated cytotoxicity. Furthermore, we found that the efficacy of ifenprodil was remarkably enhanced by metabolic modifications, such as amino acid restriction, in vitro and in vivo. Collectively, these data indicate that lysosomes as signaling hubs for metabolic conditions play critical roles in the regulation of the malignant status of GBM patients. Our findings propose that lysosomal biogenesis is a promising target to develop a novel therapeutic approach for GBM.

**LB259**

**Distinct microRNA signature and suppression of ZFP36L1 define ASCL1-positive lung adenocarcinoma.**

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Background: Achaete-scute family bHLH transcription factor 1 (ASCL1) is a master transcription factor involved in neuroendocrine differentiation. ASCL1 is expressed in approximately 10% of lung

adenocarcinomas and exerts tumor-promoting effects. However, microRNA (miRNA) profiles regulated by ASCL1 in lung adenocarcinoma cells remain unexplored.
Method: We analyzed public database of gene expression profiling (RNA-sequencing and miRNA expression data). We also studied miRNA profiles in ASCL1-positive lung adenocarcinoma cells and identified a subset of miRNAs downregulated by ASCL1 knockdown. We examined functions of genes suppressed by miRNAs in ASCL1-positive lung adenocarcinoma cell line, VMRC-LCD.
Result: We identified miRNA profiles in ASCL1-positive lung adenocarcinomas and found several miRNAs closely associated with ASCL1 expression, including miR-375, miR-95-3p/miR-95-5p, miR-124-3p, and members of the miR-17~92 family. Similar to small cell lung cancer, Yes1 associated transcriptional regulator (YAP1), a representative miR-375 target gene, is suppressed in ASCL1-positive lung adenocarcinomas. ASCL1 knockdown followed by miRNA profiling in a cell culture model further revealed that ASCL1 positively regulates miR-124-3p and members of the miR-17~92 family. Integrative transcriptomic analyses identified the RNA-binding protein zinc finger protein 36 like 1 (ZFP36L1) as a target gene of miR-124-3p, and immunohistochemical studies have demonstrated that ASCL1-positive lung adenocarcinomas are associated with low ZFP36L1 protein levels. Cell culture studies have shown that ectopic ZFP36L1 expression inhibits cell proliferation, survival, and cell cycle progression. Mechanistically, ZFP36L1 negatively regulated tumorigenic genes, including E2F transcription factor 1 (E2F1) and snail family transcriptional repressor 1 (SNAI1), indicating a tumor-suppressing action.
Conclusion: Our study revealed that suppression of ZFP36L1 via ASCL1-regulated miR-124-3p could induce tumor-promoting effects, providing evidence that ASCL1-mediated regulation of miRNAs shapes malignant features of ASCL1-positive lung adenocarcinomas.

**LB260**
**Assessing the suitability of our exosomal small ncRNAs to serve as biomarkers for breast cancer & elucidating the relationship of exosomal ncRNAs and cancer cells from t-47d and hc1143 cell lines.**
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Background: Exosomes are known to play the role of cargo, carrying biologically important materials such as non-coding RNAs to their recipient cells. Small non-coding RNAs such as miRNA have been shown to exert strong effects on tumorigenesis. The liquid biopsy, which utilizes small RNAs from plasma exosomes, is therefore a promising tool for precision oncology. The purpose of this study is to discover exosomal miRNAs that serve as detection markers of breast cancer. Our hypothesis are 1) if exosomes play the role of cargo, then there should be an abundance of upregulated ncRNAs in exosomes that are also upregulated in the cancer cells themselves. 2) It is likely that the miRNAs in exosomes that are highly expressed have critical roles in cancer cell activity. We performed small RNA-seq and examined the ncRNAs inside cancer cells and exosomal ncRNAs secreted by cancer cells into the culture media.
Methods: Two cancer cell lines T-47D and HCC1143, and primary Human Mammary Epithelial Cells (HMEC) were used. We prepared 5 samples of cellular RNA extracted from T-47D and HCC1143 and HMEC. We also extracted 8 samples of exosomal RNA from the media; which included those used for each cell culture and those never used for culture with or without FBS. Exosomes in FBS were depleted using Exosome Depletion Kits. The FASTQ files were uploaded onto QIAGEN GeneGlobe for analysis. The data was primarily normalized using geNorm.
Results: Exosomal ncRNAs from both cancer cells were compared to exosomal ncRNAs from normal cells. A total of 879 ncRNAs were detected, 108 of which were significantly upregulated and 104 of which were downregulated in cancer cells. hsa-miR-196a-5p is a ncRNA that had a fold-change of 84.08. Prior studies suggest that this miRNA is associated with MIR196A2 methylation and affects the malignancy of many types of cancers. Comparing cancer exosomal ncRNAs vs. ncRNAs inside cancer cells, a total of 879 ncRNAs were found with a FDR q-value <0.05 and cut-off of 2 fold change. 251

ncRNAs were significantly upregulated in the exosomes. hsa-miR-1-3p had a fold change value of 4025.68 and an associated p-value of 0.0002. High levels of hsa-miR-1-3p are known to be associated with large tumors and is strongly correlated to a lower breast cancer survival rate.
Conclusions: The preliminary results indicate that there were significant differences between exosomal ncRNAs from normal cells and exosomal ncRNAs from cancer cells. The data suggests that exosomal ncRNAs are a promising source of prognostic biomarkers for breast cancer as ncRNAs with a high fold change are repeatedly validated to have a significant impact on breast cancer progression. The data supports our hypothesis that the upregulation of ncRNAs in cancer cells & exosomes is linked to cancer activity. The results will be scrutinized further, as other avenues for data analysis will be explored. The next step in our experiment will be to check the cancer survival rates with the TCGA database and attempt to validate our hypothesis through further cell culture experiments.

**LB261**
**The Akt-IWS1 signaling axis regulates microRNA activity.**
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MicroRNAs are small single-stranded non-coding RNAs that regulate gene expression post-transcriptionally, by targeting the 3‘-untranslated region (3’-UTR) of mRNAs. The regulation of microRNAs at the transcriptional level is well-studied, however, how their activity is regulated remains largely elusive. The family of Akt serine/threonine protein kinases, comprised of Akt1, Akt2 and Akt3, regulates important cell functions including metabolism, survival, proliferation, and migration. We have previously shown that IWS1 (Interacts with Spt6), a factor involved in mRNA splicing and nuclear export, is phosphorylated specifically by Akt1 and Akt3 at Ser720/Thr721. Here, RNA extracts from NCI-H522 lung cancer cells, transduced with shIWS1 and reconstituted with wild type (Ser720/Thr721-IWS1) or phosphorylation-deficient IWS1 (Ala720/Ala721-IWS1) were subjected to RNA and microRNA sequencing. Bioinformatic analysis suggested that microRNAs expressed at similar levels in the two cell types have a different impact on their mRNA targets. microRNA activity reporter assays and western blot analysis for microRNA targets in these cells revealed increased microRNA activity in Ala720/Ala721-IWS1-expressing cells. Our findings were verified in a second cell line, the non-transformed immortalized colonic epithelial cells NCM460. microRNA activity reporter assays, western blot analyses and cell growth assays showed that microRNA effects are enhanced in cells expressing Ala720/Ala721-IWS1. Immunoprecipitation and proximity ligation assays showed that IWS1 interacts with proteins known to be associated with RISC, and this interaction is enhanced by Akt-mediated IWS1 phosphorylation. To evaluate the dependence of IWS1/RISC interaction on IWS1 phosphorylation, we employed *Akt1*$^{-/-}$*Akt2*$^{-/-}$*Akt3*$^{-/-}$ immortalized mouse lung fibroblasts, transduced with myc-Akt1 or myc-Akt2 or the empty retroviral vector. Immunoprecipitation experiments in these cells, before and after treatment with IGF1, revealed that IWS1 interacts with the RISC components specifically in Akt1-expressing cells upon Akt1 activation by IGF1. Overall, our data demonstrate that IWS1 interacts with RISC in a conserved Akt1-dependent manner and regulates the activity of microRNAs with significant effects on cellular properties.

**LB262**
**HSK38008: An oral AR-V7 degrader for metastatic castration-resistant prostate cancer.**
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The androgen deprivation therapy (ADT) alone and its combination with hormone therapy that block AR signaling (e.g., enzalutamide or abiraterone) are effective treatments for advanced prostate cancer. Unfortunately, patients with truncated AR splice variants which lacking the ligand-binding domain still developed resistance to these AR-targeting compounds. The truncated AR splice variant 7 (AR-V7), which is the most clinically relevant variants, remains constitutively active as a transcription factor in 75% of the patients with mCRPC, associated with shorter PFS and OS with abiraterone or enzalutamide treatment. Therefore, AR-V7 has become a promising target for mCRPC.As a novel oral AR-V7 degrader with favorable bioavailability, HSK38008 could degrade the AR-V7 protein with $DC_{50}$ of 136 nM and achieve the maximum degradation at 24h at 10 μM. In the meanwhile, HSK38008 also degrade AR with weaker $IC_{50}$ of 110 nM and the maximum degradation at 48hrs at 10 μM. By validation of protease inhibitor MG341, HSK38008 could not degrade ARV7 and AR at high concentration. Compared with enzalutamide and ARV-110, HSK38008 could block the AR-V7 pathway and AR pathway in luciferase report assay with $IC_{50}$ of 400 nM and 1913 nM respectively. Importantly, HSK38008 also showed significant inhibition of AR mutants (AR-T878A/S889G, T878S, H875Y, T878A) in luciferase assay but not ARV110 and enzalutamide. HSK38008 significantly inhibit the cell proliferation in AR-V7 positive cell line, e.g., 22RV1, but weak antiproliferation in AR and ARV7 positive cell line such as VCAP However, when combination with enzalutamide there is synergistic effect for the anti-proliferation in VCAP. It means the potential to use the compounds in a broader population of mCRPC.In the xenograft model, the mice which were orally given HSK38008 showed 22RV1 tumor growth inhibition in a dose-dependent manner, with the TGI = 89.8% at 10 mpk and the intratumoral AR-V7 protein degradation rate was 73%. 30 mpk of HSK38008 showed completely tumor regression. At the same time, abiraterone and ARV-110 did not show significantly tumor growth inhibition at 30 mpk. There is no influence on mice body weight at all HSK38008 dose groups, while 3 mice in the 30 mpk of ARV-110 group died during the experiment. These results indicated that HSK38008 achieved better therapeutic effects than enzalutamide and ARV-110 did in AR-V7 positive 22RV1 xenograft model.Rats were dosed with 10, 30 and 60 mg/kg HSK38008 once daily for up to 28 days via oral gavage and main study animals were necropsied on Day 29 or Day 57. No animal was found dead or moribund and no test article-related changes in clinical signs, body weights, food consumption, ophthalmologic examinations, clinical pathology parameters, sperm analysis, urinalysis, organ weights, histopathology. In conclusion, HSK38008 is a promising oral AR-V7 degrader with better efficacy than enzalutamide and ARV-110 in AR and AR-V7 positive, and potential AR mutants mCRPC.

**LB263**

**An *in vivo* unbiased screen identifies clathrin adaptor protein complex-2 as a novel tumor suppressor.**

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Pancreatic ductal adenocarcinoma (PDAC) is almost uniformly lethal. It is thus imperative to find new therapeutic approaches to treat this disease. While whole genome CRISPR/Cas9 screens have successfully identified new vulnerabilities in PDAC cell lines, it is becoming increasingly clear that cell culture do not faithfully recapitulate the complex nature of tumorigenesis *in vivo*, and hence may fail to identify the full spectrum of potential therapeutic targets to be explored in this devastating disease. As previous screens we performed identified phosphatidylinositol (Ptdlns) metabolism as important for pancreatic cell growth, I generated a custom sgRNA library targeting this entire form of metabolism. To identify vulnerabilities specific to tumor growth, I screened this library in five different human PDAC cell lines grown in culture versus as xenograft tumors. I found that sgRNAs targeting two genes encoding separate subunits of the Adaptor Protein complex-2 (AP2), a regulator of clathrin mediated endocytosis,

were *negatively* enriched in all five PDAC cell lines when grown in culture. In agreement, AP2 subunit genes are considered common essential by DepMap. Paradoxically however, the same sgRNAs were *positively* enriched in three of these five cell lines when grown as xenograft tumors. This begs the question how the loss of a supposably essential complex behaves like a tumor suppressor and instead enhances tumor growth when disrupted. Here, I will present data that loss of AP2 results in a substantial restructuring the plasma membrane proteome, which in cultured cells results in the loss of iron transport, one of the main functions of this complex, and cell death. Conversely, this restructuring in vivo retains receptors and adhesion proteins on the cell surface, where transcriptome and proteomic analysis indicate enhances proliferative signaling. These data support AP2 promoting cell viability of cultured cells through active iron transport, but suppresses tumor growth in vivo by endocytosing the very receptors needed for tumor growth.

**LB267**

**Nitric oxide suppression by secreted frizzled-related protein 2 drives retinoblastoma.**

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Retinoblastoma is a cancer of the infant retina primarily driven by the loss of the Rb tumor suppressor gene, which is undruggable. Here, we identified an autocrine signaling, mediated by secreted frizzled-related protein 2 (SFRP2), which suppresses nitric oxide and enables retinoblastoma growth. SFRP2 silencing resulted in rapid production of nitric oxide, leading to p53-dependent growth arrest of retinoblastoma cells. We show that coxsackievirus and adenovirus receptor (CXADR) is the cell surface receptor for SFRP2 in retinoblastoma cells, that CXADR functions as a "dependence receptor," transmitting a growth inhibitory signal in the absence of SFRP2, and that the balance between SFRP2 and CXADR determines nitric oxide production. Accordingly, high SFRP2 RNA expression correlates with high-risk histopathologic features in retinoblastoma. Targeting SFRP2 signaling by SFRP2-binding peptides or by a pharmacological inhibitor rapidly induced nitric oxide and profoundly inhibited retinoblastoma growth in orthotopic xenograft models. These results reveal a cytokine signaling pathway that regulates nitric oxide production and retinoblastoma cell proliferation, and is amenable to therapeutic intervention.

**LB269**

**Inhibition Of C-C chemokine receptor 7 extends survival rate in murine model of T-cell acute lymphoblastic leukemia.**

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C-C Chemokine Receptor 7 (CCR7) expression in pediatric T-cell acute lymphoblastic leukemia (T-ALL) promotes entry of leukemic cells into the central nervous system (CNS), where the disease finds sanctuary from systemic chemotherapies. To confirm that Notch1 drives expression of CCR7, we modified the ROSA26-PRDM14 ([PRDI-BF1 (positive regulatory domain I-binding factor 1) and RIZ (retinoblastoma interacting zinc finger) homology domain containing 14]) MX-1 Cre inducible CD8+ T-ALL mouse model. This model, upon induction with polyI:C, expresses NOTCH1 downstream of PRDM14, and the T-ALL cells enter the CNS during leukemogenesis. We crossed these mice with ROSA26-Luc2 mice, which allows for tracking of T-ALL cells during disease progression. We questioned if these T-ALL cells expressed CCR7. We found that not only do the T-ALL cells express CCR7 and enter the CNS, but that a CCR7 antagonist, $CCL19_{8\text{-}83}$ administered during leukemogenesis doubles the mean survival of the $CCL19_{8\text{-}83}$ treated mice from 45 to 90 days. These studies provide a novel platform for a potential

therapeutic that can be used to limit CNS entry and potentially recycling through the CNS.

**LB270**

**LINGO2-microRNA cluster controls oral cancer stemness ability and cancer progression.**

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Cancer recurrence and metastasis are the primary reasons for treatment failure in late-stage oral cancer. Cancer stem cells are the root of cancer recurrence and metastasis. By using the microRNAome analysis of Taiwan OSCC cohort, we found miR-876-3p was highly correlated to OSCC recurrence. The precursor miR-876 promoted *in vitro* OSCC cell proliferation, migration, and cancer stemness and regulated several cancer stemness-relative genes such as NANOG, OCT4, and LIN28A expression. miR-876 and miR-873 are located in the second intron of the 5'-untransalation region (5'-UTR) at the alternative variant leucine-rich repeat and Ig domain containing 2 (LINGO2). Interestingly, only OSCC cells presented LINGO2 protein expression but did not show in primary oral keratinocytes (HOK). The unique CpG islands at the alternative transcription start site of LINGO2 hinted at crucial epigenetic regulation that controls LINGO2 activation. Silencing of LINGO2 expression reduced OSCC cell growth and cancer stemness abilities. LINGO2 expression was higher in OSCC tumor tissue than in adjacent normal tissue in both Taiwan and The Cancer Genome Atlas (TCGA) head and neck cancer cohorts. Moreover, LINGO2 also served as a poor prognostic marker in cancer patients' overall and recurrent free survival time. In summary, we found a novel LINGO2-microRNA cluster that regulates OSCC stemness and metastasis abilities. Those cancer specific expressions may provide a new therapy niche in OSCC cancer therapy.

**LB272**

**Spatial cellular architecture predicts prognosis in glioblastoma.**

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Intra-tumoral heterogeneity and cell-state plasticity are key drivers for the therapeutic resistance of glioblastoma (GBM). Studies based on single-cell RNA-seq and spatial transcriptomics have classified GBM cells into distinct transcriptional phenotypes. However, how the transcriptional diversity and spatial cellular organization are associated with patient prognosis remains incompletely resolved. Here, we developed a deep learning model to predict spatially resolved transcriptional programs from histology images. The model was trained on spatial transcriptomics data and validated in external testing cohorts. Applying the model to two separate patient cohorts led to the discovery of conserved relationships between tumor architecture and prognosis. Patient with poor prognosis had higher proportions of GBM cells expressing a hypoxia-induced transcriptional program. In addition, high clustering patterns of reactive astrocytes were associated with a poor prognosis. Conversely, when the reactive astrocytes were dispersed and connected to other cell types, the risk was decreased. To validate our results, we developed a separate deep learning model that used histology images to predict prognosis. Applying the model to spatial transcriptomics data discovered survival-associated regional gene expression programs. Genes related to glycoprotein metabolism and injury response were significantly upregulated in tumor cells with

high aggressiveness. Our studies established a scalable approach to resolve the transcriptional heterogeneity of GBM and linked the spatial cellular architecture to clinical outcomes.

**LB273**

**An emerging paradigm of heterogeneous midkine expression in thyroid cancer.**

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Midkine (MDK) is a pleiotropic heparin-binding growth factor, contributing to both normal tissue homeostasis and disease development. MDK expression is gradually increased during carcinogenesis, acting as a mediator for the acquisition of cancer hallmarks, such as invasion/metastasis, and immunosuppression. Tissue-wide gene expression analysis using publicly available databases reveal that MDK is significantly upregulated in most human carcinomas. In the thyroid, MDK has been associated with increased metastatic potential in the context of papillary thyroid cancer, which is an otherwise relatively non-aggressive thyroid tumor. We thus theorized that more aggressive types of thyroid cancers may be linked to increased MDK expression/function from an earlier onset. To gain such insights, we developed a digital pathology infrastructure to investigate MDK expression in a characterized mouse model of Anaplastic Thyroid Carcinoma (ATC), the most aggressive and lethal type of thyroid cancer with low but increased prevalence in the human population, and an intrinsic resistance to available therapeutic options. Mice with conditional ablation of tumor suppressors, Pten and p53, in thyrocytes $[Pten, p53]^{thyr-/-}$ develop thyroid carcinomas with mixed follicular and anaplastic components, and histological hallmarks of high aggressiveness, including giant cells, bone metaplasia, and muscular/tracheal invasion, which are all recapitulated in human disease. MDK was more highly expressed in anaplastic, when compared to follicular components of the same animal tumors. When the above hallmarks were topographically and microanatomically demarcated within the anaplastic component, it was found that MDK expression was higher near and around giant cell islets, as well as within the anaplastic lesions invading beyond the cartilaginous rings of tracheal mucosal epithelium. Based on recent evidence that MDK does not solely function in the extracellular space, but can also be endocytosed within tumor cells, whereby it exerts tumor-promoting functions, we further quantified the intra/extranuclear MDK fraction using intensity thresholding immunofluorescence. Of interest, $MDK^{+}$ follicular lesions, were primarily characterized by nuclear MDK expression, whereas MDK was mostly found secreted in the extracellular space of anaplastic lesions, thus implying different modes of MDK trafficking, secretion, and possibly function in them. The take home message is to work towards establishing and developing gain- and loss-of-function experiments, to elucidate mechanistic underpinnings on the role of MDK in the development of aggressive ATC lesions.

**LB274**

**Senescent cells develop PDK4-dependent hypercatabolism and form an acidic microenvironment to drive cancer resistance.**

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Cellular senescence is a permanent state of cell cycle arrest and occurs in proliferating cells subjected to various stresses. Although senescent cells remain metabolically active, little is known about their metabolic landscape and *in vivo* pathophysiological implications. Here we show that expression of the pyruvate dehydrogenase (PDH) inhibitory enzyme, pyruvate dehydrogenase kinase 4 (PDK4), is significantly upregulated in human senescent stromal cells. Preferentially expressed upon genotoxicity-induced senescence, PDK4 is negatively correlated with posttreatment survival of cancer patients. Upon cellular senescence, PDK4 shifts glucose metabolic flux from oxidative phosphorylation (OXPHOS) to aerobic glycolysis, causing enhanced lactate production and forming an acidic microenvironment.

However, distinct from the cancer cell-featured 'Warburg effect', senescent cells maintain an intensive use of pyruvate through the tricarboxylic acid cycle (TCA), displaying increased respiration and redox activity, indicative of a special form of metabolic reprogramming. Conditioned media from $PDK4^{+}$ stromal cells change global expression and promote malignancy of recipient cancer cells *in vitro* and accelerate tumor progression *in vivo*. Nevertheless, specific targeting PDK4 curtails the adverse effects of senescent cells in cell-based assays, while promoting tumor regression and extending posttreatment survival in preclinical trials. Of note, increased levels of lactate in circulating blood after chemotherapy predicts lower survival in cancer clinics. PDK4 upregulation and ensuing lactate production are thus among key features of senescence, in addition to the established cell-autonomous and non-cell-autonomous hallmarks of senescent cells, providing a novel therapeutic target for future clinical oncology. Together, our study substantiates the hypercatabolic capacity of senescent cells, and reveals a metabolic link between senescence-associated acidic microenvironment and age-related pathologies, including but not limited to cancer.

**LB275**

**Transcriptomic reversal analysis yields cytokines and drugs mediating tumor microenvironmental reprogramming during cancer progression and therapy response.**

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Recent computational work links therapy response to a drug based on the drug's ability to reverse a tumor's transcriptome towards a healthy state. This idea of reversal has been used to mine databases of cell-line transcriptional responses against drug libraries to prioritize anti-cancer drugs. However, there are two key shortcomings in these approaches: (1) though cytokines and their receptors are proposed as modulators of therapy response, there is no reversal-based method to prioritize cytokines as potential drugs or targets, and (2) responses of microenvironmental cell types to drugs, which dictate therapy response, have not been considered. We address these limitations by exploiting recent databases of cytokine transcriptional response and single-cell RNA-seq datasets of patient responses to cancer therapies. We first used a novel approach to derive drug response signatures from LINCS data that retained the coordinated nature of gene expression changes occurring during treatment. We then used these signatures to compute a transcriptional reversal score that ranks drugs by their ability to reverse TCGA RNA-seq profiles of a tumor towards its corresponding normal in GTEx. We found that FDA-approved drugs in prostate, lung, colorectal and breast adenocarcinomas have a significantly higher reversal potential than unapproved drugs, and that these drugs are more effective at in vitro cell killing amongst CTRP and GDSC cell viability measurements. Next, we extended our signature derivation and reversal computation approach to find cytokines in the CytoSig database that can reverse TCGA RNA-seq profiles. We found that the higher the reversal potential of a cytokine, the greater its association with better overall patient survival. In particular, our approach revealed the IL10 family and IL24 as potentially therapeutic cytokines based on their predicted ability to reverse cell states across tumor types and indeed found them to be associated with better overall and progression-free survival in the TCGA cohort. Finally, in two clinical cohorts where RNA-seq was carried out on breast cancer and multiple myeloma patients before and after therapy, cell types within the tumor microenvironment of responders showed a stronger reversal towards a healthy state compared to non-responders.Altogether, our work establishes the importance of transcriptional reversal in therapy response, particularly the role of microenvironmental transcriptional alterations. This allows us to refine the reversal principle based on pan-cell type transcriptional effects and to identify cytokines that mediate patient survival as a new class of drugs and drug targets.

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**LB277**

**β1-integrin and CD44 may both be required in modulating traction force transmission in collagen-hyaluronic acid hydrogels.**

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Mechanics of the tumor microenvironment (TME) has long been recognized as a key aspect of the tumor invasion cascade. The TME contains a mesh of fibrous proteins such as collagen fibers, elastin, and fibronectin, and glycosaminoglycans (GAGs) that fill the interstitial space. As tumor cells migrate and invade throughout the extracellular matrix (ECM), they can communicate with each other mechanically by utilizing biophysical cues such as contractile forces and fiber alignment. For example, leader cells emerging from tumor spheroids can align ECM fibers to guide follower cells. In breast cancer, hyaluronic acid (HA), one of the GAGs found in the tumor stroma, is often overexpressed in pathological tissues, and stromal HA has been correlated with poor survival. Mechanically, while cells can guide their way by aligning matrix fibers through traction force generation, it is reported that HA may impact force transmission by altering matrix microstructure in the TME. It is therefore crucial to understand how HA impacts the interaction between tumor cells and the TME.

In this study, we first investigated the bulk mechanical properties and micro-architecture of collagen-HA hydrogels (Col-HA). Our rheology measurements show that HA softens the collagen network while increasing the onset strain for strain-stiffening. Structurally, together with smaller pore sizes and thinner fibers as revealed by reflectance confocal microscopy, fiber alignment in Col-HA becomes less favorable. We then questioned how MDA-MB-231 cells generate traction forces in Col-HA using 3-dimensional traction force microscopy (3D TFM). Along with less fiber alignment around cells in Col-HA, we found that force transmission distance is reduced in Col-HA, as revealed by a shorter propagation of matrix deformation. In Col-HA, while the matrix resists alignment as the fiber network becomes more isotropic, we hypothesized that force transmission is also adhesion-dependent. Specifically, we investigated the role of β1-integrin and CD44, the key adhesion molecules to collagen fiber and HA respectively, in traction force generation. Immunofluorescence staining shows a significant increase in colocalization between actin and the two adhesion molecules in Col-HA, compared to pure collagen matrix. Interestingly, CD44 is colocalized with β1-integrin in Col-HA, but not in pure collagen. While it is widely recognized that cells transmit forces via β1-integrin in collagen matrices, we suspect that, in parallel with β1-integrin, CD44 may also be involved in traction force transmission because of its linkage between actin and HA. To examine how β1-integrin and CD44 contribute to force transmission in Col-HA, we neutralized them with antibodies and performed 3D TFM experiments. Preliminary data shows that matrix deformation decreases significantly when either β1-integrin and CD44 is blocked. In conclusion, we have demonstrated that HA reduces the mechanical communication between breast tumor cells by reducing force transmission distance. And in addition to β1-integrin, CD44 may also be required in traction force transmission in Col-HA. In the future, we will explore the differential role of β1-integrin and CD44 on tumor invasion in the presence of HA.

**LB278**

**IL-12 immunotherapy prevents hepatocellular carcinoma in a murine NAFLD induced cirrhosis model.**

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**Introduction:** Interleukin 12 (IL-12) is an important bridge between the innate and adaptive immune systems. It is mainly produced by macrophages and can bind to its receptor (IL12R) on T cells. It affects

several aspects of T cell activation, including IFN-γ and perforin production. Tumor-infiltrating $CD8^+$ T cells perform important anti-tumoral actions, which need to be overcome to allow cancer cell growth. This work studied IL-12 administration as an HCC preventive strategy in cirrhotic mice. We investigated whether IL-12 can boost the recruitment of macrophages to the liver, increase their antigen-presenting ability, and activate cytolytic T cells to inhibit HCC development in cirrhotic livers.
**Method:** 8 weeks old mice were fed a choline-deficient High fat diet (CDAHFD) for 16 weeks to create NAFLD-induced HCC. A subset of CDAHFD mice was injected intravenously with IL-12 (0.5mg/gr) weekly from 12 to 16 weeks. Mice were euthanized after 16 weeks, and liver and serum were collected for further analysis.
**Results:** After tissue collection, we observed that 50% of untreated animals developed HCC lesions, while IL-12-treated mice did not have any HCC. After treatment with IL12, qPCR and western blot on total liver tissue showed up-regulation in the IL12 receptor and activation of the IL-12 pathway, evidenced by the increase in Stat4 expression and phosphorylation. Additionally, we detected an increase in IFN-γ expression by qPCR in IL-12-treated mice. Histological analysis showed a significant decrease in fat and collagen deposition in IL-12-treated livers compared to untreated livers. IL-12-treated mice had lower serum aminotransferase enzymes (AST & ALT), indicating improved liver function. Immunostaining and flow cytometry of F4/80 (macrophage marker) showed an increase in the number of macrophages in the livers treated with IL-12. Flow cytometry data showed that the majority of macrophages present in the IL-12 livers were $CCR2^+$, a marker for recruited macrophages. Notably, IL-12-treated macrophages expressed more MHCII (antigen presentation marker) and less B7H4 (inhibitory marker). In addition, we observed a significant increase in the number of cytolytic T cells ($CD8^+$) in the IL-12 treated livers, which had higher expression of the T cell activation marker (CD25) and elevated levels of perforin and IFN-γ that are nessacery for their cytotoxic activity.
**Conclusion:** There is a lack of substantial evidence to describe the underlying mechanisms supporting the idea of cirrhosis regression and HCC prevention. Our study shows that the exogenous administration of IL-12 causes an increase in migrating profibrolysis macrophages to the liver and elevates cytolytic T-cell activity. IL-12 immunotherapy can trigger cirrhosis regression and prevent HCC development.

**LB279**
**Targeting microglial metabolic rewiring synergizes with immune checkpoint blockade therapy for glioblastoma.**
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Glioblastoma (GBM) constitutes the most lethal primary brain tumor for which immunotherapy has provided limited benefit. The unique brain immune landscape is reflected in a complex tumor immune microenvironment (TIME) in GBM. Here, single cell sequencing of the GBM TIME revealed that microglia were under severe oxidative stress, which induced nuclear receptor subfamily 4 group A member 2 (NR4A2)-dependent transcriptional activity in microglia. Heterozygous *Nr4a2* (*Nr4a2*$^{+/-}$) or microglia-specific *Nr4a2* (*Nr4a2*$^{fl/fl}$*Cx3cr1*$^{cre}$) genetic targeting reshaped microglia plasticity *in vivo* by reducing alternatively activated microglia and enhancing antigen presentation capacity for $CD8^+$ T cells in GBM. In microglia, NR4A2 activated squalene monooxygenase (SQLE) to dysregulate cholesterol homeostasis. Pharmacological NR4A2 inhibition attenuated the pro-tumorigenic TIME, and targeting the NR4A2 or SQLE enhanced therapeutic efficacy of immune checkpoint blockade *in vivo*. Collectively, oxidative stress promotes tumor growth through NR4A2-SQLE activity in microglia, informing novel immune therapy paradigms in brain cancer.

**LB281**

**HIRA suppression is a mechanism of chemotherapy resistance in ovarian cancer.**

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Ovarian cancer accounts for the fifth most common cause of cancer death among women, despite being relatively rare. This is in large because majority of cases are diagnosed at advanced stage and frequently recur after primary treatment. Despite numerous efforts to develop effective targeted therapies and immunotherapies for ovarian cancer the standard of care remains chemotherapies. We postulate that part of the problem is the paucity of preclinical research and clinical trials focused on dormant persister ovarian cancer cells that remain after chemotherapy treatments. Emerging evidence supports the idea that persister cancer cells undergo a period of dormancy following chemotherapy which enables their survival and drive late recurrence. Thus, there is dire need to understand the pathways that empower the establishment of dormancy following chemotherapies in ovarian cancer. Epigenetic reprogramming is notoriously known to enable cell fate transitions and the establishment of drug resistance. An important layer of epigenetic regulation frequently overlooked in cancer is the regulation of nucleosome composition via histone variants. Importantly, analysis of data from the human protein atlas revealed that histone H3.3 chaperone, Histone Regulator Protein A (HIRA) expression inversely correlated with poor prognosis, suggesting that HIRA suppression might play a role in the development of chemotherapy resistance. Here, we demonstrate that HIRA is a major regulator of cell fate transitions in ovarian cancer cells in response to chemotherapies. Our data indicates that HIRA levels are suppressed in response chemotherapies in cancer cells. Genetic suppression of HIRA in ovarian cancer cells triggered a non-poliferative state characterized by the induction of canonical dormancy markers (p27, an increase in p38 phosphorylation) and cell cycle arrest. Accordingly, HIRA suppression in these cancer cells renders them less sensitive to the standard of care chemotherapy agent paclitaxel. Together, our data demonstrates that HIRA levels play an important role in controlling cell fate decisions in ovarian cancer and regulate chemotherapy sensitivity.

**LB282**

**RUNX2 is a master transcription factor that determines the identity of cancer-associated fibroblast subtypes with abnormal activation in the tumor microenvironment.**

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Tumor microenvironments (TME) produce molecular signals that influence therapeutic penetration, distribution, and metabolism as well as positively or negatively regulate tumor cell growth, migration, and response to therapeutics. TME mainly comprises fibroblasts, immune cells, pericytes, endothelial cells, epithelial cells, and the tumor-specific extracellular matrix (ECM). Cancer-associated fibroblasts (CAFs) are active stromal cells in the TME, acting as signaling and remodeling centers and aiding the creation of desmoplastic tumor stroma. To better understand the identities of CAF subtypes promoting tumor or abnormal activation, it is essential to find a master transcription factor (TF) that determines the identity of cancer-associated fibroblast subtypes. RUNX2 is known as a transcription factor and is rarely expressed in normal tissues; however, it is expressed in abnormally activated fibroblastic regions. In this study, we hypothesized that a master transcription factor of RUNX2 determines the phenotype of normal fibroblasts to CAF and defines its super-enhancer landscape. To identify CAF-specific and functionally important TFs, we analyzed single-cell RNA-sequencing (scRNA-seq) from various cancer patients, focusing on CAFs. Specifically, H3K27ac and RUNX2 ChIP-seq were performed on CAFs to confirm their super-enhancer profiles. To examine the biological significance of RUNX2, we established cancer-associated

fibroblasts from patients with colorectal cancer and loss of RUNX2 using shRNA-mediated methods. Here, we assessed the effect of indirect cancer cell and CAF interaction models (conditioned media) in addition to direct methods (co-culture) using a 3D spheroid. To determine the significance of RUNX2 in established CAFs and cancer cells in vivo, HCT116 colorectal cancer cells were co-grafted with RUNX2 knockdown CAFs into NOD/SCID mice. Using a combination of a direct CAF-HCT116 3D spheroid model and HCT116 cells with CAF-conditioned media, we confirmed that knockdown of RUNX2 in CAFs suppressed the migration or invasion of cancer cells and inhibited the sphere-forming ability of cancer cells by RUNX2-depleted CAFs. Knockdown of RUNX2 CAFs also failed to aggregate spherical 3D phenotypes in the direct model. Interestingly, based on the ChIP-seq analysis of CAFs with H3K27ac and RUNX2, we confirmed that RUNX2 can remodel the super-enhancer landscape in CAFs and that the gene associated with RUNX2 was enriched in myofibroblastic signatures. Using a gel contraction assay, we showed that fibroblast contractility was significantly decreased by RUNX2 knockdown. To evaluate the importance of tumor formation in vivo, we transplanted RUNX2-knockdown CAFs with cancer cells and found that the tumor burden was lower depending on RUNX2 dysfunction. We further analyzed the correlation between the expression of RUNX2 and clinical outcomes and confirmed that RUNX2 is highly expressed in CAFs and strongly correlates with poor survival across several cancer types. Our findings indicate that RUNX2 is a promising target for myofibroblastic function and pro-tumorigenic features of the cancer microenvironment.

**LB283**

**ARC2-001 inhibits cancer-associated fibroblast-dependent cancer stem cell populations by disrupting cancer-stroma interactions.**

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ARC2-001, a natural product and FDA-approved anti-leukemic drug, has shown anticancer effects in hematological malignancies. This study investigates the inhibitory effect of ARC2-001 on cancer-associated fibroblasts (CAFs). CAFs promote cancer progression and directly stimulate cancer cell proliferation. In addition, CAFs act as therapeutic barriers to prevent the penetration of anticancer drugs and immune cells in the treatment of solid tumors such as colorectal cancer. In our previous study, we found a master transcription factor PRRX1 of stromal fibroblasts for myofibroblastic lineage progression in the cancer microenvironment. After deleting the expression of PRRX1 in various CAFs using the CRISPR-CAS9-sgRNA system, we confirmed changes in gene expression patterns and performed in silico screening to derive drugs showing similar gene expression patterns. These drugs were investigated through a gel contraction assay and indirect co-culture / direct 3D spheroid co-culture systems using various cancer cells and CAFs. Among them, ARC2-001 treatment not only significantly reduced the ECM remodeling ability of CAFs but also showed strong anticancer effects in the co-culture systems. In addition, the response to ARC2-001 was much more sensitive in CAFs than in cancer cells: the IC50 values in cancer cells were30-50nM, and the IC50 values in CAFs were 10-20nM. We produced HCT116 (colon cancer cell)-Colon CAFs spheroids, treated them with ARC2-001at low concentrations that could only affect CAFs, and analyzed RNA transcriptome changes by RNA-SEQ. We confirmed that HCT116 cells underwent EMT as well as observed an increase in the cancer stem cell (CSC) population by CAFs through co-culture of HCT116-CAFs spheroids. Interestingly, treatment with a concentration of ARC2-001 sufficient to inhibit only CAFs had no significant effect on HCT116-only spheroids, and little change occurred in gene expression patterns, but in HCT116 cells in spheroid co-culture with CAFs, the aforementioned changes (EMT and CSC population increase) all collapsed. This indicates that ARC2-001 interferes with the interactions between cancer cells and CAFs. ARC2-001 damages the cancer stem cell population of HCT116 through CAFs inhibition, indicating that the CSC subpopulation of HCT116 cells is dependent on CAFs. We further hypothesized that co-administration of ARC2-001 and commonly used

anticancer drugs could be a very effective strategy, considering the ineffectiveness of commonly used anticancer drugs on the CSC subpopulation. Thus, we transplanted colon CAFs together with HCT116 cells into NOD/SCID mice and then administered 5FU, an existing anticancer drug, together with ARC2-001. For the combined treatment in the in vivo experiment, we treated ARC2-001 and 5FU at half of the commonly used concentrations. Surprisingly, we observed that both tumor growth and cancer metastasis were suppressed in the combination treatment group. This indicates that ARC2-001 dramatically increases the effectiveness of existing anticancer drugs and can be used as an additional combination therapy for colorectal cancer chemotherapy.

**LB284**

**CLAUDIN-1 targeting antibody ALE.C04 drives single activity and restores anti-PD1 efficacy in solid tumors.**

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Claudin 1 (CLDN1) is a protein confined within the normal epithelial tight junctions of different tissues. Upon malignant transformation, CLDN1 is overxpressed and epitopes become exposed outside the tight junctions (non-Junctional CLDN1). ALE.C04 is a highly specific humanized monoclonal antibody that recognizes a unique CLDN1 exposed epitope in different solid tumors. Herein we show that non-Junctional CLDN1 (NJ-CLDN1) is frequently overexpressed in solid tumors. Pre-clinically, ALE.C04 drives tumor growth inhibition in different CDXs and PDX *in vivo* tumor models. Notably, CLDN1 expression on tumor cells positively correlates with T cell exclusion, a mechanism described to hinder the efficacy of Checkpoint inhibitors (CPIs). On this line, the overexpression of Cldn1 in mouse tumor cells promoted T-cell exclusion and resistance to anti-PD1 treatment. Importantly, ALE.C04 restored both T-cell infiltration and anti-PD1 efficacy in tumors. Mechanistically, NJ-CLDN1 interacts with different components involved in extracellular matrix remodeling, thus establishing a physical barrier that exclude immune cells from the tumor nest. ALE.C04 perturbs the interface between CLDN1+ tumor cells and the stroma, thus restoring immune cell infiltration. Our pre-clinical data showed that: i) NJ-CLDN1 is a novel and druggable target in solid tumors ii) ALE.C04 is a selective anti-CLDN1 antibody capable of driving anti-tumor activity as both single agent and in combination with anti-PD1.

**LB285**

**Single cell characterization of the adjacent primed tissues and metastatic microenvironment of adrenocortical carcinoma reveals profound molecular and cellular reprograms that dictate metastatic progression and disease outcome.**

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Metastasis is the strongest predictor of outcome in cancer; and early interventions are needed to alter disease course and patient outcome. Adrenocortical carcinoma (ACC) is a rare adrenal cancer that has high mortality due to high rate of metastasis. The therapeutic options of metastatic-ACC include surgery and combination of chemotherapeutic regimens, but with very poor outcomes. The heterogeneous composition of immune and stromal cells in the metastatic microenvironment (MME) and adjacent primed tissues, and their crosstalk with malignant cells are critical determinants of cancer metastatic progression and response to therapies, which remain poorly understood for ACC. Therefore, to comprehensively characterize ACC-MME and adjacent primed tissues, and to identify global and tissue-

specific reprogramming, we performed single cell RNA sequencing of metastatic-ACC and adjacent primed tissues from the liver and lung. Our studies revealed that ACC-MME undergoes aberrant vascularization with global depletion of capillary endothelial cells (ECs) and enrichment for Tip/ Stalk-like ECs, which are significantly associated with poor disease outcome. Moreover, ECs in ACC-MME are highly pro-tumorigenic, showing global upregulation of tumor-promoting gene signatures, and key signaling pathways responsible for angiogenesis, proliferation, migration, and immune suppression, as well depletion of effective anti-tumor immune response programs such as antigen presentation and interferon gamma signaling. Furthermore, there is a major shift in tissue-specific and universal myeloid populations in ACC-MME and adjacent primed tissues, exhibiting global enrichment of dysfunctional DCs and immunosuppressive macrophages, and liver specific pDCs in MME, whereas, MDSCs are broadly enriched in both MME and adjacent primed tissues suggesting these primed for metastasis environments are immune suppressive. CD8 T-cells in ACC-MME express a dysfunctional gene signature, showing downregulation of key effector function genes. We also found an enrichment of immunosuppressive CD4-T-regs in ACC-MME that represent dysregulation of immune checkpoint molecules including CTLA4 and LAG3. Finally, immune and stroma cell populations in ACC MME and adjacent primed tissues show a commonality in reprogramming, favorable for tumor growth and metastasis, across diverse cell and tissue types in comparison to normal tissue samples. Taken together, these findings suggest that ACC-MME and its adjacent primed tissue microenvironments are reprogrammed to immunosuppressive and tumor-promoting states that orchestrate metastasis of ACC.

**LB288**

**Biomarker analysis from AMPECT correlating response to *nab*-sirolimus with *TSC1* and *TSC2* inactivating alterations.**

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*nab*-Sirolimus is an mTOR inhibitor (mTORi) approved in the US for the treatment of adult patients with locally advanced, unresectable, or metastatic malignant perivascular epithelioid cell tumor (PEComa) based on clinical efficacy and safety data from the phase 2, multicenter, open-label AMPECT trial (NCT02494570). *TSC1* and *TSC2* are tumor suppressor genes and key upstream regulators of mTOR complex 1 (mTORC1); inactivating alterations (loss-of-function mutations or deletions) in these genes lead to mTORC1 hyperactivation, which may contribute to tumor formation. Phosphorylation of S6 ribosomal protein (pS6) is a reliable surrogate for mTORC1 activity. Here we present the results of an exploratory biomarker analysis performed on samples from patients enrolled in the AMPECT study. Targeted exome next-generation sequencing (NGS) using a 500-gene OncoPanel test (Center for Advanced Molecular Diagnostics, Brigham and Women's Hospital, Boston, MA) assessed mutations, copy number changes, and translocation events. pS6 was assessed by immunohistochemistry (IHC). Twenty-five patients had tissue sufficient for NGS, 56% (14/25) had either *TSC1* (N=5, 20%) or *TSC2* (N=9, 36%) mutations: *TSC1*, 1 frameshift, 2 splice site, 1 missense, and 1 nonsense mutation; *TSC2*, 1 nonsense, 7 frameshift, and 1 homozygous deletion. In this dataset, *TSC1* and *TSC2* inactivating alterations were mutually exclusive. Patients with *TSC1* or *TSC2* inactivating alterations achieved a clinically meaningful benefit: 64.3% (9/14)

objective overall responses (including complete and partial responses) and median (95% CI) duration of response (DOR) of 45.7 (5.6, not reached [NR]) months, progression-free survival (PFS) of 41.2 (5.5, NR) months, and overall survival (OS) of NR (31.6, NR) months. Mutations in *TP53*, *RB1*, and *ATRX* were also common (48%, 24%, and 20%, respectively).
Of tissue samples evaluable for IHC (N=25), responses occurred in 58.8% (10 of 17) of patients with pS6 positive tumors versus 0% (0 of 8) with pS6 negative tumors; absent pS6 staining was negatively associated with response to *nab*-sirolimus (*P*=0.008, Fisher's exact). Median DOR for pS6 positive responders was 39.7 (5.6, NR) months. In pS6 positive vs pS6 negative patients, the median PFS was 24.4 (2.8, 53.1) vs 5.5 (2.8, NR) months, and OS was 53.1 (18.0, NR) vs 37.0 (16.6, NR) months. A variety of pathogenic inactivating alterations were observed in *TSC1* and *TSC2* genes, though *TSC2* mutations were most commonly frameshift mutations; no recurring mutations were observed. A tumor-agnostic study (PRECISION 1: NCT05103358) is now recruiting patients with pathogenic inactivating *TSC1* or *TSC2* alterations to further examine these biomarker findings.

**LB289**

**Longitudinal ctDNA levels and clinical outcomes of first-line (1L) tislelizumab (TIS) + chemotherapy (chemo) treatment for advanced non-small cell lung cancer (NSCLC) in the RATIONALE-304 and 307 studies.**

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**Background:** The role of circulating tumor DNA (ctDNA) in monitoring response to immunotherapy in NSCLC is unconfirmed. This is a retrospective analysis of association between longitudinal ctDNA levels and clinical outcomes in 1L TIS (anti-PD-1) + chemo-treated patients (pts) with nonsquamous or squamous NSCLC from RATIONALE-304 (NCT03663205) and 307 (NCT03594747), respectively.
**Methods:** Blood samples were collected at baseline (BL), first response (FR, complete or partial response assessed by investigators), and progressive disease (PD). ctDNA level was tested by OncoScreen Plus520 (Burning Rock) and the variant allele fraction categorized as undetectable (UD)/detectable (D). Paired ctDNA analysis of BL and post-BL (FR or PD) values was by Wilcoxon sign-rank test. Median PFS and OS was calculated by Kaplan-Meier methodology. PD-L1 expression stratified Cox model was used to evaluate the effect of ctDNA on PFS and OS for BL and FR (adjusted with BL ctDNA) in each study. Impact of other BL characteristics was also assessed.
**Results:** Of 217 pts treated with TIS + chemo in RATIONALE-304, 76 (35%) at BL, 40 (18%) at FR, and 30 (14%) at PD had ctDNA results. Of 238 pts treated with TIS + chemo in RATIONALE-307, 80 (34%) at BL, 65 (27%) at FR, and 33 (14%) at PD had ctDNA results. Paired ctDNA analysis showed significantly decreased ctDNA levels from BL to FR ($P<0.0001$ in 304 and 307); no obvious change was detected from BL to PD in both studies. Pts with UD ctDNA status at FR had notably longer median PFS and OS compared with pts with D ctDNA; no such association was observed using BL ctDNA status in either study (**Table**).
**Conclusions:** FR ctDNA level is decreased from BL, and seems to correlate with clinical outcomes of 1L

TIS in combination with chemotherapy in NSCLC; ctDNA has potential to be a surrogate biomarker for efficacy. This requires further prospective validation.

**Table.Analysis summary of ctDNA and PFS/OS by visit**

| | **Baseline (BL)** | | | | **First response (FR)** | | | |
|---|---|---|---|---|---|---|---|---|
| **Study** | **RATIONALE-304** | | **RATIONALE-307** | | **RATIONALE-304** | | **RATIONALE-307** | |
| **ctDNA** | **UD** | **D** | **UD** | **D** | **UD** | **D** | **UD** | **D** |
| **n** | 19 | 57 | 8 | 72 | 32 | 8 | 43 | 22 |
| **mPFS, mo (95% CI)[a]** | 9.23(5.75, 9.89) | 9.69(7.33, 14.52) | NR(4.93, NR) | 9.76(7.52, 14.55) | 17.31(9.89, NR) | 9.20(3.71, 11.99) | 20.01(9.82, NR) | 9.56(7.39, 13.9) |
| **PFS HR (95% CI), UD/D** | 1.14 (0.61, 2.21) | | 0.40 (0.09, 1.73) | | 0.16 (0.05, 0.5) | | 0.54 (0.24, 1.21) | |
| **PFS P-value[b]** | 0.6421 | | 0.2205 | | 0.0019 | | 0.1322 | |
| **mOS, mo (95% CI)** | NR(9.72, NR) | NR(14,23, NR) | NR(NR, NR) | NR(16.89, NR) | NR(NR, NR) | 18.78(9.92, NR) | NR(NR, NR) | NR(12.85, NR) |
| **OS HR (95% CI), UD/D** | 1.04 (0.48, 2.25) | | NE | | 0.16 (0.04, 0.69) | | 0.48 (0.15, 1.51) | |
| **OS P-value[b]** | 0.9254 | | NE | | 0.0147 | | 0.2079 | |

[a]Primary endpoint assessed by IRC; [b]P-values are reported for descriptive purposes only in this exploratory study.**Abbreviations:** CI, confidence interval; D, detectable ctDNA status; HR, hazard ratio; IRC, independent review committee; mo, months; mOS, median overall survival; mPFS, median progression-free survival; NE, not evaluable; NR, not reached; OS, overall survival; PFS, progression-free survival; UD, undetectable ctDNA status

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**LB290**

**Intracranial, intratumoral drug-releasing microdevices in patients with high grade gliomas may identify biomarkers of drug activity and predict tumor response to systemic chemotherapy.**

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The lack of reliable predictive biomarkers to guide effective therapy is a major obstacle for the

advancement of therapy for high grade gliomas (HGG), and particularly glioblastoma (GBM),one of the few cancers whose prognosis has not improved over the past several decades. With this pilot clinical trial we provide first in human evidence that drug-releasing intratumoral microdevices (IMD) can be safely and effectively used to obtain patient-specific, high throughput molecular and histopathological data to inform selection of drugs based on their observed antitumor effect *in situ*. The use of IMD is seamlessly integrated in standard surgical practice during tumor resection. None of the six enrolled patients experienced adverse events related to the IMD, and the retrieved tissue was usable for downstream analysis for 11 out of 12 retrieved specimens. Molecular analysis of the specimens provided, for the first time in humans, preliminary evidence of the robustness of the readout, with strong correlation between IMD analysis and clinic-radiological responses to temozolomide. We also identified novel transcriptomic and metabolomic biomarkers of response and resistance to a range of targeted and cytotoxic agents used on the IMD. From an investigational aspect, the amount of information obtained with IMD allows unprecedented characterization of tissue effects of any drugs of interest, within the physiological context of the intact tumor.

**LB291**

**Uncovering gene fusions with 3D genomics: from clinical validation to actionable insights for undiagnosable solid tumors.**

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Identifying gene fusions in tumor biopsies is critical for understanding disease etiology; however, clinical NGS panels often fail to yield clear genetic drivers. A key challenge is that RNA-seq does not perform well in FFPE tissue blocks due to RNA degradation and/or RNA panel design, and cannot detect breakpoints outside of the gene body, which are established clinical biomarkers with mechanistic significance and clinical utility in solid hematological cancers. We developed a novel DNA-based partner-agnostic approach for identifying fusions from FFPE tumors using 3D genomics based on Arima-HiC technology and NGS. We profiled 184 FFPE tumors across various tumor types. This cohort includes 33 tumors with known gene fusions detected by RNA panels for clinical concordance analysis. It also includes 151 FFPE tumors, including 62 CNS tumors, 59 gynecological sarcomas, and 22 solid heme tumors, with no detectable genetic drivers from prior DNA and RNA panel CLIA assays. For clinical concordance, Arima-HiC technology detected 33/33 fusions previously detected by RNA panels. For clinical validation and utility studies in our driver-negative cohort, Arima-HiC detected 1 or more fusions in 72% (109/151) of tumors. To attribute clinical significance, we compared the genes implicated in our fusion calls with NCCN and WHO guidelines, and OncoKB, and assigned which tumors had an FDA-approved therapeutic level biomarker (“Tier 1”), a biomarker targeted in an ongoing clinical trial (“Tier 2”), or a diagnostic/prognostic biomarker (“Tier 3”). Arima-HiC analysis found 33.8% (51/151) of tumors had Tier 1 biomarkers, 4.0% (6/151) had Tier 2 biomarkers, and a further 14.6% (22/151) had Tier 3 biomarkers, indicating an overall yield of clinically actionable biomarkers at 52.3%. Several cases with Tier 1-3 fusions underwent confirmatory immunohistochemistry testing for oncoprotein expression, and 91.6% (11/12) showed diffuse or focally positive staining. To highlight examples from prospectively analyzed cases from this cohort, we identified a novel *PD-L1* rearrangement in a pediatric glioma tumor that was not detected by DNA or RNA panels, which we confirmed by PD-L1 IHC, and the patient was administered pembrolizumab after tumor recurrence and has since exhibited stable disease. We also identified a *MYBL1* fusion in a glioma that spared the patient unnecessary chemotherapy post-resection. Our findings provide evidence of clinical concordance, validation, and utility, and underscore the need for 3D genome profiling to increase diagnostic yield by finding clinically actionable fusions in FFPE solid tumors.

**LB292**

**GCC2 on small extracellular vesicles for the early diagnosis of lung adenocarcinoma: A multicenter trial.**

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**Background:** Early detection of lung cancer is extremely important for timely and effective therapeutic interventions to increase the survival rate of patients. Emerging evidence suggests that tumor-derived small extracellular vesicle (sEV) cargo may serve as cancer-specific biomarkers. We previously reported the identification of GRIP and coiled-coil domain-containing 2-enriched sEV (sEV-GCC2) as a promising biomarker for lung adenocarcinoma in a pilot study. In this multicenter study, we explored the diagnostic and therapeutic potential of sEV-GCC2 in early-stage lung adenocarcinoma.
**Methods:** A total of 470 blood plasma samples (150 healthy controls and 320 patients with lung adenocarcinoma) were retrospectively obtained from five institutions. sEVs were isolated by size exclusion chromatography and sEV-GCC2 was quantified by enzyme-linked immunosorbent assay. Effect of sEV-GCC2 on lung cancer cell proliferation was investigated *in vitro* and *in vivo*.
**Results:** sEV-GCC2 concentration was significantly higher in patients than that in controls with an area under the curve (AUC) of 0.856 (95% confidence interval [CI], 0.820-0.886). In patients at TisN0-T1miN0 stages vs controls, the AUC was 0.802 (95% CI, 0.734-0.859). The association between sEV-GCC2 and lung adenocarcinoma remained after adjustment for pathological TNM stage and tumor location in the left lower lobe. Immunohistochemical staining revealed that GCC2 expression was significantly higher in lung adenocarcinoma tissues than in the controls ($p < 0.001$). Furthermore, sEV-GCC2 enhanced cancer cell proliferation and accelerated tumor growth and lymph node metastasis.
**Conclusions:** We identified sEV-GCC2 as a potential diagnostic marker for very early-stage lung cancer. sEV-GCC2 represents a promising therapeutic target for lung adenocarcinoma and may be quantified as key molecules in the progression of lung adenocarcinoma.

**LB293**

**The evolution of MRD assays: Moving beyond the tumor-Informed bespoke NGS panel.**

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The non-invasive detection of circulating tumor DNA (ctDNA) from plasma has been shown to have clinical value for detection of minimal residual disease (MRD), identification of emerging resistance to treatment, and predicting treatment response. The ability to detect MRD following curative treatment allows for the stratification of patients with higher risk of disease recurrence. Multiple studies, across hundreds of patients in different indications, including lung and colon cancer, have shown that tumor-informed personalized assays have high sensitivity to detect recurrence. The tumor-informed bespoke panels utilize sequencing of the tumor tissue to identify clonal somatic variants, which are then used to generate a personalized panel to track the variants in patient plasma. Next-generation MRD technologies are aiming to provide greater sensitivity by expanding methods beyond the small, personalized panels. Utilization of increased numbers of somatic variants is predicted to achieve a greater level of sensitivity for detection of MRD.
Here we describe, C2I Genomics which provides a novel platform that exploits whole genome sequencing

of the tumor tissue and plasma to define a somatic variant signature. An important difference of this method is that it develops a personalized bioinformatic signature rather than a personalized NGS panel. To understand the potential of this novel methodology, we utilized a set of 54 contrived clinical sample dilutions from three indications, with an expected (or engineered) VAF range of 0.2-0.002%, that have commutability to the intended use population.
We found that C2I Genomics technology is highly sensitive, where for all returned samples, the assay routinely achieved detection down to the lowest dilution of 0.002% allelic frequency. Overall, the assay resulted in an average detection rate of 93% across all dilutions (n = 54). The aggregate detection rate of several tumor-informed assays, examining the same dilutions, yielded a detection rate of 68%.
In summary, the C2I Genomics assay showed robust detection from contrived samples which are representative of clinical trial samples in both cfDNA yields and allele frequency.

**LB294**

**Baseline and on-treatment plasma-based genomics as a predictor of outcome in SAVANNAH: Savolitinib + osimertinib in EGFRm MET overexpressed/amplified NSCLC post-osimertinib.**

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Savolitinib, an oral, potent, and highly selective MET-TKI, in combination with osimertinib, a 3rd-generation, irreversible, oral EGFR-TKI, may overcome common acquired MET-driven resistance following osimertinib treatment. The ongoing Ph2 SAVANNAH (NCT03778229) study is investigating this combination in pts with EGFRm NSCLC who have MET overexpression and/or amplification upon progressive disease (PD) post-osimertinib. Using plasma EGFRm as an estimate of tumor burden, we report exploratory analyses of progression-free survival (PFS) by plasma EGFRm clearance during treatment at Day (D) 22. Additionally, agreement between tissue (IHC/FISH) and plasma (NGS) testing for detecting MET overexpression and/or amplification is reported. Following central MET testing on tumor tissue collected upon PD post-osimertinib, eligible pts received oral savolitinib (300 or 600 mg QD, or 300 mg BID) + oral osimertinib 80 mg QD. MET overexpression was assessed by IHC (3+ staining in ≥50% of tumor cells [IHC50+]) and amplification by FISH (MET copy number ≥5 and/or MET:CEP7 ratio ≥2 [FISH5+]). Here, PFS analysis was performed in subgroups identified by exploratory higher biomarker cut-off levels of 3+ staining in ≥90% tumor cells (IHC90+) and/or MET copy number ≥10 (FISH10+). Plasma EGFRm (Ex19del/L858R only) analysis was conducted by ddPCR at D1 and every week thereafter for 4 weeks and at week 6. Plasma EGFRm clearance was defined as undetected EGFRm ctDNA at D22, where it was detected at D1. Baseline (BL) MET amplifications were determined via ctDNA NGS at D1. Agreement analysis was performed (DCO 27Aug2021). This analysis included 192 pts who received savolitinib 300 mg QD + osimertinib and had ≥2 post-BL RECIST scans. Of those with valid tissue FISH and IHC results and evaluable ctDNA at D1 (88% [169/192]), 124 pts had detectable plasma EGFRm. Plasma EGFRm clearance was assessed in 103 pts with detectable D1 plasma EGFRm who also had evaluable ctDNA at D22. Clearance was observed more frequently in pts with IHC90+ or FISH10+ tumors (42% [28/66]) than those with IHC90- and FISH10- tumors (16% [6/37]). Median PFS (95% CI) with vs without clearance was 11.0 (7.4, 13.0) vs 4.1 (2.7, 5.5) months in pts with IHC90+ or FISH10+ tumors. Agreement between tissue FISH and plasma NGS was assessed in 72 evaluable pts. Overall, 21 (29%) pts had MET FISH10+ tumors and 14 (19%) had plasma NGS focal MET amplification, with 11 being positive by both methods (positive percent agreement [PA]: 52%, negative PA: 94%, overall PA: 67%). Plasma EGFRm clearance was observed after 3 wks of savolitinib + osimertinib, particularly in pts with MET IHC90+ or FISH10+ tumors and was associated with improved PFS. Limited positive agreement between tissue- and BL plasma-based testing suggests IHC/FISH may be able to identify more pts likely to benefit from savolitinib + osimertinib.

**LB296**

**Clinical validation of a liquid biopsy microRNA assay for diagnosis and risk stratification of invasive cutaneous melanoma.**

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**Background:** Rates of melanoma diagnoses have increased dramatically over the past 40 years, without the proportionate decrease in mortality observed in other common cancer types. Factors that make the early and accurate diagnosis of invasive cutaneous melanoma (ICM) challenging include (i) the large number of potential locations and small size of pigmented lesions, (ii) inherent subjectivity in visual diagnostic examinations, and (iii) a lack of objective molecular biomarkers. A 38-microRNA expression signature of melanoma (MEL38) was previously identified in a plasma-based pilot study and independent validated in multiple series of solid tissue biopsies.
**Patients & Methods:** Using digital microRNA expression profiling, the diagnostic and prognostic performance of MEL38 was assessed using prospectively-collected or archival plasma from 582 patients. Criteria for inclusion was histologically confirmed ICM (n=372), or other conditions including nevi, melanoma in-situ (MIS) and non-melanoma skin cancer (n=210). A second microRNA profile was also developed, optimised to predict a patient's probability of 10-year melanoma-specific survival (MSS).
**Results:** The MEL38 score correctly identified the ICM status of 551/582 (95%) plasma microRNA profiles, with an area under the curve of 0.98 ($P<0.001$). Using a MEL38 score of >5.5 (range 1 to 10) resulted an ICM detection sensitivity of 93% and specificity of 98%. Multivariate analysis indicated the MEL38 score is significantly different between ICM vs other disease states, independent of patient age, gender, biopsy site or plasma type (fresh vs archival) at $P<0.001$. In the ICM subset of patients (AJCC Stage I to 4), the MEL38 score was significantly associated with MSS (log rank $P=0.0011$).
An optimised 12-microRNA signature (MEL12) specifically for melanoma prognosis was developed to compliment the diagnostic ability of MEL38. Rates of MSS between low, standard and high-risk MEL12 groups were 94%, 78% and 58% respectively (Log rank $P<0.001$). Risk group assignment by MEL12 was significantly associated with clinical staging (Chi square $P<0.001$) and SLNB status ($P=0.027$). Melanoma was detected in the SLNB of 87% of patients with the high risk MEL12 profile. The observed differences in MSS between MEL12 risk groups remained statistically significant when adjusted for variation in patient age, gender, systemic treatment and local vs metastatic melanoma.
**Conclusion:** In a large series of clinically annotated plasma samples, the MEL38 signature was able to accurately stratify patients with ICM from those with non-ICM conditions associated with negligible or dramatically lower risk of mortality. In patients clinically or genomically-diagnosed with ICM, the MEL12 signature can provide a prognostic assessment, significantly related to SLNB status, clinical stage, and probability of 10-year MSS. Adoption of plasma microRNA profiling in the clinic may facilitate personalised, risk-informed treatment decisions, and potentially reducing the rates of melanoma under and over diagnosis.

**LB297**

**Analytical validation of a tissue-free, multi-cancer, post-diagnosis cancer research test that uses cell-free DNA methylation profiling.**

Mohini Desai, Svetlana Rakhmanova Shchegrov, Shoujie Chai, Yifan Zhou, Tracy Nguyen, Yirang Cho, Collin Melton, Eric Scott, Manami Roychowdhury-Saha, Pei-Yun Chang, Rita Shaknovich, Byoungsok Jung. GRAIL, LLC, a subsidiary of Illumina, Inc., currently held separate from Illumina Inc. under the terms of the Interim Measures Order of the European Commission dated 29 October 2021, Menlo Park, CA.

Introduction: Cancer detection blood tests have shown clinical utility after cancer diagnosis, but many evaluate only single cancers and require tumor tissue, thereby limiting their utility. We developed a versatile, tissue-free (ie, no tumor tissue required), multi-cancer detection test ("Post-Diagnosis Cancer Research Solution") based on methylation sequencing of cfDNA from blood. This technology solution can be used to evaluate cfDNA applications in cancer research, including treatment evaluation, recurrence monitoring, and prognostic guidance. We report the analytical validation of this Post-Diagnosis Cancer Research Solution, characterizing sensitivity, specificity, precision, and input range.
Methods: cfDNA samples from cancer and non-cancer donors were analyzed. Analytical sensitivity (LoD95, limit of detection with ≥95% probability) was determined as a function of methyl variant allele fraction (MVAF), a measure of circulating tumor allele fraction. A total of 6 ng of cfDNA was used (cancer cfDNA titrated into a background of non-cancer cfDNA). LoD95 was defined as the lowest observed MVAF with ≥95% cancer signal detection across tested replicates or was estimated using probit regression for eligible samples. Analytical specificity was the rate of non-cancer classification among samples from 128 non-cancer donors. Precision (defined as concordance with the expected cancer/non-cancer result) was evaluated for 15 cancer donors tested near sample LoD95 and 8 non-cancer donors, with ≥18 replicates/donor. Reliability of classification was evaluated as a function of cfDNA input mass (0.25-100 ng total cfDNA, 14 cancer and 2 non-cancer donors).
Results: A total of 12 different solid cancer types from 22 individuals with cancer were assessed in the LoD95 analysis. Median LoD95 at 6 ng total cfDNA input was 0.023% (10th percentile 0.0037%; 90th percentile 0.04%) MVAF. LoD95 estimates from *in silico* titration analyses of >200 clinical samples across a subset of the 12 cancer types were consistent with experimental LoD95 values. Analytical specificity was determined to be 98.47% (95% CI: 94.60-99.58%). Median precision across individuals was 100% (10th percentile 87%; 90th percentile 100%). Classification performance was accurate and consistent across a wide range of cfDNA input mass (100% correct cancer/non-cancer classification from 1.5-100 ng).
Conclusions: Results demonstrate that this multi-cancer Post-Diagnosis Cancer Research Solution has high analytical sensitivity, specificity, and precision, with reliable performance across a broad cfDNA input range. This technology does not require a tumor sample and provides a cancer signal estimate in terms of MVAF. These features may enable understanding of cfDNA dynamics in a wide variety of cancers for research studies.

**LB298**
**Molecular phenotype classification of metastatic prostate cancer by cell-free DNA methylation analysis.**
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Introduction: Metastatic castration-resistant prostate cancer (mCRPC) is a heterogeneous disease which can be classified into clinically relevant subtypes based on the expression of transcription factors (TF), such as the androgen receptor (AR) and neuroendocrine markers. Neuroendocrine prostate cancer (NEPC), characterized by gain of stem-like and neuroendocrine features and lack of AR expression is a clinically aggressive variant. Due to the absence of adequate biomarkers, NEPC is usually detected at a very advanced stage. There is mounting evidence that molecular subtype changes seen in NEPC are enforced by widespread epigenetic alterations, in particular DNA methylation changes. In this study, we aim to devise a novel DNA methylation-based assay for molecular subtyping and disease monitoring from cell-free DNA (cfDNA).

Methods: We analyzed genome wide methylation patterns in 60 prostate cancer patient-derived xenograft (PDX) and 133 mCRPC tumors using array- and sequencing-based assays. We integrated DNA methylation with TF cistrome data to determine the landscape of methylation alterations at key lineage TF binding sites (TFBS). A linear regression model was trained on low-pass Enzymatic Methyl-Seq (EM-seq) cfDNA data derived from PDXs to identify molecular subtype specific DNA methylation changes at these TFBS. The model performance was optimized with *in silico* admixture experiments. This model was then used to discern tumor molecular phenotypes from cfDNA in three independent cohorts of mCRPC patients using low-pass whole genome bisulfite sequencing and EM-seq.
Results: We observed a strong association between TFBS methylation and TF expression. For lineage specific TFs such as AR and ASCL1, we identified core sets of TFBSs whose differential methylation allowed for accurate assay-independent molecular subtype classification in tumor tissues. Applying an optimized quantitative model to mCRPC patients who underwent comprehensive tissue sampling by rapid autopsy we observed perfect subtype prediction from both tissue samples and cfDNA (AUC=1). A similar analytical performance was observed in additional clinical mCRPC cohorts with cfDNA.
Conclusions: We show that methylation patterns at TFBSs can determine TF activity and can be used to classify molecular subtypes from both tumor tissue and cfDNA. For prostate cancer, we demonstrate that this approach can accurately detect NEPC by cost-effective low-pass EM-seq. More broadly, this study provides a novel analysis framework for robustly assessing molecular tumor phenotypes in cfDNA with applications in solid and liquid tumor diagnostics.

**LB301**
**Molecular pharmacology and broad synergy of the novel ATR inhibitor M1774 with DNA damaging anticancer agents.**
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Ataxia telangiectasia and Rad3-related (ATR) checkpoint kinase orchestrates DNA damage response and repair pathways stimulated by replicative stresses. Recent studies have established that pharmacological inhibition of ATR is clinically promising. As M1774 is an oral ATR inhibitor in clinical development, we explored the molecular basis by which M1774 induces cancer cell death. As a single agent, we found that M1774 suppresses cancer cell viability at nanomolar concentrations with a potency higher than ceralasertib and berzosertib, but lower than gartisertib (M4344) and elimusertib in the small cell lung cancer (SCLC) cell lines H146, H82, and DMS114. We found that M1774 efficiently suppresses the ATR/CHK1 checkpoints. While M1774 alone induced apoptosis and G2/M cell cycle arrest at micromolar concentrations, at a non-toxic low dose, M1774 enhanced TOP1 inhibitor-mediated cancer cell death by preventing replication arrest and inducing DNA damage detected by EdU and γH2AX staining. Tandem mass tagging (TMT) coupled with mass spectrometry revealed that M1774 combined with SN-38 increases the expression of replication-related proteins (TIPIN, CDC45, TIMELESS, and RPA1) and G2/M-related proteins (PLK1 and CCNB1). To establish the synergistic combinations of M1774 with clinical anticancer DNA damaging agents in preclinical models, we performed experiments in cancer cell lines, patient-derived organoids, and xenograft models. Low doses of M1774 significantly synergized with the clinical TOP1 inhibitor SN-38, the TOP2 inhibitor etoposide, cisplatin, and the PARP inhibitor talazoparib in SCLC cell lines. We also found that M1774 significantly reversed chemoresistance to DNA-damaging agents in cancer cells lacking SLFN11 expression, suggesting that SLFN11 expression can be utilized for combination therapy with M1774 as a biomarker. The synergistic efficacy between M1774 and DNA-damaging agents was confirmed in SCLC patient-derived organoids, colon cancer patient-derived organoids, and H82 SCLC xenografts. Together, these results provide insights into the molecular mechanism and potential combination strategies for M1774 in cancer therapy.

**LB302**

**Radiotherapy exposure and baseline gut microbiota predict clinical outcomes of fruquintinib plus sintilimab in microsatellite-stable metastatic colorectal cancer.**

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Background: Clinical trials demonstrated that immune checkpoint inhibitors (ICIs) combined with antiangiogenic drugs had achieved promising outcomes in the third-line and further treatment in metastatic colorectal cancer (mCRC) patients (pts) with microsatellite-stable (MSS) or proficient mismatch repair. Radiotherapy (RT) may enhance the anti-tumor effect of immunotherapy. However, the outcomes of RT on pts receiving targeted therapy plus ICIs remains unclear. Therefore, this study was aimed to investigate the association between RT exposure and clinical responses to fruquintinib plus sintilimab (F&S) in previously treated MSS-CRC and explore predictive biomarkers.
Methods: This prospective observational cohort study assessed treatment outcomes of F&S as the third- or further-line therapy for advanced CRC pts at Wuhan Union Hospital from Mar 2021 to Jun 2022 (NCT05635149). The pts were divided into two cohorts according to their RT history. The objective response rate (ORR) was set as the primary endpoint. We collected pre-treatment stool samples from pts for microbiota sequencing.
Results: A total of 55 pts were enrolled, of whom 25 pts received prior RT (RT cohort, RTC) and the other 30 pts had no exposure to RT (non-RT cohort, NRTC). Globally, the objective response rate (ORR) was 18.0%, the DCR was 56.3%, and the median PFS (mPFS) was 3.6 mo. When compared between the two cohorts, the ORR was 28.0% vs. 6.7% (OR=7.344, P=0.039, RTC vs. NRTC, the same hereinafter), the DCR was 80.0% vs. 36.7% (OR=7.991, P=0.010), and the mPFS was 6.1 mo vs. 2.6 mo (HR=0.286, P<0.001). Multivariate COX regression showed that RT was an independent factor on the PFS. Overall, 27 (49.1%) pts experienced grade 1 or 2 adverse events (AEs), and grade 3 AEs were observed in 7 (12.7%) pts. The most common AEs included hepatotoxicity, hypertension and hand-foot syndrome. Moreover, analysis of gut microbiome (n=20) showed there was also significant difference in mPFS of pts in RTC (n=8) versus NRTC (n=12) (6.1 mo vs. 3.1 mo, P=0.002). Bifidobacterium and lactobacillus enriched significantly in RTC at the genus level, and further ROC curve identified the coexistence of bifidobacteria and lactobacillus enrichment predicted greater DCR (AUC=0.909), enhancing their potential as biomarkers for response. Furthermore, we found that high abundance of lactobacillus was also strongly associated with prolonged PFS (P=0.043).
Conclusion: In this study, we found that pts with RT exposure could benefit more from F&S as the third- or further-line therapy in MSS colorectal cancer. Furthermore, bifidobacteria and lactobacillus enriched in baseline stool samples from pts in RTC, which may be potential biomarkers for outcomes. Further studies with larger scale sample size are warranted to validate our findings.

**LB303**

**Tumor microenvironment modulation by SERPINE1 increases radioimmunotherapy in murine model of gastric cancer.**

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The deposition of the extracellular matrix (ECM) is associated with poor prognosis and therapy response in gastric cancer. An elevated extracellular matrix (ECM) and interstitial fluid pressure in gastric cancer limits the targeting of HER2-expressing gastric cancer cells when radioimmunotherapy (RIT) with $^{64}$Cu-trastuzumab ($^{64}$Cu-TRZ) is utilized. Here we used Losartan (LOS), which is an antihypertensive drug, to

downregulate ECM and interstitial fluid pressure in gastric cancer mice model. After 40 mg/kg of LOS treatment, a 2-fold higher Alexa-647-TRZ uptake significantly enhanced $^{64}$Cu-TRZ uptake by positron emission tomography (PET) imaging, LOS treatment decreased the tumor volume, and enhanced the survival rate. LOS-treated samples showed reduced SERPINE1 mRNA expression compared with the controls, as determined by qRT-PCR. Downregulated mRNA expression of TGFβ-1, and upregulated mRNA expression of MMP-2 in LOS-treated samples were observed. The corresponding protein expression of SERPINE1 was confirmed using western blotting. We also confirmed that silencing of SERPINE1 increased MMP2 activity. We revealed that the enhanced therapeutic effect of $^{64}$Cu-TRZ via LOS was due to the downregulation of SERPINE1, which degrades the ECM through MMP2. Our novel combinational therapy of using $^{64}$Cu-TRZ with LOS is highly effective for treatment of gastric cancer.

**LB304**
**Mechanisms of cell death escape in glioblastoma.**
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Glioblastoma is the most common brain tumor in adults and among the deadliest malignancies *per se* with a highly invasive phenotype upon presentation. To achieve rapid colonialization throughout the central nervous system, glioblastoma cells have to be equipped with a high resistance to several forms of programmed cell death, such as apoptosis. All this occurs in the absence of any tumor-initiated signature mutations. Using a comprehensive comparative analysis combining expression profiles and functional analysis of normal brain glia cells, primary tumors and tumor-derived organoids with distinct differentiation subtypes we investigated the underlying features associated with the high resistance to cell death-induced by conventional treatment. To break this resistance, we looked into the possibility to add the small molecule inhibitor Venetoclax, that targets the Bcl-2 family, to conventional therapy. The Bcl-2 family are a number of evolutionarily-conserved proteins that share Bcl-2 homology (BH) domains and are most notable for their regulation of apoptosis at the mitochondrion. Interestingly, although stem cell-like cells (SCs) express more pro-apoptotic Bcl-2 family proteins than their differentiated progeny they remain more resistant to apoptosis, suggesting that the Bcl-2 family is not the main mediator of apoptosis resistance. In stark contrast to leukemia cells, inhibition of Bcl-2 alone has no effect on glioblastoma cells, but combining this with either Temozolomide (TMZ), the standard chemotherapeutic option, or serum starvation leads to synergistic effects. These are, however, weak hence we investigated whether compensatory mechanisms are activated. Indeed, in both SCs and DCs we found an upregulation of Mcl-1, a molecule known to compensate for inhibited Bcl-2. However, additionally blocking Mcl-1 with several different small molecule inhibitors did not further sensitize primary Glioblastoma cells. This suggests that while mechanical upregulation of Mcl-1 occurs in Glioblastoma, it is of little functional consequence. The reduced importance of the Bcl-2 family is also reflected in the non-transformed astrocytes which are precursor of glioblastoma. They already display an intrinsic high resistance to apoptotic cell death. Treatment with TMZ and modulators of apoptosis does not significantly affect their viability. Our findings suggest that the Bcl-2 family has a reduced role in mediating the survival of brain cells, at least when compared to haemopoietic cells. While a certain sensitizing effect can be achieved in Glioblastoma cells by combining cellular stressors with inhibitors of the Bcl-2 family, this is unlikely to be a sufficient to overcome therapy resistance.

**LB311**
**Increased potency of CD8-targeted fusosomes enhances CAR gene delivery to resting primary T cells.**
Jesse Green, Brian Granger, Ouwen Liang, Jesus Moreno, Andrew Tucker, Aesha Vakil, Reuben Burch,

Nicholas Mozdzierz, Matthew Scott-Skandera, Stephanie Riofrio, Mike DeCosta, Amey Gaikwad, Sanket Phadke, Kaely Gallagher, Adam Charlton, Sarah Miller, Hosein Kouros-Mehr, Misha Shamashkin, Neal Van Hoeven, Kutlu Elpek, Terry Fry. Sana Biotechnology, Seattle, WA.

Introduction: Fusosomes are viral vectors pseudotyped with modified paramyxovirus envelopes targeting specific cell types. A CD8-targeted fusosome delivering CD19CAR transgene has the potential to provide an off-the-shelf therapeutic approach to generate CD19-directed chimeric antigen receptor (CAR) T cells in patients after direct intravenous delivery or extracorporeal delivery (ECD), i.e., a short-term exposure of apheresis product to fusosome before re-infusion into the patient. Here, we demonstrate that an improved manufacturing process resulted in higher fusosome titer and quality, increasing the potency of CAR T generation from resting CD8 T cells. This potency is also observed when fusosomes are evaluated in a clinically relevant mock ECD approach.
Methods: Fusosome titer was quantified by ddPCR directly for physical titer (vector genomes, GQA) or after infections of SupT1 cells for functional titer (IU/mL). Transduction of CD8 T cells was measured by flow cytometry and vector copy number analysis after short-term (1-4 hour) incubation of PBMCs with fusosome followed by wash and culture in resting or CD3/CD28-activated conditions. To assess CD19CAR T cell generation at a mock clinical setting and scale, a healthy donor was apheresed to collect PBMCs, fusosome was administered into the apheresis bag and PBMCs were sampled from the bag over a 2.5-hour time course for further analysis. *In vivo* anti-tumor efficacy of the fusosome was measured in immune-deficient NSG mice engrafted intravenously with Nalm6, then PBMCs followed by fusosome one day later. Tumor growth was monitored by bioluminescence imaging.
Results: Fusosomes produced by the improved process had both a higher functional concentration (>14-fold higher IU/mL) and better infectivity (>10-fold higher IU/GQA) in comparison to fusosomes produced by the original process. Exposure of PBMCs to fusosomes produced by the improved process led to increased fusosome binding to CD8 T cells at high IU/PBMC doses and higher transduction efficiency in CD8 T cells at the same calculated dose per cell compared to fusosomes produced by the original process (>3-fold higher mean CAR+ percentage after 2-hour exposure to 2 IU/PBMC, $n$=8). Furthermore, fusosomes produced by the improved process generated higher frequencies of CAR+ CD8 T cells and resulted in increased ability to control tumor growth at lower doses *in vivo* in a Nalm6 model. Importantly, the ECD approach translated well to a mock clinical setting, resulting in successful generation of CAR T cells (12.6% of CD8 T cells after a 2.5-hour incubation).
Conclusion: CD8-targeted fusosome encoding a CD19CAR transgene represents a potential novel therapeutic approach for cancer. This study demonstrates that improvements in the manufacturing process can significantly increase the potency of the fusosome in T cells, and we intend to move forward to clinical trials using this improved process.

**LB313**

**EGFRvIII-targeted alpha therapy shows significant therapeutic efficacy as both a single-agent and in combination with standard of care against preclinical GBM models.**

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Targeted alpha therapy (TAT) is a rapidly advancing class of radiotherapeutics that can effectively deliver potent and local radiation to cancer cells while sparing the surrounding normal cells. TATs hold great promise for treatment-resistant tumors such as glioblastoma multiforme (GBM) due to the extensive DNA damage and cell death induced by alpha particles. GBM is an aggressive and lethal primary adult brain

tumor that is highly resistant to external beam radiation and chemotherapy. Herein, we present the preclinical evaluation of a novel TAT for treatment of GBM targeting the most common tumour-specific mutant, epidermal growth factor receptor variant 3 (EGFRvIII). Our EGFRvIII TAT consists of a humanized EGFRvIII monoclonal antibody, a proprietary bifunctional chelate, and the alpha-emitting radionuclide, actinium-225 ($^{225}$Ac). *In vivo* biodistribution and efficacy of our EGFRvIII TAT was evaluated in two aggressive orthotopic EGFRvIII-expressing GBM patient-derived xenograft models (PDXs; G06 and G39) with varying degrees of blood-brain-tumor barrier (BBTB) permeability. Imaging biodistribution studies were performed using an [$^{111}$In]-anti-EGFRvIII agent analogous to our [$^{225}$Ac] therapeutic candidate. SPECT/BLT imaging 96 h after intravenous administration of the imaging agent revealed high tumor-specific uptake of 50.2 %ID/cc in G39 tumors with a disrupted BBTB, and 10.4% ID/cc in G06 tumors with a relatively intact BBTB. Normal brain showed very low uptake (1.6-2.1% ID/cc), and all other normal organs were <10 % ID/cc except for highly perfused organs such as heart and liver, indicating no significant off-target uptake. [$^{225}$Ac]-anti-EGFRvIII single-dose and fractionated multi-dose efficacy studies were conducted in G06 and G39 orthotopic PDX models, respectively. Survival analysis showed a dose-dependent increase in survival in response to treatment with [$^{225}$Ac]-anti-EGFRvIII therapy, with a greater than 3-fold increase in survival compared to vehicle controls at doses of 200-400 nCi. Furthermore, the survival benefit of the 400 nCi single-dose or fractionated equivalent dose groups were comparable, demonstrating that efficacy was dependent upon the total administered radioactivity dose. Finally, we show that combined treatment of [$^{225}$Ac]-anti-EGFRvIII with the standard of care (SoC), external beam radiation plus temozolomide, resulted in a significant survival advantage (>1.7-fold) compared to TAT or SoC therapy alone. Collectively, these results demonstrate that [$^{225}$Ac]-anti-EGFRvIII is a highly selective and potent therapeutic for GBM which holds great potential as both a single-agent and in combination with SoC.

**LB314**

**Epigenetic regulator Menin-MLL1 maintains cancer stem cells and promotes neuroblastoma growth.**

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Epigenetic regulators MLL1 and Menin plays important role in cancer progression, metastasis, and relapse. Apart from the oncogenic translocations in leukemias, MLL1 forms a COMPASS complex with Menin and other binding partners to act as an H3K4 methyltransferase. Inhibition of COMPASS complex by blocking the Menin-MLL1 interaction is reported to be effective in different cancers. In this study, we found that a specific Menin-MLL1 inhibitor, MI-503 inhibits neuroblastoma cancer stem cells (NB CSCs; CD114+ cells) to inhibit overall neuroblastoma (NB) growth in both *in vitro* and *in vivo* NB tumor models. NB is an extracranial solid pediatric cancer that accounts for about 15% of all pediatric cancer-related deaths, with the long-term survival of relapsed NB patients being less than 10%. We found that MI-503 significantly and in a dose-dependent manner inhibits cell proliferation in nine NB cell lines tested including MYCN amplified (NGP, LAN-5, IMR-32), MYCN non-amplified (SH-SY5Y, SK-N-AS, CHLA-255), and primary patient-derived NB cell lines (COG-N-415, COG-N-269, COG-N-357), in contrast to control fibroblasts. Additionally, MI-503 significantly inhibits NB 3D spheroidal tumor growth and live cell counts compared to control treatments. Further, flow cytometry analysis revealed that MI-503 specifically induces apoptosis and blocks cell cycle progression at the S phase in the NB CSCs (CD114+ cells) in comparison to non-CSCs (CD114- cells) that leads to significant inhibition of overall NB CSC levels in different NB cell lines. By inhibiting the COMPASS complex, MI-503 significantly inhibits H3K4me3 levels as determined by the histone immunoblot assays. This leads to the inhibition of multiple cancer stemness-related genes expression such as OCT4, Nanog, HOXA, AF9, and MEIS1. Further, we determined the efficacy of MI-503 in NB xenograft mouse models and found that MI-503 significantly inhibits overall NB tumor growth (12.3 fold; $p<0.001$) and tumor burden (10.2 fold; $p<0.01$)

by directly inhibiting tumor NB CSCs ($p<0.01$). We have not observed any drug-treatment-related toxicities or gender bias. Overall, our study highlights a novel epigenetic-based therapeutic approach for high-risk NB and underlines the role of epigenetic regulators in maintaining the NB CSCs and NB growth. In our future efforts, we will develop concomitant therapeutic approaches by combining MI-503 with current chemotherapies to target both NB CSCs and bulk tumor cells. This will lead to the development of effective, targeted, and clinically tractable therapies for NB patients.

**LB315**

**Inhibition of FGFR4 with futibatinib combined with inhibition of IGF1R, Src family kinases, or AKT is synergistic against rhabdomyosarcoma.**

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**Background:** FGFR4 is a receptor tyrosine kinase shown to be commonly overexpressed in rhabdomyosarcoma (RMS). In fusion positive (FP) RMS, *FGFR4* overexpression is driven by the PAX3-FOXO1 fusion oncogene. In fusion negative (FN) RMS, *FGFR4* is often overexpressed and mutationally activated in 7.5% of patients. CRISPR knockout experiments have shown that many RMS cell lines are dependent on FGFR4 for survival. Thus, targeting FGFR4 with a small molecule inhibitor is a promising approach to treat RMS. Futibatinib, a highly selective, irreversible FGFR1-4 inhibitor that was recently granted FDA approval for cholangiocarcinoma, may be effective in treating RMS. Single agent small molecule therapy with kinase inhibitors commonly leads to acquired resistance through compensatory signaling pathways. We hypothesized that inhibition of FGFR4 would lead to suppression of negative feedback loops, resulting in activation of oncogenic kinase pathways. Furthermore, we hypothesized that dual inhibition of FGFR4 and one of these upregulated kinases would be synergistic in delaying or reversing RMS progression.

**Methods:** Cell viability assay was used to determine the $IC_{50}$ of futibatinib in RMS cell lines in which *FGFR4* is mutationally activated (RMS559, FN RMS) or overexpressed (RH4, FP RMS). A high throughput kinome activity assay was used to discover kinases that were activated in RMS cells treated with futibatinib. A drug matrix screen combining futibatinib with 144 drugs was used to discover synergistic combinations. Incucyte live cell imaging was used to validate the synergy of the inhibitor combinations. Western blot was used to confirm the activity of the kinase inhibitors. Caspase activity assays were used to monitor apoptosis.

**Results:** The $IC_{50}$ of futibatinib was ~500 nM for RMS559 and ~10 μM for RH4. Western blot showed that futibatinib inhibited FGFR4 phosphorylation in a dose dependent manner. The kinome activity assay found that futibatinib treatment resulted in SFK, AKT, and IGF1R activation. Drug matrix screening in RMS559 found that futibatinib was synergistic with SFK, IGF1R, and AKT inhibitors at cell killing. Incucyte confluence measurements confirmed the synergy of futibatinib with SFK, IGF1R, or AKT inhibitors in both RMS559 and RH4. Apoptosis was significantly higher in RMS cells treated with these combinations compared to individual inhibitors.

**Conclusions and future directions:** These results suggest that treating RMS with an FGFR4 inhibitor combined with an SFK, IGF1R, or AKT inhibitor is synergistic in both *FGFR4* mutated FN RMS and *FGFR4* overexpressed FP RMS. These dual inhibitor therapies may be an effective approach in overcoming resistance to monotherapy and lead to better outcomes in FGFR4-dependent RMS patients. Additionally, our approach may be beneficial for other FGFR-dependent cancers which may develop resistance to monotherapy. Animal studies are underway to determine the *in vivo* efficacy of these combinations, and top hits will be considered for translation to the clinic.

**LB316**

**A next-generation KRAS$^{G12C}$ inhibitor ABSK071 demonstrated broad synergy with other therapeutic agents in KRAS$^{G12C}$ mutated cancer models.**

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**Background:** KRAS is frequently mutated in human cancers, including pancreatic (~90%), colorectal (~35%), and lung cancer (~25%). The KRAS$^{G12C}$ mutation (single amino acid substitution of cysteine for glycine at position 12) accounts for ~14% of lung cancer, ~4% of colorectal cancer, and ~2% of pancreatic cancer. Currently, two covalent KRAS$^{G12C}$ inhibitors, namely sotorasib (AMG-510) and adagrasib (MRTX-849), have been approved as monotherapy to treat locally advanced or metastatic NSCLC with KRAS$^{G12C}$ mutation through accelerated approval process. Despite the beneficial effects of KRAS$^{G12C}$ inhibitors in clinic for certain patients, the limited antitumor efficacy in most patients and potential drug resistance are major concerns. A next-generation inhibitor with better inhibitory activity may improve anti-tumor efficacy. Combination with other therapeutic agents may also improve the single-agent activity of KRAS$^{G12C}$ inhibitors. These approaches could both overcome the limitations of sotorasib and adagrasib and provide additional benefits to patients.
**Materials and methods:** KRAS$^{G12C}$ inhibitory activity of ABSK071 was examined *in vitro* and *in vivo* in comparison with sotorasib and adagrasib. To identify potential targeted agents synergizing with ABSK071, we tested a set of inhibitors that targeting signaling components of KRAS pathways, including EGFRi, FGFRi, SHP2i and SOS1i, in combination with ABSK071 in cellular experiments using KRAS$^{G12C}$ mutant cell lines. Cell growth inhibition was measured and synergistic effect was analyzed. *In vivo* efficacy study was also conducted to confirm the synergistic anti-tumor effect in animal models. A set of immuno-oncology reagents, including anti-PD-1/L1, CSF-1R inhibitor (ABSK021) and CD73 inhibitor (ABSK051) were also tested in combination with ABSK071 *in vivo* using mouse syngeneic models.
**Results:** ABSK071 demonstrated much stronger inhibitory activity than sotorasib and adagrasib against a variety of cell lines harboring KRAS$^{G12C}$, as well as significantly better *in vivo* anti-tumor efficacy in xenograft models that were less sensitive to sotorasib. Synergistic effects on cell growth inhibition were observed *in vitro* with ABSK071 in combination with several agents including cetuximab, afatinib, AZD4547, TNO-155, RMC-4630 and BI3406. Superior anti-tumor activities were observed *in vivo* when these agents were combined with ABSK071 than in combination with sotorasib. In mouse syngeneic models harboring Kras$^{G12C}$ mutations, ABSK071 also demonstrated synergy with anti PD-1/L1 antibody or other IO reagents.
**Conclusions:** ABSK071 is a next-generation KRAS$^{G12C}$ inhibitor with greater activity and anti-tumor efficacy *in vitro* and *in vivo*. It also demonstrated broad synergistic effects with a large set of targeted agents and immuno-oncology agents, indicating its strong potential in combinatory therapy in treating a wider range of KRAS$^{G12C}$-dependent cancers.

**LB317**

**Discovery and characterization of a next-generation FGFR inhibitor overcoming FGFR resistant mutations.**

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**Introduction:** FGFRs play important roles in cancer development and inhibition of FGFR could disrupt tumor cell proliferation and growth. Four selective FGFR inhibitors have been approved (erdafitinib, pemigatinib, infigratinib, and futibatinib) and several others are in clinical development. Unfortunately

upon treatment with these first-generation FGFR inhibitors, acquired resistance often develops and is frequently associated with the emergence of secondary FGFR2/3 kinase domain mutations. Therefore, selectively targeting FGFR2/3 as well as their resistant mutations may render a second-generation treatment approach for the refractory/relapsed patients. Using advanced computation-aided structural analysis and medicinal chemistry design, we have discovered a novel, next-generation, and highly selective FGFR inhibitor, ABSK121. This novel inhibitor demonstrated robust anti-tumor activity in FGFR-dependent tumor models with strong activities against not only *de novo* but also acquired resistant mutations.
**Method**: ABSK121 was evaluated in biochemical and cellular proliferation experiments for its inhibition on wile type FGFR enzymatic activity and FGFR-dependent cancer cell proliferation. Its potency against FGFR mutations was also analyzed in relevant biochemical and cellular experiments. Efficacy studies were conducted in multiple tumor models to confirm its in vivo activities. Preliminary selectivity profile and ADME profiles were also evaluated.
**Results**: ABSK121 inhibited wild type FGFRs with IC50<10 nM in biochemical assay. ABSK121 also displayed great potency against resistant kinase domain mutations. In cell lines harboring FGFR amplification, fusions, or resistant mutations, ABSK121 demonstrated strong anti-proliferation activity as well as strong inhibition of FGFR downstream signaling activities. In preclinical *in vivo* studies, oral administration of ABSK121 strongly inhibited the growth of subcutaneous xenograft tumors dependent on wild type or resistant mutant FGFR. Suppression of tumor growth was dose-dependent and well correlated with pharmacodynamic inhibition of FGFR signaling. ABSK121 also showed great kinase selectivity with no CYP or hERG inhibition.
**Conclusion**: ABSK121, presented here by Abbisko Therapeutics, is a highly potent, selective, and next-generation small molecule FGFR inhibitor with great potency against resistant FGFR mutations. Its superior profile supports fast-track preclinical and clinical development.

**LB318**
**SB-4826, a first-in-class oral, covalent inhibitor of SUMO E1 that induces IFN signaling and inhibits tumor growth as monotherapy and in combination with immune checkpoint blockade.**
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Sumoylation is a reversible post-translational modification of proteins by small ubiquitin-like modifier (SUMO) that regulates protein function and contributes to cellular response to stress. Sumoylation is an essential pathway for all cells and is involved in diverse processes such as inflammation, DNA damage response, signaling and cell survival/apoptosis. Due to its involvement in such cellular functions, sumoylation intersects with the majority of the hallmarks of cancer. Many cancers display elevated expression of SUMO pathway components, a mechanism co-opted by tumors to survive under stressful conditions. In addition to its tumor intrinsic functions, sumoylation has emerged as a novel target for activating anti-tumor immunity due to its role in regulating type I interferon (IFN) signaling. Targeting sumoylation is a compelling anti-cancer strategy due to the multiple mechanisms of action that may drive anti-tumor activity for cancer patients. We report the discovery of SB-4826, a novel, orally active, covalent small molecule inhibitor of the Sumo Activating Enzyme (SUMO E1), the initiating enzyme of the sumoylation cascade. SB-4826 selectively and irreversibly binds an allosteric pocket of SUMO E1 and drives potent biochemical and cellular activity. SB-4826 inhibits global sumoylation in cells and blocks proliferation across a broad panel (100+) of cancer cell lines. Cysteine-proteome profiling by mass spectrometry indicated that Cys-30 of SUMO E1 was the only cysteine-containing peptide (out of 14,541 measured) that met the criteria for covalent engagement indicating exquisite selectivity across the cellular proteome. In an in vivo pharmacodynamic model measuring IFN signaling SB-4826 treatment led to dose- and time-dependent induction of IFN regulated cytokines. SB-4826 treatment resulted in significant

tumor growth inhibition in multiple in vivo models. In a syngeneic A20 lymphoma model SB-4826 led to tumor stasis. In a human RAJI xenograft model, SB-4826 induced near-complete inhibition of tumor growth as a single agent and resulted in tumor regression when combined with rituximab. In a mouse CT-26 colorectal tumor model which is generally refractory to anti-PD-1 treatment, SB-4826 significantly inhibited tumor growth as monotherapy and led to 60% complete tumor responses when combined with anti-PD-1. SB-4826 caused increased CD8+ T cell infiltration in CT-26 tumors supporting the role of SB-4826 in inducing IFN signaling and leading to a tumor microenvironment that is more responsive to immune checkpoint inhibition. SUMO E1 is a clinically validated cancer target as evidenced by recent data showing encouraging responses to TAK-981, an IV-administered, ATP-competitive inhibitor. SB-4826 is a differentiated and optimized SUMO E1 inhibitor that is administered orally and demonstrated improved activity in preclinical models. SB-4826 has favorable properties for development, is a new and promising cancer therapy, and is entering phase 1 clinical studies in 2023.

**LB319**

**IAG933, a selective and orally efficacious YAP1/WWTR1(TAZ)-panTEAD protein-protein interaction inhibitor with pre-clinical activity in monotherapy and combinations.**

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The YAP-TEAD protein-protein interaction (PPI) is a critical event known to mediate YAP oncogenic functions downstream of the Hippo pathway. Current advanced pharmacological agents which aim at inhibiting YAP-TEAD oncogenic function do so by engaging into the lipid pocket of TEAD. Thereby the consequences of a direct pharmacological disruption of the interface of YAP and TEADs remain unexplored. Here we report the identification of IAG933, the first molecule able to potently and directly disrupt the YAP/TAZ-TEADs PPI with suitable properties to enter in clinical trial. The path to drug discovery was established by structure-based optimization of a truncated natural YAP peptide allowing the pharmacophore mapping of TEAD coil binding site. Based on *in silico* screening, validated hit was optimized using structure- and property-based lead optimization yielding IAG933, whose chemical structure will be for the first time disclosed here. Biochemical and cellular assays demonstrate that IAG933 specifically abrogates the interaction between YAP/TAZ coactivators and all four TEAD isoforms, thus selectively inhibiting TEAD-driven transcriptional activity and inducing anti-cancer effects. At the epigenome level, YAP eviction from chromatin was observed upon treatment with IAG933, while leaving TEADs genomic occupancy unaffected. Concomitantly, engagement of co-repressor VGLL4 translated to a decrease in enhancer activity with rapid and progressive changes in transcription of Hippo target genes. In preclinical experiments, IAG933 linear pharmacokinetics was consistent with dose proportional TEAD transcriptional inhibition and anti-tumor efficacy in xenograft and primary-tumor derived malignant pleural mesothelioma models. Daily treatment with IAG933 elicited complete tumor regression in the MSTO-211H xenograft model at well-tolerated doses. In line with the current clinical strategy for IAG933, robust anti-tumor efficacy in cancer models bearing NF2 loss of function or expressing TAZ-fusions was observed. Moreover, we provide evidence for combination benefits of IAG933 with several MAPK/KRAS inhibitors, both *in vitro* and *in vivo*, in non-Hippo altered models including lung, pancreatic and colorectal cancer. Overall, our results provide a rationale of progressing IAG933 as a monotherapy in patients with Hippo-mutated cancers, and as a combination partner in MAPK-dependent cancers, with the potential to treat patient populations of high unmet medical need.

**LB321**

**Discovery and characterization of QTX3046, a potent, selective, and orally bioavailable non-covalent KRAS$^{G12D}$ inhibitor.**

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The *RAS* family of proto-oncogenes are the most frequently mutated genes in cancer, in which mutations in *KRAS* account for approximately 25% of all human cancers. *RAS* oncogenes impair the ability of RAS to convert from its active GTP-bound form into its inactive GDP-bound state leading to the sustained activation of the MAPK signaling pathway and ultimately driving tumorigenesis. While recently approved covalent KRAS inhibitors have demonstrated clinical benefit in lung, colorectal, pancreatic, and other solid tumors, the requirement for the cysteine mutant limits potential applications to the approximately 10% subset of *KRAS* patients bearing a *KRAS$^{G12C}$* mutation. As the most prevalent *KRAS* mutation, *KRAS$^{G12D}$*–driven malignancies represent a high unmet need, attracting immense attention in drug discovery. However, direct KRAS$^{G12D}$ inhibitors described in the literature to date lack oral bioavailability.

Here we show that QTX3046 is a potent, highly selective, and orally bioavailable non-covalent KRAS$^{G12D}$ inhibitor. QTX3046 demonstrated picomolar binding affinity (0.01 nM) to the inactive form of KRAS$^{G12D}$ by SPR, > 400-fold affinity over the inactive KRAS$^{WT}$ protein, and inhibited SOS1/2-mediated nucleotide exchange with picomolar potency (0.1 nM). QTX3046 also displayed nanomolar binding affinity to the GppNHp-bound "ON"-state of KRAS$^{G12D}$ by SPR. Allosteric activity of QTX3046 selectively disrupted KRAS$^{G12D}$:RAF1 protein-protein interaction in a concentration-dependent manner using both biochemical and cell-based (NanoBiT) target engagement assays, while showing weak or no activity against KRAS$^{WT}$ and other KRAS mutants. Western blot analyses and CTG proliferation assays illustrated that QTX3046 treatment inhibited downstream ERK phosphorylation and cell proliferation selectively in *KRAS$^{G12D}$*-driven cancer cell lines. KRAS$^{G12D}$ selectivity was further confirmed in panels of isogenic SW48 human colorectal cancer and mouse embryonic fibroblast (MEF) cell lines harboring various single *RAS* mutations.

QTX3046 achieved sustained systemic exposure levels required for efficacy following single oral administration in mice, indicating potential durable inhibition of KRAS signaling *in vivo*. In a *KRAS$^{G12D}$*-driven xenograft model, oral administration of QTX3046 twice daily (BID) achieved tumor regression in 100% of tumors and was well-tolerated. The physiochemical profile for QTX3046 is generally favorable with good solubility. The systemic clearance in rats and dogs was high to moderate, respectively and despite low apparent permeability, demonstrated good oral bioavailability in each species. The current PK profile and preclinical proof-of-concept data support advancement of QTX3046 into IND enabling studies to support potential clinical investigation in *KRAS$^{G12D}$*-driven solid tumor indications.

**LB322**

**Antitumor activity of 1st-in-class pan-RAS inhibitor ADT-1004 in mouse models of pancreatic ductal adenocarcinoma.**

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Pancreatic ductal adenocarcinoma (PDA) is the 4th leading cause of cancer death in the U.S. with only

about a 10% five-year survival rate and an estimated 60,000 deaths/year by 2030. Poor survival is frequently due to advanced disease at the time of diagnosis, as well as the high prevalence of KRAS driver mutations. Currently, approved KRAS-targeted therapeutics are designed to covalently inactivate only KRAS-G12C mutants, whereas the most common mutations in PDA are KRAS-G12D, KRAS-G12V, and KRAS-G12R. Thus, the need for novel RAS-targeted therapeutics with efficacy in tumors with these more common mutations is of utmost urgency to reduce PDA disease burden. We have synthesized a novel chemical class of pan-RAS inhibitors with unique biological properties and attractive drug-like properties. As a prodrug, ADT-1004, generates an active metabolite, ADT-007, that potently inhibits the growth of human and murine PDA cell lines *in vitro* with single-digit nM $IC_{50}$ irrespective of specific RAS mutational codon or isozyme by inhibiting activated RAS and signaling downstream of KRAS (ERK1/2 and AKT phosphorylation). ADT-1004 is well tolerated with no discernable toxicity at oral dosages of up to 175 mg/kg bid. Pharmacokinetic studies demonstrated that ADT-1004 produces sustained plasma concentrations of ADT-007 ~50-fold above $IC_{50}$ values, with even higher concentrations in both subcutaneous and orthotopic pancreatic tumors. Once daily oral administration of ADT-1004 significantly inhibited the growth of orthotopically implanted PDA tumors with a corresponding reduction in RAS/MAPK signaling. ADT-1004 represents a 1st-in-class pan-RAS inhibitor with potential advantages over mutant-specific KRAS inhibitors for greater efficacy and ability to avert mechanisms of resistance. These data support future clinical trials of ADT-1004 as a monotherapy for the treatment of patients with PDA regardless of the underlying mutation.

**LB323**

**Inhibition of ENPP1 using small molecule, SR-8541A, enhances the effect of checkpoint inhibition in cancer models.**

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Purpose: It has become increasingly clear that the activation of both the innate and adaptive immune systems is vital to provide the best outcomes with immunotherapies. As part of the adaptive immune response, checkpoint inhibitors (CIs) have shown promise in the clinic, but seem to only work in a small subset of cancers with response rates below 20%. It is anticipated that activation of the innate immune response may help sensitize multiple cancer types to adaptive immune therapies. cGAS-STING pathway, which is activated in response to cytosolic DNA, has emerged as a key mechanism to activate innate immunity, primarily through type I interferon (IFN) signaling. Several direct STING agonists have been developed but their performance in the clinic has been dissatisfactory. A key limitation with direct STING agonists is the widespread expression of STING in normal tissues, whereby the hyperactivation of STING can lead to a systemic cytokine storm. Thus, there is a need to identify alternative approaches to activate STING in a controlled manner. ENPP1 is the only known direct negative regulator of the STING pathway that hydrolyzes 2′3′ cGAMP, the direct ligand of STING. Highest levels of 2′3′ cGAMP are found in tumors and recent evidence suggests that 2′3′ cGAMP acts locally, as a paracrine immune transmitter. Therefore, inhibition of ENPP1 may produce superior outcomes by activating STING in the tumor microenvironment. Previously, we reported the development of SR-8541A, a highly selective and potent inhibitor of ENPP1 that activates the STING pathway. Here, we show that the inhibition of ENPP1 with SR-8541A enhances the effect of CIs in breast and colon cancer models.
Methods: Immune infiltration assays were conducted using human breast cancer cell line derived organoids (MDA-MB-231 and MDA-MB-468). Co-cultures of cancer organoids and immune cells (PBMCs) were exposed to SR-8541A +/- CIs (CTLA-4 and/or PD-1) for 48 hours. Confocal Z-Stack imaging, RT-PCR, and MSD cytokine assays were performed to evaluate the effects. *In vivo* studies were conducted using syngeneic mouse models (CT-26 and EMT-6), which were engrafted subcutaneously and treated with SR-8541A +/- CIs. Tumor growth was monitored over the course of the study. IHC and RT-

PCR were conducted on the tumors.
Results: Combination of SR-8541A with CIs showed a significant increase in immune infiltration in both the MDA-MB-231 and MDA-MB-468 organoid models. Corresponding RT-PCR analysis showed activation of IFN signaling (IFN-ß, CXCL10, ISG15). *In vivo* combination with CIs also exhibited a significant increase in overall efficacy, along with increased levels of CD3+ and CD8+ T-cell infiltration into the tumors, and increased levels of IFN response.
Conclusion: In summary, we show that combination of ENPP1 inhibition with checkpoint inhibition promotes a robust antitumor activity by stimulating both innate and adaptive immune response.

**LB324**
**BB3008, a potent and selective small molecular HPK1 inhibitor effective in multiple syngeneic tumor models.**
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Introduction: Hematopoietic progenitor kinase 1 (HPK1) is a serine/threonine kinase in the MAP4K family predominantly expressed in immune cells, and has been identified as a negative regulator of T cell and B cell receptor signaling and dendritic cell function. Inhibition of the kinase activity of HPK1 results in the activation of exhausted T cell in tumor microenvironment and enhanced anti-tumor immune response. Observation of HPK1 overexpression in various tumor tissues further indicates the kinase as a novel target for immuno-oncology therapy. Here we report BB3008 as a small molecular HPK1 inhibitor.
Methods: BB3008 was developed through structure-based drug design, and optimized by SAR analysis and medicinal chemistry iteration. Biochemical assays and cell-base functional assays were applied in compound evaluation. Pharmacokinetic (PK) and preliminary toxicity studies were performed with mice and dogs. Syngeneic tumor models in mice were conducted to demonstrate the *in vivo* efficacy of BB3008 as a single agent and in combination with mouse PD-1 antibody.
Results: BB3008 shows sub-nanomolar HPK1 potency, with good selectivity against kinases in the MAP4K family and other TCR signaling-related kinases, such as FYN and ZAP70. Inhibition of HPK1 by BB3008 strongly suppresses the phosphorylation at Ser376 of the downstream biomarker protein SLP-76, with IC50 at 30 nM. With the stimulation of CD3/CD28, boost of IL-2 production was observed in Jurkat cells, purified human T cells, and human peripheral blood mononuclear cells upon treatment of BB3008 at sub-micromolar concentration. BB3008 also enhanced the killing activity of T cell against EL4 lymphoma cell *in vitro* in a concentration-dependent manner. PK profile of BB3008 showed good bioavailability (>80%) with a nearly linear dose-dependent exposure. The compound had no significant suppression on various normal cell lines proliferation, and had no hERG liability. In preliminary safety evaluation, no abnormal clinical symptoms and serum/blood tests were observed in mice and dogs dosed up to 1000mpk and 150mpk for 14 days, respectively. BB3008 showed significant tumor-growth inhibition rates in CT26, Hepa 1-6 and 4T1 mouse models as single agent, and in MC-38 mouse model in combination with mouse PD-1 antibody.
Conclusion: As a potent, selective and oral available small molecule HPK1 inhibitor, BB3008 shows good safety tolerance in preclinical animals, and promising efficacy in multiple solid tumor models, both as single agent or in combination with immune checkpoint blockade. The IND-enabling study of BB3008 is in progress.

**LB326**
**Combined inhibition of BRD4 and FAK1 as a novel therapeutic strategy for neurofibromatosis type 2 related schwannomas.**
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Neurofibromatosis type 2 (NF2) is a rare disorder that is inherited in an autosomal-dominant manner and

is attributed to the loss of heterozygosity (LOH) of the NF2 gene, which encodes for the tumor suppressor protein Merlin. Patients affected by the disease develop vestibular schwannomas (VS), meningiomas, and ependymomas resulting in high morbidity and premature mortality. To date, neurofibromatosis type 2 has no FDA-approved drug-based treatment. Merlin plays a central role in mediating cell contact inhibition (CI) of proliferation. Loss of Merlin leads to abnormal activation of multiple signaling pathways, including those regulated by small G-proteins Ras/Rac/cdc42 and the Hippo-YAP pathway. The Hippo-YAP pathway plays a major role in cell growth and organ size control and at its core is comprised of a kinase cascade that regulates the transcriptional regulator YAP. The function of YAP is crucial for VS development, and there are several mechanisms by which YAP regulates transcription. Some of these functions have been shown to involve the Bromodomain and Extra-Terminal domain (BET) proteins acting as co-factors. We have previously shown that the BET inhibitor JQ1 can selectively reduce the growth of the *NF2*-null schwannoma and Schwann cells *in vitro* and tumorigenesis *in vivo*. Additionally, evidence has shown that Merlin loss can lead to increased cell proliferation that it requires the activity of Focal Adhesion Kinase 1 (FAK1). We have previously demonstrated that FAK1 inhibition via Crizotinib has antiproliferative effects in NF2-null Schwann cells, and is currently in phase II clinical trials in NF2-related VS. In this study, wild-type and NF2-null Schwann cells and schwannoma cell lines were used to determine the impact on cell growth by employing the treatment with the BET inhibitor JQ1 and the FAK1 inhibitor Crizotinib. Our analysis in NF2-null Schwann cells and schwannoma cell lines shows that the combined inhibition exerts an antineoplastic effect at nanomolar concentrations. Moreover, this combination resulted in selective inhibition of NF2-null Schwann cell proliferation when compared to wild-type Schwann cells in vitro, when compared to either drug alone. Our preliminary data suggest that combined targeting of BET and FAK1 may offer a potential therapeutic option for the treatment of NF2-related schwannomas.

**LB327**

**Discovery and characterization of ABSK112, a next-generation and potential best-in-class EGFR Exon20 mutant inhibitor with superior selectivity and brain penetration ability.**

Mei Ning, Shuqun Yang, Juan Peng, Shuangyun Hu, Weijia Lu, Cheng Dai, Baowei Zhao, Mingming Zhang, Hongping Yu, Zhui Chen, Yao-chang Xu. Abbisko Therapeutics, Shanghai, China.

**Introduction:** EGFR Exon20 mutations are clinically validated oncogenic alterations including a wide spectrum of mutations occurring in lung cancer and various other cancer types. Although several EGFR Exon20 inhibitors have reached clinical stage or received approval, there still leave large room for improvement in safety and efficacy, likely due to their limited selectivity against wild-type EGFR or other kinases, suboptimal mutation coverage, and lack brain penetrating ability. Herein, we have discovered a novel and next-generation EGFR Exon20 mutation inhibitor, ABSK112. It showed high selectivity over wild-type EGFR and other kinases, as well as a more comprehensive coverage over majority of EGFR Exon20 mutations in comparison with other EGFR Exon20 inhibitors.
**Method**: Anti-proliferation experiments in cell lines harboring various EGFR Exon20 mutations were used to evaluate potency and spectrum of coverage for ABSK112 and other inhibitors. Efficacy studies and PK/PD study in multiple tumor models confirmed its *in vivo* activities. Cell proliferation and *in vivo* efficacies studies in models harboring mutated or wild type EGFR were used to demonstrate its selectivity. Kinome selectivity, safety profiles, PK and ADME profiles were also characterized.
**Results**: ABSK112 showed potent inhibition of proliferation in multiple EGFR Exon20 mutation cell lines and superior wild-type EGFR selectivity compare to other inhibitors including mobocertinib (TAK788). In xenograft mouse models with various EGFR Exon20 mutations, oral dose of ABSK112 showed strong and dose-dependent anti-tumor efficacy. Moreover, ABSK112 exhibited much reduced *in vivo* inhibition against xenograft tumors with wild-type EGFR, compared to mobocertinib, confirming its superior selectivity. PK/PD analysis showed good correlation between ABSK112 exposure and inhibition of EGFR signaling. ABSK112 also demonstrated excellent preclinical brain penetration, bioavailability,

and safety profile.
**Conclusion**: ABSK112 is a novel and next-generation EGFR Exon20 mutation inhibitor with improved selectivity over wild-type EGFR and strong brain penetrating ability. It has demonstrated superior *in vivo* efficacy in several xenograft models with various EGFR Exon20 mutations, and excellent drug-like properties supporting its further development into clinical studies.

**LB328**
**Discovery & characterization of a next-generation FGFR4 inhibitor overcoming resistant mutations.**
Haiyan Ying, Nannan Zhang, Haibing Deng, Fei Yang, Wenqun Xin, Bin Shen, Hongping Yu, Zhui Chen, Yao-chang Xu. Abbisko Therapeutics, Shanghai, China.

**Introduction**: Aberrant activation of FGF19-FGFR4 signaling pathway plays an essential role in the tumorigenesis of Hepatocellular carcinoma (HCC) and FGFR4 inhibitors have shown preliminary efficacy in recent clinical trials for patients with FGF19 overexpression. However, the observed responses only lasted a few months before tumors relapse. Acquired FGFR4 resistant mutations were found in ~30% of FGFR4 inhibitor responsive patients. Similar FGFR4 mutations haven also been found *de novo* in about 7-10% of Rhabdomyosarcoma (RMS) and ER-treated invasive lobular carcinoma patients. First generation FGFR4 inhibitors have minimal activity against these *de novo* or acquired resistant mutations. Therefore, next-generation of FGFR4 inhibitors are needed to overcome these resistant FGFR4 mutations to provide better treatment options for patients. Using advanced computation-aided structural analysis and medicinal chemistry design, we have discovered a next-generation small molecule FGFR4 inhibitor, ABSK012, and demonstrated its strong activities against *de novo* and acquired resistant FGFR4 mutations while retaining inhibition for wild-type FGFR4.
**Method**: Inhibitory activity of ABSK012 against FGFR4 and FGFR4 mutants was evaluated by MSA assay and its inhibition on FGFR4-dependent cell proliferation was evaluated by Celltiter-Glo assay in wile type FGFR4-dependent cancer cell lines or mutant FGFR4-dependent Ba/f3 cell lines. Its selectivity against other FGFR family member and kinases was analyzed by cellular and KinomeScan profiling. Efficacy studies were conducted in HCC xenograft models and mutant FGFR4-dependent xenograft models including a RMS PDX model harboring FGFR4 V550L mutation.
**Results**: ABSK012 demonstrated strong potency over multiple FGFR4 mutants that are insensitive to a first generation FGFR4 inhibitor BLU-554. It also inhibited wild-type FGFR4 with IC50<5 nM in biochemical assay and exhibited great selectivity against other kinases. In multiple mutant and wild-type FGFR4-dependent cell lines, ABSK012 demonstrated significantly improved anti-proliferation activity. In preclinical *in vivo* studies in HCC models, oral administration of ABSK012 strongly inhibited the tumor growth at doses without obvious toxicities. More importantly, in a RMS PDX xenograft harboring FGFR4 V550L mutation, ABSK012 also showed significant anti-tumor activity. Other ADME and safety profiling demonstrated excellent drug-like properties of ABSK012.
**Conclusion**: ABSK012, presented here by Abbisko Therapeutics, is a highly potent, selective, and next-generation small molecule FGFR4 inhibitor overcoming FGFR4 mutations resistant to first-generation inhibitors. Its superior preclinical profile supports its fast-track development into clinic.

**LB329**
**A potent and selective small molecule inhibitor of CSF-1R ABSK021 demonstrates strong efficacy in preclinical models of osteosarcoma.**
Nannan Zhang, Cheng Dai, Jiacheng Zhang, Shuqun Yang, Jie Wang, Jie Zhang, Manqi Liu, Zhui Chen. Abbisko Therapeutics, Shanghai, China.

**Introduction**: Osteosarcoma is the most common primary malignant bone tumor in children and young

adults. Surgery combined with multimodal chemotherapy remains as the standard treatment for osteosarcoma patients. However, patients with osteosarcoma metastasis have a five-year survival rate of less than 30%, and their long-term outcomes have not improved over the last 30 years. CSF-1/CSF-1R signaling is crucial for the survival, function, proliferation and differentiation of myeloid lineage cells, including osteoclasts and monocytes/macrophages. Targeting CSF-1R either on tumor cells or tumor-associated macrophages has been reported to limit osteosarcoma progression in preclinical models. ABSK021, an oral, highly potent and selective small molecule inhibitor of CSF-1R discovered by Abbisko, has entered into Phase I trial (NCT04192344) and showed significant anti-tumor activity and favorable safety profile in patients with advanced tenosynovial giant cell tumor. Here, we conducted a series of preclinical *in vitro* and *in vivo* experiments, as well as human osteosarcoma profiling, to investigate the treatment potential of ABSK021 for osteosarcoma patients.
**Methods**: Cellular potency of ABSK021 was evaluated by Celltiter-Glo assay in osteosarcoma cell lines and CSF-1R expression was evaluated by western blot. *In vivo* efficacy studies were conducted in murine osteosarcoma models and pharmacodynamics changes were measured. CSF-1R IHC staining was conducted in tissue microarray samples from osteosarcoma patients.
**Results**: ABSK021 potently inhibited the proliferation of K7M2, a murine osteosarcoma cell line with high expression of CSF-1R. In animal, ABSK021 showed strong anti-tumor activity on K7M2 syngeneic model and SA4094 osteosarcoma PDX model without causing significant body weight loss. Pharmacodynamics analysis displayed robust inhibition of macrophage infiltration and CSF-1R signaling by ABSK021, confirming its effective target engagement *in vivo*. Parallel IHC analysis of human tissue microarray samples revealed high prevalence of positive CSF-1R staining in osteosarcoma patients.
**Conclusion**: Taken together, these results demonstrate that ABSK021 has strong inhibition of CSF-1R activity and corresponding anti-tumor activity in preclinical osteosarcoma models. High prevalence of CSF-1R expression was also found in osteosarcoma patients, suggesting great potential of utilizing ABSK021 as a novel therapy to treat osteosarcoma patients in clinic.

**LB330**
**FHND5071: a selective RET inhibitor with unique pharmacokinetic profile.**
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Background: Gene rearrangements (fusions) and mutations in RET have the potential to be oncogenic drivers and have been observed in a variety of tumors types. Selective RET inhibitors selpercatinib and pralsetinib have been approved for patients with RET-altered cancers. FHND5071 is a novel kinase inhibitor which specifically targets RET activated forms and has unique pharmacokinetic profile.
Methods: The pharmacological profile of FHND5071 have been confirmed in *in vitro* and *in vivo* evaluations, including enzyme and cell-based assays, PK/PD study, and RET-dependent tumor models.
Results: In enzymatic assays, FHND5071 potently inhibited RET wild type, RET fusions and mutations with the $IC_{50}$ of 4.47~19.26 nM and demonstrated 89-fold selectivity over KDR (VEGFR-2). FHND5071-M2, the active metabolite identified in pre-clinical pharmacokinetics, had the same potency as FHND5071. In cellular settings, FHND5071 inhibited the proliferation of Ba/F3 engineered cells (RET WT, RET V804M, RET M918T) and inhibited RET phosphorylation in HEK-293 engineered cells (WT, V804M, RET M918T, KIF5B-RET, CCDC6-RET) with the similar potency compared with selpercatinib. In PK/PD study (Ba/F3 KIF5B-RET allograft model), compared with selpercatinib, FHND5071 had longer $T_{max}$ and MRT in plasma and tumor tissue. FHND5071 preferred to be distributed into tissues. The exposure of FHND5071 in tumor tissue ($AUC_{0-t}$) was ~28 times that of selpercatinib and the concentration of FHND5071 in brain tissue was ~33 times that of selpercatinib at 4 h after dosing. Meanwhile, after FHND5071 administration, RET phosphorylation and the downstream signaling protein (pAKT, pErk1/2) were significantly inhibited in tumor tissue and the inhibition was maintained for 24 h at

least. But the duration of inhibition with selpercatinib was shorter than that of FHND5071. In tumor models, FHND5071 exhibited significant anti-tumor efficacy in Ba/F3 KIF5B-RET model at oral dose levels ≥3 mg/kg once daily (QD) without inducing significant toxicity. FHND5071 QD exhibited similar anti-tumor activity as selpercatinib administrated twice a day (BID) at 30 mg/kg. 30 mg/kg FHND5071 (QD) demonstrated significant anti-tumor efficacy with 100% TGI in patient-derived xenograft models (colorectal cancer with CCDC6-RET and ovarian cancer with NCOA4-RET). In CCDC6-RET intracranial xenograft model, 30 mg/kg FHND5071 (QD) significant prolonged life time of model mice. The median survival time was 56 days and the increase in life-span was 51% ($p<0.005$ compared with the vehicle group).
Conclusions: FHND5071 demonstrated excellent selectivity and anti-tumor efficacy. The unique pharmacokinetic profile of FHND5071 supported the frequency of once-a-day dosing and better efficacy in target organ and brain tumor in clinical. FHND5071 has received IND approval from NMPA and FDA. It is currently in the dose-escalation segment of a first-in-human phase 1 trial for patients with RET-driven solid tumors.

**LB333**
**Cytokine affinity tuning using the *AlphaSeq* platform to generate targeted immuno-oncology therapeutics.**
Ryan Swanson, Davis Goodnight, Jeff Adamo, Kyle Minch, Emily Engelhart, Randolph Lopez, David Younger. A-Alpha Bio, Seattle, WA.

Here, we demonstrate a novel approach for generating affinity detuned cytokines that can be fused to antibodies for selective immune cell activation to improve anti-tumor responses and mitigate systemic toxicity. Although cytokine therapies have demonstrated curative effects in some cancer patients, clinical use remains limited with current toxicity profiles accompanying systemic administration. Next generation cytokine approaches include conditional signaling focused to sites of interest, including the tumor microenvironment or specific immune cell populations. In this presentation, we share a novel approach for generating detuned cytokine therapeutic candidates using the *AlphaSeq* platform, which involves the repurposing of yeast agglutination and mating to quantitatively measure protein-protein interactions at a library-on-library scale. We show, for the first time, how *AlphaSeq* can be used to measure interactions between cytokines and their receptors as well as generate engineered cytokines with a broad range of affinities. A saturated mutational library was created for a cytokine of interest and subsequently screened against a second library consisting of axis receptor chains, their species orthologs and off-target receptors. Cytokine variants with lower affinity than parental were recombinantly expressed as Fc fusion proteins to orthogonally measure affinity with biolayer interferometry and characterize potency with an *in vitro* human PBMC phosflow assay. Finally, detuned cytokine candidates were fused to antibodies recognizing surface antigens of interest to demonstrate cell population-specific signaling. Our results show the *AlphaSeq* platform can accurately quantitate thousands of cytokine variant affinities simultaneously against multiple relevant receptors which translates to signaling potency in primary human cells. When engineered as antibody fusion proteins, candidate immunocytokines are identified that only induce signaling in intended cell populations. *AlphaSeq's* rapid, comprehensive affinity determination can be utilized to develop a portfolio of clinically relevant therapeutic immunocytokines.

**LB334**
**Assessment of the potential of graphene oxide as an antigen delivery platform for cancer immunotherapy.**
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Introduction: Novel cancer immunotherapies are urgently needed due to a lack of definitive treatments, as well as toxicity and cost of current therapies. 2-dimensional carbon graphene oxide (GO) nanomaterials provide a large surface area, high protein loading efficiency and good biocompatibility, as well as being inexpensive. Given these properties, we investigated whether and how GO could be an effective antigen delivery system for targeted cancer immunotherapies.
Methods: GO was non-covalently complexed with the model antigen ovalbumin peptide ($OVA_{257-264}$) and the immunostimulant and toll-like receptor 3 agonist polyinosinic:polycytidylic acid (Poly (I:C)). We examined innate and adaptive immune responses after subcutaneous injection of GO complexes by histology and flow cytometry.
Results: We found that GO without Poly (I:C) had a very limited immunogenic potential. In contrast, GO complexed with Poly (I:C) significantly increased immune cell recruitment to the injection site, and enhanced activation and migration of dendritic cell subsets (cDC1s and cDC2s) in skin-draining lymph nodes 24 hours after injection. Additionally, we found that GO complexed with Poly (I:C) dramatically enhanced OVA-specific $CD8^+$ T cell expansion and cytotoxic potential in both spleen and skin-draining lymph nodes 7 days after subcutaneous complex administration to mice which had previously been injected intravenously with traceable, OVA-specific (OT-I) $CD8^+$ T cells.
Conclusion: Our results have revealed the immunomodulatory potential of GO when complexed with Poly (I:C) by attracting immune cells to the injection site and augmenting antigen-specific cytotoxic responses both locally and systemically. Ongoing work is determining the anti-tumor potential of this approach, and potential mechanisms by which GO complexes might limit tumor progression.

**LB335**

**Establishment of the effective dose of the STAT3 inhibitor WP1066 used in combination with STING activation for reprograming the preclinical glioma microenvironment.**

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Purpose: Trials of immunotherapy in diffuse glioma patients have been mostly unsuccessful. We therefore sought to determine the combined effects of the signal transducer and activator of transcription 3 (STAT3) inhibitor WP1066 and the STING agonist IACS-8803 in a preclinical model of glioma.
Experimental Design: C57BL/6 mice (n=9-10/group) with orthotopically engrafted GL261 cells were treated orally with WP1066, a blood-brain-barrier penetrant inhibitor of STAT3, in combination with the STING agonist IACS-8803 administered directly into the tumor. Analysis of treatment effects included immunoblots, ubiquitination, multiplex immunohistochemistry, and NanoString immune phenotyping of glioma-infiltrating immune cells.
Results: The STING agonist 8803 at 2.5µg/mouse, in combination with WP1066 dosed at 30mg/kg with a 12-hour delay, increased animal subject median survival (MS) to 58 days, in contrast to monotherapy (WP1066=25 days, 8803=29 days) or control (25 days) ($p=0.002$ of combo relative to all other groups). Dose escalation of WP1066 to 60 mg/kg, administered simultaneously with 8803, completely abrogated the therapeutic combinatorial effect. Five ubiquitin-binding domains were predicted on STING using the UbPred program; concentrations of 5µM or higher of WP1066 induced polyubiquitination of phosphorylated STING. Immune profiling during the therapeutic window demonstrated a multi-pathway

induction of anti-tumor immunity.
Conclusions: Co-administration of a reduced effective dose of WP1066 is necessary with STING agonist 8803 to effectively enhance the anti-glioma immune reactivity in the tumor microenvironment.

**LB336**
**pH-sensitive polymeric micelles loading IL-12 profoundly inflame the tumor microenvironment to eradicate cold tumors.**
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Background: Treatment of immunologically cold tumors is a major challenge. IL-12 can activates both innate and adaptive immunity to elicit strong antitumor immunity. Nevertheless, the poor pharmacokinetic and severe side effects of IL-12 limit the application. We have recently developed pH-sensitive polymeric micelles loading IL-12 (IL-12/m) that can selective activate in tumor tissues to invigorate immunotherapy and enhance efficacy. Herein, we elucidated the mechanisms behind the enhanced efficacy of IL-12/m and their ability to synergize with immune checkpoint inhibitors (ICIs).
Methods: To determine the contribution of the immune cells on the efficacy on IL-12/m, mice bearing orthotopic 4T1 tumor models were treated with anti-CD4 (for depleting CD4+ cells), anti-CD8 (for depleting CD8+ cells), anti-asialo GM1 (for depleting NK cells). The tumor volume was tracked after treatment with IL-12/m. The immune status of the tumors was studied by histology, flow cytometry and RNA-seq after treatment with free IL-12, IL-12/m and their combination with ICIs. The efficacy of the treatments was assessed by following the tumor growth and mice survival.
Results: IL-12 can promote the growth and function of T cells to initiate the enhanced anti-tumoral immune reactions. Flow cytometry analysis IL-12/m significantly enhanced tumor infiltration of CD8+ T cells compared to free IL-12. The immune cell depletion study revealed that the depletion of CD8+ cytotoxic T cells, CD4+ T cells and NK cells significantly lessen efficacy of IL-12/m, which is consistent with the biological function of IL-12. The depletion experiment suggests CD8+ cytotoxic T cells are the most important fraction for IL-12/m efficacy. Moreover, immunohistochemistry analysis of 4T1 revealed IL-12/m promoted high infiltration of CD8+ cells and the upregulation of Granzyme B in CD8 + Cells. IL-12/m also increased PD-L1 levels in tumors. The effects of IL-12/m on the TME was further investigated as a monotherapy and in combination with anti-PD-1 antibodies. The combination of IL-12/m with anti-PD-1antibodies presented high CD45+ cells in tumors. Lymphoid cells and T cells were upregulated by IL-12 and the anti-PD-1 antibody combination. The general CD4+ T cells also showed upregulation in the IL-12/m treated groups. While anti-PD-1 monotherapy did not show any suppression on Tregs in the tumor model, the combination of IL-12/m plus anti-PD-1 depleted Tregs. Moreover, IL-12/m increase the presence of Th cells. We then investigated the activation by transcriptome analysis. The heatmap of global gene expression indicated differentiated gene profiles. GOBP and KEGG analysis showed that IL-12/m upregulated the stimulation of proliferation, differentiation and activation of various effector immune cells, differentiation of T cells into Th cells, and T cell activation and T cell receptor signaling pathway. The enhanced the intratumoral immunity of IL-12/m plus ICIs allowed eradicating 4T1 tumors.
Conclusions: Systemically injected IL-12/m ensured strong stimulation of antitumor immunity leading to high safety and strong therapeutic outcomes in cold tumor models. The enhanced immune response of IL-12 synergize with checkpoint blockade to eradicate cold tumors. The approach has potential for developing safer and more effective immunotherapies.

**LB337**
**Inhibition of the E3 ligase CBL-B enhances the effector function and proliferation of natural killer**

**cells.**
Yilin Qi, Jun Kuai, Yingzhi Bi, David Greco, Samira Jaeger, Ken Carson, Hadi Danaee, Timothy Reilly, Geraldine Harriman, Fang Wang. HotSpot Therapeutics, Inc, Boston, MA.

The E3 ubiquitin ligase CBL-B is a master regulator of effector T cells through its downstream regulation of T-cell receptor and co-stimulatory stimulation, but it has also been shown to play a negative feedback role in NK cells. CBL-B depletion enhances the production of IFNγ and the cytotoxicity of activated NK cells. Similar to the effect of depleting CD8 T cells in mice, depletion of NK cells abolishes the tumor growth delay observed in Cbl-b-/- mice. With emerging data supporting the potential role of exogenous NK cells in anti-tumor immunity, inhibition of CBL-B represents a promising approach to enhance both adaptive and innate immunity for cancer immunotherapy. Using one of our small molecules, allosteric CBL-B inhibitors, HOT-A, we demonstrated that CBL-B inhibition enhanced NK activation, IFNγ production and granzyme B secretion in response to the various stimulation conditions, including cytokines (such as IL-15 and IL12 + IL-18) and stimulatory receptors (such as NKp30 and NKG2D + CD244). NK cell proliferation was also enhanced in the presence of HOT-A. Single cell analysis further suggested that HOT-A increased NK polyfunctional cytokine expression during IL-12 + IL-18 activation. In a coculture system of NK cells and K562 target cells, HOT-A dose-dependently promoted primary human NK cell activation, cytokine production and cytotoxicity in the K562 cells. In the *in vivo* CT-26 tumor model, granzyme B+ NK cells in the TIL were significantly increased in the HOT-A treated tumor bearing mice and NK function gene signature was also increased by Nanostring analysis, suggesting an enhanced NK function in the tumor microenvironment. Taken together, the pre-clinical data presented here demonstrate that our CBL-B inhibitor enhances NK cell function *in vitro* and *in vivo,* suggesting that its enhancement of anti-tumor immunity may be driven through both effector T cell and NK cell-driven biology.

**LB338**
**A novel class of T cell-activators targeting germline encoded TCRβ chains promotes antitumor activity in PD1-refractory models through expansion of a clonally enriched effector memory T cell subset.**
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Background: Here we describe a new class of selective T cell activator molecule that combines antibody targeting of the variable (V) β regions of the T cell receptor (TCR) with a costimulatory cytokine. STAR0602, a molecule that selectively targets T cells expressing Vβ6 and Vβ10 TCRs and delivers IL-2R co-stimulation, activates and expands human Vβ6 and Vβ10 T cells that are enriched in tumor infiltrating lymphocytes (TIL) relative to other Vβ subsets. Using this molecular design, STAR0602 and molecules targeting other Vβ T cell subsets simultaneously engage a novel, non-clonal mode of TCR activation with cytokine co-stimulation.
Methods: The effects of STAR0602 on activation and expansion of primary human T cells were assessed in vitro by flow cytometry, homogeneous time-resolved fluorescence, TCRseq, and NanoString. Several murine surrogate molecules targeting different Vβ T cell subsets with a range of baseline frequencies were tested in murine syngeneic tumor models. Tumors were excised for phenotyping of TILs including tetramer staining of antigen specific T cells using flow cytometry, and scRNAseq/TCRseq.
Results: In vitro, STAR0602 induced TCR signaling and IL-2R pathway activation in human T cells that preceded expansion of Vβ6/Vβ10 T cells that acquired an atypical central memory T cell ($T_{CM}$) phenotype. Across PD1-refractory syngeneic murine tumor models, monotherapy with fusion molecules targeting different Vβ TCR and delivering IL-2R co-stimulation induced potent anti-tumor activity that was associated with expansion and accumulation of targeted Vβ T cell subsets in blood and tumors. In

vivo anti-tumor activity was dependent on the accumulation of effector memory T or $T_{CM}$ Vβ T cells expressing a novel effector gene signature. Additional analysis of TILs showed increased clonality in *de novo* expanded Vβ T cells compared to controls in addition to increased numbers of tumor-specific T cells.
Conclusions: To our knowledge, the use of therapeutic antibodies to selectively target and expand Vβ T cell subsets and thereby modulate the germline TCR repertoire for therapeutic benefit is novel. Here we show that STAR0602 selectively binds and activates human Vβ6/Vβ10 T cells and promotes a novel $T_{CM}$ phenotype. Murine surrogate molecules targeting different Vβ T cell subsets demonstrated potent and durable single-agent anti-tumor activity even when targeting low frequency Vβ subsets. These expanded Vβ T cells appear to drive enhanced anti-tumor activity due to their increased effector potentials, novel memory phenotype, and a striking enrichment in clonal diversity. In summary, STAR0602 and similar Vβ TCR-targeting constructs have the potential to remodel the adaptive immune response to solid tumors that are refractory to anti-PD1 therapy. A Phase 1/2 clinical trial of STAR0602 is ongoing in patients with advanced metastatic cancers.

**LB339**
**PARP inhibition reprograms CD8 T cells, enhancing their function and generation of prolonged memory, leading to greater anti-tumor immune response.**
Wael Traboulsi,[1] Subhadip Kundu,[1] Pankaj Gaur,[1] Zainab Ramlaoui,[1] Dareen Sarhan,[1] Nour Shobaki,[1] Jacob Lee,[1] Nazli Jafarzadeh,[1] Simon T. Barry,[2] Viia E. Valge-Archer,[2] Vivek Verma,[1] Seema Gupta,[1] Samir N. Khleif[1]. [1]Georgetown University Medical Center, Washington, DC; [2]AstraZeneca, Cambridge, United Kingdom.

Olaparib is the most widely studied third-generation PARP inhibitor (PARPi) in clinical practice with a significant clinical outcome in BRCA deficient tumors such as breast and ovarian cancers. However, innate and adaptive resistance in patients with DNA damage repair (DDR) gene mutations were reported after treatment with PARPi, highlighting the potential implication of other compartments such as the role of immune cells in the mechanism of resistance. Importantly, PARPi is shown to have an immune-modulatory capacity within the tumor microenvironment (TME) by activating the STING pathway and increasing interferon gamma (INF-gamma) and chemokine secretion and hence increasing the recruitment and function of CD8 T cells. In addition, PARPi is shown to affect other cells within the TME such as, macrophages, myeloid-derived suppressor cells (MDSCs) and cancer associated fibroblasts (CAFs), promoting either an immune enhancement or immune suppressor milieu that could indirectly affect CD8 T cell function. However, little is known about the direct effect of PARPi on CD8 T cells. In this study using two cold tumor models, we show that PARPi controls the tumor growth and increases survival in tumor-bearing mice. This effect is due to the enhancement of number and cytotoxicity of antigen-specific CD8 T cells and the maintenance of memory population within the TME. *In vitro* study shows that PARPi activates the SIRT-1/ FOXO1 pathway, leading to the modification of gene expression related to memory and fatty acid oxidation (FAO) metabolism in CD8 T cells. As a result, a unique subpopulation of superior central memory cells with high recall responses and anti-tumor effects are generated after PARPi treatment. Interestingly this unique subpopulation was also generated after PARPi in human cells. Together, our findings highlight the direct effect of PARP inhibition on CD8 T cells and its ability to modulate and reprogram CD8 T cells within the TME, leading to a greater anti-tumor immune response.

**LB340**
**Spur cell-delivered peptide antigen generates T cell immunity to eradicate mouse $KRAS^{G12D}$ spontaneous lung cancer.**
Shuang Qu,[1] Jiafan Wang,[1] Meng Jia,[2] Hongwei Liang,[1] Yuan Liu,[3] Ke Zen[1]. [1]China Pharmaceutical University, Nanjing, China; [2]Nanjing University, Nanjing, China; [3]Georgia State University, Atlanta, GA.

Red blood cells (RBCs) have been widely used in delivering various drugs including cancer antigens. However, due to their inherent biocompatibility and low immunogenicity, RBC-based tumor antigen delivery often fails to initiate anti-tumor immunity and results in lower clinical efficacy of cancer vaccines. Here we report that tumor antigen-encapsulated RBCs can be rapidly transformed to spur cells which have much higher immunogenicity. Compared to normal RBCs, spur cells display a defused surface CD47 pattern with a lower affinity to SIRPα, and thus can be rapidly engulfed by activated macrophages and dendritic cells. The tumor antigen-loaded spur cells elicit high antigen responses both *in vitro* and *in vivo*. In combination with the SHP-1 inhibitor, spur cell-delivered $KRAS^{G12D}$ antigenic peptides markedly suppress tumor growth in $KRAS^{G12D}$ spontaneous mouse lung cancer model. In addition, a specific T cell population targeting the $KRAS^{G12D}$ mutated peptide antigen is detected in cancer-survival mice. Our study thus has developed spur cell-delivered cancer neoantigen as an effective cancer vaccine.

**LB341**

**Single-cell CRISPR screens in primary human T cells identify regulators of Th2 cell skewing.**

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CRISPR screens have become the primary discovery engine in modern biology. However, many screening workflows are still performed in cancer cell lines and coupled to simplistic read-outs such as cellular fitness. At Myllia Biotechnology, we combine CRISPR screening with single-cell RNA sequencing, leveraging two transformative technologies to enable genetic screening for complex phenotypes. We utilize the CRISPR screening workflow to map the impact of thousands of genetic perturbations on the global transcriptome at single-cell resolution. Our powerful approach has broad applications in identifying novel drug targets or elucidating unknown mechanisms of actions of drugs. Primary human T cells are currently of great interest in the scientific community. They are not only key players in autoimmunity and other inflammatory diseases, but also represent attractive targets for immunotherapy of cancer. To enable the discovery of novel targets, we built a workflow that utilizes CD4+ T cells from peripheral blood and allows functional genomic screens in these cells. Upon activation, naïve CD4+ T cells proliferate and differentiate into specific T helper cell subsets, such as Th1, Th2, or Th17 cells. Here, we present data of an experiment in which we screened for regulators of T helper cell differentiation and skewed cells towards the Th2 subset. We aimed to identify genes whose knockout boosts or attenuates the ability of primary naïve CD4+ T cells to become Th2 cells. Th2 cells support the humoral immune response, and their dysfunction has been linked to inflammatory diseases, including asthma.

In our screen, the different T cell subsets could be captured using curated transcriptomic signatures. Importantly, several gene KOs introduced in a pooled fashion using CRISPR/Cas9 accumulated in distinct subpopulations, suggesting that these genes regulate the differentiation of naïve T cells into the various T helper cell subsets.

Overall, our pooled screening approach in primary human T cells allows for novel insights in the plasticity of T cells and identifies genes that could serve as drug targets in autoimmunity, inflammation and immuno-oncology.

**LB343**

**Antitumor and metabolomic evaluation of immune checkpoint inhibition in diet-induced obese mice.**

Michael Boice, Whitney Tuoti, Logan Chesney, Maggie Murray, Paul M. Gonzales, Stephen T. Gately. Translational Drug Development, Scottsdale, AZ.

Obesity is an epidemic in the Western world and a risk factor for at least 13 types of cancer. Cancer rates are rising for several obesity-related cancers, such as liver, pancreatic, thyroid, and uterine, as well as for colorectal cancer in patients under 55. Preclinical studies for new cancer drugs are often performed in models of cancer that are hosted in mice that are metabolically healthy and of normal weight. To establish models of cancer in hosts that are more reflective of the clinical population, we established the tumor growth kinetics of commonly used murine models of cancer in diet induced obese (DIO) mice. Syngeneic tumor models, MC38 and Hepa1-6, implanted in 18-week-old DIO C57BL6 mice showed accelerated tumor growth when compared to the growth rate in age-match control C57BL6 mice. We also studied the growth of three other C57BL/6J syngeneic tumor models; MB49, TC-1 and PAN 02. Interestingly, pancreatic PAN 02 demonstrated slower growth in DIO mice compared to age matched controls. We then tested the response of anti-mPD-1 (αPD-1) against MC38 syngeneic mouse tumor model comparing response of αPD-1 in 18-week-old DIO mice versus age matched control diet (CD) mice. The αPD-1 treatment showed a strong anti-tumor response (TGI=53%) against MC38 in DIO mice in contrast to no response (TGI=0%) in CD mice. Evaluation of the tumor microenvironment (TME) on Day 13 revealed comparable numbers of $CD8^+$ T Cells in 18-week-old DIO mice tumors compared to CD tumors. Treatment with αPD-1 resulted in a statistically significant increase in $CD8^+$ T Cells in DIO mouse tumors compared to CD tumors ($p<0.008$). An increase in $LAG3^+$ cells were detected in αPD-1 treated DIO tumors compared to CD ($p<0.05$). A global metabolomics assessment of serum metabolites showed significant alterations in metabolites between CD and DIO mice. DIO αPD-1 treated mice had 88 metabolites significantly altered compared to only 16 metabolites in CD αPD-1 treated mice. Thirty-six metabolites increased after αPD-1 treatment in DIO mice but were decreased after αPD-1 treatment in CD mice. Amongst these 36 metabolites were alterations in the pentose phosphate pathway particularly with ribose 5-phospate and sedoheptulose 1-phosphate intermediates. DIO accelerated MC38 tumor growth and showed improved sensitivity to αPD-1 treatment compared to CD in 18-week-old mice. The significant increase in $LAG3^+$ cells in the TME of DIO MC38 tumors treated with αPD-1 could represent an opportunity for greater antitumor efficacy of αPD-1treatment combined with anti-LAG3 in DIO mice. Use of syngeneic tumor models in DIO mice may provide improved models for identification and development of immunomodulatory, or other cancer therapeutics in a more metabolically challenged, clinically relevant system. These models could also permit the detection of novel circulating metabolites that could be targets for therapeutic development to improve treatment efficacy.

**LB344**

**Complement-mediated signaling during cross-presentation of tumor antigen augments T cell responses.**

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Production of complement C3a and C5a locally at the immune synapse by activated dendritic cells (DCs) enhances T cell responses in multiple experimental models. Complement signaling through cognate C3a receptor (C3aR) and C5a receptor (C5aR) modulate effector T cell stability, proliferation and differentiation. We have previously shown that complement C3 plays a mechanistic role in driving synergistic anti-tumor effects of blocking indoleamine 2,3-dioxygenase (IDO) during radiation and chemotherapy in a murine brain tumor model that expresses the $gp100_{25\text{-}33}$ peptide antigen. We hypothesize that, when anti-tumor responses are allowed to happen, complement C3 production by local DCs acts as a costimulation factor, operating through C3aR and C5aR on the T cell surface to allow optimal T cell activation and proliferation. To study the role of complement C3 as a costimulation factor during T cell responses against a nominal tumor antigen ($gp100_{25\text{-}33}$ peptide, expressed by B16F10 melanoma), we used T cells from syngeneic pmel-1 mice (TCR transgenic with CD8 T cells that recognize the cross-presented $gp100_{25\text{-}33}$ peptide) co-cultured with $gp100_{25\text{-}33}$ peptide and dendritic cells from either wild-type (WT) or C3-defiecient (C3-KO) mice. In addition, we used *in vitro* culture

conditions where $gp100_{25-33}$ tumor peptide was in stringent limited supply to model homeostatic conditions of the tumor microenvironment. We found that, when stimulated by DCs from C3-KO mice, pmel-1 CD8 T cells had decreased activation marker expression, proliferation, and cytokine effector function relative to stimulation by DCs from WT mice. Next, we bred pmel-1 mice onto a syngeneic background deficient in receptors for both C3a and C5a complement factors (C3aR-KO/C5aR-KO double knock-out mice). We used CD8 T cells from the resulting mouse strain (pmel-1/C3aR-KO/C5aR-KO) co-cultured with WT DCs to study the role of complement signaling in T cells during responses to tumor antigen. Using the same stringent antigen conditions, CD8 T cells from the pmel-1/C3aR-KO/C5aR-KO mice had decreased activation marker expression and proliferation, and differences in cytokine function compared to CD8 T cells from the pmel-1 mice. These findings suggest that lack of local complement production by the DCs in the tumor microenvironment may inhibit anti-tumor immune responses, and that complement signaling through C3aR and C5aR on T cells may play an important role during such anti-tumor responses.

**LB346**

**Mapping of shared tumor antigen reactivity with nanoparticle encapsulated mRNA in prostate cancer patients.**

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Background: Prostate cancer (PC) remains refractory immune checkpoint therapies (CPT) despite having an FDA-approved cancer vaccine. PC is known to have a low mutation burden resulting in low level of neoantigens, so shared tumor associated antigens (TAAs) and translocations presumably represent a dominant source of antigens in this disease. While PC is thought to be a poorly immunogenic tumor, the frequency and breadth of tumor antigen reactivity in PC patients is unclear.
Methods: We utilized novel programmable immunogenic mRNA nanoparticle platform and created formulations encoding a range of full-length prostate TAAs ($TAA_{mRNA}$) to systemically study T cell immune responses to across a range of TAAs in PC patients. We administered 8 PC $TAA_{mRNAs}$ (NY-ESO-1, HK2, STEAP1, TMPRSS2-ERG, PAP, PSA, PSCA and PSMA) to *in-vitro* cultured peripheral blood mononuclear cells (PBMC) from 43 treatment-naïve localized PC patients compared to 17 age-matched non-cancer control patients. We assessed upregulation of early T cell activation markers and interferon-gamma (INFg) cytokine production through flow cytometry and ELISPOT, respectively.
Results: 20% of PC patients possessed TAA-specific $CD8^+$ T cell responses to at least one antigen. NY-ESO-1, HK2 and TMPRSS2-ERG had the highest T cell recall responses at an average of 35%, with NY-ESO-1 demonstrating the strongest responses per patient. Of the patients that respond to any given TAA, 47% of patients responded to three or more antigens, with 11% of PC patients responding to all TAAs. PC patients also showed heightened production of IFNg, with 35% of patients showing pan-responsiveness to three or more antigens. Particularly, HK2 stimulated the highest levels of INFg production. These levels of responsiveness are not seen in the control cohort.
Conclusions: Encapsulated mRNA nanoparticles can be used to systematically map antigen reactivity across a cohort of cancer patients and reveal frequent pan-responsiveness to multiple TAAs in what has considered to be an immunologically cold cancer These results also reveal a hierarchy in immunogenicity of candidates that can enable future vaccine development. Continual efforts to assess the immunogenicity of shared PC TAAs in more advanced disease, and how patient responses to TAAs are modulated by immunotherapies, can aid in refining combinatorial therapies for PC.

**LB347**

**A lung-mimicking synthetic metastatic niche reveals N1 neutrophils drive breast cancer metastatic dormancy in the lungs.**

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3D scaffolds mimicking the environment in the primary tumor or metastatic organs can deconstruct complex niche signals and facilitate the study of cancer progression and metastasis. Here, we reported that a subcutaneous 3D scaffold implant acted as a lung-mimicking dormant metastatic niche in mouse models of metastatic breast cancer, recruiting lung-tropic circulating tumor cells yet suppressing their growth through potent *in situ* antitumor immunity. We compared it with the immunosuppressive lungs developing lethal metastases and the dormant lungs suppressing tumor growth derived from breast cancer models with varying tumor aggressiveness and host immunity. Our data suggested that breast cancer-induced $Gr1^{+}CD11b^{+}Ly6G^{+}$ granulocytic myeloid cells (neutrophils) infiltrated the scaffold implants and lungs, secreting the same signal to facilitate the metastatic seeding of lung-tropic cancer cells in these two types of niches. However, circulating neutrophils with opposing phenotypes and functions (N1 and N2) were selectively recruited and enriched in the dormant scaffolds/lungs and immunosuppressive lungs, respectively, responding to two distinct groups of chemoattractants. N1 or N2 neutrophils established activated or suppressive immune environments in the metastatic niches, directing different fates of cancer cells. The clinical relevance of these scientific findings was validated by the strong positive correlation of a high N1-to-N2 neutrophil chemoattractant ratio with a low-grade primary tumor, a low metastases incidence, and a better prognosis in breast cancer patients. Overall, our study revealed the multifaceted roles of neutrophils in regulating lung metastasis and underscored the importance of N1 neutrophils in driving breast cancer metastatic dormancy in the lungs, inspiring next-generation immunotherapy.

**LB348**

**A controlled high fiber dietary intervention alters metabolome and gut microbiome in melanoma survivors.**

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Background: Multiple studies support the gut microbiome as playing a key role in response to immune checkpoint blockade (ICB). Habitual dietary fiber intake has been associated with improved response to ICB. We conducted a pilot feasibility study of a high-fiber dietary intervention (HFDI) in melanoma survivors.
Methods: Melanoma survivors (BMI <40 $kg/m^2$, currently not on active therapy) were enrolled to a 6-week controlled feeding study in which all meals were provided by MD Anderson Cancer Center Bionutrition Research Core. Target daily fiber intake was up to 50 g/day derived from whole foods. The primary outcome was compliance. Blood and stool were collected q 2 weeks. Whole genome shotgun sequencing (WGS) was performed on fecal specimens, and metabolomics was conducted using mass spectroscopy on blood and stool.
Results: Ten participants were enrolled, of which 60% were female. Average BMI was 31.4 $kg/m^2$, and average baseline fiber intake was 17.8 g/day (range 7.4-28.3 g/day). Overall compliance was 88% (95% CI 83%-93%) with 45.8 g/day fiber reached (range 40.0-50.0 g/day). HFDI was well-tolerated with 1 participant experiencing grade 2 diarrhea and all other AEs were grade 1: diarrhea (40%), bloating (50%), flatulence (60%) and abdominal pain (10%). Exploratory analyses of global and targeted metabolomics showed increases in the short chain fatty acid acetate, shifts in omega-3/6 PUFAs, and indole/tryptophan metabolism. Stool WGS analyses showed that the largest shifts in gut microbiome structure in those who started with the lowest baseline fiber intake.
Conclusion: A HFDI is well-tolerated and feasible in a melanoma population. The expected shifts in metabolome and gut microbiome observed with HFDI support that participants were compliant. Thus, this intervention is currently being tested in a Phase II study in metastatic melanoma patients receiving ICB.

**LB349**

**The use of PBMC humanized mice to test the efficacy and safety of antibody and cell-based cancer immunotherapeutics.**

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In the fight against cancer, immunotherapies are one of the largest growing therapeutics in development. Immunotherapeutics are designed to boost or harness the power of the immune system to prevent, control, or eliminate cancer; while many immune therapies have been found to be safe others have induced severe toxicities. For instance, even compounds that target the same molecule/antigen can have dramatically differing safety profiles. Current preclinical models evaluating these therapies are underequipped to assess the safety of these compounds: *in vitro* assays fail to predict systemic responses and traditional animal models often fail to correlate with human responses. To better meet the needs of assessing preclinical toxicity we developed a PBMC-humanized mouse model to test a variety of therapeutics, including both monoclonal and bispecific antibodies and induce human cytokine release responses which can manifest within hours or days later resulting in tissue damage and lethality of the mice. To date we have tested a variety of therapeutics, including Blinatumomab, Rituximab, EGFRxCD3 BiTE, CAR-T, and others in our platform while evaluating the ability of the therapeutic to induce human cytokines, bodyweight loss, clinical symptom assessment, and survival in the context of toxicity alone or along with the evaluation with efficacy. We found that many of the therapeutics tested in our platform showed similarities to clinical data in humans. For example, urelumab and utomilumab are both fully humanized monoclonal antibodies against 4-1BB (CD137). However, during clinical trials, urelumab was shown to induce severe liver toxicities while utomilumab was well tolerated. In our huPBMC mouse model, we likewise showed that huPBMC mice dosed with 10 mpk of urelumab experienced body weight loss, showed liver necrosis, and met the clinical criteria for early euthanasia compared to mice treated with 10 mpk utomilumab and PBS treated controls. Serum levels of enzymes associated with liver damage: AST, ALT and GLDH were significantly higher in urelumab treated mice and terminal serum cytokine analysis revealed similarities with those found to be increased in urelumab clinical trials, including elevated IFNγ, IP-10, MIG, and MIP-1α and MIP-1β. Further, HuPBMC mice are also capable of detecting variability among donors. We have screened well over 60 human PBMC donors in huPBMC mice treated with OKT3 and αCD28 and while we always see an increase in cytokines such as IFNγ - the range of induction varies greatly among donors. Further, we see PBMC-donor variability in body weight loss and survival rate after OKT3 and αCD28 treatments. We demonstrate that the PBMC humanized mouse model shows clinical relevance. The use of these models for preclinical safety assessments has the potential to become an important part of novel immunotherapeutic development for patient safety and reducing drug development costs.

**LB350**

**Novel mouse model to study HPV associated pathologies.**

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Papillomaviruses are species specific, non-enveloped, double-stranded DNA viruses that have an ~8kb circular genome enclosed by a 55 nm icosahedral capsid. Human papillomaviruses (HPV) are associated with both benign and malignant epithelial lesions. High-risk HPV subtypes are the causative agents of cervical and the majority of oropharyngeal squamous cell carcinomas. Persistent HPV infection is required for HPV-associated tumorigenesis; however, cellular factors needed to support the viral persistence and HPV genes expression are not completely understood. Several transgenic mouse models,

developed in last decades, helped tremendously to study HPV-induced carcinogenesis. However, the absence of HPV non-coding upstream regulatory region and late coding region, as well as targeted early genes expression driven by an artificial promoter - features that are shared by all existent HPV transgenic mice - limit our ability to investigate HPV-associated malignant transformation and the role of host factors in this process. Therefore, we generated HPV16 transgenic mouse by using CRISPR/Cas9-mediated genome editing to insert the full-length HPV16 genome into the Rosa26 locus. Two founder mice, with single HPV copy and with tandem HPV integration, were used to establish mouse colonies. Interestingly, regardless of HPV copy number, ~30% of the HPV knock-in mice exhibited early death during the first 2 weeks after birth, while survived pups were significantly smaller than the same age wild-type mice, and had severe hair loss. Intriguingly, the growth retardant and hair loss phenotype in the HPV16 knock-in mice disappeared in ~4 weeks, most likely indicating that viral genes compromise specific stages of mouse development. We evaluated the expression of HPV genes during development, in different mouse organs, and in established fibroblasts and epithelial cell cultures. Our newly developed HPV16 knock-in mouse model provides viral gene expression from natural viral promoters offering an essential basis to explore the role of cellular factors in HPV-driven pathologies.

**LB351**

**A novel mouse model to interrogate the functional relationship between protein synthesis and antigen presentation in cancer.**

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Antigen presentation is a fundamental component of cancer immunity. Cytotoxic $CD8^+$ T cells, the primary mediators of cell killing, recognize peptide antigens presented in the context of Major Histocompatibility Complex Class I (MHC-I). MHC-I presents an astoundingly diverse array of peptides, collectively known as the immunopeptidome. The biosynthesis of the immunopeptidome, which integrates gene expression, protein synthesis, and proteolytic processing, is not well understood. Additionally, most studies interrogating the immunopeptidome use cultured cells *in vitro*, which lack physiologically relevant stimuli. In contrast, *in vivo* studies utilize bulk tumor or tissue lysates, in which heterogeneous cell mixtures obscure the ability to understand cell type or disease specific patterns of antigen presentation. The technical limitations have hampered our understanding of the cancer immunopeptidome in physiologically relevant tumor microenvironments *in vivo*. Here we present a novel genetically engineered mouse model (GEMM) of cancer, that includes $Kras^{Lox\text{-}Stop\text{-}Lox\text{-}G12D}$; $p53^{fl/fl}$; a Cre conditionally tagged MHC-I ($K^b$Strep), and a Cre conditionally tagged ribosome ($Rpl22^{HA/HA}$), termed the "KP/RiboMHC" mouse model. The KP/RiboMHC model enables simultaneous purification of ribosomes and peptide MHC complexes to functionally interrogate translational dynamics and antigen presentation *in vivo*. We developed optimized workflows to perform simultaneous ribosome profiling and immunopeptidomics from lung and pancreas cancer cell lines *in vitro* and specifically from malignant cells *in vivo*. Thus, the KP/RiboMHC model provides an unprecedented opportunity to ask fundamental questions regarding the influence of physiological stimuli in the tumor microenvironment on the biosynthesis of the immunopeptidome. Insights gained using this mouse will pave the way forward for the development of context specific, peptide-centric cancer immunotherapies.

**LB352**

**KRAS inhibition enhances immunogenicity of $KRAS^{G12C}$ colorectal cancer.**

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RAS mutations are the most common oncogenic mutations, present in >20% of human cancers. KRAS, the predominantly mutated isoform of RAS, is mutated in 40% of colorectal cancer (CRC), leading to constitutive activation of the MAPK (RAS/RAF) pathway and other downstream effectors. Due to the difficulty of targeting RAS, the first $KRAS^{G12C}$ selective inhibitor has only been developed recently and approved by the FDA for non-small cell lung cancer. However, the inhibitor demonstrated much lower response rates in CRC (<20%) than other cancer types. Thus, developing effective therapies for KRAS mutant (KRASm) CRC still remains challenging. Oncogenic KRAS signaling has been shown to have immunosuppressive effects on tumor microenvironment, thus we hypothesized that KRAS inhibitor can indirectly enhance antitumor immunity, providing a rationale for their combination with immune checkpoint blockade. Indeed, using a syngeneic $KRAS^{G12C}$ CRC mouse model with knockout of APC, TP53, SMAD4 and expressing $KRAS^{G12C}$, we observed significant increase in the percentage of CD3+CD8+ T cells as well as changes in myeloid populations in tumors treated with $KRAS^{G12C}$ inhibitor compared with vehicle control. Combination of $KRAS^{G12C}$/PD-1 inhibition produced a more substantial and sustained reduction in tumor growth compared with KRAS or PD-1 inhibition alone. $KRAS^{G12C}$ CRC mouse model represents a proper tool to study tumor cell-intrinsic mechanism of cooperativity between KRAS inhibition and immune response, since $KRAS^{G12C}$ inhibitor targets KRASm tumor cells only without having direct effects on stromal and immune cells. Interestingly, we found tumor-intrinsic immune programs induction by KRAS/MAPK inhibition in $KRAS^{G12C}$ CRC mouse models and patient-derived organoid models. We also found that $KRAS^{G12C}$ inhibition upregulates T-cell recruiting chemokines CXCL9 and CXCL10 via *IRF1* in KRASm tumor cells, which might be one of the potential mechanisms underlying enhanced infiltration of CD3+CD8+ T cells. These data suggest a link between KRAS inhibition and anti-tumor immunity, thus provide insights into novel combination strategies for $KRAS^{G12C}$ colorectal cancers.

**LB353**

**The switch from cap-dependent to cap-independent translation reshapes the immunopeptidome of lung cancer.**

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Cancer immunosurveillance relies on the presentation of peptide antigens on human leuckocyte antigen class I (HLA-I). HLA-I molecules present a diverse array of antigens, known as the “immunopeptidome”, which are recognized by cytotoxic $CD8^+$ T cells. Recent evidence suggests that cryptic peptide antigens derived from non-canonical open reading frames (nuORFs) contribute to the immunopeptidome and drive $CD8^+$ T cell responses. Integrating ribosome profiling (Ribo-seq) with mass spectrometry (MS) analyses has enabled the identification of several cryptic peptides derived from nuORFs including from putative non-coding regions including the 5’UTR, 3’UTR, long non coding RNAs (lncRNAs) and retained introns. Notably, many nuORFs undergo cap-independent translation. Various physiological and pharmacological stressors arrest canonical cap-dependent translation initiation and induce cap-independent translation. However, how this shift in translational dynamics influences the immunopeptidome is unknown. Using lung cancer cell lines, we show that the eIF4A inhibitor silvestrol induces cap-independent translation and reshapes the landscape of nuORF-derived peptide antigens. Silvestrol induced changes in the immunopeptidome strongly contrast with the global translation inhibitor homoharringtonine, which dampens antigen presentation. These observations suggest that the presentation of nuORF antigens in lung cancer is dynamic and tightly controlled by translational dynamics. More broadly, our study provokes reconsideration of translation inhibitors as immunomodulatory drugs that can change the HLA-I antigen repertoire and generate peptides that can act as new targets for cancer immunotherapy.

**LB354**

**9p arm loss is an important predictor of immune evasion in several human cancers .**

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The identification of biomarkers that predict patient response to immune checkpoint blockade (ICB) is a key focus of current cancer research. We and others have found that high levels of Somatic Copy Number Alterations (SCNAs) in tumors are associated with decreased infiltration by cytotoxic immune cells and predict patient's response to ICB. Recently, we predicted that the strongest SCNA driver of cytotoxic T cell depletion in HPV-negative oral cancer, was the loss of the entire 9p arm and that it was a marker of poor survival in recurrent oral cancers treated by anti-PD1 therapy. 9p arm loss is among the most common SCNAs in solid tumors and we revealed its importance beyond 9p21.3 deletion (i.e., *CDKN2A* and the *IFNA* cluster).To systematically investigate the immune-related role of 9p deletion and other SCNAs in cancer, we have developed a computational pipeline to identify the genes altered by SCNAs predicting the strongest risk of cancer immune evasion. We analyzed 7 solid tumor types and identified thousands of tumor-specific SCNAs predicting an immune cold phenotype but only ~100 SCNAs were conserved across all tumor types. Strikingly, 60% of the conserved deletions were localized on the 9p arm and the strongest 9p candidates included some known cancer drivers but also new candidates such as *RANBP6* and *TOPORS*, that have been suggested to decrease proinflammatory and pro-immunogenic molecules. We have identified a 17-gene cluster deletion in 9p13 composed mostly of genes involved in proteasomal degradation or membrane trafficking, suggesting that its deletion might disturb MHC-mediated antigen presentation. We are now engineering human and mouse oral cancer cell lines to recapitulate 9p deletion and decipher its role in immune evasion through in vitro and in vivo immunological assays. We are also using the parameters we determined in our computational pipeline to develop a machine learning method improving our prediction of SCNAs driving immune evasion. Our findings identified 9p deletion as a novel biomarker for predicting clinical benefit of ICB and will allow a better understanding of the SCNA-mediated mechanisms suppressing tumor immune response.

**LB357**

**CRISPR-Cas9 targeting of somatic mutations selectively kills cancer cells.**

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Pancreatic ductal adenocarcinoma (PDAC) accounts for 3% of all cancers but is the 3rd leading cause of cancer death in the United States. This staggering discrepancy is partly because most pancreatic cancers evade early detection and patients most commonly present with metastatic disease. As cancer initiates from a single transformed cell, passenger mutations that accumulate over the course of the patient's lifetime in that cell are then clonally propagated to the growing tumor and metastases, providing a targetable molecular signature throughout the cancer. Here we propose a CRISPR-Cas9 gene therapy aimed at these tumor-specific somatic mutations. Using whole genome sequencing of rapid autopsy tumor and normal samples from five patient cases, we identified single base substitutions present in tumor tissues and absent in patient-matched normal tissue. A subset (6.6% on average) of these mutations lead to novel protospacer adjacent motif (PAM) sites. These tumor specific PAM sites are necessary for Cas9 binding and DNA scission and are therefore selectively targetable by CRISPR-Cas9 systems. Using a hybrid capture/NGS approach, we demonstrate in patients with multiple metastases that the majority of these novel PAM sites, ~70%, are maintained among the different metastases. Using multitarget sgRNA which cut at a known number of sites in the human genome, we determined a relationship between number of CRISPR-Cas9 cuts and growth inhibition in PDAC cells. We found that 8-10 cut sites in non-coding regions are sufficient to induce >95% cancer cell death. Finally, using cocultures of fluorescently

labeled PDAC cells and a multiplexed sgRNA array targeting multiple tumor specific PAMs, we demonstrate selective cell killing of the targeted cell line. Importantly, deep sequencing of patient-matched normal lymphocytes lacking the tumor-specific PAMs treated with the same sgRNA array exhibited no editing at the targeted loci. This work establishes a bioinformatic platform for the discovery of tumor-specific CRISPR targets in the context of metastatic disease and demonstrates selective killing to cancer cells while sparing the patient's normal cells. These data establish the proof of principle for a gene therapy approach targeting metastatic disease.

**LB358**

**Efficacy of a functional precision medicine approach in relapsed/refractory pediatric cancer patients: results from a prospective clinical study.**

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Personalized medicine often serves as the first salvage therapy strategy when standard oncology treatments fail. However, most precision oncology approaches rely on molecular profiling which, unfortunately, provides therapeutic options for less than 10% of cancer patients. Functional precision medicine (FPM) complements molecular profiling by combining it with rapid, high-throughput drug testing on live patient cells to identify promising treatment options. In this study, we investigated the efficacy of FPM in the management of pediatric patients with recurrent and/or refractory cancers. We enrolled 25 pediatric/young adult patients with refractory solid or liquid cancers in this clinical trial (number NCT03860376). Enrolled patients represented the breadth of cancer indications generally presenting in pediatric patients: acute lymphoblastic leukemia (3 patients), acute myeloid leukemia (3 patients), astrocytoma (1 patient), ependymoma (1 patient), Ewing's sarcoma (4 patients), glioblastoma (1 patient), malignant rhabdoid tumor (1 patient), medulloblastoma (1 patient), neuroblastoma (1 patient), osteosarcoma (4 patients), rhabdomyosarcoma (4 patients) and Wilms' tumor (1 patient). We used a functional *ex vivo* drug sensitivity test (DST) panel encompassing 40 formulary drugs frequently used at Nicklaus Children's Hospital and 47 non-formulary drugs approved by FDA for cancer treatment, as well as drugs from phase III and IV clinical trials. Drug sensitivity scores (DSS) were calculated for each drug based on cancer cells' responses. DST results were then combined with results from targeted mutation profiles to match actionable mutations with selective targeted therapies. FPM and molecular data were prospectively generated and treatment recommendations were provided to an FPM Molecular Tumor Board (MTB) of clinicians. In total, 19 of 25 patients (76%) had complete functional and molecular profiling data provided to the MTB to support clinical decision-making. Six patients had their subsequent treatment guided by FPM recommendations, of which 83% (five of six patients) demonstrated greater than 1.3x increased progression-free survival compared to their previous therapy. Interestingly, hierarchical clustering analysis of DST results shows that patients with the same subtype of cancer do not cluster together, and no cancer subtype is differentially represented in either high-level cluster, suggesting the importance of functional profiling to provide deeper insight into individual patient pharmacological response. This FPM study is the first pediatric cancer study to enroll both solid and hematologic cancers regardless of tumor type, and the first FPM study in the United States to generate prospective treatment data on pediatric oncology patients. We illustrate the feasibility and efficacy of FPM to meet the needs of cancer patients with both liquid and solid tumors, especially for high-risk populations such as pediatric cancer patients.

**LB360**

**Type-1 interferon sensing is critical for CD8+ resident memory (TRM) cell generation in tumor-draining lymph nodes.**

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CD8+ tissue resident memory T cells ($T_{RM}$) are potent mediators of anti-tumor immunity. We have previously identified a population of lymph node resident memory T cells (LN $T_{RM}$) that are critical for protection against melanoma growth in tumor-draining lymph nodes (TDLN). However, the precursor population that gives rise to these cells, and mechanisms supporting their generation are not understood. Here we sought to define the features of tumor-specific T cells that govern their fate as $T_{RM}$ cells in TDLNs. We employed our previously described model wherein depletion of CD4+ T cells induces the generation of protective melanoma Ag-specific $T_{RM}$ cells throughout skin and LNs of B16 tumor-excised mice. To map the fate of $T_{RM}$ precursor cells (pre-$T_{RM}$), we implemented paired scTCR/RNA sequencing of antigen-experienced (CD44+ CD8+) T cells from surgically harvested TDLNs of individual mice and identified clonotypes that matched to differentiated $T_{RM}$ cells in LNs and skin of the same mice 30 days later. Additionally, we tracked gp100-specific CD8+ transgenic T cells (pmel cells) *in vivo* to define requirements for LN $T_{RM}$ generation. Clonal tracing revealed that expanded clonotypes in TDLNs differentiated into $T_{RM}$ cells in LNs and skin of tumor-excised mice. Interestingly, there were no apparent differences in the transcriptional properties of pre-$T_{RM}$ clonotypes (in early TDLNs) that acquired a $T_{RM}$ fate in the skin versus the LN. These pre-$T_{RM}$ clonotypes in TDLNs clustered into three major subsets, one of which appeared terminally differentiated with high expression of cell cycle genes, and two of which had high expression of genes associated with memory (*Il7r, Tcf7, Slamf6*) and tissue residency (*Cd69, Itgae, Cxcr6, Cxcr3*). One of the two memory-like precursor clusters additionally expressed high levels of interferon responsive genes (*Isg15, Isg20, Ifit1, Ifi47*). Experiments involving pmel cell adoptive transfer and FTY720 treatment showed that pre-$T_{RM}$ cells seeded LNs early, underscoring the existence of a committed $T_{RM}$ precursor population. Based on the pronounced IFN-sensing signature in pre-$T_{RM}$ clonotypes, we sought to define a role for IFN in $T_{RM}$ formation. Using Mx-1-GFP reporter pmel cells, we found that type-1 IFN sensing reached peak levels in TDLNs during tumor growth. To test whether type-1 IFN sensing was required for $T_{RM}$ generation, responses were assessed in IFNAR1 KO mice and additionally in mice that received wild-type vs. IFNAR1 KO pmel T cells. Interestingly, in both cases the absence of IFNAR1 resulted in a reduction in $T_{RM}$ generation TDLNs but not in skin. Overall, these results reveal the features of tumor-specific pre-$T_{RM}$ cells in TDLNs, and demonstrate that early type 1 IFN sensing dictates tissue-specific $T_{RM}$ fate decisions, predisposing to $T_{RM}$ generation in LNs.

**LB361**

**Li-Fraumeni syndrome-associated dimer-forming mutant p53 promotes transactivation-independent mitochondrial cell death.**

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Mutations that occur within the oligomerization domain (OD) of the tumor suppressor p53 generally abolish p53 tetramerization and are associated with increased cancer susceptibility in Li-Fraumeni syndrome (LFS). Despite their prevalence, the impact of OD-mutant p53 proteins in cancer, especially

beyond a loss of canonical p53 activity, has not been well elucidated. We sought to delineate the gain-of-function (GOF) vs. loss-of-function (LOF) activities of OD-mutant p53, specifically focusing on the LFS tumor-derived p53(A347D) variant. We obtained LFS patient-derived dermal fibroblasts heterozygous for the mutation and generated an allelic series of U2OS cancer cell lines expressing wild-type p53, heterozygous ($p53^{+/AD}$) or homozygous ($p53^{AD/AD}$) mutant p53 or no p53 (p53 KO). In contrast to wild-type p53, mutant p53(A347D) exclusively forms dimers in both fibroblasts and cancer cells and has lost the ability to bind and transactivate the majority of canonical p53 target genes, which yields comparable tumorigenic properties between mutant p53 and p53 KO cells. Mutant p53(A347D), however, displays neomorphic activities. Glycolysis and oxidative phosphorylation pathways are enriched in cells bearing p53(A347D) relative to both wild-type p53 and p53 KO cells. Furthermore, dimeric mutant p53 induces striking mitochondrial network aberration and preferentially associates with mitochondria to drive apoptotic cell death upon topoisomerase II inhibition in the absence of transcription. Ultimately, we describe dimeric mutant p53 as wielding a double-edged sword: driving tumorigenesis through LOF while gaining enhanced apoptogenic activity, thereby providing a potential basis for select therapeutic approaches.

**LB362**

**Epigenome-wide DNA methylation alterations precede diagnosis since birth and affect prognosis of pediatric B-cell acute lymphoblastic leukemia.**

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**Purpose of the study:** This study was aimed at identifying epigenome signature associated with risk of pediatric leukemia and uncovering molecular precursors of leukemia at birth in the blood of children before they develop the disease. Pediatric cancer is the leading cause of disease-related mortality in children and adolescents, with increasing incidence worldwide and lifelong sequelae in survivors. The most common form is leukemia, the causes of which are largely unknown. Growing evidence points to an origin *in utero*, when global redistribution of the epigenome modifications occurs driving tissue differentiation. Here, we sought to identify genome-wide differentially methylated genes at birth in

newborns who later developed pediatric precursor B-cell ALL (pre-B ALL), compared with those who did not.
**Experimental procedures:** Epigenome-wide DNA methylation was profiled in neonatal blood, with follow-up to pediatric pre-B ALL, using double-blinded analyses between prospective cohorts extending from birth to diagnosis and retrospective studies backtracking from clinical disease to birth. Validation was done using an independent technology and population (totaling 317 cases and 483 control) and complemented with pan-tissue methylation-stability (n=5,023 tissues; 30 types) and methylation-expression (n=2,294 tissues; 26 types) analyses. At diagnosis, methylation analysis was performed in leukemia tissues from pre-B ALL patients (n=644) with at least ten-year follow-up.
**Results:** We found a limited number of loci (among which an imprinted tumor suppressor gene) as being significantly hypermethylated at birth in nested cases relative to controls in all tested populations, including European and Hispanic ancestries. Some DMRs were found to be stable over follow-up years after birth and across surrogate blood and target bone marrow tissues. Differential methylation was found to be associated with a change in gene expression and with worse pre-B ALL patient survival, supporting a functional and translational role for differential methylation.
**Conclusions:** Our results provide proof-of-concept to detect at birth epigenetic alterations predisposing to childhood leukemia, reproducible in three continents and two ethnicities. DNA methylation alterations evident before diagnosis could be precursors of pediatric pre-B ALL development and actionable targets for risk assessment and prognosis.

**LB363**
**Patient-derived iPSCs faithfully represent the genetic diversity and cellular architecture of human acute myeloid leukemia.**
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The reprogramming of malignant cells to pluripotency has presented considerable challenges, hindering the application of induced pluripotent stem cell (iPSC) modeling technology to human cancers. In addition, how well iPSC-derived cells resemble their primary counterparts is currently largely unknown. We developed a reprogramming method tailored to human malignancies, “Complete Capture of Mutational Burden” (CCoMB), that combines comprehensive genetic characterization of the starting sample and inference of its clonal architecture with large-scale screening of clonally reprogrammed colonies. Using this method we were able to generate a panel of iPSC lines representing all major genetic groups of acute myeloid leukemia (AML). Specifically, we derived iPSC lines from 15 patients representing all major genetic groups of AML - PML-RARA; chromatin-spliceosome; TP53-mutated/aneuploidy; AML1-ETO, MLL-rearranged; NPM1-mutated and others - collectively capturing 21 distinct genotypes and 24 driver genetic lesions (mutations, translocations, deletions). Matched normal iPSCs were derived from 7 of these patients. Reprogramming to iPSCs captured both preleukemic (CH/initiating mutation only) and fully leukemic clones (baring the full set of patient mutations). In almost all cases, reprogramming informed reconstruction of the evolutionary hierarchy of the AML, with unexpected hierarchies unveiled in 4 of the cases. These AML-iPSCs retain genetic fidelity and, upon in vitro hematopoietic differentiation, produce hematopoietic cells with hallmark phenotypic leukemic features, including serial engraftment of a lethal myeloid leukemia into immunocompromised mice and extended self-renewal in vitro. Transplantation of cells derived from iPSCs representing two distinct AML clones from each of 3 patients revealed that these mimic the clonal dynamics in the patients, with more advanced clones showing increased representation in the xenografts, compared to the earlier clones. To compare the iPSC-derived to the primary leukemias, we performed single-cell transcriptomics analyses in patient-matched iPSC-derived and primary leukemic cells from 3 patients, both ex vivo/in vitro and after transplantation into NSGS mice. Clustering analyses identified cell types corresponding to primitive hematopoietic stem cell (HSC)/ multipotent progenitor (MPP), hematopoietic progenitor cells (HPCs) and

more mature myelomonocytic lineage cells in all samples from all patients, at varying frequencies. Leukemias derived through in vitro differentiation from iPSC lines exhibited both similarities, as well as differences, in their cellular composition and transcriptome, compared to the patient-matched ex vivo leukemias. However, upon transplantation of the same leukemias into NSGS mice, iPSC-derived xenografts were strikingly similar to the patient-derived xenografts. iPSC-derived leukemic cells exhibited a more stem/progenitor cell phenotype in vitro, with progressive maturation along the myeloid lineage upon primary and, even more, upon secondary transplantation, mimicking primary xenografts. In summary, our results reveal very few true biological barriers to the reprogramming of AML cells and show that AML-iPSC-derived leukemias faithfully mimic the primary patient leukemias upon xenotransplantation.

**CT001**

**A personalized cancer vaccine, mRNA-4157, combined with pembrolizumab versus pembrolizumab in patients with resected high-risk melanoma: Efficacy and safety results from the randomized, open-label Phase 2 mRNA-4157-P201/Keynote-942 trial.**

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**Background:** Targeting of mutation-derived epitopes (neoantigens) by T cells has been demonstrated to drive anti-tumor immune responses. mRNA-4157 is a novel mRNA-based personalized cancer vaccine, which encodes up to 34 patient-specific tumor neoantigens. It was hypothesized mRNA-4157 could synergize with adjuvant pembrolizumab to improve recurrence free survival (RFS) in patients with resected stages IIIB/IIIC/IIID and IV melanoma.

**Methods:** Eligible patients with completely resected, high-risk cutaneous melanoma were randomly assigned 2:1 (stratified by stage) to receive mRNA-4157 in combination with pembrolizumab or pembrolizumab alone. mRNA-4157 (1mg) was administered intramuscularly every 3 weeks for a total of 9 doses and pembrolizumab (200mg) intravenously was given every 3 weeks for up to 18 cycles. Safety was evaluated as a secondary endpoint. RFS in the overall intention-to-treat population was the primary end point. The study was designed with 80% power to detect a hazard ratio (HR) of 0.5 with an overall 1-sided type I error of 0.1 when a total of 40 RFS events were observed. The primary analysis for RFS was specified to occur after all patients completed a minimum of 12 months on study and at least 40 RFS events were observed.

**Results:** 107 patients received the combination of mRNA-4157 with pembrolizumab and 50 patients were treated with pembrolizumab monotherapy. Recurrence or death was reported in 24 of 107 patients (22.4%) in the combination arm and in 20 of 50 patients (40%) in the monotherapy arm, at a median follow-up of 101 and 105 weeks respectively. 18-month RFS rates (95% CI) were 78.6% (69.0%, 85.6%) vs 62.2% (46.9%, 74.3%) in the combination and monotherapy arm respectively. The combination showed protocol defined statistical significance and a clinically meaningful improvement in RFS compared to pembrolizumab, with a reduction in the risk of recurrence or death by 44% (HR = 0.561; 95% CI: (0.309, 1.017); stratified log-rank test 1-sided p-value of 0.0266. The majority of treatment related adverse events were Grade 1/2. The number of patients reporting treatment related Grade $\geq 3$ adverse events was generally similar between the arms (25% vs 18%, respectively). The most common mRNA-4157 related Grade 3 event was fatigue. No Grade 4 or Grade 5 events related to mRNA-4157 were reported. No potentiation of immune-mediated adverse events were observed with the addition of mRNA-4157 to pembrolizumab.

**Conclusions:** mRNA-4157 in combination with pembrolizumab as adjuvant therapy for resected high-

risk melanoma significantly prolonged RFS compared to pembrolizumab without an increase in clinically meaningful adverse events. These results are the first to demonstrate improvement of RFS over adjuvant standard of care PD-1 blockade in resected high-risk melanoma and provide the first randomized evidence that a personalized neoantigen approach is potentially beneficial for cancer patients. A phase 3 study will be initiated in patients with melanoma.

**CT002**

**KEYMAKER-U02 substudy 02C: neoadjuvant pembrolizumab (pembro) + vibostolimab (vibo) or gebasaxturev (geba) or pembro alone followed by adjuvant pembro for stage IIIB-D melanoma.**

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Background: The phase 1/2 KEYMAKER-U02 substudy 02C (NCT04303169) is evaluating neoadjuvant pembro + investigational agents or pembro alone followed by adjuvant pembro in stage IIIB-D melanoma. Results from patients treated with neoadjuvant pembro (anti-PD-1) + vibo (anti-TIGIT; arm 1), pembro + geba (coxsackievirus A21; arm 2), or pembro alone (arm 3) are presented.
Methods: Eligible patients were ≥18 y with resectable stage IIIB, IIIC, or IIID melanoma per AJCC 8th ed criteria, ≥1 measurable lesion per RECIST v1.1, and ECOG PS ≤1. Patients were randomly allocated across open investigational arms. Before resection, patients in arm 1 received 2 administrations of pembro 200 mg Q3W + vibo 200 mg Q3W (cycle 1, day 1; cycle 2, day 1); patients in arm 2 received 1 administration of pembro 400 mg (cycle 1, day 8) + 5 administrations of geba at a fixed dose of $3 \times 10^8$ tissue culture infectious dose 50% during cycle 1 (days 1, 3, 5, 8, and 22); and patients in arm 3 received 1 administration of pembro 400 mg. Surgical resection was performed at week 6. At week 12, patients started adjuvant pembro 400 mg Q6W for ≤8 administrations (total treatment duration, ~1 y). Primary end points were safety and tolerability and pCR rate by central review. Secondary end points were near pCR rate and pPR rate by central review and RFS by investigator review. ORR per RECIST v1.1 and EFS by investigator review were exploratory.
Results: At data cutoff (September 9, 2022), 66 patients had been assigned to treatment (arm 1 [pembro + vibo], n = 26; arm 2 [pembro + geba], n = 25; arm 3 [pembro alone], n = 15). Median follow-up was 14.1 mo (range, 8.0-26.1). Treatment-related AEs occurred in 92% of patients in arm 1, 84% in arm 2, and 80% in arm 3. Grade 3/4 treatment-related AEs occurred in 8%, 24%, and 7% of patients, respectively, with no grade 5 treatment-related AEs. 3 patients (12%) in arm 1, 5 (20%) in arm 2, and 0 in arm 3 discontinued any drug because of treatment-related AEs. Immune-mediated AEs or infusion reactions

were reported in 31% of patients in arm 1, 32% in arm 2, and 27% in arm 3. The pCR rate was 38% (95% CI, 20-59) in arm 1, 28% (12-49) in arm 2, and 40% (16-68) in arm 3; the near pCR rate was 12% (2-30), 12% (3-31), and 7% (<1-32), respectively; and the pPR rate was 31% (14-52), 12% (3-31), and 27% (8-55), respectively. Median RFS was not reached in any arm; 18-mo RFS rates were 95% (95% CI, 70-99) in arm 1, 87% (56-97) in arm 2, and 73% (24-93) in arm 3. ORR was 50% (95% CI, 30-71), 32% (15-54), and 27% (8-55), respectively. Median EFS was not reached in any arm; 18-mo EFS rates were 81% (95% CI, 60-92), 61% (38-78), and 79% (47-93), respectively.
Conclusions: Neoadjuvant pembro + vibo, pembro + geba, and pembro alone followed by adjuvant pembro had manageable safety and promising antitumor activity in patients with stage IIIB-D melanoma. Of the combination treatments, pembro + vibo showed the most promise.

**CT003**

**IMbrave050: Phase 3 study of adjuvant atezolizumab + bevacizumab versus active surveillance in patients with hepatocellular carcinoma (HCC) at high risk of disease recurrence following resection or ablation.**

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Background: The risk of HCC recurrence after liver resection or ablation with curative intent is 70-80% within 5 years, indicating an unmet need for effective adjuvant therapies. Atezolizumab (atezo) with bevacizumab (bev) is the standard of care for unresectable HCC based on the IMbrave150 study, which demonstrated statistically significant and clinically meaningful improvements in overall survival (OS), progression-free survival, and objective response rate versus sorafenib (Finn NEJM 2020, Cheng J Hepatol 2022). On the basis of the antitumor activity of atezo + bev and its capacity to positively modulate the tumor microenvironment, IMbrave050 was designed to evaluate the efficacy of adjuvant atezo + bev in delaying or preventing recurrence in patients (pts) with high-risk HCC.
Methods: IMbrave050 (NCT04102098) enrolled pts with HCC at high risk of recurrence following resection or ablation. High-risk criteria were based on tumor burden (tumor size and number), vascular invasion, and tumor differentiation. Pts were randomized to Arm A (atezo + bev) or Arm B (active surveillance). Stratification factors included geographic region (Asia-Pacific excluding Japan vs rest of world) and a composite factor encompassing the number of high-risk features, curative procedure, and use of optional adjuvant TACE (allowed for one cycle following resection). Pts in Arm A received atezo 1200 mg + bev 15 mg/kg IV q3w for a period of one year or 17 cycles. Pts in Arm B underwent active surveillance for one year and were eligible to crossover to atezo + bev following independent review facility (IRF) confirmation of recurrence. The primary endpoint was IRF-assessed recurrence-free survival (RFS). Secondary efficacy endpoints included OS; investigator-assessed (INV) RFS; RFS and

OS according to PD-L1 status; and time to extrahepatic spread and/or macrovascular invasion.
Results: The ITT population included 334 pts each in Arms A and B. Baseline demographics were well balanced between arms. At interim analysis, with a median follow-up of 17.4 mo (cut off date: Oct 21, 2022), the primary endpoint was met with an IRF-RFS HR of 0.72 (95% CI, 0.56, 0.93; *P*=0.0120), and results were generally consistent across clinical subgroups. INV-RFS was similar (HR, 0.70; 95% CI, 0.54, 0.91). The safety of atezo + bev was generally manageable and consistent with the well-established safety profile of each therapeutic agent and with the underlying disease.
Conclusions: Atezo + bev is the first adjuvant regimen to demonstrate a statistically significant and clinically meaningful improvement in RFS vs active surveillance in pts at high risk of disease recurrence following resection or ablation. The benefit:risk profile of atezo + bev favors the use of this regimen as an adjuvant therapy and has potential to set a new standard of care in adjuvant HCC.

**CT004**

**Intratumoral (IT) MEDI1191 + durvalumab (D): Update on the first-in-human study in advanced solid tumors.**

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**Background**: MEDI1191 is an IT-administered lipid nanoparticle-formulated mRNA encoding IL-12. This phase 1, open-label study (NCT03946800) showed that MEDI1191 in sequential (seq) or concurrent (conc) combination with IV D (anti-PD-L1) was safe and had preliminary antitumor activity in pts with advanced/metastatic solid tumors who had progressed on SoC. Here we present updated analyses, including the first report of pts with deep-seated lesions.
**Methods:** In Part 1A (subcutaneous/cutaneous [SC/C] lesions), pts received MEDI1191 on Days 1 and 22, followed by D 1500 mg on Day 43 then Q4W. In Parts 1B (SC/C) and 1D (deep-seated lesions), pts received MEDI1191 on Days 1, 29 and 57 then Q8W, with D on Day 1 then Q4W. Treatment continued for up to 2 years, until progression or unacceptable toxicity. Primary objectives were safety, tolerability and maximum tolerated dose (MTD); secondary objectives included preliminary antitumor activity per RECIST v1.1 in injected/non-injected lesions.
**Results:** As of October 5, 2022, 61 pts (40 had prior anti-PD-[L]1) received seq (Part 1A 0.1-12.0 μg, n=25 [4/25 had MEDI1191 only]) or conc (Part 1B 1.0-12.0 μg, n=27; Part 1D 1.0-3.0 μg, n=9) MEDI1191 + D. The most commonly represented cancer was melanoma (n=14). Pts in Part 1D had pancreatic (n=2), colorectal (n=2), gastric, anal, melanoma, neuroendocrine or unknown primary cancer (each n=1); all had hepatic metastases. There were no dose-limiting toxicities for MEDI1191 and no MTD was identified. Gr ≥3 MEDI1191-related AEs occurred in 3 pts (4.9%; Gr 3 asthenia and pyrexia, each n=1; Gr 4 lymphocyte count decreased, n=1); 2 pts (3.3%) had a MEDI1191-related serious AE (SAE; Gr 2 pyrexia and confusion, each n=1). Gr ≥3 D-related AEs occurred in 3 pts (4.9%; Gr 3 asthenia, pyrexia [both also MEDI1191-related] and pruritus; each n=1); 1 pt (1.6%) had a D- and MEDI1191-related SAE (Gr 2 pyrexia). In Parts 1A and 1B, 7 pts had partial responses (PRs): 5 confirmed (cPRs) in melanoma (n=2), sarcoma, breast and neuroendocrine cancer (each n=1), and 2 unconfirmed (uPRs) in melanoma and head and neck cancer (each n=1); no pts had PRs in Part 1D. 3/5

pts with cPR had prior anti-PD-(L)1; 2/3 also had prior anti-CTLA-4. For the 5 cPRs, DoR was 1.9-22.3 months (median not reached); 3 had ongoing PRs and 2 had stable disease (SD) at data cutoff. Non-target injected lesions shrank in 4 pts with cPRs. Overall, 15 pts had SD (including the 2 uPRs). MEDI1191 induced pharmacodynamic changes in the periphery and tumor microenvironment. A ≥2-fold increase in serum IL-12 levels was seen in 42/46 pts, with increases in serum IFNγ in 37/46; 9/22 pts had ≥2-fold increases in CD8+ T cell tumor infiltration and tumoral PD-L1 expression.
**Conclusions**: MEDI1191 + D was safe and tolerable in pts with SC/C or deep-seated lesions. Antitumor activity was seen in injected and distant lesions, and pharmacodynamic activity was consistent with expectations based on mechanistic biology.

**CT005**

**AEGEAN: A phase 3 trial of neoadjuvant durvalumab + chemotherapy followed by adjuvant durvalumab in patients with resectable NSCLC.**

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**Background:** Recent trials have demonstrated the clinical benefit of immunotherapy in either the neoadjuvant or adjuvant resectable (R) NSCLC setting. AEGEAN (NCT03800134) is a randomized, double-blind, placebo (PBO)-controlled trial assessing neoadjuvant durvalumab (D) + chemotherapy (CT) followed by surgery (Sx) and adjuvant D in patients (pts) with R-NSCLC.
**Methods:** Adults with treatment (Tx)-naïve R-NSCLC (stage II-IIIB[N2]; AJCC 8th ed) and ECOG PS 0/1 were randomized (1:1) to receive D 1500 mg or PBO IV + platinum-based CT (every 3 weeks [Q3W] for 4 cycles) before Sx, then further D 1500 mg or PBO IV (Q4W, up to 12 cycles). Pts were stratified by disease stage (II vs III) and PD-L1 tumor cell expression (<1% vs ≥1%, Ventana SP263). Pts with documented EGFR/ALK aberrations were excluded from the modified intent-to-treat (mITT) population for efficacy analyses. The primary endpoints were pathological complete response (pCR), evaluated centrally, and event-free survival (EFS; using RECIST v1.1), evaluated by BICR. Safety was assessed in all pts who received ≥1 study Tx dose.
**Results:** Between Jan 2, 2019, and Apr 19, 2022, 802 pts were randomized to the ITT population (n=740

in the mITT population) of whom 799 received Tx (D arm, n=400; PBO arm, n=399). Baseline characteristics were largely balanced (mITT). Overall, 84.7% in the D arm and 87.2% in PBO arm completed 4 cycles of platinum-doublet CT and 77.6% and 76.7%, respectively, completed Sx (mITT). As of Nov 10, 2022 (data cutoff), median EFS follow-up in censored pts was 11.7 months (mITT). The pCR rate was significantly higher and EFS significantly prolonged in the D vs PBO arms (mITT) (Table). In the safety analysis set, maximum grade 3/4 any-cause AEs occurred in 42.3% vs 43.4% in the D and PBO arms, respectively, during the overall Tx period.
**Conclusions:** The AEGEAN trial met both of its primary endpoints of improved pCR and EFS. Perioperative D plus neoadjuvant CT was associated with a manageable safety profile.
Clinical trial identification: NCT03800134 (release date: January 11, 2019)

| Endpoint | D arm | PBO arm | Tx effect | P value |
|---|---|---|---|---|
| pCR | n/N: 63/366 (17.2%) | n/N: 16/374 (4.3%) | Difference in pCR (95% CI), %: 13.0 (8.7-17.6)[a] | 0.000036 (assessed at IA)[b] |
| EFS | n events/N: 98/366 (26.8%) | n events/N: 138/374 (36.9%) | HR (95% CI): 0.68 (0.53-0.88)[d] | 0.003902[e] |
| | Median (95% CI), months: NR (31.9-NR)[c] | Median (95% CI), months: 25.9 (18.9-NR)[c] | | |

[a]CIs by stratified Miettinen and Nurminen's method. [b]Statistical significance was achieved at the IA (402 pts; data cutoff, Jan 14, 2022); no testing was performed at FA. The statistically significant p-value of 0.000036 was based on a Cochran-Mantel-Haenszel test. [c]Kaplan-Meier method. [d]Stratified Cox proportional hazards model. eStratified log-rank test. CI, confidence interval; FA, final analysis; HR, hazard ratio; IA, interim analysis; NR, not reached.

**CT006**

**First-in-class, first-in-human phase 1 trial of VT3989, an inhibitor of yes-associated protein (YAP)/transcriptional enhancer activator domain (TEAD), in patients (pts) with advanced solid tumors enriched for malignant mesothelioma and other tumors with neurofibromatosis 2 (NF2) mutations.**

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Background: Inactivation of the Hippo pathway is a common finding in MM and other cancers, leading to constitutive YAP activation, but has thus far been undruggable. *NF2* encodes Merlin that activates Hippo kinase resulting in inactivation of YAP, which is the major effector of Hippo signaling. *NF2*-deficient tumors, often due to inactivating *NF2* mutations (*NF2*m), are dependent on YAP for growth. VT3989 is an oral, highly potent and selective inhibitor of TEAD palmitoylation, which blocks YAP function and has shown promising preclinical activity.
Materials and Methods: This FIH trial used a 3 + 3 dose escalation design and evaluated VT3989 from 25 to 200 mg QD continuously and 50 to 200 mg intermittently. Pts with refractory solid tumors, ECOG PS 0-1, serum albumin > 2.5, urinary protein creatinine ratio $\leq$ 0.5 mg/mg and albumin creatinine ratio $\leq$ 100 mg/g were enrolled. Objectives were to determine the safety, tolerability and recommended Phase 2 dose (RP2D) of VT3989; to evaluate antitumor activity, pharmacokinetics (PK) and correlate *NF2*m and other

alterations in tumor and ctDNA with response.
Results: 67 pts have been enrolled. Median age 63.5y (21-83y), ECOG PS 0:1 13:54 pts. Median prior therapies 3 (range 0-8). Tumor types: 42 MM (29 pleural and 13 non-pleural) and 25 solid tumors including 9 meningioma and 4 sarcoma pts. 34 pts had *NF2*m (29 somatic and 5 germline). All MM pts had progressed on prior platinum/pemetrexed; all but 2 had prior immune checkpoint inhibitors. VT3989 is safe and well tolerated with no dose limiting toxicities. No MTD was defined up to 200 mg QD. The most common AEs are proteinuria, albuminuria and peripheral edema, mainly observed with the continuous schedule. There were 7 possibly related G3 AEs (fatigue, ALT, AST, dehydration, dyspnea, hypotension and peripheral edema) and 1 G4 cardiomyopathy. No pt developed decreased renal function or nephrotic syndrome. VT3989 has a 12-15 day half-life, dose proportional PK and reaches steady state within 2 weeks.
Seven pts (6 refractory MM and 1 *NF2*m sarcoma) achieved RECIST v1.1 partial responses (PRs) with 4 confirmed, 3 unconfirmed (1 pending). 3 PRs in MM pts are ongoing up to 18+ months. The clinical benefit response rate (PR + SD > 8 weeks, per protocol) in MM pts is 57%.
Of the 6 MM pts (1 pericardial, 1 pleural/peritoneal and 4 peritoneal) with PR, 2 have *NF2*m, 3 are wildtype, and 1 is unknown.
Conclusions: VT3989 is safe and well tolerated with durable RECIST v1.1 antitumor responses in pts with advanced MM and *NF2*m cancers, providing the first early clinical proof-of-concept for effectively drugging the Hippo-YAP-TEAD pathway. RP2D optimization including further schedule evaluation and expansion are ongoing in pts with MM and *NF2*m tumors.

**CT007**

**Phase 2-3 trial of pegargiminase plus chemotherapy versus placebo plus chemotherapy in patients with non-epithelioid pleural mesothelioma.**

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Background: Arginine deprivation with ADI-PEG20 (pegargiminase) alone or combined with chemotherapy displayed antitumor activity in early phase clinical trials of argininosuccinate synthase 1 (ASS1)-deficient cancers, including pleural mesothelioma (PM). The non-epithelioid subtype of

mesothelioma is particularly aggressive and arginine-auxotrophic due to frequent ASS1 loss. ATOMIC-meso is a pivotal phase 2-3 trial comparing standard of care (SOC) chemotherapy plus pegargiminase or placebo for patients (pts) with non-epithelioid PM.
Methods: In this double-blind, placebo-controlled, phase 2-3 trial, we randomly assigned ECOG PS 0-1 pts with chemonaïve non-epithelioid PM in an intention-to-treat 1:1 ratio to receive q3 week SOC chemotherapy with pemetrexed (500mg/$m^2$ i.v.) and cisplatin (75mg/$m^2$ i.v.) with q1 week pegargiminase (36 mg/$m^2$ i.m.) or placebo i.m. up to 6 cycles. Carboplatin (AUC5) was permitted in the phase 3 portion instead of cisplatin. Maintenance therapy with pegargiminase or placebo was continued until progression, toxicity or 24 months. The primary end point was median OS (mOS) and secondary end points included median PFS (mPFS), safety, pharmacodynamics and immunogenicity. ORR was assessed by blinded independent central review (BICR) for the phase 2 portion only using modified RECIST or RECIST v1.1.
Results: 249 pts with non-epithelioid PM (median age 71, range 28-86; male 82.7%; 48.2% biphasic and 51.8% sarcomatoid) from 5 countries were randomized between Aug 2017 and Aug 2021: 125 pts were assigned to the pegargiminase-chemotherapy group and 124 pts to the placebo-chemotherapy group. The experimental arm showed a superior mOS respect to SOC: 9.3 months (95% confidence interval [CI], 7.9-11.8) vs. 7.7 months (95% CI, 6.1-9.5) (hazard ratio [HR], 0.71; 95% CI, 0.55-0.93; p=0.023). Also, the mPFS was higher in the experimental vs. control arm: 6.2 months (95% CI, 5.8-7.4) vs. 5.6 months (95% CI, 4.14-5.91) ([HR], 0.65; 95% CI, 0.46-0.90; p=0.019). The ORR by BICR was 13.8% in the pegargiminase-chemotherapy group vs. 13.5% in the placebo-chemotherapy group (p=0.95) with more stable disease in the former (71.3% vs. 62.9%). Plasma arginine declined with a reciprocal increase in citrulline. Anti-ADI-PEG20 antibodies were detected in 97.4% of patients by week 25 on pegargiminase. Grade ≥ 3 treatment-related adverse events to pegargiminase occurred in 28.8% and to placebo in 16.9%; grade ≥ 3 drug hypersensitivity and skin reactions occurred in 3.2% and 1.6% in the experimental arm, respectively, and none on placebo. Post-study drug treatment was comparable in both arms (45.6% post-pegargiminase vs. 46.8% post-placebo).
Conclusions: In this first randomized trial of an arginine-depleting chemotherapy in cancer, the pegargiminase-pemetrexed-platinum triplet prolonged survival and had a favorable safety profile in pts with non-epithelioid PM (ClinicalTrials.gov Identifier NCT02709512)

**CT008**

**Pembrolizumab (pembro) in combination with gemcitabine and cisplatin (gem/cis) for advanced biliary tract cancer (BTC): Phase 3 KEYNOTE-966 study.**

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**Introduction**: BTC has a poor prognosis despite available treatment. KEYNOTE-966 is a randomized,

double-blind, phase 3 trial of pembro versus placebo in combination with gem/cis as first-line therapy for unresectable locally advanced or metastatic BTC (NCT04003636).
**Methods**: Eligible patients (pts) with histologically confirmed, RECIST v1.1-measurable disease with no prior systemic therapy in the unresectable or metastatic setting were randomized 1:1 to pembro 200 mg or placebo administered IV on day 1 Q3W for ≤35 cycles added to gemcitabine 1000 mg/m$^2$ administered IV on days 1 and 8 Q3W until disease progression and cisplatin 25 mg/m$^2$ administered IV on days 1 and 8 Q3W for ≤8 cycles. Randomization was stratified by region (Asia vs non-Asia), stage (metastatic vs locally advanced), and tumor origin (gallbladder vs intrahepatic vs extrahepatic). The primary end point was OS. Secondary end points were PFS, ORR, and DOR per RECIST v1.1 by blinded independent central review and safety. Per protocol, the final analysis of PFS and ORR was at the first interim analysis (IA1; data cutoff, December 15, 2021). All other data are from the protocol-specified final analysis (FA; data cutoff, December 15, 2022). One-sided *P*-value boundaries for significance were 0.0200 for OS, 0.0125 for PFS, and 0.0125 for ORR.
**Results**: 1069 pts were randomized to pembro + gem/cis (n = 533) or placebo + gem/cis (n = 536). Baseline characteristics were generally balanced between arms; 45.5% of pts were from Asia, 88.2% had metastatic disease, and 59.2% had intrahepatic origin. Median study follow-up was 25.6 mo (range 18.3-38.4) at FA. At FA, median (95% CI) OS was 12.7 mo (11.5-13.6) for pembro + gem/cis vs 10.9 mo (9.9-11.6) for placebo + gem/cis (HR 0.83; 95% CI 0.72-0.95; *P* = 0.0034); 24-mo OS was 24.9% vs 18.1%. OS results were generally consistent across subgroups. At IA1, median (95% CI) PFS was 6.5 mo (5.7-6.9) for pembro + gem/cis vs 5.6 mo (5.1-6.6) for placebo + gem/cis (HR 0.86; 95% CI 0.75-1.00; *P* = 0.0225); 12-mo PFS was 25.4% vs 19.8%. At IA1, ORR (95% CI) was 28.7% (24.9-32.8) for pembro + gem/cis vs 28.5% (24.8-32.6) for placebo + gem/cis (difference 0.2; 95% CI -5.2 to 5.6; *P* = 0.4735); median (range) DOR was 9.7 mo (1.2+ to 22.7+) vs 6.9 mo (0.0+ to 19.2+). Grade 3-5 AEs occurred in 85.3% of 529 pts treated in the pembro + gem/cis arm vs 84.1% of 534 treated in the placebo + gem/cis arm (drug related, 71.3% vs 69.3%). Grade 5 AE incidence was 5.9% vs 9.2% (drug related, 1.5% vs 0.6%). Potentially immune-mediated AEs and infusion reactions occurred in 22.1% vs 12.9%.
**Conclusion:** Pembro + gem/cis provided a statistically significant, clinically meaningful OS improvement versus placebo + gem/cis in pts with previously untreated metastatic or unresectable BTC. The safety profile of pembro + gem/cis was as expected and manageable. These data support pembro + gem/cis as a new first-line treatment option in this setting.

**CT009**

**S1512: High response rate with single agent anti-PD-1 in patients with metastatic desmoplastic melanoma.**

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**Background**: Desmoplastic melanoma (DM) is a subtype of melanoma with high tumor mutation burden (TMB) due to ultraviolet light damage. A previously published retrospective review of patients with PD-1 blockade treated DM suggested this cancer may be highly responsive to PD-1 blockade (Eroglu et al. Nature 2018). S1512 is the first prospective study investigating PD-1 blockade with pembrolizumab in patients with DM. 1512 Cohort A results were presented and demonstrated a 59% complete pathologic response rate with neoadjuvant pembrolizumab in patients with resectable DM (Kendra et al. ASCO 2022). Herein, we present results from S1512 Cohort B in patients with metastatic DM.
**Experimental procedures**: Patients aged $\geq$18 with metastatic DM received pembrolizumab 200 mg q3 weeks. Eligibility criteria included: Zubrod PS 0-2, no central nervous system metastases, autoimmune disease, or steroid $\geq$10 mg of prednisone. The primary endpoint was complete response rate (CR) assessed per RECIST 1.1, with the assumption that CR of 20% or higher indicates that the treatment is of interest. A single stage design with 21 eligible patients would have an alpha of 8.5% (when the true CR is 5%) and a power of 82%. Tumor assessments were performed every 9 weeks. Secondary endpoints included progression free survival (PFS), overall survival (OS), and toxicity assessment. Biopsies were analyzed by whole exome sequencing (WES) for mutational load and oncogenic driver mutations.
**Summary of data**: 27 eligible patients were enrolled in Cohort B. 93% were male, 70% had Zubrod PS 0, median age 75 (range 59-90). Median number of cycles received: 15 (range 1-34). At the time of data cutoff, 3 patients remain on protocol treatment with pembrolizumab. Treatment was discontinued due to: 2 years of therapy (4), adverse event (AE) (8), physician and patient choice (6), progressive disease (4), and under review (2). Objective response rate (including both confirmed and unconfirmed partial and complete responses) was 85% (95% CI: 66%-96%), with 7/27 CR (26%) and 16/27 PR (59%). Grade 3 or higher adverse events were reported in 10 patients. WES analysis was available for 16 patients. Both baseline (N=10) and on-treatment biopsies (N=12) were evaluated for canonical genetic drivers of melanoma; 7 patients had tumors with loss of function (LOF) mutations in *NF1*, and all had mutations in *TP53*, with no case having activating mutations in *BRAF* or *NRAS*, consistent with the known genetic alterations in DM. The median TMB in baseline biopsies was 79.4 Mut/Mb (range 34.3-159).
**Conclusion:** Patients with metastatic DM are exceptional responders to single agent PD-1 blockade with pembrolizumab. Based on this data, the pathologic subtype of melanoma can be used as a predictive biomarker of response to single agent pembrolizumab. Funding: U10CA180888 and U10CA180819; and in part by Merck Sharp & Dohme LLC., a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

**CT011**

**A phase 1 multicenter study (TRAVERSE) evaluating the safety and efficacy of ALLO-316 following conditioning regimen in pts with advanced or metastatic clear cell renal cell carcinoma (ccRCC).**

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Background: While doublet therapies provide important first-line treatment options in metastatic renal cell carcinoma (mRCC), novel and mechanistically distinct agents are needed for pts who are not cured by/intolerant of such therapies. Due to the high incidence (~80%) of CD70 antigen expression in primary and mRCC, yet limited expression in normal tissue, ccRCC is an attractive proof-of-concept tumor for CD70 directed allogeneic CAR T. TRAVERSE (NCT04696731), a first-in-human trial, seeks to identify a maximum tolerated dose (MTD) of ALLO-316 after conditioning with fludarabine/cyclophosphamide with/without ALLO-647 in pts with advanced or metastatic ccRCC. ALLO-316 is an anti-CD70

allogeneic CAR T cell product that utilizes TALEN® gene editing to knock out TCRα constant gene to reduce the risk of graft-versus-host disease (GvHD) and knock out CD52 gene to permit use of ALLO-647 (a humanized anti-CD52 mAb) to selectively deplete host T cells without affecting allogeneic CAR T cells.
Methods: This multicenter, single-arm, open-label, 3+3 dose-escalation trial enrolls adults with advanced or metastatic ccRCC and ≥1 measurable lesion and ECOG Performance Status 0 or 1. Prior treatment with an immune checkpoint inhibitor and a vascular endothelial growth-factor targeted therapy was required, with evidence of progression on/after treatment or discontinuation due to toxicity. ALLO-316 is administered at escalating doses (40 - 240 X $10^6$ allogeneic CAR+ cells IV) on Day 0 after conditioning. The primary endpoint is a target incidence rate for dose-limiting toxicities (DLTs) <33% in the first 28 days after infusion of ALLO-316.
Results: By 12/3/2022, 18 pts with ccRCC (median age: 63 yrs; 82% male) were enrolled; all (100%) 17 pts who received ALLO-316, had metastatic disease with 3 lines (median) of prior therapy. Eleven (65%) of these pts experienced CRS, all low Gr except one (6%) Gr 3. No ICANS or GVHD was observed. One (6%) DLT (elevated LFT) was observed and required dose expansion. MTD has not yet been reached. Three pts achieved best overall response of PR at all time points with two PRs confirmed at subsequent visits; ORR = 12% and disease control rate (DCR) = 71%. In pts with confirmed CD70+ tumors (n=9), confirmed ORR = 22% (unconfirmed ORR = 33%) and DCR = 100%. High CAR T cell expansion was observed in peripheral blood (median $C_{max}$ > 35,000 copies/μg) and high VCN in available tumor aspirates (n=3).
Conclusions: ALLO-316, an allogeneic CAR T cell product targeting CD70 in advanced mRCC, is demonstrating encouraging antitumor activity and a manageable safety profile. A single administration of ALLO-316 could be an effective treatment for pts with CD70+ solid tumors, including RCC, and hematologic malignancies. The MTD for ALLO-316 in TRAVERSE will support Phase 2 trial design. Enrollment of pts with CD70+ tumors is ongoing.

**CT012**

**Clinical activity of MCLA-158 (petosemtamab), an IgG1 bispecific antibody targeting EGFR and LGR5, in advanced head and neck squamous cell cancer (HNSCC).**

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EGFR is a known driver of cancer growth, and leucine-rich, repeat-containing, G-protein coupled receptor 5 (LGR5) is a transmembrane receptor expressed on cancer stem cells. Petosemtamab is a human, common light chain, IgG1 bispecific antibody with ADCC-enhanced activity, targeting EGFR and LGR5, which has shown potent antitumor activity in patient (pt)-derived HNSCC xenograft models. In the dose escalation part of an ongoing phase 1/2 study, the RP2D was established at 1500 mg Q2W, 4 week cycle (JCO 2021:39.3 Sup 62). Petosemtamab is being investigated in the expansion part in pts with selected advanced solid tumors. Promising activity was seen in HNSCC pts previously treated with both platinum-based chemotherapy and checkpoint inhibitors (MCT 2021:20 Sup12 P185). We present data of

the expanded HNSCC cohort treated at the RP2D. Primary objective (expansion): investigator-assessed ORR per RECIST 1.1. Secondary objectives: ORR, DOR, PFS (per investigator and central review), OS and safety/tolerability. Key eligibility criteria: advanced/metastatic HNSCC, prior standard therapy, ECOG PS 0-1, measurable disease (RECIST 1.1), baseline tumor biopsy. At the data cutoff date (28 Nov 2022), 49 HNSCC pts were treated. Median age was 63 years (range 31-77), ECOG PS 0/1: 13/35 pts, and 78% were male. Most frequent primary tumor locations were oropharynx (35%), oral cavity (31%), and larynx (16%). Pts received a median of 2 (range 1-4) lines of prior systemic therapy, including anti-PD-1/PD-L1 in 96% of pts and platinum-based chemotherapy in 92% of pts; 2 pts received prior cetuximab. A median of 4 treatment cycles (range 1-21) was administered, with 17 pts continuing on therapy at the cutoff. Among 42 pts evaluable for efficacy (≥2 cycles and ≥1 postbaseline scan, or early PD), ORR per investigator was 35.7% (15/42), including 1 CR (ongoing after 18 months), 12 PRs, and 2 unconfirmed PRs with treatment ongoing at the cutoff. 15 pts had SD and DCR was 71.4%. Median DOR was 6.0 months (95%CI=3.3-not calculable). Median PFS was 5.0 months (95%CI=3.2-6.8). Of 78 pts treated at the RP2D (escalation and all expansion cohorts), the most frequent AEs regardless of causality (all grades/G3-4) were rash (33%/0%), hypotension (26%/6%), dyspnea (26%/4%), nausea (26%/1%), dermatitis acneiform (24%/1%), blood magnesium decreased (19%/5%), erythema (19%/0%), diarrhea (19%/0%); IRRs (composite term) were reported in 74%/21% of pts, mostly at the first infusion, and all resolved. 5 pts (6%) discontinued treatment due to IRRs on Day 1. 1 esophageal cancer pt died due to unrelated G5 GI bleeding. Retrospective biomarker analyses will be presented. Petosemtamab demonstrates promising clinical efficacy with a manageable safety profile in pretreated HNSCC pts. Further clinical development in HNSCC is planned with petosemtamab monotherapy and in combination with SOC.

**CT013**

**Safety and efficacy from the phase 1/2 first-in-human study of REGN5459, a BCMA×CD3 bispecific antibody with low CD3 affinity, in patients with relapsed/refractory multiple myeloma.**

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Background: MM remains incurable and new tx options are needed, especially in heavily pre-treated R/R pts. REGN5459, a BCMAxCD3 bispecific Ab, binds to BCMA on MM cells and with low affinity to CD3 on T cells, triggering T-cell activation and plasma cell depletion with low cytokine release preclinically. This first-in-human study aimed to assess the safety, tolerability, and preliminary anti-tumor activity of REGN5459 monotherapy in pts with R/R MM.
Methods: Eligible pts had received ≥3 prior lines of tx including an anti-CD38 Ab, proteasome inhibitor, and immunomodulatory drug, and had exhausted all available tx options. Pts could receive REGN5459 until progression/intolerable toxicity. Primary objectives were to assess safety, tolerability, dose limiting toxicities (DLTs), and determine the recommended Ph 2 dose (RP2D) of REGN5459 (Ph 1) and assess efficacy of REGN5459 (Ph 2) per ORR.
Results: As of June 9, 2022, 43 pts were enrolled (Ph 1, 33; Ph 2, 10): median age 67 yrs (range, 26-85), 51% female, 16% high cytogenetic risk, 14% extramedullary plasmacytoma, 37% R-ISS Stage III disease, 61% triple-class refractory, and median of 5 (range, 2-9) prior lines of tx. In Ph 1, one DLT was reported in a pt receiving the highest dose (900 mg; Gr 3 hypoxia, pt later found to have primary lung cancer). RP2D was identified as 480 mg. All pts enrolled had ≥1 TEAE, 74% had Gr ≥3 TEAEs. TEAEs

all-Gr in ≥30% of pts were CRS (54%), fatigue (44%), neutropenia (37%), anemia (35%), cough (30%), and diarrhea (30%). Gr ≥3 TEAEs in ≥15% of pts were neutropenia (37%), anemia (26%), lymphopenia (23%), thrombocytopenia (19%), and hypertension (16%). CRS Gr 1, 2, and 3 were reported in 47%, 2%, and 5%, respectively; there was no Gr 4 or 5 CRS. No Gr 3 CRS with RP2D. Tocilizumab was used in 19% and steroids in 9% of pts. One pt developed ICANS (2%, Gr 2). Incidence of serious TEAEs and TEAEs leading to tx discontinuation was 63% and 16%. Infections occurred in 61% (37% Gr ≥3). After data-cutoff, two deaths due to COVID pneumonia and COVID infection have been reported. ORR was 67% (58% ≥VGPR) for the entire cohort and 100% (85% ≥VGPR; 15% sCR; 39% CR) among pts receiving the RP2D (n=13). Median follow-up was 7 mos (range, 1-26) with longest response ongoing for 22+ mos. Median time to response was 0.8 mos. Median DOR was NR (95% CI, 12-NE); 12-mo DOR for RP2D was 66.7% (95% CI, 5.4-94.5). Of pts in ≥CR with available MRD results (n=8), 50% were MRD negative at the $10^{-5}$ threshold.
Conclusion: These initial data show that REGN5459 has acceptable safety/tolerability in R/R MM with most CRS of low grade and low incidence of ICANS. Modulation of CD3 affinity on bispecific Abs to maximize tumor killing, and mitigate CRS and T-cell exhaustion, warrants further research. Efficacy in this heavily pre-treated cohort was encouraging, with 100% ORR with the RP2D. Updated data will be presented at the meeting.

**CT014**

**Phase I dose-escalation study of fractionated dose 225Ac J591 for metastatic castration resistant prostate cancer.**

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Background: PSMA-based targeted radionuclide therapy is now a standard of care for mCRPC since approval of 177Lu-PSMA-617. Use of antibodies (e.g., J591) to target PSMA with higher potency radionuclides (e.g., 225Ac) impacts kinetics, biodistribution, clinical efficacy, and toxicities. In our first-in-human ph I dose-escalation study of single dose 225Ac-J591 in patients with mCRPC, no MTD was reached (max dose 93.3 KBq/kg). Following this, we developed ph I/II parallel dose-escalation studies of fractionated (D1, D15) single cycle and multiple (q6w) dose regimens. Here we present safety data from the initial fractionated study in predominantly 177Lu-naive.
Methods: Eligible patients had adequate organ function, ECOG performance status 0-2, and progressive mCRPC following potent AR pathway inhibitor (ARPI) and chemo (or unfit/refusing). Prior 177Lu-PSMA was allowed until an amendment developed a separate study for post-177Lu-PSMA. Baseline 68Ga-PSMA PET scans were performed, but not used to determine eligibility. A 3+3 dose-escalation design was used. Phase I primary objective: determination of dose-limiting toxicity (DLT) and recommended phase II dose (RP2D). DLT was defined as within 8 weeks of first dose: neutropenia (Gr 4 or febrile neutropenia), thrombocytopenia (TCP) (Gr 4, or Gr 3 with clinically significant bleeding), any Gr >2 non-hematologic toxicity deemed to be at least possibly related to 225Ac-J591, or any attributable toxicity precluding or delaying the second dose by >2 weeks. Secondary/exploratory objectives: efficacy measures (e.g., PSA decline, radiographic RR, biochemical/radiographic PFS, OS, CTCs, patient reported outcomes), safety (CTCAE v5), and correlatives (plasma and tissue genomics, PSMA imaging).
Results: 24 patients were enrolled in phase I. Median age 73.5 (57-91), PSA 25.78 (3.39-2133.41); 53% (n=13) >1 prior ARPI, 58% (n=14) taxane chemo, 8% (n=2) anti-PSMA therapy, 12.5% (n=3) prior 223Ra. CALGB prognostic groups: Good 4 (16%), Intermediate 8 (33%), Poor 12 (50%). No DLTs were observed in Cohort 1 (n=3) or 2 (n=6). In C3, 2/6 subjects experienced DLTs (Gr 3 weakness, Gr 2 TCP

with >2 week delay in second fraction). 8 patients were enrolled in an intermediate dose cohort (2.5) with 1 DLT (Gr 4 TCP). Two patients withdrew before the second dose (intercurrent illness; interruption of 225Ac supply). Most common low gr non-hematologic treatment emergent AEs: fatigue (95%), xerostomia (69%), and nausea (57%). Among evaluable patients for PSA change (n=22), 21 (95%) experienced PSA decline with 14 (67%) with decline of 50% and 6 (37%) with decline of 90%. 13/21 patients had CTCs samples collected at baseline and 12 wks; 5 were unfavorable at baseline (≥5/7.5 mL); 10/13 (77%) remained favorable or converted from unfavorable to favorable; 6/12 (50%) had 50% decline in CTC count; and 5/13 (38%) converted from detectable to undetectable.
Conclusions: A single fractionated cycle of 225Ac-J591 was delivered with few high grade AEs and with evidence of preliminary efficacy by PSA and CTC changes across all dose levels.

**CT016**

**Clinical and translational findings of pemigatinib in previously treated solid tumors with activating *FGFR1-3* alterations in the FIGHT-207 study.**

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**Background:** Fibroblast growth factor receptor (*FGFR*) alterations are promising targets in different tumors. We report results of FIGHT-207, an open-label, single-arm phase 2 study of pemigatinib, a potent, selective FGFR1-3 inhibitor, in patients with previously treated unresectable or metastatic *FGFR*-altered solid tumors (NCT03822117).
**Methods:** Patients were assigned to cohorts A (*FGFR1-3* fusions), B (*FGFR1-3* activating non-kinase domain mutations), and C (*FGFR1-3* kinase domain mutations and alterations of unknown significance) and received continuous 13.5 mg pemigatinib once daily. Prior FGFR inhibitor treatment was prohibited. The primary endpoint was objective response rate (ORR) per RECIST v1.1 or RANO as confirmed by independent central review.
**Results:** 107 patients were enrolled and assigned to cohorts A (n=49), B (n=32), and C (n=26). ORR in cohorts A, B, and C was 26.5%, 9.4%, and 3.8%, respectively. Responses were observed for multiple tumor types, including gliomas and gynecologic and pancreatic tumors (**Table**). Notably, we saw responses for several infrequent *FGFR* alterations, including an *FGFR1* kinase domain mutation (K656E) and an *FGFR2* extracellular in-frame deletion (*FGFR2* I291_Y308Del). Among patients in cohorts A+B with centrally confirmed *FGFR* alterations, *BAP1* co-alterations were observed in 47% (7/15) of responders, and *TP53* co-alterations were present in 43% (23/53) of nonresponders. Safety was consistent with prior reports.
**Conclusions:** In addition to cholangiocarcinoma, pemigatinib showed clinical activity in gliomas, gynecologic tumors, and pancreatic cancer and safety was consistent with prior reports. We observed responses in patients with previously unreported *FGFR* alterations, suggesting that a broader population of patients may benefit from FGFR inhibitors. Further correlative work to predict response to therapy is needed to better identify these patients.

**Table. Characteristics of Patients With Best Percentage Change From Baseline in Target Lesion Size ≥30%**

| Patient | Tumor Type | *FGFR* alteration* | BOR | Best % Change From BL in Target Lesion Size | PFS, mo |
|---|---|---|---|---|---|
| | **Gliomas** | | | | |
| 1 | Glioblastoma | *FGFR3* fusion | CR | −100.0 | 20.2† |
| 2 | Glioblastoma | *FGFR3* fusion | PR | −100.0 | 8.4† |
| 3 | Diffuse astrocytoma | *FGFR1* K656E mutation | PR | −80.7 | 8.0 |
| | **Gynecologic** | | | | |
| 4 | Cervical cancer | *FGFR3* fusion | PR | −100.0 | 6.4 |
| 5 | Cervical cancer | *FGFR3* fusion | PR | −32.1 | 6.3 |
| 6 | Endometrial cancer | *FGFR1* rearrangement | SD | −100.0 | 1.9† |
| 7 | Ovarian cancer | *FGFR2* fusion | SD | −44.0 | 2.1† |
| 8 | Cervical cancer | *FGFR2* C382R mutation | SD | −35.6 | 5.6 |
| | **Pancreatic** | | | | |
| 9 | Pancreatic adenocarcinoma | *FGFR2* fusion | PR | −72.1 | 8.4† |
| 10 | Pancreatic adenocarcinoma | *2 FGFR2* fusions | PR | −54.9 | 8.9† |
| 11 | Pancreatic adenocarcinoma | *FGFR2* fusion | PR | −33.3 | 6.3† |
| 12 | Pancreatic adenocarcinoma | *FGFR2* fusion | SD | −100.0 | 2.7 |
| | **Cholangiocarcinoma** | | | | |
| 13 | Cholangiocarcinoma | *FGFR2* fusion | PR | −64.6 | 6.2 |
| 14 | Cholangiocarcinoma | *FGFR2* fusion | PR | −61.4 | 9.9 |
| 15 | Cholangiocarcinoma | *FGFR2* fusion | PR | −53.5 | 8.8 |
| 16 | Cholangiocarcinoma | *FGFR2* C382R mutation | PR | −49.7 | 6.0 |
| 17 | Cholangiocarcinoma | *FGFR2* fusion | PR | −46.2 | 5.9† |
| 18 | Cholangiocarcinoma | *FGFR2* fusion | PR | −43.2 | 10.5 |
| 19 | Cholangiocarcinoma | *FGFR2* I291, Y308 deletion | PR | −42.5 | 14.8 |
| 20 | Cholangiocarcinoma | *FGFR2* fusion | PR | −34.1 | 11.2† |
| 21 | Cholangiocarcinoma | *FGFR2* fusion | SD | −42.2 | 2.5† |
| 22 | Cholangiocarcinoma | *FGFR2* Y375C mutation | SD | −37.5 | 3.7† |
| 23 | Cholangiocarcinoma | *FGFR2* W290C mutation | SD | −34.0 | 10.5 |
| | **Urothelial tract/bladder cancer** | | | | |
| 24 | Urothelial tract/bladder cancer | *FGFR3* Y373C mutation | PR | −50.8 | 6.2† |

| | | | | | |
|---|---|---|---|---|---|
| 25 | Urothelial tract/bladder cancer | *FGFR3* Y373C mutation | SD | −30.0 | 3.9 |
| | **Other** | | | | |
| 26 | Non-small cell lung cancer | *FGFR2* fusion | SD | −72.0 | 4.1 |
| 27 | Prostate cancer | *FGFR2* fusion | SD | −32.5 | 6.4 |

BL, baseline; BOR, best overall response; CR, complete response; FGFR, fibroblast growth factor receptor; PFS, progression-free survival; PR, partial response; SD, stable disease. *
Includes *FGFR* alterations confirmed through both local and central genetic testing reports. † Censored.

**CT017**

**Pan-mutant and isoform selective PI3Kα inhibitor, RLY-2608, demonstrates selective targeting in a first-in-human study of *PIK3CA*-mutant solid tumor patients, ReDiscover trial.**

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**BACKGROUND**: Oncogenic activation of PI3Kα via mutation of *PIK3CA* is the most common kinase driver event across solid tumors, particularly breast cancer (BC). Alpelisib, an orthosteric inhibitor, validated mutant-PI3Kα as a therapeutic target; however, toxicity from non-selective inhibition of WT PI3Kα (hyperglycemia) and other PI3K isoforms limits its clinical activity. RLY-2608, a novel oral allosteric PI3Kα inhibitor, is designed to overcome these limitations via mutant- and isoform-selective PI3Kα inhibition. We initiated a first-in-human study (NCT05216432) to define the MTD, safety, pharmacokinetics (PK), and anti-tumor activity of RLY-2608 in *PIK3CA*-mutant solid tumor patients (pts) and of RLY-2608 plus fulvestrant (combo) in pts with *PIK3CA*-mutant, HR+HER2-BC, who had received prior CDK4/6 inhibitor and endocrine therapy.
**METHODS**: Adult pts with no prior PI3Ki/mTORi therapy received RLY-2608 QD or BID on a 4-week (wk) cycle following a Bayesian Optimal Interval dose escalation. Eligible BC pts received fulvestrant. PK, pharmacodynamics (ex vivo pAKT inhibition; tumor markers; ctDNAs), treatment emergent adverse events (TEAEs), and anti-tumor activity per RECIST 1.1 were assessed.
**RESULTS**: As of 31Jan23, 40 pts (21 BC combo pts) with *PIK3CA* mutations (12 kinase, 18 helical, 10 other) received RLY-2608 at doses of 100-1600 mg/day. Median number of prior treatments was 4 (1-11). An MTD has not been reached and dose escalation continues. RLY-2608 PK were favorable with BID dosing providing low steady-state peak-to-trough variability for mutant-selective inhibition. This enabled dose-dependent increase in exposure and target inhibition with doses ≥600 mg BID and pAKT suppression (≥80%) in the expected therapeutic range with minimal/no impact on glucose homeostasis. Across arms, safety was consistent: most frequent TEAEs (>15%) were low grade (≤Gr 2) nausea (30%), fatigue (22.5%), headache (22.5%), hypokalemia (22.5%), and diarrhea (17.5%). No DLTs were observed, and no pts discontinued due to related AEs. Initial anti-tumor activity was observed in BC combo pts including declines in tumor markers, *PIK3CA*/*ESR1* ctDNAs and radiographic tumor reduction

in 7 of 10 RECIST measurable pts. At 600 mg BID, 6/6 BC combo pts had disease stabilization and remain on treatment, including all RECIST-measurable pts with tumor reduction. Overall, 17 (81%) BC combo pts remain on treatment with median duration of 12 (1-40) wks.
**CONCLUSION**: Across *PIK3CA* genotypes, RLY-2608 demonstrated target inhibition and anti-tumor activity with minimal impact on glucose homeostasis. These proof-of-mechanism data indicate that RLY-2608 is the first allosteric, pan-mutant selective PI3Kα inhibitor and that RLY-2608 has broad therapeutic potential in *PIK3CA*-driven cancers.

**CT018**

**Safety and efficacy of three PARP inhibitors (PARPi) combined with the ataxia telangiectasia- and Rad3-related kinase inhibitor (ATRi) camonsertib in patients (pts) with solid tumors harboring DNA damage response (DDR) alterations.**

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Introduction: ATRi and PARPi combinations kill tumor cells via synergistic modulation of complementary DDR pathways but clinical utility is limited by overlapping toxicities. A genome-wide CRISPR-Cas9 screen identified DDR alterations that sensitize tumors to the ATRi camonsertib (cam) plus PARPi. Based on preclinical models, tolerability and efficacy of cam plus PARPi given intermittently at low doses in pts with solid tumors with DDR alterations, including BRCA-mutated PARPi-resistant cancers, was evaluated.
Methods: Pts (≥ 18 yrs) with relapsed/refractory (r/r) solid tumors with DDR alterations were treated with cam plus talazoparib (tala), niraparib (nira), or olaparib (ola) in 2 phase I trials (NCT04497116, NCT04972110). An adaptive BOIN design was used to optimize dose/schedule. Endpoints were safety, recommended phase II dose (RP2D), pharmacokinetics (PK), response (RECIST v1.1 [confirmed/unconfirmed], CA 125, or PSA), clinical benefit rate (CBR; RECIST/tumor marker response or treatment duration ≥ 16 wk), and ctDNA molecular response rate (MRR; best reduction in mean variant allele frequency [mVAF] ≥ 50%).
Results: As of Nov 2022, 99 pts were enrolled (61 with ≥ 3 prior lines of therapy, 36 PARPi-pretreated); 43, 27, 29 pts in tala, nira, and ola combinations, respectively. Tumors included ovarian (n=20), prostate (n=13), breast (n=17), bile duct (n=3); most common genotypes were *ATM* (n=26), *BRCA1* (n=19), and *BRCA2* (n=33). Grade 3+ toxicities included reversible myelosuppression (neutropenia, anemia, and thrombocytopenia in 35%, 28%, and 14% of pts overall; 33%, 16%, and 11% at preliminary RP2Ds),

fatigue (3%), and alkaline phosphatase increase (2%). Two pts discontinued treatment due to drug-related toxicity. Forty pts remain on therapy, up to 63 wks. Preliminary RP2Ds: cam 80 mg QD (d 5-7) + tala 0.25 mg QD, 1 wk on/1 wk off; cam 80 mg QD + nira 100 mg QD, 2 d on/5 d off; cam 50 mg QD + ola 100 mg BID, 3 d on/4 d off. As of abstract submission, response rate was 13% in 85 evaluable pts; CBR was 49%. Responders included pts with ovarian (n=6), bile duct (n=2), and breast, pancreatic, and carcinoma of unknown primary (n=1 each) cancers harboring alterations in *BRCA1/2* (n=9), *IDH1* (n=1), and *ATM* (n=1). Of 30 evaluable PARPi-pretreated pts, response rate was 23% and CBR was 52%. MRR was 64% (14/22 evaluable); change in mVAF correlated with a change in target lesion size (r=0.63, P=0.001). PK of each drug in the combination was consistent with respective monotherapy PK. Conclusion: Combination of cam plus PARPi at low doses on intermittent schedules was generally well-tolerated with clinical activity in DDR-aberrant tumors, including those pretreated with PARPi. RP2D optimization and translational correlative studies are ongoing.

**CT019**

**Phase I/II study of the PARP inhibitor olaparib and ATR inhibitor ceralasertib in children with advanced malignancies: Arm N of the AcSé-ESMART trial.**

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Background: AcSé-ESMART is a European proof-of-concept, phase I/II, platform precision cancer medicine trial designed to explore targeted agents in a molecularly enriched population. Arm N explores the PARP inhibitor (PARPi) olaparib (ola) in combination with the Ataxia telangiectasia mutated rad3 related (ATR) inhibitor (ATRi) ceralasertib (cer). In pediatric cancer, the rationale for this combination is twofold: i) Many cancers show evidence of a constitutively active ATR pathway due to endogenous replication/transcription stress (RS) which can be targeted by ATRi. PARPi in this setting serves as additional replication stressor/ATRi sensitizer. ii) Classical BRCA alterations are rare and alterations conferring homologous recombination deficiency (HRD) likely require a combination approach including PARPi. This is a first-in-child study and we here report the results of the Phase I dose escalation part. Methods: Children and adolescents with relapsed or refractory cancer and advanced molecular profiling at relapse were eligible. All patients had to have molecular alterations consistent with HRD or RS in their tumor. Ola was administered orally twice daily (BID) continuously and cer orally BID on days 1-14 of a 28-day cycle. Dose level (DL) 1 was ola 150/100/50 mg BID and cer 80/40/30 mg BID in age group 12-18/6-<12/3-<6 years, respectively. Dose-escalation followed a Boin design, 12 patients treated at the recommended Phase II dose (RP2D) were required to conclude the Phase I part. Plasma for pharmacokinetics (PK) of both drugs was collected during Cycle 1.
Results: Eighteen patients (8 sarcomas, 5 central nervous system tumors, 4 neuroblastomas, 1 carcinoma)

with a median age of 16 years (range, 4;24) were enrolled over 3 dose levels between Feb 2021 and Sep 2022 and received a median of 3.5 cycles (range 1-12+; data cut-off Jan 2023). The combination was well tolerated overall, toxicity was mainly hematologic (thrombocytopenia, neutropenia, anemia) and gastrointestinal (nausea/ vomiting). Five patients encountered dose-limiting toxicities (thrombocytopenia grade 3/4 requiring platelet transfusions > 7 days in all, and grade 4 neutropenia for > 7 days or with documented infection in 1 patient each; 0/3 DL-1, 2/12 in DL1 and 3/3 in DL2), suggesting DL1 as RP2D. One confirmed partial response (PR) was observed in a patient with pinealoblastoma (*FANCA* VUS), a prolonged stable disease converted to a PR after cycle 9 in a neuroblastoma (11q loss, *ATRX* VUS, in cycle 10). Four patients are ongoing in cycle 5/6/10/12, respectively. PK and retrospective biomarker analysis are ongoing to identify factors associated with clinical benefit and data will be presented.
Conclusions: Ola and cer in combination are well tolerated. Recruitment in two expansion cohorts (HRD and RS) is ongoing at the RP2D, including confirmation of RP2D in younger children.

**CT021**

**Assessing clinical and pharmacodynamic (PD) profiles of patients (pts) with chronic lymphocytic leukemia (CLL) on ianalumab (VAY736) + ibrutinib.**

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**Introduction** VAY736 is an afucosylated, human monoclonal antibody engineered to enhance antibody-dependent cellular cytotoxicity that targets BAFF-R+ B cells for elimination. In preclinical CLL models, VAY736 showed antileukemic activity and, when combined with ibrutinib, significantly reduced disease burden, which may allow some pts to discontinue ibrutinib.
**Methods** This Phase Ib dose-escalation (ESC)/-expansion (EXP) trial (NCT03400176) enrolled pts with CLL who did not achieve a complete response (CR) after >1 year of ibrutinib or had developed a resistance mutation to ibrutinib. Pts received IV VAY736 (ESC: 0.3-9 mg/kg; EXP: 3 mg/kg) once every 2 weeks and oral ibrutinib (420 mg) once daily for up to 8 28-day cycles. Pts achieving undetectable MRD (uMRD) at C9D1 could discontinue ibrutinib at investigator discretion. The study aimed to characterize the safety and tolerability of VAY736 + ibrutinib, assess antitumor activity, PK, and characterize PD profiles.
**Results** By Jul 29, 2022, 39 pts were enrolled (ESC: n=15; EXP: n=24). **Table 1** shows pt characteristics, safety, and efficacy data. The overall response at C9D1 for 37 evaluable pts was 40.5% CR + CRi and 16.2% PR (1L: 63.6% CR + CRi and 18.2% PR). At C9D1, 17 pts (45.9%) had uMRD in blood or bone marrow (BM). In the 2-year follow-up period, 16 pts discontinued ibrutinib and were off therapy for 4.9-19.8 months. Frequency of peripheral NKp46+ NK cells increased at least 50% after VAY736 in over 50% of pts. Preliminary coverage-based limiting-cell experiment analysis of RNAseq (CLEAR) data from 10 pts supports peripheral NK cell activation with VAY736.
**Conclusions** VAY736 + ibrutinib appears highly active and has an acceptable safety profile. Multiple pts attained uMRD in blood or BM. Biomarker data suggest NK cell activation with VAY736. More pts will be included in the RNAseq analysis at presentation. Future development of VAY736 for CLL is strongly indicated based on these promising data.

Table 1. Patient characteristics, safety, and efficacy results.

| | **All patients (N=39)** |
|---|---|

| **Patient demographics and prior treatment** | | | |
|---|---|---|---|
| Median age, years (range) | 65.0 (39-82) | | |
| ECOG performance status, n (%) | | | |
| 0 | 36 (92.3) | | |
| 1 | 3 (7.7) | | |
| No prior regimens excluding ibrutinib, n (%) | 12 (30.8) | | |
| Median number of prior regimens, n (range) | 1.0 (0.0-14.0) | | |
| Median duration of ibrutinib, years (range) | 2.95 (0.2-8.3) | | |
| **Patient baseline characteristics** | | | |
| Dohner risk by FISH,[a] n (%) | | | |
| 17p deletion | 6 (15.4) | | |
| 11q deletion | 9 (23.1) | | |
| Trisomy 12 | 3 (7.7) | | |
| 13q deletion | 10 (25.6) | | |
| *IGHV* mutant status, n (%) | | | |
| Non-mutant | 32 (82.1) | | |
| Complex karyotype, n (%) | | | |
| Yes | 20 (51.3) | | |
| **Safety** | | | |
| Dose-limiting toxicities, n (%) | 0 | | |
| Patients with at least one AE, any grade, n (%) | 38 (97.4) | | |
| Patients with at least one Grade ≥3 AE, n (%) | 13 (33.3) | | |
| Most common (occurring in ≥2 patients) Grade ≥3 AEs, n (%) | | | |
| Neutrophil count decreased | 5 (12.8) | | |
| Lymphocyte count decreased | 2 (5.1) | | |
| Hypophosphatemia | 2 (5.1) | | |
| Lipase increased | 2 (5.1) | | |
| **Efficacy** | **1L[b] n=11** | **R/R n=26** | **Evaluable patients N=37** |
| Overall response at C9D1 or before discontinuation,[c] n (%) | | | |
| Complete response | 6 (54.5) | 8 (30.8) | 14 (37.8) |
| Complete response with incomplete marrow recovery | 1 (9.1) | 0 | 1 (2.7) |
| Partial response | 2 (18.2) | 4 (15.4) | 6 (16.2) |
| Stable disease | 2 (18.2) | 8 (30.8) | 10 (27.0) |
| Progressive disease | 0 | 5 (19.2) | 5 (13.5) |

| | | | |
|---|---|---|---|
| uMRD response at C9D1 or before discontinuation,[c] n (%) | | | |
| Bone marrow uMRD | 6 (54.5) | 6 (23.1) | 12 (32.4) |
| Blood uMRD | 7 (63.6) | 10 (38.5) | 17 (45.9) |
| Blood or bone marrow uMRD | 7 (63.6) | 10 (38.5) | 17 (45.9) |
| Patients elected to discontinue ibrutinib after achieving CR or uMRD, n (%) | 16 (43.2) | | |
| [a]The categories were: patients with a 17p deletion; patients with an 11q deletion without a 17p deletion; patients with trisomy 12 without a 17p deletion or an 11q deletion; and patients with a 13q deletion without a 17p deletion, trisomy 12, or an 11q deletion; [b]Patients with no prior therapies excluding ibrutinib; [c]For evaluable patients (N=37). | | | |
| 1L, first line; AE, adverse event; CR, complete response; C, cycle; D, day; ECOG, Eastern Cooperative Oncology Group; FISH, fluorescence in situ hybridization; IGHV, immunoglobulin heavy chain variable region; R/R, relapsed/refractory; uMRD, undetectable minimal residual disease. | | | |

**CT022**

**Five-year efficacy and safety of tafasitamab in patients with relapsed or refractory DLBCL: Final results from the phase II L-MIND study.**

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Background: Tafasitamab, an anti-CD19 immunotherapy that enhances antibody-dependent cellular cytotoxicity and phagocytosis, received accelerated approval in the USA and conditional authorization in Europe in combination with lenalidomide (LEN) for patients (pts) with relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL) ineligible for autologous stem cell transplant (ASCT) based on the results of the open-label, multicenter, single-arm, Phase II L-MIND study (NCT02399085; Salles G, et al. Lancet Oncol 2020, Duell J, et al. Haematologica 2021). Here, we report the final, 5-year follow-up of L-MIND. Data cut-off was Nov 14, 2022.
Methods: Pts were aged ≥18 years with ASCT-ineligible R/R DLBCL, 1-3 prior systemic therapies (including a CD20-targeting regimen), and ECOG PS 0-2. Tafasitamab (12 mg/kg) was given for up to 12

cycles in combination with LEN (25 mg), then as monotherapy until disease progression (PD) or unacceptable toxicity. The primary endpoint was best objective response rate (ORR; complete response [CR] or partial response [PR], by independent radiology committee). Secondary endpoints included duration of response (DoR), progression-free survival (PFS), overall survival (OS), and incidence and severity of adverse events (AEs). Exploratory analyses evaluated efficacy endpoints by prior lines of therapy (pLoT).
Results: Of 81 pts enrolled, 80 were treated (full analysis set [FAS]). The ORR (FAS) of 57.5% [95% CI: 45.9-68.5], with CR of 41.2% [30.4-51.6] (n=33) and PR of 16.2% [8.9-26.2] (n=13), was generally consistent with the primary and 3-year analyses. Median DoR was not reached (NR) with median follow up (mFU) of 44.0 months [29.9-57.0]. Median PFS was 11.6 months [5.7-45.7] (mFU 45.6 [22.9-57.6]) and median OS was 33.5 months [18.3-NR] (mFU 65.6 [59.9-70.3]). At data cut-off, OS was >60 months in 21 pts (18 with best response of CR, 1 PR, 1 stable disease and 1 PD), including 14 with 1 pLoT and 7 with ≥2 pLoT. Pts with 1 pLoT (n=40) in the FAS had higher ORR (67.5%; 52.5% CR [n=21] and 15% PR [n=6]) compared to pts with ≥2 pLoT (n=40; 47.5%; 30% CR [n=12] and 17.5% PR [n=7]). However, median DoR was not reached for both subgroups, indicating similar long-term efficacy for responders. AEs were consistent with previous reports and manageable; incidence declined after transition from combination to tafasitamab monotherapy and again with monotherapy >2 years.
Conclusion: The final, 5-year analysis of L-MIND showed prolonged durable responses with tafasitamab + LEN combination therapy, followed by long-term tafasitamab monotherapy, in pts with R/R DLBCL ineligible for ASCT, with median DoR not reached after 44 months mFU. No new safety signals were identified, confirming the tolerability profile observed with earlier data cuts. These long-term data suggest that this immunotherapy may have curative potential that is being explored in further studies.

**CT023**

**Phase 1 trial of CD19/CD20 bispecific chimeric antigen receptor-engineered naïve/memory T cells for relapsed or refractory non-Hodgkin lymphoma.**

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Despite excellent responses to anti-CD19 chimeric antigen receptor (CAR)-T cell therapy in patients with relapsed/refractory (R/R) B-cell non-Hodgkin lymphoma (NHL), more than half of patients will relapse due to poor CAR-T persistence or CD19 antigen escape. Engineering naïve/memory T ($T_{N/MEM}$) cells with a bispecific anti-CD19/CD20 CAR could improve outcomes by mitigating these limitations. Here we report an update of the first-in-human phase 1 clinical trial with $T_{N/MEM}$ cells expressing a bispecific anti-CD19/CD20 CAR (CART19/20) for patients with R/R NHL (NCT04007029). Eligible patients were ≥18 years old with R/R diffuse large B-cell lymphoma (DLBCL) or primary mediastinal B-cell lymphoma (PMBCL) after ≥2 prior lines of therapy, or with mantle-cell lymphoma (MCL), follicular lymphoma (FL), or CLL/SLL after ≥3 prior lines of therapy. Prior CAR-T cell therapy was excluded, but other forms of CD19- or CD20-targeted therapies were allowed. Autologous leukocytes were obtained by leukapheresis and sorted for CD14-/CD25-/CD62L+ $T_{N/MEM}$ cells, followed by lentiviral transduction of the bispecific CD19/CD20 CAR. Bridging therapy after leukapheresis was allowed. Lymphodepletion chemotherapy with fludarabine 30 mg/m$^2$/day and cyclophosphamide 500 mg/m$^2$/day was administered for 3 days from Day −5 to Day −3 prior to CAR-T cell infusion. The primary endpoint was safety and the secondary endpoints were ORR, progression free survival (PFS), overall survival (OS), and CART19/20 transgene persistence. As of December 19, 2022, 11 patients have been treated with CART19/20 cells (7

DLBCL, including 4 transformed FL and 1 PMBCL; 3 FL; and 1 MCL). Patients were enrolled at two dose levels, including 50 x $10^6$ CAR+ cells (8 patients) and 200 x $10^6$ CAR+ cells (3 patients). The median age was 58 (range: 34 to 70). All patients had stage IV disease and 9 of 11 patients received bridging therapy. Six patients had grade-1 cytokine release syndrome (CRS), without any occurrence of higher-grade CRS. Neurotoxicity was not observed. Ten of the 11 patients achieved an objective response (91% ORR), with 8 patients (73%) achieving complete response (CR). One patient with FL relapsed at 18 months, received a second CART19/20 cell infusion (112 x $10^6$ CAR+ cells), and re-achieved a CR that has persisted for >6 months at last follow-up. With a median follow-up of 20.9 months (range: 3.4 - 37 months), 7 patients remain in CR with a median PFS of 18.2 months (95% CI 3.4 months - not estimable) and the median OS was not reached. This phase 1 study demonstrates robust safety and tolerability of CART19/20 T-cells in patients with R/R NHL, without occurrence of severe CRS or neurotoxicity. CART19/20 cells, utilizing a bispecific CAR and $T_{N/MEM}$ cells, may be an effective strategy to overcome the challenges of poor CAR T-cell persistence and antigen escape.

**CT024**

**REDIRECT: A Phase 2 study of AFM13 in patients with CD30-positive relapsed or refractory (R/R) peripheral T cell lymphoma (PTCL).**

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PTCLs are aggressive hematologic malignancies often with poor prognoses; for patients with R/R PTCL there is no standard-of-care therapy and novel treatments are required. AFM13 is a tetravalent, bispecific Innate Cell Engager (ICE®) that binds CD30 when expressed on PTCL cells, and CD16A on innate effector cells, redirecting and enhancing the innate immune response to $CD30^+$ tumor cells. Phase 1 clinical studies of AFM13 in patients with R/R Hodgkin lymphoma and cutaneous $CD30^+$ lymphomas showed a tolerable safety profile and clinical activity; early correlative science data in a small group of patients with HL showed increased natural killer (NK) cell activity in responsive patients. A Phase 2, open-label, multi-cohort study (NCT04101331) assessing the efficacy of AFM13 in patients with R/R PTCL was initiated in 2019. Patients had histologically confirmed CD30 expression in ≥1% of tumor cells and had received ≥1 prior systemic therapy. The primary endpoint was to assess the overall response rate (ORR) based on FDG-PET per independent review committee. Secondary endpoints included safety, complete response rate (CRR), duration of response (DoR), pharmacokinetics, immunogenicity, and quality of life. Progression-free survival (PFS) and overall survival (OS) were exploratory endpoints. Patients received 200 mg AFM13 intravenously once weekly until disease progression, intolerable toxicity, termination at the investigator's discretion, or withdrawal of consent. A total of 108 patients (age 21-93; 61% male) received AFM13, with a median (min, max) number of infusions of 9.0 (1, 116). Numbers of patients per PTCL subtype assessed were: PTCL not-otherwise-specified (PTCL-NOS), 41; angioimmunoblastic T cell lymphoma (AITL), 30; anaplastic large cell lymphoma (ALCL), 26; other, 11. Patients received a mean number of 2.7 prior treatment lines; 46.3% received prior brentuximab vedotin

(BV), 17.6% received prior auto-transplant. The ORR was 32.4% (CRR was 10.2%); ORR in each subgroup was 22.0% (PTCL-NOS), 53.3% (AITL), 23.1% (ALCL), and 36.4% (other). Median DoR, PFS, and OS were 2.3 months, 3.5 months, and 13.8 months, respectively. Reported treatment emergent adverse events (TEAEs) were as anticipated based on previous studies. AFM13-related TEAEs occurred in 79/108 patients (73.1%), with 14 events in 9 patients (8%) considered serious. The most frequent TEAE was infusion-related reactions, observed in 34/108 patients (31.5%), including 12 Grade 3 events in 6 patients (5.7%). Neutropenia was the most frequent TEAE with Grade ≥3 occurring in 9.3% of patients. AFM13 monotherapy was well managed and showed robust clinical activity in selected R/R PTCL subtypes. These data, together with encouraging preliminary efficacy seen in AFM13 combination studies in HL, support further evaluation of AFM13 in combination with NK cells to augment the innate immune response to CD30+ tumors.

**CT025**

**NMS-03592088, a novel, potent FLT3, KIT and CSF1R inhibitor with activity in FLT3 positive acute myeloid leukemia patients with prior FLT3 inhibitor experience.**

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Background: FLT3 mutations occur in approximately 30% of AML patients and are associated with aggressive disease. Despite the approval of midostaurin and gilteritinib, the prognosis for FLT3+ patients with relapsed or refractory disease is poor. NMS-088 is a novel, potent FLT3, KIT and CSF1R inhibitor with superior preclinical activity compared with approved FLT3 inhibitors in different FLT3-driven models. In addition, NMS-088 is active on FLT3 resistance mutation F691L. Dose escalation results from a Phase I/II study to establish safety, dose selection and preliminary clinical activity for NMS-088 in patients with R/R AML and CMML are described.

Methods: In the Phase I 3+3 escalations, NMS-088 is administered daily for 21 of 28 days (schedule A) or continuously (schedule B). Patients must have R/R AML or CMML unsuitable for standard therapy. The primary objective is the MTD or MAD as assessed by DLTs. Secondary endpoints include safety, PK and ELN response.

Results: as of January 26, 44 R/R AML or CMML patients were treated at doses from 20 to 360 mg/day in A or from 120 to 250 mg/day in B. Median age was 64 yrs, 41 pts had AML and 3 pts had CMML, median number of prior lines was 2 (range 1 to 10). FLT3 mutations were present in 24 out of 41 AML pts (20 FLT3-ITD, 2 FLT3 D835 and 2 FLT3-ITD and D835). The majority of pts with FLT3+ AML had received prior FLT3 inhibitors (86.4%). NMS-088 showed manageable safety with no MTD characterized. One pt had DLT (abnormal posture, decreased activity, dyspnea G3 and eyelid ptosis G1) at 360 mg in A (at day 21) and one pt had DLT (eyelid ptosis G3) at 180 mg in B (at day 29), both suggestive for myasthenic syndrome. Three additional pts experienced possible myasthenic syndrome at

doses ≥ 180 mg. Overall the most frequent treatment emergent related adverse events (≥10%) were nausea (any grade 20.5%), vomiting (13.6%), asthenia (11.4%). Discontinuations due to related AEs were as follows: 2 DLT pts per protocol, 2 pts due to nausea (G1; day 161 at 270 mg) and myasthenia gravis (G3; day 39 at 300 mg in a pt with baseline AChR antibodies). There was a dose-dependent trend for response. A total of 5 out of 12 evaluable pts with FLT3+ AML treated at dose ≥ 300 mg achieved a response with 2 CRi, 1 CRi/MLFS and 2 MLFS. Remarkably, all these pts had received prior midostaurin and 2 pts received both midostaurin and gilteritinib. Two pts with response withdrew from treatment to receive HSCT (DoR 1.0+ mos each). For other responding pts DoR was 1.3, 2.8 and 7.9 mos. Conclusions: NMS-088 showed clinical efficacy in pts with FLT3+ R/R AML, including pts who have failed prior FLT3 inhibitors. Together with the manageable safety observed, these results warrant further development of this drug including potential as a novel valuable therapeutic option for pts who have exhausted available treatments. The trial is currently opened for enrollment (NTC03922100).

**CT026**

**A first-in-human phase 1 study of LY3410738, a covalent inhibitor of mutant IDH, in advanced myeloid malignancies.**

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**Background:** LY3410738 is a potent, selective, covalent, dual inhibitor of IDH1/2 mutations (IDH1/2m). LY3410738 binds covalently at a novel binding site, enabling continued potency in preclinical models in the setting of second site IDH resistance mutations. We present initial results from the first-in-human phase 1 dose escalation study of oral LY3410738 in patients (pts) with R/R IDH1/2m hematologic cancers.
**Methods**: Dose escalation (3+3 design) evaluated LY3410738 monotherapy in IDH1/2m R/R AML (NCT04603001). Key objectives included determining the RP2D, safety, PK, PD (inhibition of plasma D-2-HG), and preliminary antitumor activity.
**Results:** As of 28 July 2022, 114 pts including 108 R/R AML pts received LY3410738 dosed at 5-600 mg QD or 40-300 mg BID. Pts were median 73 years of age (range, 22-92) with a median of 2 prior therapies (range, 1-10); 29% received a prior IDH inhibitor and 58% a prior BCL2 inhibitor. Median time on treatment was 2.3 months (range, 0.1-15). No DLTs or treatment related deaths were observed. Treatment emergent adverse events ≥20% were diarrhea (22%), fatigue (21%), and anemia (20%). Differentiation syndrome was reported in 11 pts (10%); 4 grade 1/2 (4%), 7 grade 3 (6%). LY3410738 exposure was dose proportional. In pts with IDH1m cancers, LY3410738 achieved sustained D-2-HG inhibition at all dose levels including in pts who received prior IDH1 inhibitor. In pts with IDH2m cancers, a higher dose (≥150 mg daily dose) was required for D-2-HG inhibition. Responses were observed in both IDH1m and IDH2m AML (Table). Higher doses were required for IDH2m AML, especially IDH2 R140m pts. Efficacy appears higher in venetoclax naïve pts and limited in IDH inhibitor pre-treated pts.
**Conclusions:** LY3410738 demonstrated a favorable safety profile with potent and sustained D-2-HG inhibition in pts with IDH1m R132, IDH2m R172, and IDH2m R140 mutations. Preliminary efficacy was also seen in all genotypes, in a dose dependent manner. RP2D evaluation is ongoing.

**Table: Response in R/R AML**

| **IDH Inhibitor Naive (N=68)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **IDH1 R132** | | | **IDH2 R172** | | | | **IDH2 R140** | | | |
| | **No prior Venetoclax (n=13)** | **Prior Venetoclax (n=19)** | **Total (N=32)** | **Low Doses[a] (n=5)** | **(High Doses[b])** | | **Total (N=13)** | **Low Doses[a] (n=9)** | **(High Doses[b])** | | **Total (N=23)** |
| | | | | | **No prior Venetoclax (n=3)** | **Prior Venetoclax (n=5)** | | | **No prior Venetoclax (n=6)** | **Prior Venetoclax (n=8)** | |
| **CR+CRh, n (%)** | 5 (38%) | 2 (11%) | 7 (22%) | - | 3 (100%) | 2 (40%) | 5 (38%) | - | 2 (33%) | - | 2 (9%) |
| **CR, n (%)** | 3 (23%) | 2 (11%) | 5 (16%) | - | 2 (67%) | 2 (40%) | 4 (31%) | - | 2 (33%) | - | 2 (9%) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **CRh, n (%)** | 2 (15%) | - | 2 (6%) | - | 1 (33%) | - | 1 (8%) | - | - | - | - |
| **CRc (CR+CRh+CRi/CRp), n (%)** | 6 (46%) | 6 (32%) | 12 (38%) | - | 3 (100%) | 3 (60%) | 6 (46%) | - | 2 (33%) | - | 2 (9%) |
| **MFLS, n (%)** | 1 (8%) | 3 (16%) | 4 (13%) | 2 (40%) | - | - | 2 (15%) | 1 (11%) | - | - | 1 (4%) |

**IDH Inhibitor Pre-Treated (N=33)**

| | **IDH1 R132** | | | **IDH2 R172** | | | | **IDH2 R140** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No prior Venetoclax (n=2)** | **Prior Venetoclax (n=8)** | **Total (N=10)** | **Low Doses[a] (n=6)** | **(High Doses[b])** | | **Total (N=8)** | **Low Doses[a] (n=4)** | **(High Doses[b])** | | **Total (N=15)** |
| | | | | | **No prior Venetoclax (n=1)** | **Prior Venetoclax (n=1)** | | | **No prior Venetoclax (n=3)** | **Prior Venetoclax (n=8)** | |
| **CR+CRh, n (%)** | - | - | - | - | - | - | - | - | - | 1 (13%) | 1 (7%) |
| **CR, n (%)** | - | - | - | - | - | - | - | - | - | - | - |
| **CRh, n (%)** | - | - | - | - | - | - | - | - | - | 1 (13%) | 1 (7%) |
| **CRc (CR+CRh+CRi/CRp), n (%)** | - | - | - | - | - | - | - | 1 (25%) | - | 1 (13%) | 1 (7%) |
| **MLFS, n (%)** | - | - | - | - | - | - | - | - | - | - | - |

Among the 108 treated R/R AML pts, 101 were efficacy evaluable (42 IDH1 R132, 21 IDH2 R172, 38 IDH2 R140); 68 pts were IDH inhibitor naïve and 33 had received a prior IDH inhibitor treatment. Efficacy evaluable pts are those who had completed the first bone marrow assessment or had discontinued treatment prior to first bone marrow assessment

[a] Total daily low doses: ≤75 mg Arm A, ≤30 mg Arm B

[b] Total daily high doses: ≥150 mg Arm A, ≥60 mg Arm B

Arm A: not requiring a strong CYP3A4 inhibitor

Arm B: requiring a strong CYP3A4 inhibitor

**CT028**

**A first-in-human phase 1 study of LY3537982, a highly selective and potent KRAS G12C inhibitor in patients with KRAS G12C-mutant advanced solid tumors.**

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**Background:** Mutations in *KRAS* are among the most frequent oncogenic drivers with the G12C mutation found in up to ~13% of NSCLC. LY3537982 is an oral, highly selective, and potent inhibitor of KRAS G12C, which preclinically delivers >90% sustained target occupancy. We present the initial results from LOXO-RAS-20001, a phase 1 study of LY3537982 in patients (pts) with KRAS G12C-mutant advanced solid tumors (NCT04956640).

**Methods**: LY3537982 monotherapy dose escalation followed a mTPI-2 method. Dose expansion cohorts included combinations with pembrolizumab (NSCLC) and cetuximab (CRC). Key objectives were to determine the RP2D of LY3537982, safety, PK, and antitumor activity per RECIST v1.1.

**Results:** As of 17 August 2022, 56 pts with NSCLC (16), CRC (17), PANC (8), and other tumor types (15) were treated with LY3537982 monotherapy on doses from 50-200 mg BID. Median number of prior systemic therapies was 2 (range, 0-8). No DLTs were observed, MTD was not reached, and RP2D determination is ongoing. Median time on treatment was 3 months (range, 0.3-13), 33 pts are ongoing, and 23 pts discontinued (none due to a related AE). TEAEs observed in ≥10% of pts were diarrhea (38%), constipation (16%), fatigue (16%), peripheral oedema (13%), and nausea (11%), mostly grade 1. The only grade ≥3 treatment related AE was neutropenia (n=1), and no pneumonitis or grade ≥2 transaminitis was observed. There were no treatment related serious AEs or deaths. Dose proportional steady-state exposures were observed through 150 mg BID. Table shows preliminary efficacy data.

**Conclusions:** LY3537982 demonstrated a favorable safety profile, including the absence of high-grade liver toxicity, and tolerance in pts previously intolerant to other KRAS G12C inhibitors. Preliminary efficacy was observed with LY3537982 monotherapy across multiple tumor types. Updated data in more than 100 pts, including data in combination with pembrolizumab and cetuximab will be presented.

**Table: Preliminary LY3537982 Monotherapy Efficacy**

| | **NSCLC (KRAS G12Ci naïve)(N=5)** | **NSCLC(Prior KRAS G12Ci treated) (N=11)** | **CRC[d](N=17)** | **PANC[d](N=8)** | **Other tumor types[d,f](N=15)** |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Efficacy Evaluable[a], n | 5 | 9 | 15 | 8 | 11 |
| ORR, n (%) | 3 (60%) | 0 | 1 (7%) | 3 (38%) | 4 (36%) |
| BOR, n (%) | | | | | |
| PR, n (%) | 3 (60%)[c] | 0 | 1 (7%)[e] | 3 (38%)[e] | 4 (36%)[g] |
| SD, n (%) | 1 (20%) | 6 (67%) | 13 (87%) | 4 (50%) | 6 (55%) |
| PD, n (%) | 1 (20%) | 3 (33%) | 1 (7%) | 1 (13%) | 1 (9%) |
| DCR[b], n (%) | 4 (80%) | 6 (67%) | 14 (93%) | 7 (88%) | 10 (91%) |

Abbreviations: CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PANC, pancreatic cancer

[a] Efficacy evaluable pts are those who had at least one post-baseline response assessment or had discontinued treatment

[b] DCR includes PR+SD

[c] 3 NSCLC pts have unconfirmed PRs, ongoing and pending confirmation as of the data cut-off date

[d] All pts KRAS G12C inhibitor naïve, prior KRAS G12C inhibitor therapy not permitted for tumor types other than NSCLC

[e] 1 CRC pt and 1 PANC pt have unconfirmed PR, ongoing and pending confirmation as of the data cut-off date

[f] Other tumor types include cholangiocarcinoma (n=4), chondrosarcoma (n=1), jejunal adenocarcinoma (n=1), large cell neuroendocrine of lung (n=1), nasal malignant melanoma (n=1), ovarian cancer (n=3), salivary adenoid cystic carcinoma (n=1), small intestine cancer (n=1), tracheal basaloid squamous cell carcinoma (n=1), and upper tract urothelial carcinoma (n=1)

[g] PRs observed in pts with tracheal basaloid squamous cell carcinoma, cholangiocarcinoma, nasal malignant melanoma, and ovarian cancer (1 each)

**CT029**

**Phase Ib study of GDC-6036 in combination with cetuximab in patients with colorectal cancer (CRC) with *KRAS G12C* mutation.**

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**Background:** GDC-6036 is an oral, highly potent and selective KRAS G12C inhibitor that demonstrated anti-tumor activity in patients with *KRAS G12C*-positive advanced solid tumors, including CRC. In a previously reported single-agent cohort, GDC-6036 achieved a best response of partial response or complete response in 35% (19/55) of patients, with a confirmed overall response rate (ORR) of 24% (13/55 patients) in *KRAS G12C*-positive CRC patients (Desai et. al., ESMO 2022). EGFR blockade may sensitize tumors to KRAS G12C inhibition and the combination of an anti-EGFR antibody (cetuximab) with a KRAS G12C inhibitor showed greater anti-tumor activity than single-agent KRAS G12C inhibition in preclinical models (Amodio et. al., 2020). **Methods:** In an ongoing Phase I study (NCT04449874), patients with advanced or metastatic *KRAS G12C*-positive CRC were administered GDC-6036 (200-400 mg orally once a day) with cetuximab (400 mg/m$^2$ intravenously initially, then 250 mg/m$^2$ weekly) until intolerable toxicity or disease progression. Endpoints included safety (NCI-CTCAE v5), pharmacokinetics (PK), and preliminary anti-tumor activity (RECIST v1.1). **Results:** As of the clinical data cut-off date of 21 Nov 2022, 29 patients (enrolled by 07 Oct 2022) had received GDC-6036 and cetuximab. The median lines of prior metastatic therapy was 2 (range 1-8) and the median time on study treatment was 5.2 (range 1.4-11.2) months. All patients experienced at least one treatment-related adverse event (TRAE); the most common TRAEs (≥15%) were rash (grouped terms), diarrhea, nausea, vomiting, dry skin, and paronychia. Grade 3-4 TRAEs occurred in 11 patients (38%). Five patients (17%) experienced at least one serious AE, none of which were treatment-related (including 2 patients who died of CRC progression during the safety follow-up). AEs led to GDC-6036 modifications (interruptions and/or reductions) in 13 (45%) patients, dose reduction in 3 (10%) patients, and no patients discontinued due to AEs. Eleven patients discontinued from study treatment (10 due to disease progression and 1 due to physician's discretion). The PK profile of GDC-6036 (400 mg once a day) was similar in combination with cetuximab when compared with single-agent. A partial response was achieved in 66% (19/29) of patients, with a confirmed ORR of 62% (18/29 patients). **Conclusions:** GDC-6036 in combination with cetuximab demonstrated a manageable safety profile and promising clinical activity. These data support that the addition of anti-EGFR therapy to GDC-6036 may lead to robust clinical benefit in patients with *KRAS G12C*-positive CRC.

**CT030**

**Phase I study of IBI351 (GFH925) monotherapy in patients with advanced solid tumors: Updated results of the phase I study.**

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**Background:** IBI351 (GFH925) is an irreversibly covalent inhibitor of $KRAS^{G12C}$. Data from prior data cutoffs (primary: Feb, 2022; initial update: July, 2022) showed that IBI351 (GFH925) was well-tolerated and demonstrated promising efficacy in patients (pts) with advanced solid tumors harboring KRAS p.G12C mutation. Here, we update results of this phase I study evaluating IBI351 (GFH925) in pts with advanced solid tumors again.
**Methods:** Pts with locally advanced, recurrent or metastatic solid tumors with $KRAS^{G12C}$ mutation for whom standard therapy had failed were enrolled. Phase I dose escalation had an accelerated titration design for dose level 250mg once daily (QD) and a Bayesian optimal interval (BOIN) design with 450-900mg QD and 450-750mg twice daily (BID). The primary endpoints were safety and tolerability. The secondary endpoints were pharmacokinetics (PK), anti-tumor activity of IBI351 (GFH925) monotherapy per RECIST v1.1, and overall survival.
**Results**: As of November 30th, 2022, 74 pts (1 at 250mg QD, 3 at 450mg QD, 9 at 700mg QD, 5 at 900mg QD, 21 at 450mg BID, 31 at 600mg BID and 4 at 750mg BID; 62 men, 12 women; median age: 64 yrs, range: 42-76 yrs) were enrolled, among whom 67 pts had non-small cell lung cancer (NSCLC). Among 67 NSCLC pts, 44.8% pts received ≥2 prior lines of treatment (tx), 38.8% pts had brain metastases; adenocarcinoma was the most common histology (n=66, 98.5%). All 74 pts were included for safety analysis. No dose-limiting toxicity (DLT) were observed in any dose cohorts. The overall safety profile was consistent with the latest previous report, with no new safety signals identified. As of December 15th, 2022, among 67 response-evaluable NSCLC pts across all dose levels, the ORR (by investigator assessment) was 58.2% (95% CI, 45.5-70.2), and the confirmed ORR was 44.8% (95% CI, 32.6-57.4); the disease control rate (DCR) was 92.5% (95% CI, 83.4-97.5). At the 600mg BID dose level (RP2D), the ORR was 63.3% (95% CI, 43.9-80.1), and the confirmed ORR was 50.0% (95% CI, 31.3-68.7), the DCR was 96.7% (95% CI, 82.8-99.9). With a median progression-free survival (PFS) follow-up of 5.5 months (95% CI, 5.3-6.8) for NSCLC pts at the 600mg BID dose level, 21 (70%) pts were continuing treatments, and the median duration of response (DoR) and PFS were not reached. Thirteen out of 15 confirmed responders were still in response.
**Conclusion**: IBI351 (GFH925) was well-tolerated across all doses explored in patients with advanced solid tumors harboring KRAS p.G12C mutation. The data also demonstrated promising efficacy and durable response of IBI351 (GFH925) in previously treated advanced NSCLC.
**Clinical trial information:** NCT05005234.

**CT031**

**A first-in-human, phase 1a/1b, open-label, dose-escalation and expansion study to investigate the safety, pharmacokinetics, and antitumor activity of the RAF dimer inhibitor BGB-3245 in patients with advanced or refractory tumors.**

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**Background:** BGB-3245 is a RAF dimer inhibitor with preclinical activity in MAPK-altered tumor models harboring BRAF V600 mutations, atypical BRAF mutations/fusions, and RAS mutations. This

study is investigating the safety, pharmacokinetics, and preliminary antitumor activity of BGB-3245 in patients (pts) with advanced or refractory MAPK-altered solid tumors.
**Methods:** Eligible pts were ≥18 yrs old with ECOG 0-1 and had solid tumors harboring MAPK pathway alterations. Dose-escalation and cohort-size decisions were made using the Modified Toxicity Probability Interval Design. The starting dose was 5 mg QD. Treatment emergent adverse events (TEAEs) were graded per NCI CTCAE v5.0. Tumor responses were assessed by investigators using RECIST v1.1.
**Results:** As of 1 Sep 2022, 42 pts were treated across 6 cohorts (5-60 mg QD). The median age was 60 yrs; pts had received a median of 3 prior lines of treatment. All pts had TEAEs; 79% had treatment-related (TR) AEs. The most common TRAEs (≥ 10%) were rash acneiform (33%), rash maculopapular (24%), fever (17%), ALT elevations and nausea (both 12%). Gr ≥3 TRAEs were reported in 29% of pts, events in ≥ 2 pts included decreased platelet count and rash maculopapular (3 each), ALT and AST elevations, and fever (2 each). Dose reductions occurred in 5 pts: rhabdomyolysis (1), LVEF decreased (1), hand-foot syndrome (1), rash maculopapular (1), and liver function abnormalities (1). Dose interruptions due to AEs occurred in 60% of pts. Dose discontinuations occurred in 79% of pts, 57% due to disease progression or death, 21% due to AE. Dose limiting toxicities were observed at 10 mg, 40 mg, and 60 mg. 40 mg QD was determined as the MTD. PK results showed generally dose-proportional increases in exposure. Tmax was at ~2 h; BGB-3245 had a long terminal half-life and 7.4-fold average accumulation in exposure at steady-state. 79% of pts were efficacy evaluable. The disease control rate was 48% with 1 CR, 5 cPR, 2 uPR and 8 SD≥24 wks. Objective responders included BRAF V600E melanoma pts post-BRAF/MEK and checkpoint inhibitors (2; 1 CR, 1 cPR), 1 NRAS G12S melanoma and 1 NRAS Q61K melanoma (post checkpoint inhibitors), 1 BRAF V600E LGSOC (progressed on BRAF inhibitor), 1 BRAF V600E cholangiocarcinoma (progressed on BRAF/MEK inhibitors), 1 BRAF K601E/PIK3CA endometrial cancer, and 1 KRAS G12D appendiceal cancer. Preliminary analysis of circulating tumor DNA showed correspondence to clinical response.
**Conclusions:** BGB-3245 has a manageable safety profile and a generally dose-proportional PK. Antitumor activity was observed in pts with no approved targeted therapy options. The safety and early efficacy profile of BGB-3245 supports further investigation in selected MAPK-altered tumors.

**CT032**

**Trials in progress: a global phase 1/1b clinical trial evaluating exarafenib (KIN-2787), a highly selective pan-RAF inhibitor, in adult patients with BRAF-altered solid tumors and NRAS mutant melanoma.**

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Background: Patients with cancers driven by BRAF Class II (C II) or C III alterations and/or NRAS mutations are unlikely to benefit from approved BRAF-targeted therapies and have few other treatment options. Exarafenib is a potentially best in class orally available pan-RAF inhibitor optimized for potency & high selectivity. It has differentiated dose-dependent activity across a broad range of cell lines & models driven by BRAF C I (inc. those with acquired resistance to 1$^{st}$ gen RAFi), C II, C III or NRAS

alterations.
Methods: KN-8701 (NCT04913285) is an ongoing Phase 1 dose escalation & dose expansion study at 35 sites in 7 countries evaluating exarafenib monotherapy in participants (pts) with advanced solid tumors harboring oncogenic BRAF or NRAS alterations.
Results: Preliminary results as of 13 Dec 2022 are reported for 52 pts (median age 63, 51.9% male) with a median of 3 prior therapies treated in 6 dose levels. Treated pts included those with solid tumors driven by BRAF C I (38.5%), C II (19.2%), C III (28.8%) alterations or melanoma pts with NRAS mutations (13.5%). Steady state exarafenib exposure ($C_{max}$ and AUC) increased dose proportionally. Pathway inhibition & decreases in ctDNA were observed across BRAF classes & tumor types. Dose limiting toxicities observed at the highest dose level were Grade 3 (Gr3) acneiform rash & Gr3 macular rash. The maximum tolerated dose (MTD) was determined to be 300 mg bid. Treatment-related adverse events (TRAEs) (any grade) occurred in 69.2% of pts with 13.5% of pts having Gr ≥3 events. Skin AEs (any grade) were the most common TRAEs observed in 48.1% of pts with 7.7% of pts having Gr ≥ 3 skin events. GI TRAEs occurred in 19.2% pts (Gr 1-2 only), including diarrhea (1 pt, Gr 1). Reversible, asymptomatic Gr 3 increased ALT and/or increased AST AEs in 3 pts were the only non-skin Gr ≥ 3 TRAEs reported in ≥ 2 pts. At therapeutically relevant exposures, there was no cutaneous evidence of paradoxical activation. Of the 34 pts across all dose levels with at least 1 post-baseline tumor assessment, 6 pts (17.6%) had a partial response (5 confirmed at submission date) & 16 pts (47.1%) had stable disease including 8 pts (23.5%) with objective tumor shrinkage (up to 20%). The 6 responders all continue exarafenib therapy (treatment duration up to 12 months) and include 3 pts with BRAF C I alteration-driven cancers (inc. 1 RAFi + MEKi pre-treated melanoma pt) and 1 pt each with BRAF C II, BRAF C III-driven & NRAS-mutation driven cancers. For pts who achieved or confirmed their best response at MTD, the response rate was 33% (4/12) & included responses in pts with BRAF C I, C II, C III, and NRAS mutant tumors.
Conclusions: Exarafenib achieves therapeutically meaningful drug exposures and demonstrates promising tolerability & clinical activity in BRAF or NRAS alteration-driven solid tumors. Pt enrollment & exarafenib treatment at 300 mg bid continues.

**CT033**

**Safety, pharmacokinetics, and antitumor activity findings from a phase 1b, open-label, dose-escalation and expansion study investigating RAF dimer inhibitor lifirafenib in combination with MEK inhibitor mirdametinib in patients with advanced or refractory solid tumors.**

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Background: RAF dimer inhibition can suppress RAF-dependent MEK reactivation leading to sustained MAPK pathway inhibition. RAF dimer inhibitor lifirafenib (L) synergized with MEK inhibitor mirdametinib (M) in *RAS*-mutated cancer models. In this ongoing Phase 1b study of L+M in patients (pts) with advanced/refractory solid tumors harboring MAPK pathway aberrations, we investigate preliminary safety, PK, and efficacy.
Methods: Pts were enrolled by a 3+3 design and treated with L (15-20 mg QD) + M (2-4 mg QD or BID) across 9 dose levels (DLs). Primary objectives were to evaluate safety/tolerability, estimate MTD, and identify recommended Phase 2 dose (RP2D). Tumor responses were investigator assessed using RECIST v1.1. AEs were graded per NCI CTCAE v5.0.
Results: Table 1 presents demographic, efficacy, and safety results as of 01 Sep 2022. Objective responses

(1 CR, 14 PRs) were achieved in 15/54 (27.8%) efficacy-evaluable pts, including 10/17 low-grade serous ovarian cancer (LGSOC) (58.8%; median exposure ~23 mo), 2 NSCLC (1 *NRAS Q61K*, 1 *BRAF-V600E*), 2 endometrial cancer (1 *BRAF ZC3HAv1* fusion, 1 *KRAS G12A*), and 1 LG serous adenocarcinoma of Mullerian origin (*KRAS G12V*). For L and M, plasma maximum drug concentration ($C_{max}$) and exposure (AUC) were comparable to that of each compound at same DL in monotherapy studies, suggesting low likelihood of drug-drug interaction. L+M was generally well tolerated, with limited DLTs and discontinuations. There were 2 deaths due to TEAEs considered unrelated to L+M. The MTD/RP2D were not yet determined.
Conclusions: L+M demonstrated a favorable safety profile and showed antitumor activity in pts with various *KRAS*, *NRAS*, and *BRAF* mutations across several solid tumor types, including LGSOC, NSCLC, and endometrial cancer. The combination warrants further clinical investigation.

Table 1

| **Demographics (N=56)** | | | |
|---|---|---|---|
| Age (y), median (range) | 59.5 (29-78) | | |
| ECOG PS 0/1, n (%) | 56 (100.0) | | |
| Prior lines of therapy, median (range) | 1 (1-6) | | |
| **Efficacy Set (N=54), n (%)** | | | |
| | LGSOC (n=17) | Other than LGSOC (n=37) | All malignancies (n=54) |
| ORR (CR+PR) | 10 (58.8) | 5 (13.5) | 15 (27.8) |
| CR | 1 (5.9) | 0 | 1 (1.9) |
| PR | 9 (52.9) | 5 (13.5) | 14 (25.9) |
| SD | 6 (35.3) | 18 (48.6) | 24 (44.4) |
| DCR (CR+PR+SD) | 16 (94.1) | 23 (62.2) | 39 (72.2) |
| **Safety Set (N=56), n (%)** | | | |
| TEAE[a] | 55 (98.2) | | |
| SAE | 23 (41.1) | | |
| Grade 3 TEAE | 24 (42.9) | | |
| TEAE leading to treatment discontinuation | 3 (5.4) | | |
| DLT | 6 (10.7) | | |
| CR, complete response; DCR, disease control rate; DLT, dose-limiting toxicity; ECOG PS, Eastern Cooperative Oncology Group performance status; LGSOC, low-grade serous ovarian cancer; ORR, objective response rate; PR, partial response; SAE, serious adverse event; SD, stable disease; TEAE, treatment-emergent adverse event. [a] Commonly reported (>40%): fatigue (55.4%), dermatitis acneiform (46.4%), and diarrhea (44.6%). | | | |

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**CT034**

**GLIMMER-01: initial results from a phase 1 dose escalation trial of a first-in-class bi-sialidase (E-602) in solid tumors.**

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Background: Hypersialylation (excessive sialoglycans) on tumor cells has been known to be associated with poorer cancer outcomes for more than 40 years. Sialoglycans are immune suppressive and promote tumor immune evasion by binding to sialoglycan receptors (e.g. Siglecs) expressed on immune cells. However, redundant immune cell expression among Siglecs has posed a challenge for receptor-targeting therapeutic approaches. E-602 is a first-in-class fusion protein of engineered human sialidase (Neu2) and a human IgG1 Fc region. The dimeric sialidase moieties of E-602 circumvent the redundancy of this biology by directly cleaving terminal sialic acid residues from sialoglycans on immune and tumor cells. In preclinical studies, E-602 enhanced immune function by augmenting antigen-specific priming and activation of T cells and restoring function of exhausted-like T cells, and E-602 demonstrated antitumor activity as monotherapy in multiple mouse tumor models.
Methods: A Phase 1/2 first-in-human dose escalation study is evaluating the safety, pharmacokinetics (PK), pharmacodynamics (PD), and antitumor activity of E-602 in patients with advanced cancers. Eligible patients with select advanced solid tumors were treated with E-602 IV once weekly at dose levels between 1 and 30 mg/kg. Circulating immune cells were analyzed for changes in sialylation and immunophenotyping by flow cytometry. Changes in circulating cytokines were measured by immunoassays.
Results: As of January 6, 2023, 32 patients were treated with at least one dose of E-602. The most common tumors treated were colorectal (n=18) and pancreatic (n=6). Doses up to 30 mg/kg were tolerated with no dose-limiting toxicities. The most frequent adverse event was infusion-related reactions which were primarily grade 1-2 and clinically manageable. PK was linear across the evaluated dose range with an estimated T1/2 of 9-24 hours. Dose dependent PD observations included (1) desialylation of peripheral CD8+ T, CD4+ T, and NK cells, remaining detectable in some patients at 7 days post-dose; (2) increases in the immune activation marker, CD69, on peripheral CD8+ T, CD4+ T, and NK cells; and (3) increases in the IFNγ-dependent chemokine CXCL10, TNF-α, and MIP1-β. Thus far, response-evaluable patients have had stable or progressive disease. Additional safety, PK, PD, and clinical outcomes for other patients treated as part of backfill at multiple dose levels will be reported at the meeting.
Conclusion: E-602 is tolerated at doses up to 30 mg/kg. Consistent with preclinical findings, dose-dependent desialylation and immune system activation were observed. Based on the observed tolerability and PD effects, the Phase 2 portion of the study to evaluate clinical activity of E-602 monotherapy in patients with checkpoint-inhibitor resistant NSCLC and melanoma will proceed.

**CT035**

**Addition of Salmonella-IL2 to FOLFIRINOX for metastatic stage 4 pancreatic cancer nearly doubles median survival.**

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Oncology, Jewish General Hospital and McGill University, Montreal, Quebec, Canada; [2]Salspera, Cambridge, MA.

**Introduction and Background:** Salmonella-IL2 (Saltikva) is an attenuated strain of orally administered Salmonella that carries the human gene for IL-2 that colonizes the tumor microenvironment and locally releases IL-2 without any untoward effects. Following significant anti-tumor responses during preclinical study in multiple tumor models; a Phase I trial in humans found a single dose to be not toxic and elevated the Natural Killer T cell populations in patients with advanced gastrointestinal (GI) cancers. Additionally, a Phase I multiple dose trial in companion dogs with metastatic osteosarcoma demonstrated a 22% complete response rate. A Phase II trial was performed studying the addition of Salmonella-IL2 to standard of care chemotherapy for stage 4 metastatic pancreatic cancer.
**Patients and Methods:** A Health Canada and local IRB approved, non-randomized, two-arm study in patients with Stage 4 metastatic adenocarcinoma of the pancreas is currently underway. The goal is to recruit 30 patients per arm; Arm 1: Salmonella-IL2 with FOLFIRINOX and Arm 2: Salmonella-IL2 with Gemcitabine/Abraxane. The primary outcome is overall survival with secondary outcomes of response determined by CT imaging using RECIST 1.1 criteria and biomarker data. Outcomes were compared to patients with stage 4 metastatic pancreatic cancer administered FOLFIRINOX (n=37) or Gemcitabine/Abraxane (n=24) from 4 years prior to starting this trial (2016-2020) at the study site and to historical controls from the published literature. Salmonella-IL2 is orally administered concomitantly with the administration of the prescribed chemotherapy according to arm of enrollment. $10^9$ cfu of attenuated Salmonella-IL2 was administered orally after ingestion of a gastric acid neutralizing agent and followed with 200ml of an isotonic crystalloid fluid.
**Results:** A total of 28 patients have been enrolled into this clinical trial. 20 patients were administered Salmonella-IL2 orally every 2 weeks with FOLFIRINOX; mean age 59.4 (32-73) years; 12(60%) women, and 8 (40%) men. 8 patients were administered Salmonella-IL2 with Gemcitabine/Abraxane every 2/3 weeks; mean age is 68.8 (56-82); 6 (66.7%) women and 3 (33.3%) men. Median survival in the FOLFIRINOX arm was 24 months compared to 11.1 months in published historical controls and 13.1 months site specific controls (n=37) ($P<0.05$; Log Rank Test). In addition, CT imaging using RECIST 1.1 criteria demonstrated a 73% partial response rate compared to 31% in historical controls. There have been 24 serious adverse events; none of which have been attributed to the study drug. No survival advantage was observed in the Salmonella-IL2 with Gemcitabine/Abraxane arm.
**Conclusion:** Addition of Salmonella-IL2 to FOLFIRINOX for stage 4 metastatic pancreatic cancer results in the near doubling of median survival when compared to historical and site-specific controls receiving FOLFIRNOX only. These findings strongly indicate that a larger pivotal phase 3 multicenter trial is warranted.

**CT036**
**Safety and immunogenicity of a first-in-human mutant KRAS long peptide vaccine combined with ipilimumab/nivolumab in resected pancreatic cancer: Preliminary analysis from a phase I study.**
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**Background:** Novel strategies are needed to decrease the high recurrence rates of pancreatic ductal adenocarcinoma (PDAC) after curative-intent surgery. Mutant KRAS (mKRAS) is an oncogenic driver found in approximately 90% of PDAC that has emerged as a target for neoantigen-specific vaccination.

**Methods:** This is a single-arm, open-label, first-in-human phase I study of a pooled synthetic long peptide (SLP) vaccine targeting six mKRAS subtypes (G12D, G12R, G12V, G12A, G12C, G13D) combined with ipilimumab/nivolumab (ipi/nivo) in patients with resected PDAC (NCT04117087). Key inclusion criteria: presence of a vaccine-targeted KRAS mutation, disease-free status after completing adjuvant chemotherapy within 6 months of enrollment. Priming phase: mKRAS vaccine given on days 1, 8, 15, and 22 along with ipi 1 mg/kg every 6 weeks for 2 doses and nivo 3 mg/kg every 3 weeks for 4 doses. Boost phase: mKRAS vaccine given on weeks 13, 21, 29, 37, and 45 along with nivo 480 mg every 4 weeks for 10 doses. Co-primary endpoints: safety, mKRAS-specific T cell response. Secondary endpoints: disease-free survival (DFS), overall survival (OS).
**Results:** At the time of data cutoff (December 1, 2022), 11 patients were treated with a median follow-up time of 10.7 months. 8/11 patients achieved a mKRAS-specific T cell response, defined as >5-fold change in IFN-gamma-producing mKRAS-specific T cells within 17 weeks post-vaccination, as assessed by serial ELISpot. The median fold change in IFN-gamma-producing mKRAS-specific T cells within 17 weeks post-vaccination was 10.2 (range: 1.5-686.3). The majority of all adverse events were grade 1 (79.5%) or grade 2 (15.2%) in severity per NCI CTCAE v5.0. Four grade 3 immune-related adverse events (pneumonitis, adrenal insufficiency, arthralgias, myalgias) and discontinuation of checkpoint blockade occurred in 2/11 patients. The median DFS for the entire cohort was 6.4 months and not reached for OS. Patients who mounted a mKRAS-specific T cell response demonstrated a significant improvement in median DFS compared to immune non-responders (not reached vs 2.8 months, $p$ = 0.045).
**Conclusions:** The combination of a pooled SLP mKRAS vaccine and dual checkpoint blockade is tolerable and immunogenic in patients with resected PDAC. Induction of an mKRAS-specific T cell response is associated with improved DFS in this cohort. Ongoing correlative studies will apply multi-omic approaches to identify novel biomarkers of immune response and resistance.

**CT037**

**Nivolumab plus ipilimumab vs chemotherapy as first-line treatment for advanced gastric cancer/gastroesophageal junction cancer/esophageal adenocarcinoma: CheckMate 649 biomarker analyses.**

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**Background:** First-line nivolumab (NIVO) + chemotherapy (chemo) showed superior overall survival (OS) vs chemo, but NIVO + ipilimumab (IPI) vs chemo did not meet the prespecified OS boundary for significance in patients with advanced gastric cancer/gastroesophageal junction cancer/esophageal adenocarcinoma (GC/GEJC/EAC) in CheckMate 649. However, NIVO + IPI resulted in more durable responses and higher 24-month OS rates vs chemo. We present exploratory biomarker analyses of NIVO + IPI vs chemo.
**Methods:** Tumor mutational burden (TMB) was derived from whole exome sequencing of baseline tumor tissue and matching blood. TMB-high (TMB-H) was defined as ≥ 199 mutations/exome. Gene expression signatures (GES), including 12-gene chemokine, 2-gene regulatory T cell (Treg), 15-gene fibroblast, and 5-gene endothelial, were assessed via RNA sequencing of baseline tumor tissue. GES subgroups were defined by signature score tertiles.
**Results:** 813 patients were randomized to receive NIVO + IPI or chemo; 366 were evaluable for TMB (NIVO + IPI 45%, chemo 45%), and 402 were evaluable for GES (NIVO + IPI 49%, chemo 50%). OS HR for NIVO + IPI vs chemo was numerically lower in the TMB-H (6% of evaluable patients, HR 0.31) vs TMB-low (94% of evaluable patients, HR 0.87) subgroup (**Table**). Multiple GES subgroups showed OS benefit with NIVO + IPI vs chemo, including chemokine-high, Treg-high, fibroblast-low, and endothelial-low subgroups (**Table**). Additional analysis on microsatellite instability and GES in programmed death ligand-1 combined positive score subgroups will be presented.
**Conclusions:** This exploratory analysis suggests that there are patient subgroups with GC/GEJC/EAC that may benefit from dual immune checkpoint blockade. Clinical utility of these biomarkers should be prospectively validated in future trials.

| **Overall survival** | | | | | |
|---|---|---|---|---|---|
| | | **All randomized patients** | | | |
| HR (95% CI) | | 0.89 (0.77-1.03) | | | |
| | | **All evaluable** HR (95% CI) | **High, n (%)** HR (95% CI) | **Medium, n (%)** HR (95% CI) | **Low, n (%)** HR (95% CI) |
| **TMB (n = 366)** | | 0.81 (0.65-1.00) | 21 (6) 0.31 (0.10-0.95) | - | 345 (94) 0.87 (0.70-1.09) |
| **GES (n = 402)** | **Chemokine** | 0.82 (0.67-1.01) | 134 (33) 0.59 (0.40-0.86) | 134 (33) 0.92 (0.65-1.32) | 134 (33) 0.98 (0.68-1.40) |
| | **Treg** | | 134 (33) 0.59 (0.41-0.86) | 134 (33) 0.91 (0.64-1.30) | 134 (33) 1.07 (0.75-1.52) |
| | **Fibroblast** | | 134 (33) 0.98 (0.69-1.40) | 134 (33) 0.94 (0.66-1.35) | 134 (33) 0.63 (0.43-0.91) |
| | **Endothelial** | | 134 (33) 0.97 (0.68-1.38) | 134 (33) 0.94 (0.65-1.34) | 134 (33) 0.65 (0.45-0.94) |
| OS HR data are unstratified for NIVO + IPI vs chemo. | | | | | |

**CT038**
**Comprehensive molecular characterization of clinical response to durvalumab plus pazopanib combination in patients with advanced soft tissue sarcomas: A phase 2 clinical trial.**
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We aimed to determine the activity of the anti-VEGF receptor tyrosine-kinase inhibitor, pazopanib, combined with the anti-PD-L1 inhibitor, durvalumab, in soft tissue sarcoma (STS). Further, we performed molecular characterisation with whole exome and transcriptomic sequencing to identify the determinants of response. In this single-arm, phase 2 trial (NCT#03798106), we enrolled patients with metastatic and/or recurrent STS who had received up to two previous lines of systemic anticancer therapy and had at least one measurable lesion. Treatment consisted of pazopanib 800 mg orally, administered once a day, continuously, and durvalumab 1500 mg administered via intravenous infusion once every 3 weeks. The primary endpoint was the overall response rate. Between September 2019 and October 2020, fourteen (30.5%) of the 46 evaluable patients showed an objective response, including in alveolar soft-part sarcoma, angiosarcoma, undifferentiated pleomorphic sarcoma, and desmoplastic small round cell tumour. During a median follow-up period of 18.4 months, the median progression-free survival (PFS) was 7.7 months (95% confidence interval: 5.7-10.4). The common treatment-related adverse events of grades 3-4 included neutropenia (n = 9), elevated aspartate aminotransferase (n = 7) and alanine aminotransferase (n = 5) levels, and thrombocytopenia (n = 4). In the exploratory analysis, the B lineage signature was a significant key determinant of overall response ($P$=0.014). In situ analysis also showed that tumours with high $CD20^+$ B cell infiltration and vessel density had a longer PFS than those with low B cell infiltration and vessel density ($P$=$6.5\times10^{-4}$) as well as better response **(**50% *vs* 12%, $P$=0.019). In conclusion, durvalumab combined with pazopanib demonstrated promising efficacy in an unselected STS cohort, with a manageable toxicity profile. Our findings provide insights into combined high B cell infiltration and vessel density as potentially relevant biomarkers for the selection of patients who may benefit to a greater extent from PD-L1 blockade and VEGF inhibitor combination.

**CT039**

**Immunomodulatory effects of ceralasertib in combination with durvalumab in patients with NSCLC and progression on anti-PD-(L)1 treatment (HUDSON, NCT03334617).**

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**Background**: Promising clinical activity has been described with the combination of ceralasertib (oral ATR inhibitor) and durvalumab (anti-PD-L1 mAb) in patients with NSCLC who have progressed on prior anti-PD-(L)1 immunotherapy (HUDSON, NCT03334617) (Besse et al., OA15.05, IASLC 2022 WCLC). Similarly promising clinical benefit has been seen with this combination in patients with immunotherapy-resistant melanoma, prompting ongoing Phase2/Phase3 trials in both melanoma (NCT05061134) and NSCLC (NCT05450692). Preliminary preclinical data showed that ceralasertib can modulate innate and adaptive immunity; here we extend these findings in the peripheral blood of HUDSON patients.
**Methods**: Longitudinal blood samples were collected at baseline, on-therapy samples after 7 days of ceralasertib alone and after durvalumab treatment to evaluate gene expression analyzed using a linear mixed effects model, and T-cell receptor (TCR) repertoire sequencing were analyzed using paired

Wilcoxon analyses.
**Results**: Matched pre- and on-treatment blood samples were available for analysis from 48 patients for gene expression analysis and 62 patients for TCR repertoire sequencing. Gene expression analysis revealed dynamic, reversible changes upon ceralasertib treatment for 7 days (prior to durvalumab). Consistent with pre-clinical data, changes in innate and adaptive immunity-relevant signatures such as monocyte lineage (adjusted p-value [p.adj]=1.1e-23), cytotoxic (p.adj=7.4e-04), exhausted T-cell signatures (p.adj=1.5e-05) and increases in the IFNγ response (p.adj=2.0e-03), IFNα response (p.adj=3.7e-05), and TNFα signaling (p.adj=2.3e-13) signatures were seen, which reverted to baseline levels after ceralasertib followed by durvalumab treatment. Longitudinal TCR sequencing demonstrated similar cyclical changes, most notably reductions in clonality after ceralasertib treatment for 7 days (p=1.1e-06), with a return to baseline after ceralasertib followed by durvalumab treatment. An increase in peripheral TCR clonality was observed in most patients (n=48/58) after the addition of durvalumab, followed by an increase in expanded (p=3.29e-13) and newly detected expanded TCR clones (p=1e-15). The net result of TCR changes after ceralasertib followed by durvalumab treatment was an overall increase in clonality in most patients, without a significant difference in the composition of the most abundant T-cell clones, as measured by the Morisita index.
**Conclusions**: We found that ceralasertib, combined with durvalumab treatment in relapsed/refractory NSCLC patients, decreased signatures associated with exhausted T-cells and, conversely, increased interferon pathway activation. Furthermore, we observed an expansion and maintenance of abundant T-cell clones indicative of an anti-tumor response.

**CT040**
**A first-in-human phase 1 trial of IO-108, an antagonist antibody targeting LILRB2 (ILT4), as monotherapy and in combination with pembrolizumab in adult patients with advanced relapsed or refractory solid tumors: Dose escalation study.**
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**Background:** IO-108 is a fully human IgG4 monoclonal antibody with high affinity and specificity towards LILRB2 (also known as ILT4). It blocks the interaction of LILRB2 with the known ligands for LILRB2, including HLA-G, ANGPTLs, SEMA4A and CD1d.
**Methods:** IO-108-CL-001 is a first-in-human, Phase 1a/1b study in patients with relapsed/refractory solid tumors (NCT05054348). The dose escalation phase enrolled patients into escalating dose cohorts of IO-108 (60-1800 mg, Q3W IV) as monotherapy or in combination with pembrolizumab. Patients with disease progression on monotherapy could crossover to the combination arm. Primary objectives were safety and tolerability, and identification of the recommended phase 2 dose (RP2D). Secondary and exploratory objectives include evaluation of pharmacokinetics (PK), immunogenicity, pharmacodynamic (PD) biomarker effects and anti-tumor activity as measured by objective response rate (RECIST 1.1).
**Results:** Twenty-five relapsed/refractory solid tumor patients (median 4.5 prior lines of therapy for monotherapy and 3.6 for combination therapy) were treated with IO-108 monotherapy (n=12) or IO-108 + pembrolizumab (n=13). IO-108 was well-tolerated up to the maximal administered dose of 1800 mg Q3W as monotherapy and in combination with pembrolizumab, with no SAEs related to IO-108 and no DLTs observed. Maximum tolerated dose (MTD) was not reached. Full receptor occupancy through 21 days was achieved at ≥600 mg. The preliminary RP2D is 1200 mg Q3W. Dose-expansion cohorts of IO-108 monotherapy and IO-108 + anti-PD-1 are ongoing.Twenty-three patients were efficacy evaluable (11 monotherapy, 12 combination therapy plus 1 crossover). Overall response rate was 9% in monotherapy

cohort (1 Merkel cell carcinoma, prior pembrolizumab followed by nivolumab/ipilimumab) and 23% in combination therapy (2 cholangiocarcinoma, 1 MSS CRC with neuroendocrine features). The best overall response was 1 CR, 4 SD among monotherapy, and was 3 PR, 4 SD among combination therapy. The 4 responding patients remain on study with an on-going treatment duration of 8 to 12 months as of abstract submission.Consistent with the MOA, clinical benefit correlated with baseline characteristics and post-treatment changes in PD biomarkers including reprogramming of myeloid cells and activation of T cells.
**Conclusion:** The initial encouraging data support further development of IO-108, both as monotherapy and in combination with anti-PD-(L)1 for patients with advanced solid tumors.

**CT043**

**Feasibility study to evaluate performance of the LUM Imaging System for intraoperative detection of residual tumor in breast cancer patients receiving and not receiving neoadjuvant therapy.**

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**Background:** Microscopically tumor-free lumpectomy margins are critical for safe breast conserving surgery (BCS). With current tools, 15%-25% of BCS have positive margins that require second surgical procedures and increase cost and patient discomfort. Better detection tools are needed to identify residual cancer during the initial BCS and reduce second operations. LUM015 is a protease-activated fluorescent imaging agent that accumulates in tumor cells and tumor associated macrophages after preoperative intravenous injection. The LUM Imaging System visualizes activated LUM015 in the lumpectomy cavity via a hand-held detector and tumor detection software. This system has been tested in multiple single-site studies and two prospective multi-site studies enrolling >600 patients and demonstrated successful detection of residual BCS cavity tumor. Initial studies excluded the ~20% of patients receiving neoadjuvant therapy. Patchy tumor cell death with preoperative therapy can leave small, multifocal deposits of tumor invisible on pre-operative imaging and not palpable or visible during surgery. We now evaluate the LUM Imaging System in patients with and without neoadjuvant therapy.
**Design:** This prospective, multi-center study tests the LUM Imaging System in BCS after neoadjuvant therapy to evaluate potential impact of treatment-related tissue changes and tumor cell death on tumor detection algorithms. An initial cohort of 10 patients addressed the objective of algorithm development. A second cohort of 104 patients will further evaluate the feasibility of the LUM Imaging System after neoadjuvant therapy. A third cohort will enroll 208 patients who have not received neoadjuvant therapy. All cohorts are evaluated for safety and for reduction in residual tumor after LUM Imaging System guidance compared to standard BCS. After excision of the main lumpectomy specimen, patients are randomized 3:1. In the device arm, the cavity is imaged and margins with LUM015 signal are excised. Final comprehensive shaved margins are removed in both arms to evaluate extent of residual disease after the use of the LUM Imaging System or after standard lumpectomy. No LUM Imaging is performed in the control arm, however, all patients are injected with LUM015 to evaluate drug safety. Patient reported outcomes assessing re-excision concerns, breast appearance and preferences for treatment type are collected.
**Eligibility:** This study enrolls women with histologically confirmed primary invasive breast cancer (IBC), ductal carcinoma in situ (DCIS) or a combination of IBC/DCIS undergoing a BCS for their breast malignancy who have received any form of neoadjuvant treatment prior to surgery or who have not received any therapy prior to surgery. Patients allergic to polyethylene glycol or intravenous contrast agents are excluded. Use of blue node mapping dyes before imaging with the LUM015 is not allowed.
**Progress:** Cohort 1 has completed enrollment and interim analysis. No new risks specific to the

neoadjuvant population were identified. LUM015 fluorescent signals measured in neoadjuvant patients were within the expected range, and no changes to the tumor detection algorithm were required. Cohorts 2 and 3 have enrolled a total of 84 patients. This trial is registered as NCT04440982.

**CT044**

**KGOG2031 A phase II trial of repeated high dose luteal hormone therapy for intrauterine recurrence following fertility preserving therapy for atypical endometrial hyperplasia or endometrial cancer.**

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Background: Atypical endometrial hyperplasia (AEH) and early endometrial cancer (EEC) are common disease in menopausal women, however, are gradually increasing in women aged under 40 years. The standard treatment for AEH/EC is hysterectomy, but hormonal therapy is performed for preserving fertility. In general, fertility-preserving treatment is considered for AEH or EEC with well-differentiation and lesions confined to the endometrium without myometrial invasion. According to previous studies, hormonal therapy in these patients responded relatively well. Nevertheless, recurrence was not uncommon, accounting for 26.0% in AEH and 40.6% in EC. The standard treatment for these recurrent patients is hysterectomy, but if there is no myometrial invasion and no extrauterine lesions, repeated hormonal treatment can be considered. However, studies targeting these patients have been very scarce. The purpose of this study was to evaluate the efficacy and safety of high-dose progesterone therapy in recurrence following fertility preserving therapy for AEH or EEC.
Methods: KGOG2031 is a multi-center and prospective phase 2 trial. The primary endpoint is a 2-year disease free survival. The secondary endpoints were duration of disease-free survival, overall survival, response rate, adverse events, and infertile rates. Patients with recurrent AEH or EEC without myometrial invasion or extrauterine lesions who underwent high-dose MPA therapy for primary lesions were included. The inclusion criteria are limited to patients with less than two recurrences. The pathologic type for EEC was confined to endometrioid type. The patients should be confirmed histologically at least one complete remission after first treatment. Patients with myometrial invasion, cervical involvement, or extrauterine lesions observed on abdominal/chest computed tomography or pelvic magnetic resonance imaging were excluded. Furthermore, grade 2 or 3 of endometrioid or any grade of other pathologic type of endometrial cancer or non-atypical endometrial hyperplasia were excluded. The enrolled patients should take medroxyprogesterone acetate 500mg every day. D&CB will be performed after anesthesia every 8 weeks to determine the effectiveness, and treatment will be continued for up to 40 weeks until complete remission is achieved. The target number of enrollment is 115 cases, and the recruitment period is 3 years. The follow-up duration is 2 years after the end of treatment, and the total study duration was up to 5 years.

**CT045**

**A randomized phase II trial of TG4001 plus avelumab versus avelumab alone in recurrent/metastatic (R/M) human papilloma virus (HPV)-16 positive anogenital cancers.**

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Background: Immune checkpoint inhibitors targeting the PD1/PD-L1 axis have made a breakthrough in advanced solid tumors, leading to improved overall survival (OS) in several cancer types. However, only a minority of patients experience durable responses, requiring further development of anti-tumoral immunotherapies to overcome tumor immune escape. We hypothesized that priming the immune system with TG4001, a vaccine using the highly attenuated Modified Vaccinia virus Ankara as vector expressing the HPV16 E6 and E7 proteins (rendered non-oncogenic) and interleukin-2, would increase the clinical benefit associated with PD-L1 blockade in HPV16+ cancer patients. In the single-arm part of the trial evaluating the combination of TG4001 and avelumab in terms of response rate, the overall response rate (ORR) was 22%. Presence of liver metastases had a profound impact on outcome in terms of ORR and Progression Free Survival (PFS). In patients (pts) without liver metastases an ORR of 32% was observed versus 0% in pts with liver metastases, warranting further development of this combination in this patient population *(SITC 2020, Abstract ID 793).*
Methods: We have designed a randomized open-label two-arms trial evaluating the efficacy of the combination of TG4001 and avelumab versus avelumab alone (NCT03260023). Eligible pts have R/M HPV-16 positive anogenital cancer including cervical, vulvar, vaginal, penile, and anal cancers with no more than one prior line of chemotherapy for R/M disease and no previous exposure to cancer immunotherapy. HPV-16 positivity is determined in a central laboratory. Patients are randomly assigned 1:1 to receive either TG4001 plus avelumab or avelumab alone. Randomization is stratified by primary tumor type (cervical, anal, genital). TG4001 is administered subcutaneously (SC) at $5x10^7$ plaque forming units (pfu) Q1w for 5 weeks, Q2w until month 6 and Q12w until progressive disease and avelumab intravenously (IV) at 800 mg Q2w until progressive disease. A total of up to 140 pts are currently being recruited into two cohorts (A and B) in France, Spain, and USA. For Cohort A, consisting of pts without liver metastases at Baseline (BL), a 2-stage sample size adjustment design is used with PFS by RECIST 1.1 as primary endpoint. Plans are to accrue 120 patients following Interim Analysis and Independent Data Monitoring Committee (IDMC) review. Cohort B recruits pts with liver metastases at BL. To be eligible, hepatic disease is restricted in terms of extent and number of lesions. A total of 20 pts will be recruited and analyzed for the percentage of early progressors. Secondary endpoints include overall survival, objective response rate according to RECIST1.1, duration of response, disease control rate. Exploratory endpoints comprise analyses of blood-based parameters and tumor-based proteins and RNA expression and their impact on clinical outcomes.

**CT046**

**A randomized, phase 2 study of pembrolizumab plus chemotherapy with or without anti—immunoglobulin-like transcript 4 monoclonal antibody MK-4830 as neoadjuvant treatment for high-grade serous ovarian cancer.**

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Inc., Rahway, NJ; [12]Sheba Medical Center⌐Tel HaShomer Hospital, Ramat Gan, Israel.

Background: Combination therapy with the anti-PD-1 monoclonal antibody (mAb) pembrolizumab and chemotherapy in advanced solid tumors has shown efficacy in the (neo)adjuvant and first-line setting, including high-grade serous ovarian cancer (HGSOC). The immunoglobulin-like transcript 4 (ILT4) receptor expressed on myeloid cells is involved in immune tolerance and suppression within the tumor microenvironment, and its blockade may improve treatment outcomes with combination therapies. Pembrolizumab plus chemotherapy with or without MK-4830 (a novel humanized anti-ILT4 mAb) will be investigated as neoadjuvant therapy for HGSOC in a randomized, open-label, phase 2 study (NCT05446870). Specifically, this trial is designed to evaluate the utility of circulating tumor DNA (ctDNA) as a biomarker of response and minimal residual disease in patients with treatment-naive HGSOC.
Methods: Key eligibility criteria include adult patients with treatment-naive International Federation of Gynecology and Obstetrics Stage IIIA-C/IV HGSOC, primary peritoneal cancer, or fallopian tube cancer who are candidates for neoadjuvant and adjuvant chemotherapy and interval debulking surgery (IDS), and have an ECOG performance status of 0 or 1. Approximately 160 patients will be randomly assigned (1:1) to receive 3 cycles each of neoadjuvant and adjuvant therapy with patients in arm 1 receiving chemotherapy (carboplatin AUC 5 or 6 plus paclitaxel 175 mg/m$^2$ or docetaxel 75 mg/m$^2$) + pembrolizumab 200 mg and patients in arm 2 receiving chemotherapy + pembrolizumab + MK-4830 800 mg. Bevacizumab (or biosimilar) may be added to either arm in the adjuvant setting. All treatments will be given on day 1 of each 3-week cycle. For patients who undergo IDS, the last dose of neoadjuvant therapy will be administered 3-6 weeks before IDS, and adjuvant therapy will commence <7 weeks after IDS. Adjuvant therapy will be administered for up to 3 cycles or until disease progression, unacceptable toxicity, or withdrawal. Upon completion of adjuvant therapy, patients may undertake maintenance therapy per investigator discretion and local guidelines. Tumor imaging will be performed at baseline, ≥21 days after neoadjuvant therapy or prior to IDS, after IDS or prior to the start of adjuvant therapy, and at the end of treatment. The primary objective is to evaluate the change from baseline in ctDNA, defined as the continuous mean mutant/tumor molecules/mL as measured in a blood sample, at cycle 3. Secondary objectives are to evaluate the association between change from baseline in ctDNA at cycle 3 and surgical outcomes (including pathologic complete response and chemotherapy response score), and safety and tolerability. Enrollment is ongoing.

**CT047**

**A phase 2 study of ipatasertib in combination with pembrolizumab for first line treatment of recurrent or metastatic squamous cell cancer of the head and neck.**

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Background: Single agent pembrolizumab in relapsed / metastatic head and neck squamous cell carcinoma (R/M HNSCC) has an ORR of 19% and mOS of 13.6 months. There are many factors which may influence which patients respond to antibodies targeting the PD-1 axis. Regulatory T cells (Tregs) play a significant role in an immunosuppressive tumor microenvironment (TME). Low Tregs in the TME have been correlated with clinical efficacy with PD-1 blockade in various solid tumors. Tregs play an active role in HNSCC. Anti-PD-1 antibodies induce Treg activation in part through AKT pathway activation, which may contribute to low response rates to checkpoint inhibitor therapy. AKT blockade selectively inhibits the proliferation of human Tregs. Additionally, inhibition of the PI3K-AKT-mTOR pathway limits myeloid-derived suppressor cells (MDSC) infiltration and differentiation, and boosts CD8+ T cell memory and effector function. Ipatasertib is an oral highly selective small-molecule inhibitor

of all three isoforms of AKT. In the Ice-CAP phase I solid tumor trial, combination PD-L1 antibody and Ipatasertib demonstrated reduction of Tregs and increased CD8+ effector T cells in paired biopsy specimens. This trial is designed to compare the efficacy of combination ipatasertib plus pembrolizumab compared to pembrolizumab monotherapy in R/M HNSCC.
Methods: In this prospective, two-arm, phase II, multicenter trial, patients with R/M HNSCC will be treated with pembrolizumab 200mg on day 1 with or without ipatasertib 400mg QD days 1-14 of 21-day cycles. Patients must have PD-L1 CPS score ≥ 1, have measurable disease per RECIST 1.1, and consent to on-treatment biopsy. Patients will be excluded if they have received prior systemic therapy for R/M HNSCC, cannot swallow a pill, or require insulin for diabetes. The primary objective is to compare the PFS between the two arms. We will estimate the relative hazard ratio associated with Ipatasertib plus pembrolizumab compared to pembrolizumab using the Cox Model where randomized treatment assignment is the only variable in the model. A total of 48 patients will be enrolled, with 24 patients in each cohort. Secondary objectives include ORR, safety and tolerability of the combination, and changes in tumor immune cell infiltration, AKT signaling, and changes in peripheral blood immune cells. Accrual is ongoing (NCT05172258).

**CT048**

**A Phase II trial of JDQ443 in *KRAS G12C*-mutated NSCLC with PD-L1 expression <1% or PD-L1 expression≥1% and an *STK11* co-mutation.**

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**Background** Kirsten rat sarcoma viral oncogene homolog (*KRAS*) is the most frequently mutated oncogene in non-small cell lung cancer (NSCLC). *KRAS G12C*, the most frequent *KRAS* variant, is found in ~13% of patients (pts) with NSCLC. KRAS is a GTPase that regulates cell signaling pathways necessary for proliferation, differentiation, and survival. *KRAS* mutation reduces the intrinsic GTPase activity of the enzyme, allowing for the accumulation of active, GTP-bound KRAS and hyperactivation of downstream signaling, driving tumorigenesis.
JDQ443 is a potent, selective $KRAS^{G12C}$ inhibitor that irreversibly traps $KRAS^{G12C}$ in its inactive, GDP bound state and blocks downstream signaling. In preliminary data from the Phase Ib part of the KontRASt-01 study (NCT04699188), JDQ443 showed promising antitumor activity and an acceptable safety profile in previously treated pts with *KRAS G12C*-mutated advanced NSCLC.
Pts with *KRAS G12C*-mutated NSCLC currently receive the same first-line (1L) treatment as those without driver mutations, consisting of immunotherapy alone or combined with chemotherapy; however, ~30% of pts with NSCLC present with programmed death-ligand 1 (PD-L1) expression <1%, and ~10-20% of pts harbor an *STK11* mutation, both indicators of poor response to immunotherapy. Therefore, alternative 1L treatment options are needed for these pts. Of note, PD-L1 expression and *STK11* mutation do not affect responsiveness to $KRAS^{G12C}$ inhibitors, raising interest in the evaluation of these targeted therapies as 1L alternatives to immunotherapy for pts with *KRAS G12C*-mutated NSCLC.
**Methods** KontRASt-06 (NCT05445843) is an open-label, Phase II, single-arm, multicenter study evaluating JDQ443 monotherapy (200 mg JDQ443 twice daily in 21-day cycles) as a 1L treatment for two cohorts of adult pts with locally advanced or metastatic, *KRAS G12C*-mutated NSCLC. Cohort A (n=90) includes pts whose tumors have PD-L1 expression <1%, regardless of *STK11* mutation status, while Cohort B (n=30) includes pts whose tumors have PD-L1 expression ≥1% and an *STK11* co-mutation. Local testing for PD-L1 status and *KRAS* and *STK11* mutations is

accepted; *KRAS* and *STK11* mutations may be assessed in blood samples. A tissue sample is required for retrospective biomarker status confirmation and exploratory study. The study is currently enrolling pts into both cohorts.
The primary endpoint is the overall response rate (ORR) per RECIST version 1.1, assessed by a blinded independent review committee, in Cohort A. Key secondary endpoints are ORR in Cohort B and duration of response in both cohorts. Other secondary endpoints include progression-free survival, overall survival, safety, pharmacokinetics, and pt-reported outcomes. A comprehensive biomarker strategy aims to investigate predictors of treatment response and resistance in the study population.

**CT049**

**VELOCITY-Lung: A phase 2 study evaluating safety and efficacy of sacituzumab govitecan (SG) + zimberelimab (zim) + etrumadenant (etruma) in patients (pts) with advanced or metastatic non-small cell lung cancer (mNSCLC) progressing on or after platinum (PT)-based chemotherapy and checkpoint inhibitors (CPI).**

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Background: Single-agent chemotherapy is the standard of care (SOC) in pts with mNSCLC progressing on or after PT-based chemotherapy and CPI but is associated with poor outcomes. SG is a Trop-2-directed antibody-drug conjugate. SG monotherapy demonstrated an objective response rate (ORR) of 17%, with a manageable safety profile in 54 pts with mNSCLC who had multiple prior therapies (Heist RS, et al. J Clin Oncol. 2017), and a phase 3 study is currently ongoing in this population (NCT05089734). Zim (anti-PD-1) and etruma (dual adenosine receptor antagonist) are under clinical investigation for anti-tumor activity. Zim+etruma and SG+CPI have been previously studied, with overall manageable safety profiles. Here we describe the design of substudy-02 of the VELOCITY-Lung phase 2 platform study, which will evaluate novel treatment combinations, including SG+zim+etruma, in pts with advanced or mNSCLC progressing on or after PT-based chemotherapy and CPI.
Methods: VELOCITY-Lung (NCT05633667) is an open-label, multicenter, randomized, phase 2 platform study, interrogating multiple diverse mechanisms of targeting tumor cells. Eligibility criteria include pathologically documented stage IV NSCLC, and progression after PT-based chemotherapy and CPI given either in combination or sequentially, including pts who received maintenance CPI for stage III disease. Pts with actionable genomic alterations must have received ≥1 previous targeted treatment. Pts will be enrolled into the preliminary stage treatment arm, SG+zim+etruma. After new treatment arms are added to this stage, or when the study proceeds to the expansion stage, patients will be randomized. The randomization ratio will be determined by the sponsor depending on the number of experimental arms initiated in the expansion stage, the comparator arm, and any newly added preliminary stage treatment arms. Randomization will be stratified by histology and prior therapy for actionable genomic alterations. Dosing is per recommended phase 2 dose or maximum tolerated dose for study agents and pts will continue to receive treatment until progressive disease, death, unacceptable toxicity, or initiation of a subsequent anticancer therapy. The primary endpoint is ORR assessed by investigator per RECIST v1.1. Secondary endpoints include progression-free survival, duration of response, OS, and safety. During the preliminary stage, efficacy will be compared against historical SOC; during the expansion stage, efficacy will be compared with an active comparator arm within the study. Depending on the number of treatment arms being tested in the expansion stage, this study plans to enroll ~23 to 133 patients globally and is open for recruitment.

**CT050**

**A phase 2, multi-center, open-label, dose-finding study evaluating telomere targeting agent THIO sequenced with cemiplimab in patients with advanced NSCLC.**

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Background: The modified nucleoside 6-thio-2'-deoxyguanosine (THIO) is a first-in-class direct telomere-targeting agent that is preferentially incorporated in telomeres of telomerase-positive cells, leading to telomere uncapping and cancer cell death. Preclinical evidence indicates that THIO pretreatment sensitizes non-small cell lung cancer (NSCLC) cells to cemiplimab, a PD-1 inhibitor approved as 1L treatment for patients with locally advanced/metastatic NSCLC with ≥50% PD-L1 expression. The THIO-101 trial is designed to evaluate safety and efficacy of THIO followed by cemiplimab in patients with advanced NSCLC who have developed resistance or relapsed after prior immune checkpoint inhibitor (ICI) therapy.
Trial Design: THIO-101 enrolls adult patients with stage 3/4 NSCLC, who have progressed or relapsed after treatment with an ICI alone or in combination with platinum-based therapy. Using a 3+3 design, the safety lead-in (Part A) is enrolling up to 6 patients in the first cohort to receive a total dose of THIO 360 mg IV (120 mg QD, D1-3) followed by a fixed dose of cemiplimab 350 mg on D5 Q3W. If ≥2 patients experience dose-limiting toxicities, a second cohort will receive a total dose of THIO 180 mg IV (60 mg QD, D1-3) followed by cemiplimab. In the dose- finding portion of the study (Part B), patients will be randomized 1:1:1 in a Simon 2-stage design (n=41 per arm) to receive THIO 360 mg [if cleared in Part A], 180 mg, or 60 mg followed by cemiplimab. Sequential THIO and cemiplimab treatment may be continued Q3W for up to 1 year, or until disease progression, unacceptable toxicity, or withdrawal of consent. The primary endpoints are safety, objective response rate, and disease control rate (CR, PR, and SD). Secondary endpoints include duration of response, progression-free survival, and overall survival; exploratory endpoints include PK/PD parameters (type I IFN, IL-6, CRP) and assessment of tumor telomerase status by IHC. Investigators will assess disease progression per RECIST v1.1 and/or iRECIST, with radiographic scans performed on D1 of cycles 3 and 5 and every 9-12 weeks thereafter. Adverse events are evaluated according to NCI CTCAE v5.0. The trial is enrolling patients at sites in Europe and Australia. The trial has completed enrollment in Part A without DLTs and opened enrollment in Part B. NCT05208944; EUDRA CT Number, 2021-005136-34

**CT051**

**Double lung transplant registry aimed for lung-limited malignancies (DREAM) - a prospective registry study of bilateral lung transplantation for medically refractory cancers confined to the lungs.**

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Background: According to a consensus document from the International Society for Heart and Lung Transplantation (ISHLT), adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) are listed as ‘special circumstances’ for lung transplantation (Weill D et al., 2015). There have been five reported studies of lung transplantation in carefully selected subsets of patients with multifocal lung adenocarcinoma (Raemdonck DV et al., 2016; Glanville AR et al., 2018). The 5-year survival rate of bilateral sequential lung transplantation was estimated at over 50% in patients with multifocal BAC, invasive adenocarcinoma, and NSCLC (Ahmad U et al., 2012; Paloyan EB et al., 2000; Zorn GL et al., 2003; de Perrot M et al., 2004). In 1999, a case series reported a post-transplant recurrence-free survival of 23 to 56 months in three patients (Garver RI et al., 1999). There is also an unmet need for patients who

have lung-limited metastasis after successful treatment for primary tumors such as sarcomas or colorectal cancer (CRC). As with liver transplantation for CRC patients who have liver-confined metastases (Dueland S et al., 2020; Hernandez-Alejandro R et al., 2022), the applicability of lung transplantation in lung-limited metastasis patients should be explored.
Methods: This is a prospective registration trial to evaluate outcomes of patients who undergo lung transplantation for the treatment of the select groups of medically refractory cancers (primary lung cancers or metastatic cancers in lungs). Overall survival (OS), disease-free survival (DFS), allograft rejection (AR) and allograft survival (AS) will be monitored as well as molecular and genetic biomarkers to investigate the correlation with prognosis. The study duration will be 10 years including surveillance. Recruitment to occur during the first 5 years of the study. The goal is to enroll 175 participants through the Lurie Comprehensive Cancer Center of Northwestern University. Essential Criteria:

1. The tumor should be without any involvement of mediastinal lymph nodes involvement confirmed by endobronchial ultrasound (EBUS) or mediastinoscopy.
2. The patient who are resistant or refractory to or without available standard of care treatment options or experimental treatment options that are known to increase survival outcome.

Study cohorts: • Cohort A: Primary lung cancers - Examples include, but not limited to, invasive mucinous/non-mucinous non-small cell lung cancers and multifocal carcinomas. • Cohort B: Metastatic cancers to the lung only - Examples include, but not limited to, germ cell tumors, head & neck tumors, colorectal tumors, renal cell tumors, testicular cancers. Clinical trial registry number : NCT05671887. Enrollment began November 16, 2022. Trial is open and recruiting as of January 12, 2023.

**CT052**

**A phase 1/2 randomized study of imvotamab monotherapy and in combination with loncastuximab tesirine in relapsed/refractory non-Hodgkin lymphomas.**

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Background: Recent advances in the treatment of non-Hodgkin lymphoma (NHL) have transformed the landscape and provided significant benefits to patients. Novel agents that target CD19 and CD20 on B cells, such as bispecific T-cell engagers and chimeric antigen receptor T-cell (CAR-T) therapy, have demonstrated benefit for patients in the relapsed and refractory setting. However, these agents can often be associated with significant toxicity and there is a need for new therapies that can provide clinical

benefit with a superior safety profile. Imvotamab is a novel CD20 x CD3 bispecific antibody utilizing an IgM backbone. This allows targeting of up to 10 CD20 binding sites for every CD3 site. In preclinical models, imvotamab demonstrated encouraging antitumor activity and stimulated T-cells in a more physiologic manner than IgG-based antibodies, which may reduce adverse events typically associated with T-cell engagers and CAR-T, such as CRS and neurotoxicity. Loncastuximab tesirine is a CD19-targeting antibody-drug conjugate approved by the US FDA and EMA for relapsed DLBCL after 2 lines of systemic therapy. Loncastuximab tesirine has shown activity in both DLBCL and FL. Combining therapies such as loncastuximab tesirine, which targets CD19 and induce apoptosis of cancer cells, with imvotamab's ability to eliminate CD20+ tumor cells by engagement with T-cells, may improve treatment outcomes among patients with NHL via synergistic mechanisms of action.
Methods: This study is a Phase 1/2, multicenter, single-arm clinical trial of imvotamab as monotherapy and in combination with loncastuximab tesirine for patients with relapsed/refractory NHL. Phase 1a Dose Escalation is complete with no DLTs or neurotoxicity AEs up to 1000mg dose titration. Phase 1b Combination will evaluate imvotamab and loncastuximab tesirine in patients with R/R 2$^{nd}$ line or later NHL. Phase 2 monotherapy Dose Selection is currently ongoing. The Phase 2 component randomizes patients at two different dose levels (100 mg and 300 mg plateau dose) in two separate indications (R/R DLBCL and R/R FL). Patients receive weekly dosing on Day 1, 8, and 15 of each 21- day cycle. Dosing begins at 15 mg and is increased weekly for 3-4 weeks to reach the plateau dose. Patients then stay at the plateau dose until disease progression or unacceptable toxicity. Patients who achieve a response by Week 12 may switch to a less frequent dosing interval of every 3 weeks. Primary endpoints include frequency and severity of adverse events and objective response rate (ORR) based on Lugano criteria. Correlative biomarker studies will evaluate the relationship of clinical benefit with blood and tissue biomarkers. The study is currently open with patients enrolling in Phase 2 at time of submission. Phase 1b Combination is expected to begin enrollment in the first quarter of 2023. Clinical trial information: NCT04082936.

**CT053**

**Merlin_001: a prospective registry study of a primary melanoma gene-signature to predict sentinel node (SN) status and determine its prognostic value for more accurate staging of SN-negative melanoma patients.**

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Background: Sentinel lymph node biopsy (SLNB) provides important staging and prognostic information that guides surveillance and adjuvant systemic therapy decisions for patients with cutaneous melanoma. At most centers, SLNB is indicated for patients with cutaneous melanoma with at least a 5% risk of having nodal metastases, typically melanomas ≥0.8 mm in thickness or selected thinner lesions with high-risk features such as elevated mitotic rate and/or ulceration. However, up to 85% of patients who undergo SLNB have a negative result. Currently, there is an unmet clinical need to better identify patients with a low risk of nodal metastasis who may safely forgo SLNB, but otherwise meet established criteria for undergoing SLNB. Previously, a model using primary tumor gene expression profile (GEP) data combined with clinicopathological features (CP) was developed to identify melanoma patients with a low risk of having a positive SLN. The model has been externally validated in multiple retrospective studies. The aim of the MERLIN_001 observational registry study is to prospectively validate the CP-GEP model in an independent multicenter cohort of primary cutaneous melanoma patients, who undergo lymphatic mapping and SLNB per current clinical guidelines.
Methods: A total of 9 centers across the US are included in the study with planned enrollment of 2,340

patients, allowing for a loss of up to 30% due to screen failure, tissue loss, low RNA yield, no gene expression profile or failure to perform the planned SLNB. Patients with clinically node-negative cutaneous melanoma and planned SLNB using current guideline indications are eligible for the study and will be followed for five years. Both patients and investigators are blinded to the results (NCT04759781). FFPE material from the initial melanoma biopsy is collected and the GEP of the primary melanoma is assessed. Subsequently, CP-GEP probability scores are calculated and expressed as a binary classification (Low Risk or High Risk for nodal metastasis), which will be compared to SLNB pathology results. Performance metrics for CP-GEP will be evaluated and will include: SLNB Reduction Rate based on Negative Predictive Value, Positive Predictive Value, Sensitivity and Specificity, and the corresponding 95% confidence intervals. Finally, the performance of CP-GEP to stratify patients according to risk of recurrence (recurrence-free survival, distant metastasis-free survival, overall survival) will also be assessed, based on five-year outcomes data. Additional analyses will be performed using the data collected throughout the study. Enrollment of patients started in September 2021 and is ongoing. As of January 2023, 890 patients have been enrolled, representing 46% of the targeted number of patients with a successful SLNB and CP-GEP test result.

**CT054**

**Phase 2 study to assess the efficacy, safety, and tolerability of the GDF-15 inhibitor ponsegromab in patients with cancer cachexia.**

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**Background:** Cancer cachexia is a multifactorial metabolic syndrome of wasting characterized by anorexia, unintended weight loss, and decreased skeletal muscle mass, leading to progressive functional impairment, fatigue, diminished quality of life, poor response to anticancer therapy, and reduced survival. One of the biomarkers associated with cancer cachexia is cytokine growth differentiation factor 15 (GDF-15), which is secreted by tumor cells. Preliminary phase 1 data suggest that suppression of GDF-15 may lead to improvement in cachexia-related symptom burden. Ponsegromab is a potent and selective humanized monoclonal antibody that inhibits GDF-15-mediated signaling. The primary objective of this study (NCT05546476) is to assess the effect of ponsegromab on body weight in patients with cancer, cachexia, and elevated circulating GDF-15 concentrations. Secondary objectives include assessing physical activity, gait, anorexia/appetite, nausea and vomiting, fatigue, and safety. Exploratory objectives include evaluating pharmacokinetics, pharmacodynamics, and immunogenicity.
**Trial design**: This phase 2 study will enroll approximately 168 adults with non-small cell lung, pancreatic, or colorectal cancers who have cachexia and elevated GDF-15 concentrations. The study will be conducted in 2 parts. The initial 12-week treatment period will be a randomized, double-blind, placebo-controlled study wherein participants who meet eligibility criteria will be randomized 1:1:1:1 to one of 3 dose groups of ponsegromab (administered subcutaneously [SC] every 4 weeks [Q4W]) or placebo. The double-blind period will be followed by optional open-label treatment (OLT) with ponsegromab Q4W SC for up to 1 year. Upon completion of the optional OLT period, there will be a follow-up visit at Week 72. Participants who do not proceed with the optional OLT period will complete the Week 12 visit and a follow-up visit at Week 16. The primary endpoint is the mean change from baseline in body weight at Week 12. A mixed model for repeated measures followed by a Bayesian $E_{max}$ model will be used for the primary analysis. Secondary endpoints include physical activity and gait

measured by remote digital sensors; patient-reported appetite-related symptoms assessed by Functional Assessment of Anorexia-Cachexia Therapy scores; anorexia/appetite, nausea, vomiting, and fatigue evaluated according to questions from the Cancer-related Cachexia Symptom Diary; and incidence of adverse events, safety laboratory tests, vital signs, and electrocardiogram abnormalities.
**ClinicalTrials.gov identifier:** NCT05546476

**CT055**
**PAVO: A phase-II, open label, single arm study of niraparib in patients with locally advanced/metastatic *PALB2* mutated tumors.**
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**Background**
Poly (ADP-ribose) polymerase inhibitors (PARPi) have demonstrated efficacy in treating solid tumors with Homologous Recombination Deficiency (HRD), the inability to repair DNA double-stranded breaks through the Homologous Recombination Repair (HRR) pathway. Specific genetic and epigenetic alterations result in defective HRR function. Bi-allelic loss of *BRCA1* or *BRCA2* principally drives HRR deficiency. While *BRCA1/2* are instrumental to HRR, multiple genes, including *PALB2*, impact the HRR pathway. *PALB2* mutations occur in an estimated 0.97% to 3.66% of solid tumors [AACR GENIE PALB2] and are associated with susceptibility to various cancers. No clinically approved therapies specifically targeting PALB2 currently exist. Emerging evidence suggests that patients with germline or somatic *PALB2* mutations may benefit from PARPi treatment, which has potential to be a new tumor agnostic therapy option across a wide range of solid tumors.
**Methods**
PAVO is a pan-tumor, single-arm, multicenter Phase-II study assessing the safety and efficacy of niraparib (a PARPi) in patients who harbor a confirmed *PALB2* mutation. The study plans to enroll up to 110 adult subjects. Eligible participants must have: locally advanced or metastatic solid tumor(s); confirmed pathogenic or likely pathogenic somatic or germline *PALB2* mutation; received all standard of care (SOC) therapy for their tumor type, or are unlikely to derive benefit from SOC therapy in the opinion of the treating physician; ECOG performance status of 0 or 1; life expectancy of ≥ 12 weeks with adequate organ/bone marrow function. Exclusion criteria include a confirmed somatic or germline *BRCA1/2* mutation, prior treatment with any PARPi, ovarian or prostate cancer, or rapid progression while on platinum-based therapy in the metastatic setting.
Niraparib will be administered in 28 day cycles with daily dosing, as outlined in the protocol. Participants will continue study treatment until documented radiographic progression, unacceptable toxicity, death, or consent withdrawal.
The primary study endpoint is objective response rate (ORR), defined as the proportion of participants who have partial or complete response to therapy as assessed by Independent Central Review. Secondary

endpoints include DOR, PFS, and CBR.
PAVO is sponsored by Tempus with support from GSK (GlaxoSmithKline). The trial opened in March 2022. Tempus molecular data tracking (integrated NGS and EMR data) and the TIME Trial program (rapid match of patients to Just in TIME sites for clinical trials), enable patient identification and prescreening. Enrollment occurs through a combination of TIME and prospective clinical sites, where individualized prescreening models are in development. New site identification and referral of molecularly eligible patients to enrolling centers are ongoing.
**Clinical Trial Registry:** NCT05169437

**CT056**
**A multicenter, open-label phase 1/2 trial evaluating the safety, tolerability, and efficacy of MORAb-202, a folate-receptor-alpha-targeting antibody-drug conjugate in patients with selected tumor types.**
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**Background:** MORAb-202 (farletuzumab ecteribulin) is an antibody-drug conjugate (ADC) comprised of the humanized antifolate receptor-alpha (FRα) monoclonal antibody, farletuzumab, and the cytotoxic microtubule inhibitor, eribulin, conjugated by a cathepsin B-cleavable linker. MORAb-202 targets the eribulin payload to tumor cells expressing FRα, where internalization leads to lysosomal cleavage of the ADC and intracellular release of eribulin, causing apoptosis, cell-cycle arrest, and bystander effects in adjacent cells. A previous phase 1 study in Japan of MORAb-202 (NCT03386942) demonstrated antitumor activity across multiple tumor types and identified interstitial lung disease (ILD) as an adverse event of interest (Shimizu *CCR* 2021). An expansion cohort (doses: 0.9, 1.2 mg/kg) in patients with platinum-resistant ovarian cancer (OC) found meaningful efficacy across FRα-expression levels and ILD/pneumonitis (mainly low grade) was the most common adverse event (Nishio ASCO 2022).
**Methods:** This multicenter phase 1/2 study (NCT04300556) consists of Dose-Escalation and Dose-Confirmation cohorts. In the Dose-Escalation phase, the primary objectives were to evaluate safety/tolerability and determine the recommended phase 2 dose of MORAb-202 in patients with OC, endometrial cancer (EC), non-small cell lung cancer (NSCLC), or triple-negative breast cancer (TNBC). In the ongoing Dose-Confirmation phase, the primary objectives are (1) to further evaluate safety/tolerability and (2) to evaluate preliminary efficacy (Objective Response Rate) in patients with OC or EC. Based on a population pharmacokinetics model (Hayato ASCO 2022), body-surface-area-based dosing is utilized. The initial cohort has enrolled 7 patients at a MORAb-202 25 mg/m$^2$ IV Q3W dose and is ongoing; further enrollment of patients at 25 mg/m$^2$ and 33 mg/m$^2$ will occur following ILD safety evaluation. Tumor assessments will be conducted by investigators using RECIST v1.1 at screening, every 6 weeks for 24 weeks, then every 12 weeks or as needed. Assessments of computed tomography scans for ILD will be conducted by a central expert review board.

**CT057**
**Phase 2, multicenter open-label basket trial of *nab*-sirolimus for patients with inactivating alterations in *TSC1* or *TSC2* (PRECISION I).**
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*nab*-Sirolimus is a novel albumin-bound mTOR inhibitor (mTORi) approved in the US for adult patients with malignant PEComa. In an exploratory analysis of the pivotal AMPECT trial of *nab*-sirolimus in advanced malignant PEComa (NCT02494570), 8/9 (89%) and 1/5 (20%) patients with inactivating alterations in *TSC1* and *TSC2*, respectively, had confirmed response (Wagner, *J Clin Oncol*, 2021). *TSC1* and *TSC2* alterations have been observed in patients with a broad variety of cancers. Most treatment-related adverse events in AMPECT were grade 1/2 (none were grade ≥4) and were consistent with long-term treatment of *nab*-sirolimus. PRECISION I (NCT05103358) will evaluate efficacy and safety of *nab*-sirolimus in patients with *TSC1* (Arm A) and *TSC2* (Arm B) alterations. Eligible patients are ≥12 years old and mTORi-naïve, possess malignant solid tumors with *TSC1* or *TSC2* inactivating alterations (confirmed by central review of sequencing reports), and have received appropriate standard treatments, as determined by the investigator. *nab*-Sirolimus 100 mg/m$^2$ will be given weekly intravenously over 30 minutes on Days 1 and 8 of each 21-day cycle. The primary endpoint is overall response rate per independent radiographic review (IRR) using RECIST v1.1. Other endpoints include duration of response, time to response, progression-free survival by IRR, overall survival, patient-reported quality of life, and safety. Enrollment began March 2022. Collaboration with leading next-generation sequencing vendors will expedite the identification of patients with qualifying *TSC1* or *TSC2* mutations; study access will be facilitated through a "just-in-time" approach to trial location activation. Based on the prevalence of *TSC1* or *TSC2* inactivating alterations, the most frequent tumor types expected are bladder, hepatobiliary, endometrial, soft tissue sarcoma, ovarian, and esophagogastric (**Table**).

Est incidence of patients with TSC1 or TSC2 alterations available for 1L therapy in 2030

| **Tumor Type** | ***TSC1* Mutations, %[a]** | ***TSC2* Mutations, %[a]** | **Eligible *TSC1* or *TSC2* Combined, %** |
|---|---|---|---|
| Bladder | 6.33 | 1.70 | 8.03 |
| **Hepatobiliary** | **1.27** | **3.31** | **4.58** |
| Endometrial | 2.10 | 1.22 | 3.32 |
| Soft tissue sarcoma | 1.28 | 1.71 | 2.99 |
| Ovarian | 1.85 | 0.92 | 2.77 |
| **Esophagogastric** | **0.65** | **1.46** | **2.11** |
| **Colorectal carcinoma** | **0.99** | **0.39** | **1.38** |
| **Pancreatic** | **0.57** | — | **0.57** |

Note: Estimated incidence of patients in the United States with definite impact *TSC1* or *TSC2* alterations available for 1L therapy in 2030. All gastrointestinal tumors (bolded) with known incidence of *TSC1* or *TSC2* and tumor types with combined incidence of *TSC1* or *TSC2* alterations of >2% are listed. [a]The proportion of patients with definite impact mutations (ie, mutations known to have a biological impact, including frameshift, nonsense, and splice-site mutations and deep deletions) was

derived from the NIH NCI Genomic Data Commons Data Portal (NIH NCI Genomic Data Commons). 1L, first-line.

**CT058**

**TROPION-PanTumor03: Phase 2, multicenter study of datopotamab deruxtecan (Dato-DXd) as monotherapy and in combination with anticancer agents in patients (pts) with advanced/metastatic solid tumors.**

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Background: Pts with metastatic epithelial tumors who progress after initial therapy have a poor prognosis; new therapeutic agents could potentially improve outcomes. Trophoblast cell surface protein 2 (TROP2) is a transmembrane glycoprotein that is over-expressed on the cell surface of many epithelial cancers representing a promising target for surface-directed therapeutic modalities. Dato-DXd is an antibody-drug conjugate consisting of a humanized anti-TROP2 IgG1 monoclonal antibody covalently linked to a highly potent topoisomerase I inhibitor payload via a stable, tumor-selective, tetrapeptide-based cleavable linker. Dato-DXd monotherapy demonstrated encouraging efficacy and safety in pts with lung and breast cancer (TROPION-PanTumor01 study; NCT03401385).
Methods: The phase 2, multicenter, open-label TROPION-Pantumor03 study (NCT05489211) is exploring safety and efficacy of Dato-DXd as monotherapy and in combination with various anticancer agents (e.g. durvalumab, AZD5305, nivolumab, bevacizumab, chemotherapies) that may be active in the tumor types being evaluated. TROPION-Pantumor03 is a master trial comprising independent substudies to enable simultaneous evaluation of the recommended phase 2 dose (RP2D), safety, and preliminary efficacy of Dato-DXd across several tumor types and treatment combinations. Pts with endometrial, gastric, metastatic castration-resistant prostate (mCRPC), ovarian, and colorectal (CRC) cancer are being enrolled. Eligible pts: age ≥18 years, histologically/cytologically documented advanced or metastatic disease, ≥1 measurable target lesion (RECIST 1.1) not previously irradiated (mCRPC substudy: non-measurable bone metastatic disease permitted), and adequate bone marrow and organ function. An FFPE tumor sample must be available for all pts. Prospective selection for TROP2 expression is required in pts with CRC; in all other cohorts, TROP2 expression will be analyzed retrospectively. In all monotherapy cohorts, pts will receive Dato-DXd 6 mg/kg IV infusion Q3W; in the combination cohorts, Dato-DXd 4 or 6 mg/kg Q3W, as appropriate for each combination. Pts will receive treatment until disease progression, unacceptable toxicity, or withdrawal.
Primary Endpoints: Objective response rate, PSA50 response (mCRPC substudy only), and safety/tolerability.
Secondary Endpoints: Progression-free survival, duration of response, disease control rate, best % change in tumor size, pharmacokinetics, and immunogenicity (anti-drug antibodies). Exploratory endpoints incl. overall survival, pt-reported outcomes, and biomarker analysis (incl. TROP2 and PD-L1 expression, tumor mutational profiling, proteomics and ctDNA profiling). Recruitment is ongoing as of Jan 2023.

**CT059**

**StrataPATH™: a multicohort, non-randomized, open-label phase 2 trial to explore efficacy and safety of FDA-approved cancer therapies in novel biomarker-guided patient population.**

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Purpose: The Strata Precision Indications for Approved Therapies (Strata PATH; NCT05097599) trial is a non-randomized open-label, prospective pan-tumor therapeutic trial designed to evaluate the efficacy and safety of multiple FDA-approved cancer therapies in new, biomarker-guided patient populations. Enrollment into a cohort of this basket trial is based on novel biomarkers including DNA, quantitative RNA expression and novel multivariate algorithms that account for both tumor and tumor microenvironment variables hypothesized to predict improved therapeutic response. The therapeutic classes evaluated in Strata PATH include targeted therapies, antibody-drug conjugates, immunotherapies, and angiogenesis inhibitors.

Trial Design: Key eligibility criteria are pathologically confirmed solid tumor. Patients are assigned to treatment by tumor type and biomarker status (Table). All patients will be treated until disease progression, unacceptable toxicity, patient/physician decision to withdraw, or 3 years of treatment from the date of consent. Primary Outcome measure includes overall response rate (ORR) as assessed by the investigator according to RECIST version 1.1. Secondary outcome measures include ctDNA response, Duration of Response (DoR), Time to Treatment Discontinuation (TTD), Time to Next Treatment (TTNT), Overall Survival (OS), safety, and ctDNA Response Rate. Enrollment is ongoing with 35 participants per cohort for up to 20 cohorts (700 participants total).

Summary: Strata PATH provides the next important step forward in precision medicine, guiding clinical trial and therapy selection by expanding biomarker testing to quantifiable RNA expression and multivariant algorithm based molecular testing.

Table. Strata PATH Biomarker-guided cohorts for expanded treatment of already FDA-approved drugs outside approved tumor indications

| **Biomarker** | **Molecular Test** | **Therapy** |
|---|---|---|
| ALK ROS1 | DNA | Lorlatinib |
| BRAF | DNA | Binimetinib + Encorafenib |
| BRCA, PALB2 | DNA | Talazoparib |
| HER2 overexpression | RNA | fam-Trastuzumab deruxtecan-nxki |
| Trop2 targeting algorithm | Multivariate Algorithm | Sacituzumab govitecan-hziy |
| Immunotherapy Response Score (IRS) | Multivariate Algorithm | PD-1 inhibitor |
| Angiogenesis Response Score (ARS) | Multivariate Algorithm | Axitinib |

**CT060**

**ACTION: A randomized phase 3 study of dordaviprone (ONC201) in patients with newly diagnosed H3 K27M-mutant diffuse glioma.**

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**Background:** H3 K27M-mutant diffuse midline glioma is a universally fatal malignancy primarily affecting children and young adults. While radiotherapy (RT) provides transient benefit, no effective systemic therapy is currently available and current standard of care is RT followed by monitoring. Dordaviprone (ONC201), a first-in-class imipridone, is an oral, blood-brain barrier penetrating, selective small molecule antagonist of dopamine receptor D2/3 (DRD2) and agonist of the mitochondrial protease ClpP. An integrated pooled analysis of five open-label trials previously demonstrated efficacy in dordaviprone-treated patients with recurrent disease. This phase 3 trial will be the first randomized, controlled study evaluating dordaviprone in patients with H3 K27M-mutant disease.
**Methods:** ACTION (NCT05580562) is a randomized, double-blind, placebo-controlled, parallel-group, international Phase 3 study of dordaviprone in patients with newly diagnosed H3 K27M-mutant diffuse glioma. Patients who have completed standard frontline radiotherapy will be randomized 1:1:1 to receive placebo, once-weekly dordaviprone, or twice-weekly dordaviprone on two consecutive days. Primary efficacy endpoints are overall survival (OS) and progression-free survival (PFS) in all participants; PFS will be assessed by response assessment in neuro-oncology-high grade glioma by blind independent central review. Other objectives include assessments of safety, additional efficacy endpoints, clinical benefit, quality of life, pharmacokinetics, biomarkers, and healthcare resource utilization. Eligible patients will have histologically confirmed H3 K27M-mutant diffuse glioma, a Karnofsky/Lansky performance status ≥70, and completed first-line radiotherapy. Eligibility will not be restricted based on age; however, patients must be ≥10 kg at time of randomization. Patients with a primary spinal tumor, diffuse intrinsic pontine glioma, leptomeningeal disease, or cerebrospinal fluid dissemination are not eligible. ACTION is currently enrolling in the United States, with additional sites to be open internationally in 2023.

**CT061**

**TRIDENT phase 3 study (EF-32): First-line Tumor Treating Fields (TTFields; 200 kHz) therapy concomitant with chemo-radiation, followed by maintenance TTFields/temozolomide in newly diagnosed glioblastoma.**

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**Background:** Tumor Treating Fields (TTFields) therapy is a loco-regional, noninvasive treatment approved for newly diagnosed (nd)/recurrent (r) glioblastoma (GBM) and mesothelioma. Approval for ndGBM was based on the pivotal phase 3 EF-14 study where TTFields therapy/temozolomide (TMZ), administered in the adjuvant setting, significantly improved PFS and OS vs TMZ monotherapy. TTFields therapy-related adverse events (AEs) were mostly mild-to-moderate skin reactions, with no evidence of TTFields therapy-related systemic toxicity. Radiotherapy (RT) concomitant with TTFields therapy demonstrated an increased therapeutic effect in preclinical models. Supplementary evidence from two pilot phase 2 studies demonstrated that concomitant administration of TTFields therapy with standard of care RT/TMZ was feasible and well-tolerated. Here we present a phase 3 study examining the efficacy and safety of TTFields therapy concomitant with RT/TMZ in patients with ndGBM.

**Materials and Methods:** TRIDENT (EF-32; NCT04471844) is a global, randomized, phase 3 study of patients ≥18 years of age (≥22 years in the US) with histologically confirmed ndGBM, ≥3 months life expectancy, ≥70 Karnofsky Performance Status, and adequate organ function. Patients will be stratified by the extent of resection and methylation status of the *MGMT* promoter. Approximately 950 patients will be assigned 1:1 to continuous TTFields therapy (200 kHz, ≥18 h/day) concomitant with RT/TMZ (experimental arm) or RT/TMZ alone (control arm). Patients will first receive 6 weeks of experimental or control therapy, TTFields therapy will then be added to the control group and all patients will receive 6 cycles of maintenance TMZ and continuous TTFields therapy. Once initiated, TTFields therapy use will continue until second disease progression (PFS2) or until 24 months (if clinically able) from the time of randomization. The primary endpoint is median OS; secondary endpoints include 1- and 2- year OS rates, PFS, 6- and 12-month PFS rates, and PFS2 (all per Response Assessment in Neuro-Oncology [RANO]), overall radiological response (per RANO), severity and frequency of AEs, quality of life (QoL; per European Organisation for Research and Treatment of Cancer QoL Questionnaires), neurological function (per Neurological Assessment in Neuro-Oncology scale), and tumor pathology post study treatments (when available). The ability of TTFields therapy to prolong OS in a dose-dependent manner is an exploratory endpoint. The primary endpoint is based on the hypothesis that TTFields therapy/RT/TMZ can significantly improve OS (vs RT/TMZ) and will be tested using a stratified log-rank test. The sample size is calculated for a hazard ratio of <0.8 with a 5% type I error. The study is expected to enroll patients at 150 sites and is currently open in nine countries.

**CT062**

**GBM AGILE: A global, phase 2/3 adaptive platform trial to evaluate multiple regimens in newly diagnosed and recurrent glioblastoma.**

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Background: GBM AGILE (Glioblastoma Adaptive, Global, Innovative Learning Environment) is a biomarker based, multi-arm, international, seamless Phase 2/3 Response Adaptive Randomization platform trial designed to rapidly identify experimental therapies that improve overall survival and confirm efficacious experimental therapies and associated biomarker signatures to support new drug approvals and registration. GBM AGILE is a collaboration between academic investigators, patient organizations and industry to support new drug applications for newly diagnosed and recurrent GBM. Methods: The primary objective of GBM AGILE is to identify therapies that improve the overall survival in patients with newly diagnosed or recurrent GBM. Operating under a Master Protocol, GBM AGILE allows multiple drugs from different pharmaceutical/biotech companies to be evaluated simultaneously and/or over time against a common control. New experimental therapies are added as new information about promising new drugs is identified while other therapies are removed as they complete their evaluation. Bayesian response adaptive randomization is used within subtypes of the disease to assign

participants to investigational arms based on their performance.
GBM AGILE has screened over 1300 patients and enrollment rates are 3 to 4 times greater than traditional GBM trials, with active sites averaging 0.75 to 1 patients/site/month. There are 41 active sites in the US, 4 active sites in Canada and 3 active sites in Europe with 12 more sites anticipated to open across 3 countries in Europe. Expansion to Australia is in progress. Stratification of the newly diagnosed patients is based on methylation status per the MGMT assay provided by a central CLIA lab. Maintaining reasonable timelines for MGMT read out to support eligibility and screening requires ongoing operational oversight. In order to continue to improve MGMT testing times, the percentage discordance between results from central testing and local lab testing is being evaluated to assess if local lab testing can be utilized for randomization within GBM AGILE.
Exploratory analyses are ongoing to expand knowledge of GBM. For example, information from imaging and clinical assessments will be used to build a longitudinal model, which may inform randomization by providing earlier and continuous information regarding how a given experimental arm is performing.

**CT063**

**A Phase 3 study of gedatolisib plus fulvestrant with and without palbociclib in patients with HR+/ HER2- advanced breast cancer previously treated with a CDK4/6 inhibitor plus a non-steroidal aromatase inhibitor (VIKTORIA-1).**

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Patients who receive frontline CDK4/6 inhibitor (CDK4/6i) therapy eventually experience disease progression. Resistance to CDK4/6i is likely a transient adaptive mechanism that may be reversed by inhibition of the PI3K/mTOR pathway. Thus, combination of CDK4/6i and PI3K/mTORi after disease progression on CDK4/6i could restore sensitivity to CDK4/6i and prevent activation of the PI3K/mTOR pathway. This hypothesis was evaluated in a Phase 1b study (Layman SABCS 2021) of gedatolisib (geda), a potent inhibitor of PI3K and mTOR. Subjects with HR+/HER2- ABC with prior CDK4/6i received geda (180 mg IV weekly for 3 weeks, then one week off) with palbociclib (palbo) and fulvestrant (FUL). Median PFS was 12.9 months with 63% overall response rate. Efficacy was observed regardless of *PIK3CA* mutation status (Wesolowski SABCS 2022). Geda was well tolerated, with few discontinuations due to treatment-related adverse events (4%). The most common AE was stomatitis; hyperglycemia of any grade occurred in 26% of patients. This preliminary data, dosing schedule, and study population characteristics form the basis for the Phase 3 trial, VIKTORIA-1 (NCT05501886). This Phase 3 multinational clinical trial will evaluate geda and FUL with or without palbo in patients with HR+/HER2- ABC previously treated with any CDK4/6i in combination with a non-steroidal aromatase inhibitor (AI) therapy. Those without tumor *PIK3CA* mutations will be assigned to Study 1 (n=351) and randomized to Arm A (geda, palbo, and FUL), Arm B (geda and FUL), or Arm C (FUL). Those with *PIK3CA* mutations will be assigned to Study 2 (n=350) and randomized to Arm D (geda, palbo, and FUL), Arm E (alpelisib and FUL), or Arm F (geda and FUL). Key eligibility criteria include adults with confirmed metastatic or locally advanced breast cancer, any menopausal status, radiologically evaluable disease, and prior CDK4/6i treatment in combination with a non-steroidal AI. Prior hormonal therapy,

including SERDs, is allowed. Key exclusion criteria include prior treatment with a PI3K, Akt, or mTOR inhibitor, prior treatment with chemotherapy for advanced disease, more than two lines of prior endocrine therapy, bone only disease with no soft tissue components, active CNS metastases, and type 1 diabetes or uncontrolled type 2 diabetes.
The primary endpoint is PFS assessed by blinded independent central review per RECIST v1.1. Secondary endpoints included overall survival, safety and tolerability, ORR, duration of response, time to response, CBR, quality of life, and pharmacokinetics. The trial is open for enrollment. This trial abstract was previously presented at the 2022 San Antonio Breast Cancer Symposium, December 6-10, 2022.

**CT064**

**Phase III study to evaluate the efficacy and safety of GLSI-100 (GP2 + GM-CSF) in breast cancer patients with residual disease or high-risk PCR after both neo-adjuvant and postoperative adjuvant anti-HER2 therapy, Flamingo-01.**

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**Background:** GP2 is a biologic nine amino acid peptide of the HER2/*neu* protein delivered in combination with Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) that stimulates an immune response targeting HER2/neu expressing cancers, the combination known as GLSI-100. In a prospective, randomized, single-blinded, placebo-controlled, multicenter Phase IIb study, no recurrences were observed in the HER2+ population after 5 years of follow-up, if the patient was treated with GLSI-100, survived, and was followed for more than 6 months ($p = 0.0338$). Immunotherapy elicited a potent response measured by skin tests and immunological assays. Of the 146 patients that have been treated with GLSI-100 over 4 clinical trials, GLSI-100 was well-tolerated and no serious adverse events observed were considered related to the immunotherapy.
**Method:** This Phase III trial is a prospective, randomized, double-blinded, multi-center study. After 1 year of trastuzumab-based therapy, 6 intradermal injections of GLSI-100 or placebo will be administered over the first 6 months and 5 subsequent boosters will be administered over the next 2.5 years for a total of 11 injections over 3 years. The participant duration of the trial will be 3 years treatment plus 1 additional year follow-up for a total of 4 years following the first year of treatment with trastuzumab-based therapy. Patients will be stratified based on residual disease status at surgery, hormone receptor status and region.
**Study Size - Interim Analysis:** Approximately 498 patients will be enrolled. To detect a hazard ratio of 0.3 in invasive breast cancer free survival (IBCFS), 28 events will be required. An interim analysis for superiority and futility will be conducted when at least 14 events have occurred. This sample size provides 80% power if the annual rate of events in placebo patients is 2.4% or greater. Up to 100 non-HLA-A*02 subjects will be enrolled in an open-label arm.
**Eligibility Criteria:** The patient population is defined by these key eligibility criteria: 1) HER2/neu positive and HLA-A*02; 2) Residual disease or High risk pCR (Stage III at presentation) post neo-adjuvant therapy; 3) Exclude Stage IV; and 4) Completed at least 90% of planned trastuzumab-based therapy.
**Trial Objectives:** 1) To determine if GP2 therapy increases IBCFS; 2) To assess the safety profile of GP2; and 3) To monitor immunologic responses to treatment and assess relationship to efficacy and safety.
**Study Status:** The study has been initiated at a number of sites in the US. The study is also expected to be opened in Spain, Germany, and France.
**Funding:** This trial is supported by Greenwich LifeSciences.

**CT065**

**Phase 3 study of tucatinib or placebo in combination with trastuzumab and pertuzumab as maintenance therapy for HER2+ metastatic breast cancer (HER2CLIMB-05, trial in progress).**

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Background: The current first-line (1L) standard of care (SOC) for human epidermal growth factor receptor 2-positive (HER2+) metastatic breast cancer (MBC) is trastuzumab (T) plus pertuzumab (P) and a taxane. Despite advances in 1L SOC, most patients (pts) progress during maintenance therapy with T+P. Tucatinib is a tyrosine kinase inhibitor (TKI) approved in combination with T and capecitabine for adults with HER2+ MBC, with and without brain metastases (BM). In HER2CLIMB, the addition of tucatinib significantly prolonged progression-free survival (PFS) and overall survival (OS) in pts with HER2+ MBC and was well tolerated. Adding tucatinib also reduced the risk of disease progression or death in pts with untreated and/or active BM (Murthy et al. 2020, Curigliano et al. 2021). HER2CLIMB-05 investigates whether adding tucatinib to 1L SOC as maintenance therapy will extend PFS while maintaining quality of life (QOL).
Methods: HER2CLIMB-05 (NCT05132582) is a phase 3, randomized, double-blind study evaluating tucatinib plus T+P as maintenance therapy for HER2+ MBC. Approximately 650 pts will be enrolled. Eligible pts will have advanced HER2+ disease, no progression on 4-8 cycles of prior 1L SOC, ECOG Performance Status of 0 or 1, and no or asymptomatic BM. Exclusion criteria include prior treatment with anti-HER2 and/or anti-epidermal growth factor receptor TKI (prior SOC for early BC is permitted) or inability to undergo contrast magnetic resonance imaging of the brain. Pts will be randomized 1:1 to receive either tucatinib or placebo twice daily, with T+P once every 21 days. Pts with HR+ disease may receive endocrine therapy. The primary endpoint is investigator-assessed PFS. Secondary endpoints include OS (key endpoint), PFS by blinded independent central review (BICR), time to deterioration of health-related QOL, central nervous system PFS, safety, and pharmacokinetic (PK) parameters. PFS and OS will be compared using a 2-sided stratified log-rank test between treatment groups. Time-to-event endpoints will be summarized using the Kaplan-Meier method. PK and safety data will be summarized using descriptive statistics. Enrollment is ongoing in the US, Canada, and several EU and APAC countries, with additional sites planned. This abstract was previously presented at ESMO-BC 2022, FPN (Final Publication Number): 415, by Veronique Dieras (reused with permission).

**CT066**

**HERTHENA-Lung02: A randomized Phase 3 study of patritumab deruxtecan vs platinum-based chemotherapy in locally advanced or metastatic *EGFR*-mutated NSCLC after progression with a third-generation EGFR tyrosine kinase inhibitor.**

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**Background:** Standard therapies for patients with epidermal growth factor receptor-mutated (*EGFR*m) non-small cell lung cancer (NSCLC) that has progressed after treatment with third-generation EGFR

tyrosine kinase inhibitors (TKIs) offer only limited benefit. Human epidermal growth factor receptor 3 (HER3) is often expressed in primary NSCLC tumors, and HER3 expression is commonly observed in patients with *EGFR* mutations. HER3-DXd is a novel antibody-drug conjugate composed of a human anti-HER3 monoclonal antibody (patritumab) linked to a topoisomerase I inhibitor payload via a tetrapeptide-based cleavable linker. HER3-DXd demonstrated efficacy and safety in a phase 1 study in patients with *EGFR*m NSCLC that progressed following an EGFR TKI and platinum-based chemotherapy (PBC) (U31402-A-U102; NCT03260491). In previously reported results from dose-escalation/expansion, HER3-DXd 5.6 mg/kg demonstrated a manageable safety profile and promising efficacy (confirmed objective response rate by blinded independent central review [BICR] of 39%, median duration of response of 7.0 months, and median progression-free survival of 8.2 months) in a subset of patients (n=44) with advanced *EGFR*m NSCLC that progressed after ≥1 line of PBC and a third-generation EGFR TKI.
**Trial Design:** HERTHENA-Lung02 (NCT05338970) is a global, open-label, randomized, phase 3 trial evaluating the efficacy and safety of HER3-DXd vs PBC in patients (≈560) with metastatic or locally advanced nonsquamous NSCLC with an *EGFR*-activating mutation (exon 19 deletion or L858R) who have received 1 or 2 lines of EGFR TKI treatment including a third-generation EGFR TKI and had disease progression following treatment with a third-generation EGFR TKI. Patients are randomized 1:1 to receive either HER3-DXd 5.6 mg/kg every 3 weeks or 4 cycles of PBC containing pemetrexed (can be continued as maintenance) with cisplatin or carboplatin. Patients are stratified by prior third-generation EGFR TKI treatment (osimertinib vs other), line of prior third-generation EGFR TKI use (first vs second line), region (Asia vs rest of world), and presence of stable brain metastases (yes vs no). The primary endpoint is progression-free survival by BICR (per RECIST v1.1). The key secondary endpoint is overall survival. Other secondary endpoints include investigator-assessed progression-free survival, objective response rate, duration of response, clinical benefit rate, disease control rate, time to response (all assessed by investigator and BICR per RECIST 1.1), safety, and patient-reported outcomes. Enrollment began May 2022 and is ongoing, with sites in Asia, Australia, Europe, and North America.

**CT067**

**Pembrolizumab with and without sacituzumab govitecan as first-line treatment for metastatic non-small-cell lung cancer (NSCLC) with PD-L1 TPS ≥50%: phase 3 KEYNOTE-D46/EVOKE-03 study.**

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Background: Sacituzumab govitecan is an antibody-drug conjugate comprising an anti-trophoblast cell-surface antigen 2 (Trop-2) antibody coupled to a potent payload, SN-38, via a proprietary, hydrolysable linker. In heavily pretreated patients with metastatic NSCLC, sacituzumab govitecan showed a durable antitumor response and was well tolerated with a 10-mg/kg dose regimen. Monotherapy with the anti-PD-1 monoclonal antibody pembrolizumab is a standard-of-care therapy for patients with previously untreated advanced/metastatic NSCLC with no sensitizing *EGFR* or *ALK* alterations and a PD-L1 tumor proportion score (TPS) ≥50%. KEYNOTE-D46 evaluates whether adding sacituzumab govitecan to pembrolizumab monotherapy can improve outcomes in patients with metastatic NSCLC with PD-L1 TPS ≥50%.
Trial Design: In this phase 3, open-label, active comparator-controlled, randomized study, ~614 eligible adults with previously untreated metastatic NSCLC; no *EGFR*, *ALK*, or *ROS1* alterations; PD-L1 TPS ≥50%; and measurable disease per RECIST version 1.1 will be randomized 1:1 to receive either pembrolizumab 200 mg Q3W for up to 35 cycles plus sacituzumab govitecan 10 mg/kg on days 1 and 8

of each Q3W cycle (no maximum treatment duration) or pembrolizumab 200 mg Q3W for up to 35 cycles. Randomization is stratified by ECOG PS (0 vs 1), histology (squamous vs nonsquamous), and geographic region (East Asia vs North America/Western Europe/Australia vs Rest of World). Treatment continues until PD, death, unacceptable toxicity, or another treatment discontinuation criterion is met, or (for pembrolizumab) completion of 35 cycles. Dual primary endpoints are PFS per RECIST version 1.1 by blinded independent central review (BICR) and OS. Secondary endpoints include ORR and duration of response per RECIST version 1.1 by BICR, safety, and patient-reported outcomes. Radiographic imaging occurs at baseline; weeks 6, 12, 18, and 24 from randomization; every 9 weeks thereafter through week 51; and then once every 12 weeks until BICR-confirmed PD or the start of new anticancer treatment, pregnancy, withdrawal of consent, or death. PD-L1 expression status is assessed at a central laboratory using PD-L1 IHC 22C3 pharmDx (Agilent, Santa Clara, CA). Health-related quality of life is assessed using validated patient-reported outcome instruments including the EORTC Quality of Life Questionnaire-Core 30 and Quality of Life Questionnaire-Lung Cancer 13. AEs are assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0. Enrollment started on December 9, 2022 and is currently ongoing.

**CT068**

**CASCADE-LUNG: validation of a blood-based assay that evaluates cell-free DNA fragmentation patterns to detect lung cancer.**

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Rationale: Despite longstanding national recommendations for lung cancer screening by low-dose computed tomography (LDCT), annual participation rates are below 15%. Cost, access, and uncertainty over individual-level benefits and harms of screening preclude greater uptake. The development of a low-cost initial blood test that detects lung cancer with high sensitivity could boost LDCT screening rates and improve screening efficiency, by identifying within the screening-eligible population those at relatively higher risk of lung cancer. DELFI (DNA evaluation of fragments for early interception) is a technology that uses low-coverage, whole-genome sequencing and machine learning to detect cancer signals in the blood. In an early study, DELFI demonstrated high sensitivity to detect stage I/II lung cancers [PMID 34417454]. CASCADE-LUNG (NCT05306288) is an ongoing study to clinically validate a DELFI-based test to detect lung cancers that would be found by chest LDCT. Study Design and Methods: CASCADE-LUNG is an event-driven, multisite, prospective, cross-sectional, observational, blood specimen collection study. Eligible individuals are ≥50 years old with ≥20 pack-years history of smoking who are scheduled for a first-time or annual lung cancer screening by chest CT scan within ~30 days after enrollment. Individuals are excluded for any history of hematologic malignancy or organ transplantation, prior cancer diagnosis or prior cancer treatment within 2 years before enrollment, and blood transfusion within 120 days before enrollment. Blood samples are collected within 30 days after enrollment for DELFI analysis. Medical record reviews are conducted at 4 months and 12 months post-enrollment, during which medical history (including social and family history and cancer screening history), demographics, imaging reports, diagnostic reports, surgery reports, pathology reports, and other diagnostic information are collected. Objectives are to evaluate the sensitivity and specificity of a DELFI-based test to detect lung cancer (primary), including within clinically relevant subgroups such as stage, nodule size, age, and race/ethnicity (secondary). Clinical endpoints include the presence or absence of histopathologically confirmed lung cancer diagnosed between the time of the enrollment chest CT scan and the 4-month follow-up visit (primary) and the 12-month follow-up visit (secondary), and adverse events associated with blood specimen collection (secondary). About 15,000 participants are expected to

enroll to reach ~70 lung cancers that are suitable for analysis. Enrollment began in April 2022 and is expected to close in 2023.

**CT069**

**Randomized phase 3 study (STARBOARD) evaluating encorafenib (enco) + binimetinib (bini) + pembrolizumab (pembro) for first-line treatment of unresectable locally advanced or metastatic BRAF V600-mutant melanoma.**

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**Background** *BRAF* V600 mutations are frequently found in metastatic melanoma. This mutation constitutively activates the MAPK pathway, which leads to melanoma progression. Patients with BRAF V600-mutant metastatic melanoma typically receive BRAF inhibitors (BRAFi) + MEK inhibitors (MEKi), such as enco + bini, or immune checkpoint inhibitors (CPIs; eg, pembro). However, these have limitations. BRAFi and MEKi may increase BRAF V600-mutant tumor sensitivity to CPIs. Previous studies reported improved progression-free survival (PFS) in patients with advanced BRAF V600-mutant melanoma receiving BRAFi + MEKi + CPI compared with targeted therapy alone. This phase 3 trial will evaluate the efficacy and safety of enco + bini + pembro vs placebo + pembro for unresectable locally advanced or metastatic BRAF V600-mutant melanoma. A safety lead-in (SLI) was built in to determine the recommended phase 3 dose (RP3D) prior to starting phase 3.

**Trial design** STARBOARD (NCT04657991) is a randomized, double-blind, placebo-controlled, phase 3 study evaluating approximately 600 patients with BRAF V600 advanced melanoma. Patients will be stratified by prior systemic adjuvant treatment (CPI, BRAFi/MEKi, or none) and by disease stage (per AJCC 8$^{th}$ edition; IIIB, IIIC, IIID, IV M1a[0], and IV M1b[0] vs IV M1a[1], IV M1b[1], IV M1c[0], IV M1c[1], IV M1d[0], and IV M1d[1]). Patients must have measurable disease (per RECIST 1.1); ECOG performance status of 0 or 1; and adequate bone marrow, hepatic, and renal function. Patients must not have received prior systemic therapy for unresectable or metastatic melanoma; adjuvant treatment with BRAFi/MEKi, anti-PD-1, or anti-CTLA-4 is permitted. Patients cannot have symptomatic brain metastases. Study treatments and end points are shown in Table 1. RP3D was established in May 2022; phase 3 enrollment began in June 2022.

**Table 1. Study treatments and end points**

| | **Phase 3** |
|---|---|
| **Treatment (21-day cycle)** | **RP3D from SLI:** enco + bini + pembro **Control:** placebo + pembro |
| **Primary** | PFS (RP3D vs control; by BICR) |
| **Secondary** | **Key:** OS (RP3D vs control) **Other**: PFS by investigator assessment, objective response rate, duration of response, disease control rate, and time to response, all by BICR and investigator assessment, PFS2, quality of life, safety, pharmacokinetics |

BICR, blinded independent central review; OS, overall survival.

**CT070**

**Double-blind placebo-controlled trial of AL102 for treatment of progressing desmoid tumors: the RINGSIDE phase 3 study design.**

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Background: There is an unmet need for effective systemic therapy for desmoid tumors (DT, aggressive fibromatosis) that provides durable tolerability, symptom improvement, and tumor regression. Gamma secretase inhibitors (GSIs) demonstrate antitumor activity against DT. AL102 is a potent, orally available, selective GSI under investigation as an antineoplastic agent.
Methods: RINGSIDE (AL-DES-01) is a 2-part Phase 2/3 study. In the open-label Phase 2 study (Part A), adults with progressing DT (≥10% unidimensional growth within 18 months or DT-related pain requiring non-opioid medication) were randomized to three dosing regimens: 1.2 mg once daily, 2 mg intermittent BIW (2 days on 5 days off), or 4 mg intermittent BIW. RINGSIDE Phase 3 (Part B) is a double-blind, placebo-controlled study evaluating the chosen dose regimen from Phase 2 (1.2 mg once daily) in adults and adolescents (≥12 years of age) with recurrent or treatment-naïve histologically confirmed progressing DT per investigator. For eligibility, progression is defined as ≥20% measured by MRI or CT scan according to RECIST v1.1 within 12 months of the screening visit. Planned enrollment is for ≈156 subjects globally to be randomized 1:1 to either AL102 1.2 mg once daily or placebo. Randomization will be stratified according to tumor location: intra-abdominal vs. extra-abdominal (including abdominal wall). Subjects will undergo MRI or CT scans (using the same modality throughout the study) every 12 weeks to assess tumor response according to RECIST v1.1 by blinded independent central review (BICR). The primary endpoint is progression-free survival (PFS) by BICR based on RECIST v1.1. Primary and secondary endpoints are summarized in Table 1. The trial is currently enrolling.

Table 1. RINGSIDE Phase 3 Study Endpoints

| Objectives | Endpoints |
|---|---|
| Primary | |
| Evaluate effects of AL102 on disease progression | PFS defined as time from randomization until date of assessment of progression (assessed by BICR based on RECIST v1.1) or death by any cause |
| Secondary | |
| Evaluate additional effects of AL102 on tumor response | • ORR (CR and PR) by BICR based on RECIST v1.1 • DOR defined by time from CR or PR (by BICR based on RECIST v1.1) until first documentation of disease progression or death from any cause |
| Evaluate effects of AL102 on quality of life | • Change from baseline in quality of life as determined by: • GOunder/Desmoid Tumor Research Foundation (DTRF) DEsmoid Symptom Scale and Impact Scale (GODDESS) • Patient-Reported Outcomes Measurement Information System (PROMIS) Physical Function • EuroQol 5-Dimensional (EQ-5D-3L) questionnaire • Patient's Global Impression of Change (PGI-C) • Change from baseline in pain assessment using Brief Pain Inventory (BPI) short form |
| Evaluate safety and tolerability of AL102 | • Frequency, duration and severity of treatment-emergent adverse events (TEAEs) and serious AEs • Time to treatment discontinuation due to TEAE |

BICR: blinded independent central review; CR: complete response; DOR: duration of response; ORR: objective response rate; PR: partial response; PFS: progression-free survival; RECIST: Response Evaluation Criteria in Solid Tumors

**CT073**

**Efficacy of novel oral SERD elacestrant in fulvestrant-refractory hormone receptor-positive (HR+) breast cancer: a translational investigation.**

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Purpose: Metastatic HR+ breast cancer initially responds to serial courses of endocrine therapy but ultimately becomes refractory Elacestrant, a new generation oral selective estrogen receptor degrader (SERD) and antagonist, has demonstrated efficacy in a subset of women with advanced HR+ breast cancer (Bidard FC, et al *J Clin Oncol* 2022;40:3246-3256), but there are few patient-derived models to characterize its effect in advanced cancers with diverse treatment histories and acquired mutations In this translational study, we tested the efficacy of elacestrant on ex vivo circulating tumor cells (CTCs) and patient-derived xenograft (PDX) mouse models with serial exposures to fulvestrant and conducted a reanalysis of the EMERALD trial to evaluate the efficacy of elacestrant vs standard endocrine therapy among patients who had received prior fulvestrant.

Methods: Using PDX models and cultured CTCs isolated from metastatic breast cancer patients, we analyzed sensitivity to elacestrant, compared with the currently approved SERD, fulvestrant We further analyzed clinical responses to elacestrant, compared with endocrine therapy, among women who had previously been treated with a fulvestrant-containing regimen from the recent phase 3 EMERALD Study (NCT03778931),

Results: In a patient-derived HR+ breast cancer PDX model, we demonstrate that elacestrant, at a clinically relevant dose, is highly effective in tumors extensively pretreated with fulvestrant In cultured CTCs isolated from HR+ breast cancer patients who had received multiple prior treatments, including fulvestrant, elacestrant is active, independent of mutations in the estrogen receptor (*ESR1*) or Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (*PIK3CA)* genes In these cells, elacestrant suppresses estrogen-receptor (ER) signaling at concentrations below those required for protein degradation Post-hoc analysis of the EMERALD clinical trial confirms that elacestrant is effective in patient populations previously treated with fulvestrant-containing regimens, irrespective of *ESR1* mutation status.

Conclusion: In the EMERALD study, elacestrant showed significantly prolonged PFS compared with SOC (including fulvestrant) Using a PDX model and CTCs isolated from patients with MBC from the EMERALD study, this translational investigation demonstrates that elacestrant retains efficacy in breast cancer cells that have acquired resistance to currently available ER-targeting therapies Elacestrant may be an option for patients with HR+/HER2- breast cancer whose disease progressed while receiving fulvestrant in the metastatic setting.

**CT075**

**Updated overall survival outcomes from a randomized, double-blind phase III study of sintilimab versus placebo in combination with chemotherapy as first-line treatment for advanced esophageal squamous cell carcinoma (ORIENT-15).**

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**Background:** The ORIENT-15 study (NCT03748134) evaluated sintilimab (anti-PD-1 antibody) plus chemotherapy (Sin+Chemo) versus placebo plus chemotherapy (Chemo) as first-line (1L) treatment of unresectable locally advanced, recurrent, or metastatic esophageal squamous cell carcinoma (ESCC). At the prespecified interim analysis, this study met the primary endpoints of overall survival (OS) in all patients (pts) (hazard ratio [HR] 0.63; 95% confidence interval [CI] 0.51-0.78; P<0.001) and the pts with PD-L1 combined positive score (CPS) ≥10 (HR 0.64; 95% CI 0.48-0.85; P=0.002) (Lu, et al. BMJ 2022). Here we report the updated OS results with an extended follow-up time.
**Methods:** Eligible pts were randomized 1:1 to receive sintilimab/placebo (3 mg/kg in pts weighing <60 kg or 200 mg in pts weighing ≥60 kg, IV Q3W) for up to 24 months plus chemo (paclitaxel 175 mg/m$^2$ Q3W plus cisplatin 75 mg/m$^2$ Q3W as TP regimen, or cisplatin 75 mg/m$^2$ Q3W plus 5-FU 800 mg/m$^2$ on days 1-5 Q3W as CF regimen). Stratification factors were PD-L1 expression (tumor proportion score <10% or ≥10%), ECOG PS (0 vs 1), liver metastasis (presence vs absence), and chemo regimen (TP vs CF). The primary endpoints were OS in the pts with PD-L1 CPS ≥10 and all pts.
**Results:** Overall, 690 pts (median age 63.0; 85.5% male; 93.3% Asian and 4.9% White; 24.3% liver metastasis; 90.7% TP regimen) were randomized and received Sin+Chemo (341 pts) or Chemo (349 pts). As of data cutoff date (Aug 28, 2022), the median follow-up was 32.2 months (interquartile range 28.0-35.8); 231 OS events were observed in Sin+Chemo group and 278 in Chemo group. Sin+Chemo continued to demonstrate an OS benefit vs Chemo in all pts (median OS 17.4 [95% CI 16.0-19.8] vs 12.8 [95% CI 11.3-14.5] months; HR 0.661 [95% CI: 0.554-0.788]; P<0.0001), and the pts with PD-L1 CPS ≥10 (18.4 [95% CI 16.2-24.6] vs 14.5 [95% CI 11.7-16.4] months; HR 0.635 [95% CI 0.503-0.803]; P=0.0001). Estimated OS rates at 12 and 24 months for Sin+Chemo vs Chemo in all pts were 64.0% vs 53.5% and 41.4% vs 22.9%, respectively. Subgroup analyses of OS in all pts were generally consistent with the previous report, showing homogeneity in OS outcomes. The CTCAE grade ≥3 treatment-related adverse events occurred in 60.7% pts in Sin+Chemo group and 56.2% pts in Chemo group. No new or unexpected safety signals were observed.
**Conclusions:** Sin+Chemo continued to demonstrate significant OS benefits in advanced ESCC in both overall and PD-L1 CPS ≥10 populations with an acceptable safety profile over time. These data further support the use of Sin+Chemo as a standard of care for 1L treatment in these patients.

**CT076**

**Randomized, global, phase 3 study of tislelizumab plus chemotherapy versus chemotherapy as first-line treatment for advanced or metastatic esophageal squamous cell carcinoma (RATIONALE-306): China subgroup analysis.**

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**Background:** Tislelizumab, an anti-programmed cell death protein 1 (PD-1) antibody, + chemotherapy (chemo) demonstrated a statistically significant and clinically meaningful improvement in overall survival (OS) vs placebo + chemo, with a manageable safety profile, as a first line (1L) treatment for patients (pts) with advanced or metastatic esophageal squamous cell carcinoma (ESCC) at interim analysis of the phase 3 RATIONALE-306 study. We report data from the China subgroup analysis.
**Methods:** In this randomized, double-blind, global study, adults with unresectable locally advanced or metastatic ESCC, with no prior systemic treatment for advanced disease were enrolled regardless of programmed death-ligand 1 (PD-L1) expression status. Pts were randomized (1:1); stratified by region, prior definitive therapy, and investigator-chosen chemo (platinum + fluoropyrimidine or platinum + paclitaxel). Pts received tislelizumab (T) 200 mg intravenously + chemo (C) (Arm T+C) or placebo (P) + chemo (Arm P+C) once every three weeks; treatment continued until disease progression by investigator per RECIST v1.1, intolerable toxicity, or withdrawal. The primary endpoint was OS in the intent-to-treat (ITT) population. Secondary endpoints included investigator-assessed progression-free survival (PFS) per RECIST v1.1, objective response rate (ORR), and duration of response (DoR), in addition to safety.
**Results**: Of 649 pts in the overall population, 370 (57.0%) were enrolled from China. At data cutoff (Feb 28, 2022), the median study follow-up in the China subgroup (ITT population) was 15.8 months (mo) in Arm T+C (n=182) and 10.6 mo in Arm P+C (n=188). Longer OS (median OS 16.6 mo vs 11.2 mo; unstratified hazard ratio [HR] 0.69, 95% confidence interval [CI] 0.54, 0.89) and PFS (median PFS 8.3 mo vs 5.6 mo; unstratified HR 0.58, 95% CI 0.45, 0.75) indicate survival benefit in Arm T+C vs P+C, respectively. Arm T+C had higher response rates and more durable responses than Arm P+C; ORR was 64.8% vs 44.1% (odds ratio 2.33 [95% CI 1.53, 3.55]) respectively, and median DoR was 7.4 mo (95% CI 5.6, 9.5) vs 5.7 mo (95% CI 4.3, 7.5), respectively. Similar proportions of pts in Arm T+C vs P+C had ≥1 treatment-related adverse event (TRAE; 98.8% vs 98.9%) and ≥grade 3 TRAEs (72.9% vs 73.4%). Serious TRAEs occurred in 27.6% vs 21.2% of pts in Arm T+C vs P+C, and TRAEs leading to death occurred in 2.9% vs 1.6% of pts, respectively. Treatment-emergent adverse events leading to discontinuation occurred in 28.2% vs 17.4%, in Arm T+C vs P+C.
**Conclusions:** In the China subgroup, 1L tislelizumab + chemo demonstrated clinically meaningful improvement in OS, PFS, ORR, and DoR vs placebo + chemo in pts with advanced or metastatic ESCC, with a manageable safety profile, consistent with published results in the overall population.
**Acknowledgments:** This study was sponsored by BeiGene, Ltd. Medical writing support, under the direction of the authors, was provided by Emma Ashman, BSc, of Ashfield MedComms, an Inizio company, and was funded by BeiGene, Ltd.

**CT077**

**Association of tumor mutational burden (TMB) and clinical outcomes with tislelizumab versus chemotherapy in esophageal cell carcinoma (ESCC) from RATIONALE-302.**

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**Background:** Programmed cell death protein 1 (PD-1) inhibitors are approved as second-line (2L) therapy for patients (pts) with ESCC. TMB is a predictive biomarker of response to immune checkpoint blockade in multiple cancers, but its role in ESCC is unclear. Here, we retrospectively investigated the association between TMB and clinical outcomes in the phase 3 RATIONALE-302 study of anti-PD-1 antibody tislelizumab (TIS) vs investigator-chosen chemotherapy (ICC) as 2L treatment for advanced unresectable/metastatic ESCC (NCT03430843).

**Methods:** Genomic profiling was assessed on tumor tissues collected at baseline using BurningRock OncoScreen Plus 520 NGS panel to determine TMB status. Median progression-free survival (PFS) and overall survival (OS) were calculated using the Kaplan-Meier method. Objective response rate (ORR) was calculated using the binomial exact method. Cox model was applied to assess the effect of TMB on survival outcomes. Hazard ratio (HR) and 95% confidence interval (CI) for OS and PFS in TMB subgroups were estimated.

**Results:** Of 512 pts enrolled, 209 had evaluable tumor samples (TIS, n=105; ICC, n=104). Using the widely used cutoff of 10 mutations per megabase (mut/Mb), numerically higher ORR and survival benefit with TIS over ICC were observed in the high TMB (TMB-H) vs the low TMB (TMB-L) subgroup (Table). The predictive effect of TMB on survival outcomes was not significant (interaction p-value = 0.0537 for PFS, 0.5374 for OS); however, the effect became significant using the increased cutoff of 12 mut/Mb (TMB-H prevalence = 16.7%; interaction p-value = 0.0267 for PFS, 0.0175 for OS).

**Conclusions:** TMB status may play a role in predicting clinical outcomes in pts with advanced ESCC treated with TIS versus ICC, especially when a higher TMB cutoff is chosen. These findings need further prospective validation.

**Table.** Clinical outcomes by TMB status (cutoff 10 mut/Mb)

| **TMB status** | **TMB-H** | | **TMB-L** | |
|---|---|---|---|---|
| **Treatment** | **TIS** | **ICC** | **TIS** | **ICC** |
| **n (% in TMB BEP, N=209)** | 27 (12.9) | 31 (14.8) | 78 (37.3) | 73 (34.9) |
| **ORR, % (95% CI)** | 33.3 (16.5, 54.0) | 6.5 (0.8, 21.4) | 16.7 (9.2, 26.8) | 17.8 (9.8, 28.5) |
| **Median PFS, months (95% CI)** | 2.4 (1.4, 5.5) | 2.3 (1.3, 2.9) | 1.4 (1.3, 2.7) | 2.7 (1.5, 3.3) |
| **PFS HR (95% CI)** | 0.52 (0.28, 0.97) | | 1.06 (0.73, 1.53) | |
| **Interaction p-value** | 0.0537 | | | |
| **Median OS, months (95% CI)** | 6.1 (4.2, 18.6) | 4.7 (3.4, 7.0) | 8.6 (4.6, 11.8) | 7.0 (4.6, 8.6) |

| | | |
|---|---|---|
| **OS HR (95% CI)** | 0.58 (0.32, 1.04) | 0.72 (0.50, 1.03) |
| **Interaction p-value** | 0.5374 | |
| **TMB-adjusted OS HR (95% CI)** | 0.68 (0.5, 0.92) | |

BEP, biomarker evaluable population; CI, confidence interval; HR, hazard ratio; ICC, investigator chosen chemotherapy; OS, overall survival; PFS, progression-free survival; ORR, objective response rate; TIS, tislelizumab; TMB-H, high tumor mutational burden; TMB-L, low tumor mutational burden; TMB-adjusted OS HR, overall HR adjusted for the impact of TMB on OS

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**CT078**

**First-line treatment with sintilimab (sin) vs placebo in combination with chemotherapy (chemo) in patients (pts) with unresectable gastric or gastroesophageal junction (G/GEJ) cancer: Final overall survival (OS) results from the randomized, phase III ORIENT-16 trial.**

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**Background:** The phase III ORIENT-16 trial evaluated sin (a PD-1 inhibitor) versus placebo plus chemo as first-line (1L) treatment in pts with advanced G/GEJ adenocarcinoma. Sin+chemo previously showed a significant improvement in OS vs chemo in pts with PD-L1 combined positive score (CPS) ≥5 (HR 0.660; 95% CI 0.505-0.864; P=0.0023) and in all pts (HR 0.766; 95% CI 0.626-0.936; P=0.0090), with a median follow-up of 18.8 months (m) at interim analysis (Xu, et al. Ann Oncol 2021). Here we report the results from the final analysis (NCT03745170).

**Methods:** This double-blind, phase III trial enrolled 650 pts ≥18 years of age with untreated, unresectable, locally advanced, or metastatic G/GEJ adenocarcinoma, regardless of PD-L1 expression. Pts were randomized 1:1 to receive sin (3 mg/kg in pts weighing <60 kg or 200 mg in pts weighing ≥60 kg, IV Q3W) or placebo plus chemo (oxaliplatin 130 mg/m$^2$ IV Q3W for up to 6 cycles, capecitabine 1000 mg/m$^2$ PO Bid d1-14 Q3W) for 24 months. The primary endpoints were OS in pts with CPS ≥5 and all randomized pts. The data cutoff date for the final analysis was Sep 2, 2022.

**Results:** With a median follow-up of 33.9 m, sin+chemo continued to show OS benefit over chemo in all pts (15.2 vs 12.3 m; HR 0.681 [95% CI: 0.571, 0.812]; P<0.0001); estimated OS rates at 24 and 36 m for sin+chemo vs chemo were 37.6% vs 20.6% and 26.0% vs 10.7%, respectively. OS benefits with sin+chemo vs chemo across subgroups were generally consistent with the previous report. The updated PFS was superior with sin+chemo vs chemo (HR 0.638, 95% CI 0.530-0.768; P<0.0001). The updated

confirmed ORR per RECIST v1.1 was 58.2% vs 48.8% in all pts with measurable disease at baseline, with a median DoR of 9.9 vs 7.0 m, respectively; 47.8% of responders in sin+chemo group and 25.9% of responders in chemo group had DoR ≥12 m. In pts with CPS ≥5, significant OS benefit (median OS 19.2 vs 12.9 m; HR 0.587 [95% CI: 0.467, 0.738]; P<0.0001) and superior PFS (HR 0.621, 95% CI 0.490-0.787; P<0.0001) with sin+chemo over chemo remained. A delayed numerical OS improvement was observed for subgroup pts with CPS <5. No new or unexpected safety signals were identified.
**Conclusion:** The previously reported significant OS benefit with sin+chemo vs chemo was more evident in CPS ≥5 pts and in all pts with 15 m of additional follow-up, further confirming sin+chemo as a standard of care for 1L treatment of G/GEJ adenocarcinoma.

**CT079**

**Efficacy and safety of sintilimab (anti-PD-1 mAb) for advanced cervical cancer: Results from a Phase II trial.**

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**Background:** Patients (pts) with advanced cervical cancer who progressed on first-line treatment have no standard therapy and derive limited benefit from currently available treatment. More effective therapeutic strategies are required. This phase II trial was conducted to evaluate the efficacy and safety of IBI310 (anti-CTLA-4 mAb) plus sintilimab (sint) versus sint in pts with recurrent/metastatic cervical cancer. Here we present the efficacy and safety results for pts in sint plus placebo group.
**Methods:** Pts aged 18-75 years, with histologically or cytologically confirmed cervical cancer who had progressed on or been intolerant to first-line or above platinum-based chemotherapy were enrolled. Pts in sint plus placebo group received sint (200mg) plus placebo IV Q3W for 4 cycles followed by sint monotherapy till disease progression, intolerable toxicity, withdrawal of informed consent, death, or for up to 24 months. The primary endpoint was objective response rate (ORR) assessed by IRRC per RECIST V1.1. The data cutoff date was April 20, 2022.
**Results:** Overall, 101 pts were enrolled and received at least one dose of assigned treatment (median age of 53.0 years, 71.3% pts with PD-L1 CPS ≥1, 91.0% pts with squamous-cell carcinoma, and 36.6% pts with ≥2 lines of prior systemic therapy). The median treatment exposure was 18.0 weeks. The IRRC-assessed confirmed objective response rate (ORR) was 24.5% (95%CI: 16.4%-34.2%), disease control rate was 56.1% (95%CI: 45.7%-66.1%), and median duration of response was not reached. Pts with PD-L1 CPS ≥1 showed numerically higher ORR versus those with CPS <1 (32.9% vs 17.2%). With a median follow-up of 8.3 months, median PFS was 2.7 months (95%CI: 1.5-4.3). Median overall survival (OS) was not reached; OS rate was 89.6% (95%CI: 80.9%-94.5%) at 6 months and 65.5% (95%CI: 50.9%-76.7%) at 12 months. Treatment-related adverse events (TRAEs) occurred in 75.2% pts, with the most

common being anaemia (13.9%), hypothyroidism (12.9%), white blood cell count decreased (12.9%), and hyperthyroidism (10.9%). 18.8% pts experienced CTCAE Grade 3 or higher TRAEs (no TRAE leading to death occurred). TRAEs leading to drug discontinuation occurred in 1 pt (myocarditis, grade 2).
**Conclusion:** This study demonstrated favorable antitumor activity and acceptable safety with sintilimab alone over available therapies in ≥2 line advanced cervical cancer.
**ClinicalTrials.gov identifier:** NCT04590599

**CT080**

**Tremelimumab (T) + durvalumab (D) + chemotherapy (CT) in 1L metastatic NSCLC: Outcomes by blood tumor mutational burden (bTMB) in POSEIDON.**

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**Background:** In the Phase 3 POSEIDON study (NCT03164616), 1L T plus D and platinum-based CT demonstrated statistically significant improvements in both PFS (HR, 0.72; 95% CI, 0.60-0.86; p=0.0003; data cutoff [DCO] July 24, 2019) and OS (HR, 0.77; 95% CI, 0.65-0.92; p=0.0030; DCO March 12, 2021) vs CT in patients (pts) with *EGFR*/*ALK* wild-type metastatic (m) NSCLC. On the basis of these results, T+D+CT was approved by the FDA in November 2022 for use in this setting. Here we report outcomes in POSEIDON pt subgroups defined by a range of bTMB values, including a pre-specified cutoff of 20 mutations/megabase (mut/Mb).
**Methods:** Pts were randomized 1:1:1 to 1L T+D+CT (platinum-based), D+CT or CT, with stratification by tumor cell (TC) PD-L1 expression (TC ≥50% vs <50%; VENTANA PD-L1 [SP263] assay), disease stage (IVA vs IVB) and histology (squamous vs non-squamous). bTMB was assessed using the GuardantOMNI platform. OS, PFS and ORR with T+D+CT vs CT were determined for pts with bTMB ≥ vs <20 mut/Mb. Outcomes across additional bTMB cutoffs (10, 12 and 16 mut/Mb) were also explored.
**Results:** Plasma samples were available for 958/1013 pts from the intention-to-treat (ITT) population. Of those with available plasma samples, 81.8% (784/958) were evaluable for bTMB, including 277 in the T+D+CT arm and 241 in the CT arm. Similar demographic characteristics and OS were observed in the bTMB evaluable vs ITT populations. Consistent with previous reports, the proportion of never smokers was higher in the bTMB <20 mut/Mb subgroup than in the bTMB ≥20 mut/Mb subgroup. Across all bTMB cutoffs analyzed, OS and PFS benefit for T+D+CT vs CT were generally consistent with the ITT population in both bTMB high and bTMB low subgroups. However, at each bTMB cutoff, OS and PFS benefit appeared more prominent among pts in the bTMB high subgroups. Median OS (mOS) was longer with T+D+CT vs CT in both the bTMB ≥20 mut/Mb and <20 mut/Mb subgroups and HRs suggested more pronounced benefit in the bTMB high group. mOS was 13.5 months with T+D+CT vs 10.3 months with CT (unstratified HR, 0.61; 95% CI, 0.42-0.88) for pts with bTMB ≥20 mut/Mb and 12.6 months vs 10.9 months (unstratified HR, 0.79; 95% CI, 0.63-0.99) for pts with bTMB <20 mut/Mb. PFS and ORR showed similar trends to OS (data will be presented). In both subgroups with PD-L1 TC ≥1% or <1%, HRs suggested more pronounced OS/PFS benefit in pts with bTMB ≥20 (vs <20) mut/Mb (data will be presented).
**Conclusions:** In pts with mNSCLC, treatment with a limited course of T plus D (until progression) and four cycles of CT consistently improved clinical outcomes vs CT alone in both bTMB high and low subgroups, supporting the use of this regimen as a 1L treatment option for pts with mNSCLC. The clinical benefit vs CT appeared to be greater in pts with higher bTMB over a range of cutoffs, consistent with expectations based on mechanistic biology and previous clinical data.

**CT081**

**Neoadjuvant nivolumab (NIVO) + chemotherapy (chemo) vs chemo in Chinese patients (pts) with resectable NSCLC in CheckMate 816.**

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**Background:** In the global, randomized phase 3 CheckMate 816 study (NCT02998528), neoadjuvant NIVO + chemo demonstrated statistically significant and clinically meaningful improvements in event-free survival (EFS) and pathologic complete response (pCR) vs chemo in pts with resectable NSCLC. Here, we report results from the Chinese subgroup of this study.

**Methods:** Adults with stage IB (≥ 4 cm)-IIIA (per AJCC 7th edition) resectable NSCLC, ECOG PS ≤ 1, and no known *EGFR*/*ALK* mutations were randomized to NIVO 360 mg + chemo Q3W or chemo Q3W for 3 cycles followed by surgery. EFS and pCR (per blinded independent review) were primary endpoints; major pathologic response (MPR) and time to death or distant metastasis (TTDM) were secondary endpoints. Presented results are from an updated analysis of EFS (DBL, Oct 14, 2022), and the final analysis of pCR (DBL, Sep 16, 2020).

**Results:** The Chinese subgroup comprised 97 pts (NIVO + chemo, 44; chemo, 53). Baseline characteristics were generally balanced between treatment (tx) arms, except for a higher proportion of pts with stage IIIA NSCLC in the chemo arm. At 38.2 mo's minimum follow-up, NIVO + chemo improved EFS vs chemo (HR, 0.47; 95% CI, 0.25-0.88). The pCR rate (95% CI) was higher with NIVO + chemo (25.0% [13.2-40.3]) vs chemo (1.9% [0.0-10.1]); MPR and TTDM also favored NIVO + chemo (**table**). Overall, 36 (81.8%) and 41 (77.4%) pts in the NIVO + chemo and chemo arms, respectively, had definitive surgery. Grade 3-4 tx-related and surgery-related adverse events occurred in 18 (41.9%) and 5 (13.9%) pts in the NIVO + chemo arm, respectively, vs 22 (41.5%) and 9 (22.0%) in the chemo arm.

**Conclusions:** Consistent with results in the global population of CheckMate 816, neoadjuvant NIVO + chemo improved EFS and pCR vs chemo in Chinese pts. The addition of NIVO to neoadjuvant chemo maintained tx tolerability and did not impact the feasibility of surgery. These results support neoadjuvant NIVO + chemo as a tx option for Chinese pts with resectable NSCLC.

| | **NIVO + chemo (n = 44)** | **Chemo (n = 53)** |
|---|---|---|
| **Median EFS (95% CI), mo** | NR (23.4-NR) | 13.9 (8.3-34.3) |
| HR (95% CI) | 0.47 (0.25-0.88) | |
| **3-year EFS rate (95% CI), %** | 58.7 (40.6-73.0) | 35.4 (21.8-49.2) |
| **pCR rate (95% CI), %** | 25.0 (13.2-40.3) | 1.9 (0.0-10.1) |
| Difference[a] (95% CI), % | 20.9 (7.7-34.1) | |

| | | |
|---|---|---|
| Odds ratio (95% CI) | 11.05 (1.41-86.49) | |
| **MPR rate (95% CI), %** | 34.1 (20.5-49.9) | 7.5 (2.1-18.2) |
| Difference[a] (95% CI), % | 28.1 (11.9-44.3) | |
| Odds ratio (95% CI) | 7.42 (1.92-28.65) | |
| **Median TTDM (95% CI), mo** | NR (NR-NR) | 28.8 (14.8-46.8) |
| HR (95% CI) | 0.27 (0.12-0.60) | |
| [a]Calculated using the stratified Cochran-Mantel-Haenszel method.<br>NR, not reached. | | |

**CT082**

**Next-generation sequencing (NGS) and cytokine assessment from a phase III study of copanlisib in combination with rituximab in patients with indolent non-Hodgkin lymphoma (iNHL) - associations with survival endpoints.**

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Introduction: The PI3K inhibitor copanlisib (C) plus the anti-CD20 antibody rituximab (R) was superior to R plus placebo (P) in patients (pts) with relapsed iNHL (Matasar et al. *Lancet Oncol* 2021). We previously reported that C+R improved median progression-free survival (PFS) in the iNHL, follicular lymphoma (FL), and non-FL groups, with a statistically significant improvement in the PTEN-positive population (Shalini et al., *AACR* 2022). Here we conducted NGS analysis from pts treated with either C+R or P+R for possible impact on PFS outcomes. We also determined if there was an association between plasma cytokine levels at baseline with survival.
Methods: Adult pts with relapsed B-cell iNHL were randomly assigned 2:1 to either C+R or P+R; standard dosing on a 28-day cycle applied. Either fresh or archival tumor tissues were collected for central pathology review and biomarker analysis. NGS was conducted using the TSO500 platform for 476 genes. Analyses included *EZH2* and *BCL2* mutation status in FL subset, and other genes for FL subsets and overall iNHL, as prognostic and predictive markers. The effect of mutations on PFS and overall survival (OS) outcomes was assessed. Cytokines from baseline plasma were assessed using the multiplex MSD platform (71-plex). Association between cytokine levels and PFS and OS were explored.
Results: Baseline tumor samples were evaluable by NGS from 200 pts; 127 C+R and 73 P+R. Variant alleles with frequencies >10% were analyzed. One quarter (25.7%) of FL pts were determined to have *EZH2* mutations. C+R treatment significantly improved PFS for FL pts with in mutant *EZH2* (p=0.0066) compared to P+R. FL pts with mutant *BCL2* had a statistically improved PFS compared to WT *BCL2* when receiving C+R treatment. Plasma samples were available for 71-plex assessment in 373 pts; 246 C+R and 127 P+R. Pts were assigned as high or low baseline cytokine groups based on the median values. For majority of cytokines, the low cytokine group correlated with better PFS for all pts, FL and Other iNHL pts in the C+R arm. For OS, there were too few events at the time of primary data cutoff to make any associations. However, with longer follow up (2 years from primary

completion) there was a significant improvement in OS in iNHL pts with low IL2 values at baseline receiving C+R compared to pts receiving P+R; HR 0.495 (95%CI: 0.270,0.908), p=0.0230. This association was only seen for pts with low (or undetected) levels of IL2 at baseline.
Conclusions: Mutation status was associated with improvements in PFS in FL pts with mutant *BCL2* or mutant *EZH2* treated with C+R. iNHL pts with low plasma levels of IL2 levels treated with C+R showed a significant improvement in OS.

**CT083**

**Tumor-agnostic efficacy and safety of dabrafenib plus trametinib in BRAFV600E-mutated rare cancers: ROAR basket study.**

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Beyond melanoma, BRAFV600E alterations are prevalent across multiple tumors. We conducted a basket trial (NCT02034110) to assess efficacy and safety of oral BRAF inhibitor dabrafenib (150 mg BD) plus MEK inhibitor trametinib (2 mg OD) in 206 patients (eight cohorts) with BRAFV600E-mutated advanced rare cancers. Patients with anaplastic carcinoma thyroid, biliary tract cancer, gastrointestinal stromal tumor, adenocarcinoma of small intestine, low-grade (eight histologies)/high-grade (seven histologies) glioma, hairy cell leukemia, and multiple myeloma were treated until unacceptable toxicity, disease progression, or death. Overall, median duration of exposure to dabrafenib and trametinib was 12.5 (1–82) and 12.0 (1–84) months, respectively. Primary endpoint was tumor response; secondary endpoints were duration of response, progression free and overall survival, and safety (investigator-assessed). Overall response rate was 56% (38.1%, 72.1%), 53% (37.7%, 68.8%), 0%, 67% (9.4%, 99.2%), 54% (25.1%, 80.8%), 33% (20.0%, 49.0%), 89% (77.8%, 95.9%), and 50% (18.7%, 81.3%), respectively. Median (95% confidence interval) duration of response was 14.4 (7.4, not reached), 8.9 (5.6, 13.7), not reached, 7.7 (not reached, not reached), not reached (5.5, not reached), 31.2 (7.4, 44.2), not reached, and 11.1 (5.6, not reached) months, respectively. Durable and clinically meaningful responses were observed in solid and hematological malignancies (21 histologies). Safety profile was acceptable; most frequent (≥20% patients) treatment-related adverse events were pyrexia (40.8%), fatigue (25.7%), chills (25.7%), nausea (23.8%), and rash (20.4%). The encouraging tumor-agnostic activity of dabrafenib plus trametinib is a promising approach in patients with BRAFV600E-mutated advanced rare cancers. These results supported the accelerated FDA approval for a tumor-agnostic BRAF+MEK inhibitor combination representing a precision medicine milestone.

| **Median progression free and overall survival in BRAFV600E-mutated advanced rare cancer patients** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **ATC(N=36)** | **BTC(N=43)** | **LGG(N=13)** | **HGG(N=45)** | **ASI (N=3)** | **HCL(N=55)** | **MM(N=10)** |
| **PFS** | 6.7 (4.7, 13.8) | 9.0 (5.5, 9.4) | NE | 5.5 (1.8, 13.7) | 9.5 | NE | 6.3 (2.3, 12.9) |
| **OS** | 14.5 (6.8, 23.2) | 13.5 (10.4, 17.6) | 17.6 (9.5, 32.2) | NE | 21.8 (3.4, NR) | NE | 33.9 (2.9, 44.6) |
| Patient in the GIST cohort (n=1) did not attain a complete or partial response. ASI, adenocarcinoma of small intestine; ATC, anaplastic thyroid cancer; BTC, biliary tract cancer; GIST, gastrointestinal stromal tumor; HCL, hairy cell leukemia; HGG, high grade (WHO G3/G4) glioma; LGG, low (WHO G1/G2) grade glioma; MM, multiple myeloma; NE, not evaluable; NR, not reached; OS, overall survival; PFS, progression free survival; WHO, World Health Organization. | | | | | | | |

**CT084**

**Targeted intra-arterial gemcitabine vs. continuation of IV gemcitabine plus nab-paclitaxel following Induction with sequential IV gemcitabine plus nab-paclitaxel and radiotherapy for unresectable locally advanced pancreatic cancer (TIGeR-PaC) - phase III trial interim analysis.**

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Background: Treatment of locally advanced pancreatic cancer (LAPC) remains a clinical challenge with a median survival of 16-18 months[1]. Control of local disease, beyond systemic therapy, is part of the treatment paradigm being investigated in this patient population. Double balloon mediated local delivery of intra-arterial gemcitabine (IAG) into the tumor has been demonstrated to be safe in this patient population[2]. TIGeR-PaC is an ongoing Phase III clinical trial comparing the efficacy of this approach compared to standard of care IV gemcitabine/ nab-paclitaxel (GN) for patients with LAPC.
Methods: The trial is designed with an induction phase for all patients of upfront systemic chemotherapy and radiation prior to randomization to IAG or continuation of GN. ECOG 0-1 LAPC patients receive 3 cycles of GN and 1 cycle of radiation (SBRT, 33 Gy in 5 fractions). Following induction, patients with non-progressive disease are randomized to receive IAG (8 treatments bi-weekly for 16 weeks) or continuation of GN for 4 cycles over 16 weeks. After the 16 weeks of randomized therapy, patients with non-progressive disease receive continuation systemic therapy of GN or low-dose oral capecitabine, per investigator's preference until disease progression and are followed for survival only. The primary endpoint is the overall survival (OS), and the study is powered at an 80% power to detect a hazard ratio of 0.6 between the two arms. It is assumed that the hazard functions are proportional only during the randomized treatment period, after which the two hazard functions become approximately identical during the continuing treatment period. The primary endpoint of OS for the two treatment arms will be

compared using a 2-sided Wilcoxon test. The first interim analysis of the trial for the ITT population is performed after the 26th out of an expected 86 events have occurred in the trial (30%).
Clinical Endpoint Analysis: The first interim analysis of the primary endpoint of OS was conducted on Feb 2nd, 2023. This was based on a data lock on survival status of all patients after the sponsor became aware that the 26th event in the trial occurred on Dec. 21st, 2022. As of that date, 45 patients had been randomized in the trial and the survival status of all 45 subjects was used as of the date for analysis. Of the 45 patients randomized, 23 were randomized to IAG and 22 to continuation of IV GN. There were equal number of primary events, 13, in each arm. The median survival in the control arm is 10 months, versus 16 months in the IAG arm. The p-values based on Wilcoxson non-proportional hazard ratio is 0.051 between the 2 arms. The study continues to accrue patients with a second interim analysis (after 52 events) expected at end of 2024.
1. Hammel P, Huguet F, van Laethem JL, et al. Effect of Chemoradiotherapy vs Chemotherapy on Survival in Patients With Locally Advanced Pancreatic Cancer Controlled After 4 Months of Gemcitabine With or Without Erlotinib. *JAMA*. 2016;315(17):1844.
2. Rosemurgy AS, Ross SB, Vitulli PL, et al. Safety Study of Targeted and Localized Intra-Arterial Delivery of Gemcitabine in Patients with Locally Advanced Pancreatic Adenocarcinoma. *J Pancreat Cancer*. 2017;3(1):58-65.

**CT085**

**A phase 2/3, multicenter trial of lenzilumab and azacitidine in chronic myelomonocytic leukemia: The PREACH-M trial.**

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Background: Chronic myelomonocytic leukemia (CMML) is a rare, aggressive cancer for which no targeted therapy exists. Standard of care (SOC) includes azacitidine (A), with complete and partial response (CR and PR) rates ranging between 10-17%. The pro-inflammatory cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) plays a central role in stimulating leukemic monocyte proliferation. Lenzilumab (LENZ) is a proprietary Humaneered® first-in-class monoclonal antibody with best-in-class specificity and affinity that neutralizes GM-CSF to prevent signaling through its receptor. The PREcision Approach to CHronic Myelomonocytic Leukemia (PREACH-M) trial assesses the efficacy of LENZ in CMML (ACTRN12621000223831p) to improve outcomes beyond those afforded by SOC.
Methods: PREACH-M is a Phase 2/3 non-randomized, open-label precision medicine trial in 72 adults aged at least 18 years, newly diagnosed with WHO 2016 criteria for CMML; cytopenia (hemoglobin < 100 g/L, platelets < 100 x $10^9$/L or absolute neutrophil count < 1.8 x $10^9$/L): white blood cell count ≥ 13 x$10^9$/L; as well as *TET2* and/or RAS pathway mutations (*NRAS*, *KRAS*, *CBL*). Key exclusion criteria include prior treatment with investigational agents; radiotherapy within 28 days before treatment; treatment with G-CSF within 7 days of screening; GM-CSF within 28 days of screening; and uncontrolled medical conditions. Subjects exhibiting RAS pathway mutations, with or without *TET2* mutations, receive 24 cycles (28 days) of A (SC; 75 mg/$m^2$ for 7 days) and LENZ (IV; 552 mg; d1 & d15 of cycle 1 and d1 only for all subsequent cycles); while those with non-RAS pathway mutations receive the same A regimen and sodium ascorbate (IV; 30 g for 7 days [15 g for 1st dose only, 30 g thereafter if no evidence of tumor lysis syndrome]; PO; 1.1g on all other days). Subjects who complete 24 cycles of treatment are followed every 6 months for 24 months for survival, disease status, and CMML-related therapy. The primary endpoint is the frequency of CR or PR at any time during the first 12 cycles according to Savona Criteria. Secondary endpoints include overall survival and progression-free survival at 2 years; proportion of subjects with clinical benefit at any point during the 24 cycles; impact on physical and functional

capacity; social well-being according to Multidimensional Geriatric Assessment and quality of life; as well as hematological and non-hematologic safety.
Results: As of December 31, 2022, eight subjects were treated with A and LENZ (5 females, mean age of 67 years; 3 males, mean age of 69 years); among them 6 were evaluable based on at least 3 months of follow-up. CR or objective responses were observed in all evaluable patients including 2 with high risk based on molecular profiling. 10 grade 3/4 Serious Adverse Events were observed of which 2 were assessed by the investigator as possibly related to LENZ.
Conclusion: The ongoing PREACH-M trial evaluates GM-CSF neutralization with LENZ in addition to SOC, in the treatment of CMML with RAS pathway mutations.

**CT086**

**H3.3-K27M neoantigen vaccine elicits CD4$^+$ and CD8$^+$ T cells immunity and improved prognosis against diffuse intrinsic pontine glioma.**

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**Background:** Diffuse intrinsic pontine glioma (DIPG) harboring H3.3-K27M mutation is a malignant pediatric brain tumor with a >90% mortality rate within two years of diagnosis. Aiming to improve therapeutic outcomes, we herein describe a neoantigen peptide vaccine against H3.3-K27M which effectively triggers both CD8$^+$ and CD4$^+$ T cell responses.
**Methods:** A neoantigen vaccine was designed to trigger T cell immunity against DIPGs harboring the H3.3-K27M mutation. ENACTING (NCT04749641) was then initiated as an open-label, single center, two-armed phase 1 trial to assess T cell immune responses and vaccine safety. The vaccine was administered intramuscularly with poly-ICLC adjuvant until tumor progression or untolerated toxicity. PBMCs before and after each vaccine treatment were collected for TCR repertoire analysis and immune response assessment.
**Results:** As of November 2022, 10 patients have been treated. No grade 3-4 treatment-related adverse events have been observed, with fever (80%) and injection site pain (60%) being the most common AEs. On a per patient basis, vaccines induce a landscape change of TCR repertoire in patients' PBMC after 4-6 times of dosing, indicating multiple dosing is required to trigger extensive T cell responses. T cell responses against neoantigens were detected and H3.3-K27M mutation-specific CD4$^+$ and two CD8$^+$ clones were validated. Among 9 efficacy-assessable patients, the one-year overall survival rate was 71.4%. The mPFS has reached 11.7 months and increasing. One patient reached complete response. As this trial remains ongoing, subgroup analysis will be reported in the future.
**Conclusion:** The H3.3-K27M neoantigen vaccine was well tolerated and elicited mutation-specific CD4$^+$ and CD8$^+$ T cell responses in patients. Initial results from this ongoing study suggest that, compared with other current immunotherapies against DIPG, H3.3-K27M peptide vaccination may provide superior patient outcomes, for both life qualities and survival outcomes.

**Table 1. Clinical efficacy and adverse events**

| **Efficacy** | |
|---|---|
| Complete response (CR) | 1 (11.1%) |
| Partial response (PR) | 0 (0) |
| Stable disease (SD) | 8 (88.9%) |
| Progressive disease (PD) | 0 (0) |

| | | | |
|---|---|---|---|
| Disease control rate (DCR) | | 100% | |
| 12-month overall survival | | 71.4% | |
| Median progression-free survival (mPFS) | | 11.7 months (95% CI, 7.0-NR) | |
| Median overall survival (mOS) | | 15.7 months (95% CI, 10.0-NR) | |
| **Treatment-Related Adverse Events** | | | |
| | All grades | Grade 3 | Grade 4 |
| Fever | 9 (90.0%) | 0 | 0 |
| Injection site pain | 6 (60.0%) | 0 | 0 |
| Bloating | 1 (10.0%) | 0 | 0 |
| Abdominal pain | 1 (10.0%) | 0 | 0 |
| Vomiting | 1 (10.0%) | 0 | 0 |
| Increased blood LDH | 1 (10.0%) | 0 | 0 |
| Proteinuria | 1 (10.0%) | 0 | 0 |
| Hypocalcemia | 1 (10.0%) | 0 | 0 |

**CT087**

**Phase I/II study of the WEE1 inhibitor adavosertib in combination with carboplatin in children with advanced malignancies: arm C of the AcSé-ESMART trial.**

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Background: AcSé-ESMART is a proof-of-concept, phase I/II platform trial designed to explore targeted agents in a molecularly enriched pediatric population. WEE1 plays a role in DNA repair and cell cycle control and is overexpressed in pediatric cancers. Adavosertib combinations resulted in enhanced

antitumor activity compared to single agent in neuroblastoma, rhabdomyosarcoma, medulloblastoma and high-grade glioma *in vivo* models. The efficacy and safety of the adavosertib-carboplatin combination has been established in adults with focus on *TP53* mutated ovarian cancer. Arm C of AcSé-ESMART applied this regimen to children with advanced malignancies enriched for alterations in *TP53*, DNA repair/replication stress and cell cycle control.
Methods: Adavosertib was administered orally, twice daily on Days 1 to 3 and carboplatin intravenously on Day 1 of a 21-day cycle. Dose finding used the continuous reassessment method starting at adavosertib 100 mg/m$^2$/dose and carboplatin AUC 5. Pharmacokinetic (PK) and retrospective molecular bioinformatic analysis was performed.
Results: Twenty patients (median age: 14.0 years, range 3.4-23.5) were included, 18 received a total of 69 cycles. Seven dose-limiting toxicities (DLTs) were observed leading to two de-escalations to adavosertib 75 mg/m$^2$/dose and carboplatin AUC 4. All patients with DLT had thrombocytopenia grade 3/4 requiring transfusions for >7 days and/or neutropenia grade 4 for >7 days. Main overall treatment-related toxicities were hematologic and gastrointestinal. Based on the identified DLT risk, no recommended Phase 2 dose was defined. PK analysis demonstrated equivalent adavosertib exposure in children to that in adults and both doses (75 and 100 mg/m$^2$) achieved the cell kill target. Two patients with neuroblastoma achieved partial response (PR), one with medulloblastoma unconfirmed PR, and five had stable disease (SD) >4 cycles. Patients with PR/SD >4 cycles were considered as clinical benefit (CB) for retrospective molecular analysis. There was no correlation between *TP53* genomic alteration alone and response. However, 7 of 8 patients with CB but none of the 10 patients without CB had 1 to 3 genomic alterations in the DNA repair (*BRCA2* mutation, *11q* loss containing *ATM*, *MRE11A*, *CHEK1*), cell cycle control/replication stress (*CCNE1* amplification, *RB1* mutation/loss, *SETD2* mutation/loss) and *RAS* pathway (*KRAS* mutation and amplification, *NF1* loss, *PTPN11* mutation) in their tumor.
Conclusions: Adavosertib combined with carboplatin exhibited significant hematologic toxicity. Activity signals and identified potential molecular biomarkers suggest further combination studies with less hematotoxic DNA damaging therapy in molecularly enriched pediatric cancers.

**CT088**

**Phase I/II study of the PARP inhibitor olaparib in combination with irinotecan in children with advanced malignancies: arm D of the AcSé-ESMART trial.**

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Background: AcSé-ESMART is a proof-of-concept, phase I/II, platform trial, designed to explore targeted agents in a molecularly enriched relapsed/refractory pediatric population. Arm D was evaluating the PARP inhibitor (PARPi) olaparib (ola) in combination with irinotecan (iri). In contrast to other PARPi/chemotherapy combination studies, we opted for a prolonged course of PARPi and low dose irinotecan as sensitizer. The Phase I part previously established the recommended Phase II dose (RP2D) (Gatz ASCO 2019). This is the report of the Phase II part of the trial assessing the activity in two separate expansion cohorts: cohort 1: homologous recombination repair defect (HRD) and cohort 2: Ewing sarcoma (ES).
Methods: Ola was administered orally twice daily at 90 mg/m$^2$ on Days 1 to 10 and iri intravenously at 20 mg/m$^2$ on Days 4 to 8 of a 21-day cycle. Activity assessment followed a Minimax Simon 2-stage design. Each cohort was to progress to the second stage (additional 9 patients) if 2 or more confirmed responses were observed in the first 16 patients. Patients treated in the Phase I part at the RP2D were counting towards the respective expansion cohorts.
Results: Seventy patients (median age: 14 years, range 5-23) were included in the whole study, 67 received treatment; 27 patients were treated in the dose escalation part, including 10 at the RP2D (8 in cohort 1 and 2 in cohort 2). Both cohorts passed the 1$^{st}$ stage and a total of 24 and 26 patients were recruited to cohort 1 and 2, respectively. Main diagnoses in cohort 1 were sarcoma (n=10), brain tumor (n=9), neuroblastoma (n=4). In cohort 1, 15 of 24 patients were considered enriched based on molecular alteration at relapse (*ATM* n=6; *BRCA1* n=2; DNA signature 3 n=3; *FANCD2*, *CHEK2*, *FANCA, ATRX* all n=1); all patients in cohort 2 had presence of a ES fusion (ESWR1::FLI1 n=22; EWSR1::ERG n=4). Median number of treatment cycles were 2, range 1;51 in cohort 1 and 1;32+ in cohort 2. In cohort 1, 3 patients had a partial response (PR) (pinealoblastoma, neuroblastoma, choroid plexus carcinoma; treated with 12, 51, 25 cycles), 1 patient an unconfirmed PR (rhabdomyosarcoma, 6 cycles) and 7 patients stable disease (SD) (2 prolonged with 6 and 8 cycles). In cohort 2, 1 patient had a complete response (10 cycles) and 1 a PR (32+ cycles), 7 patients had SD (3 prolonged with 6, 10, 16 cycles). Molecular enrichment did not predict response. Retrospective correlative analysis of the molecular profiling data and tumor tissue expression analysis are ongoing to identify predictive biomarkers for PARPi combination trials and data will be presented.
Conclusions: Encouraging clinical benefit was observed with the protracted ola-iri schedule in a subset of patients. Current molecular hypothesis is insufficient for patient selection and better biomarkers are needed.

**CT089**

**Molecular-guided therapy for the treatment of patients with relapsed and refractory childhood cancers: a Beat Childhood Cancer Research Consortium trial.**

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Background: Children with relapsed CNS tumors, neuroblastoma, sarcomas, and other rare solid tumors face poor outcomes. Here we describe a study to determine the feasibility of leveraging genomic profiling results within a molecular tumor board (MTB) to make real-time treatment decisions for children with relapsed/refractory solid tumors.
Methods: Subjects were divided to 3 strata: Strata 1: Relapsed/refractory neuroblastoma, Strata 2: Relapsed/refractory CNS tumors, and Strata 3: Relapsed/refractory rare solid tumors. Samples were sent for tumor/normal whole exome (WES) and tumor whole transcriptome sequencing, and the genomic data were used in a MTB to make real-time treatment decisions. The MTB recommended plan allowed for a combination of up to 4 agents. Feasibility was measured by time to completion of genomic sequencing, MTB review and initiation of treatment. Response was assessed after every 2 cycles using Response Evaluation Criteria in Solid Tumors (RECIST). Patient benefit was calculated by the sum of the CR, PR, SD, and NED subjects divided by the sum of CR, PR, SD, NED, and PD subjects. Grade 3 and higher related and unexpected adverse events (AEs) were tabulated for safety evaluation.
Results: 144 eligible subjects were enrolled with 144 evaluable for safety and 124 evaluable for response. Tumor types included neuroblastoma (n=31), CNS tumors (n=41), and rare tumors (n=72). Sarcomas (n=40) were the most common tumor type in the rare tumor stratum. The average time from biopsy to completion of DNA/RNA sequencing was 10 days (range 2-31 days); to completed analysis and drug prediction report, 17 days (8-41); 23 days (10-66) to MTB decisions; and 38 days (18-146) to initiation of the 4-drug combination agreed upon by the MTB. Treatments were selected on DNA and RNA findings in 19.5% of cases and in 80.5% on RNA alone. Patient benefit was exhibited in 70% of all subjects, 85% of neuroblastoma subjects, 73% of CNS tumor subjects, and 62% of rare tumor subjects. AEs occurred in <1% of the subject cohort. Grade 3 leukopenia was the only unexpected hematologic AE. There were 7 occurrences of unexpected non-hematologic AEs: grade 4 elevated ALT (1), grade 3 elevated AST (1), grade 3 dehydration (1), grade 3 infection (2), grade 3 oral mucositis (1), and grade 3 pancreatitis (1).
Conclusions: It has been well-established that comprehensive genomic sequencing is the future of precision medicine. Here, we have demonstrated the feasibility, efficacy, and safety of a comprehensive sequencing model to guide targeted therapy for patients with any relapsed/refractory solid malignancies. Targeted therapy was well tolerated, and the response benefit rate of 70% in these heavily pretreated populations suggests that this treatment could be an effective option for relapsed and refractory pediatric cancers.

**CT090**

**PEPN2011: a phase 1/2 study of tegavivint in children, adolescents, and young adults with recurrent or refractory solid tumors, including lymphomas and desmoid tumors: a report from the pediatric early phase clinical trials network.**

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Introduction: Tegavivint is a first in class small molecule inhibitor of Wnt-ß-catenin signaling that functions by disrupting the interaction of ß-catenin and TBL1/TBLR1 resulting in degradation of nuclear ß-catenin. Aberrant Wnt signaling has been identified as a key mechanism of cancer biology, resulting in uncontrolled transcription of pro-oncogenic Wnt target genes. Pre-clinical in vivo studies of tegavivint demonstrated anti-tumor activity in a variety of pediatric solid tumors, including osteosarcoma, Ewing sarcoma, and lymphoma.
Methods: We conducted a phase 1 dose-escalation study of single agent tegavivint in children aged ≥12 months to ≤21 years with relapsed or refractory solid tumors including lymphoma and desmoid tumors. The dose escalation was conducted using a rolling six design starting with the recommended phase 2 dose (RP2D) in adults, 5 mg/kg, administered intravenously over four hours on days 1, 8 and 15 of a 28 day cycle. A single de-escalation to 4 mg/kg and escalations to 6.5 mg/kg and 8 mg/kg if pharmacokinetic (PK) parameters indicated were planned. Once the RP2D is defined a PK cohort and phase 2 cohorts including Ewing sarcoma, desmoid tumor, osteosarcoma, liver tumors, Wilms tumor and a disease agnostic cohort including cancers with Wnt alterations will be evaluated.
Results: A total of 15 patients were enrolled on the phase 1 part of the study. Two patients were not treated due to pre-therapy DEXA scan demonstrating grade 1 osteoporosis; therefore, 13 patients received treatment with tegavivint on 2 dose levels (5 mg/kg and 6.5 mg/kg). The median age of treated patients was 16 years (range 3-21). Patient diagnoses included desmoid tumor (5), Wilms tumor (3), and one each of Ewing sarcoma, osteosarcoma, *CIC::DUX4* sarcoma, neuroblastoma, and hepatocellular carcinoma. There were no dose limiting toxicities among 4 DLT-evaluable patients at DL1 and 6 at DL2. The maximum tolerated dose was not determined. Grade three or higher adverse events at least possibly related to treatment included anemia (2) and lymphopenia (2). A single patient had a grade 3 QT prolongation requiring cessation of tegavivint after cycle 3. At 5 mg/kg, the mean AUC was 94,900 hr*ng/mL (range 52,000-136,000), and at 6.5 mg/kg was 91,200 hr*ng/mL (range 28,400-144,000). The pediatric RP2D dose was determined to be 6.5 mg/kg based on the pre-specified objective of achieving pharmacologically relevant plasma concentrations and less than 20% difference in mean AUC between the two tested dose levels.
Conclusions: Tegavivint is well tolerated in children with refractory solid tumors, the pediatric RP2D is 6.5 mg/kg demonstrating pharmacologically relevant plasma concentrations. The study is currently enrolling patients to a pharmacokinetic expansion cohort and will then proceed to phase 2.

**CT093**

**Preliminary evidence of antitumor activity of Ipatasertib (Ipat) and Atezolizumab (A) in glioblastoma (GBM) patients (pts) with PTEN loss in the Phase 1 Ice-CAP trial (NCT03673787).**

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Background PTEN loss of function is frequent in GBM correlating with poor prognosis, impaired antitumor responses and reduced efficacy of Immune Checkpoint Inhibitors (ICI). Ipat is a potent, selective, small-molecule inhibitor of Akt. Ipat efficiently depletes $FOXP3^+$ regulatory T cells from the tumor microenvironment (TME) resulting in increased infiltration of effector T cells in solid tumors

(Lopez 2020, AACR). We hypothesize that Akt inhibition in PTEN loss glioblastomas may deplete the TME of suppressive immune cells, and render malignant brain tumors more responsive to ICIs.
Methods Relapsed WHO grade IV GBM pts with stable neurological symptoms ≥5 days prior to enrollment, requiring <3mg Dexamethasone were recruited into a dose determination cohort (A2; n=12) and an expansion cohort (B3; n=11) of this early phase, open-label trial studying the combination of Ipat and A. Primary objectives were to determine the safety and tolerability of the combination (A2) and preliminary efficacy (B3).
Results 23 recurrent GBM pts (median age 55 yrs (25-71 yrs; 74% male) were enrolled. Median ECOG PS 1. All pts had surgery followed by radical-chemoradiotherapy. Median prior lines of therapy 1 (range 1-4). 15 pts had PTEN loss by IHC (H<30) indicative of biallelic loss of function, 1 had deleterious PTEN mutations detected by next generation sequencing. No DLTs, no significant treatment-related (TR) serious adverse events (SAEs), or immune-related AEs were observed. Most common TR AEs were G1-2 diarrhea (61%), rash (26%) and mucositis (22%). Clinical benefit rate (CR, PR and SD> 6 cycles) in 19 efficacy evaluable pts was 32% (6/19 overall) and 28.6% (4/14) for pts with PTEN loss. Multiplex IHC of archival samples (n=19) showed PTEN loss tumors had significantly greater numbers of CD3+ infiltrating T-lymphocytes within the TME compared to PTEN wild type tumors (median 53.64/mm2 vs 1.94/mm2; p=0.0021). Within the PTEN loss cohort (n=13), pts with clinical benefit had significantly higher baseline number of CD8+ effector T cells as compared to non-responders (median 46.50/mm2 vs 8.21/mm2; p=0.0336). Two pts with PTEN loss proceeded to re-resection on trial. One exceptional responder, a 58yr male refractory to radical chemo-radiotherapy and Bevacizumab had a resection of enhancing disease after 5 cycles that showed >70% depletion of CD4+ T regulatory cells with an increase in CD8+ lymphocyte infiltration and no residual evidence of tumor (pathological CR). In contrast, a non-responding patient who proceeded to debulking surgery had no change in infiltration of CD8+ lymphocytes, but a marked increase (>27-fold) in CD4+ regulatory T cells.
Conclusion The RP2D of 400mg Ipa OD + 1200mg A Q3W was well tolerated in GBM pts. Early efficacy signals were detected with PTEN loss being a promising predictive biomarker for response to combination.

**CT094**

**Phase I dose-escalation study evaluating the safety and tolerability of ginisortamab (UCB6114), a first-in-class anti-gremlin-1 monoclonal antibody (mAb), as monotherapy in advanced solid tumors.**

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Effective treatment for gastrointestinal (GI) cancers is a significant unmet need. Gremlin-1, secreted by cancer-associated fibroblasts, downregulates bone morphogenetic proteins (BMP), members of the transforming growth factor β superfamily, resulting in tumor stemness, hyperproliferation, and invasiveness. Ginisortamab, a fully human IgG4P mAb, neutralizes gremlin-1, thus restoring BMP signaling. Preclinical studies showed that ginisortamab binds to gremlin-1 and has antitumor activity in several mouse models, including GI cancers. Here we report the initial findings from Part A (monotherapy dose escalation) of the first-in-human study. ONC001 (NCT04393298) is an ongoing multi-part,

multicenter, nonrandomized, open-label, Phase I/II study assessing the safety, PK/PD, and antitumor activity of intravenous (IV) ginisortamab as monotherapy or in combination with selected standard of care regimens in patients (pts) with advanced solid tumors. ONC001 includes 3 dose escalation modules (Parts A-C) and a dose adaptation module (Part A1). To be eligible for Part A, pts had to be aged ≥18 years (y) with advanced disease, resistant or refractory to standard therapy, with ECOG PS ≤1. We present the primary (safety, tolerability), secondary (PK/PD), and exploratory (antitumor activity) endpoints, as well as the monotherapy RP2D from Part A. At data cutoff (June 20, 2022), 25 pts received ≥1 dose of ginisortamab (median [range] age: 64 [37-74] y; 68% male; 88% White; 36% had >3 lines of prior therapy). The most common tumor type enrolled was colorectal adenocarcinoma (44%). Ginisortamab was escalated using a modified rolling 6 design through 5 levels (100, 250, 500, 1000, and 2000 mg IV Q2W) in 28-day cycles. No DLTs, Grade ≥3 AEs related to ginisortamab, or serious related AEs were observed; the 2000 mg dose was deemed safe for further development. Temporary treatment interruption and study discontinuation due to AEs unrelated to ginisortamab occurred in 7 (28%) and 2 pts (8%), respectively. Ginisortamab PK aligned with expectations from preclinical models for an IgG4 mAb, allowing favorable dosing intervals. Confirmation of target occupancy was demonstrated by increases in total circulating gremlin-1. Antidrug antibody formation was not observed. The best overall response of stable disease was observed in 7/24 pts (29%); 4 pts had a reduction of the sum of the dimensions of their target lesions relative to baseline. Favorable safety and PK/PD data at the maximum dose of 2000 mg and preliminary antitumor activity were observed with the first-in-class anti-gremlin antibody ginisortamab. These data support further evaluation of the efficacy and safety of ginisortamab monotherapy at 2000 mg Q2W, or in combination with standard of care regimens (FOLFOX, trifluridine/tipiracil) in advanced solid tumors.

**CT095**

**Update: a phase 1/2, open-label study to assess safety, tolerability, biodistribution, radiation dosimetry and PET imaging characteristics of [$^{18}$F]FPyGal in comparison to in-vitro diagnostic for the assessment of senescence in oncological patients (NCT04536454).**

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It is well established that senescence of cancer cells can be induced by treatment with classical cytotoxic therapies but also by molecularly targeted therapies or immunotherapies, a phenomenon referred to as therapy induced senescence (TIS). Occurrence of intratumoral senescence harbors broad therapeutic implications and may limit the prognosis of cancer patients, as senescent cells, via their senescence associated secretory phenotype (SASP), can suppress anti-tumor immune responses and may increase the metastatic potential of non-senescent cancer cells. Preclinical data suggest that an eradication of senescent cells (senolytic therapies) or an application of SASP modulating therapies might improve the outcome of systemic cancer therapies, however, as a prerequisite to guide such approaches, robust and non-invasive modalities to visualize and quantify intratumoral senescence are needed. We recently reported on the safety evaluation of the novel Positron-Emission-Tomography (PET) tracer [$^{18}$F]FPyGal, a radiolabeled substrate of senescence-associated β-galactosidase in healthy volunteers (AACR 2022, #7958). In

summary, our data showed that the use of [$^{18}$F]FPyGal is safe and well-tolerated in the described human study population. In the second stage of our trial, we are now investigating the concordance of histopathological senescence markers with [$^{18}$F]FPyGal senescence signals in i) rectum cancer-, ii) non-small cell lung cancer- and iii) adenocarcinoma of the esophagogastric junction (AEG) patients, who are undergoing neoadjuvant therapy. Here, we report on patients from the rectum cancer cohort of the trial. [$^{18}$F]FPyGal PET imaging was done before and after administration of neoadjuvant radiochemotherapy (RCTx). [$^{18}$F]FPyGal PET signal was correlated with immunohistochemical senescence markers (p16, p21, p53) and quantification of SA-ß-gal (Senescence-associated beta-galactosidase) positive cells in biopsy materials (pre-therapy) and surgical resection materials (post-therapy). Expectedly, senescent cells were only detected at very low frequency in therapy naïve tumors and the lack of histological detection of senescence correlated with negativity in [$^{18}$F]FPyGal imaging. In stark contrast, homogenous senescence, quantified with the indicated senescence markers, was detected in the majority of cancer cells after RCTx. Interestingly, in one rectum carcinoma, which underwent a full remission after RCTx, senescence of stroma cells in the area of the former tumor bed was detected by PET imaging and histologically. Collectively, our data indicate the utility of [18F]FPyGal PET imaging to non-invasively detect senescence. Future trials using [18F]FPyGal to guide oncological therapy, e.g. to apply senolytic drugs in a personalized manner, are warranted.

**CT096**

**Phase 1 study of RO7119929 (TLR7 agonist prodrug) in patients (pts) with advanced primary or metastatic liver cancers.**

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**Background** Agonists for TLR7, a major regulator of the innate immune response, have been tested in oncology across several nonclinical and clinical investigations due to their ability to stimulate T-cell mediated cancer cell killing. The orally available TLR7 agonist prodrug RO7119929 is converted to active drug predominantly in the liver. It was hypothesized to effectively reprogram the local immune microenvironment and thus enhance anti-tumor activity in liver tumors.
**Methods** In this phase 1, first-in-human, open-label, dose-escalation study (NCT04338685), eligible pts had histologically confirmed advanced or metastatic primary liver cancers or solid tumors with predominant liver involvement. Pts received oral RO7119929 weekly in 3-week cycles in Part A (dose-escalation: Flat dosing [FD][A1], step-up dosing [SUD][A2] or FD with tocilizumab pre-treatment [A3]) and Part B (FD dose expansion with paired biopsies). Participants received treatment until disease progression, unacceptable toxicity or withdrawal. Primary endpoints were maximum tolerated dose (MTD) and/or optimal biologic dose and safety.
**Results** At data cut-off (22 April 2022), 55 pts were enrolled (A1: n=27; A2: n=9; A3: n=1; B1: n=18). Median age: 58 years; 76% male; 51% Asian; 52% received ≥3 prior lines of therapy; 31% HCC as primary tumor type. Most pts discontinued treatment due to disease progression (75%). Among 17 HCC pts, a durable complete response was observed in one pt and 10 (59%) pts had stable disease. Treatment-related AEs were reported in 91% of pts, most common event was CRS (87%). Fever following drug administration was generally reported as CRS, and most CRS events were Grade 1 (fever). With FD, Grade 2 CRS occurred in 20 (44%) pts and Grade 3 CRS in 6 (13%) pts. With SUD, Grade 2 CRS

occurred in one (11%) pt and no Grade 3 CRS was reported. The incidence and severity of CRS events was dose-dependent. Events generally had a predictable onset within 12h post-dosing. All events resolved, the majority within 1-2 days. CRS events of Grade 2/3 commonly occurred in Cycle 1, with later events rarely exceeding Grade 1. Increases in the TLR7-related peripheral pharmacodynamic (PD) biomarkers IFNα, ISG15 and IL6 were associated with higher doses, with particularly IL6 correlating with CRS severity. With SUD, high levels of IFNα and ISG15 levels were maintained while IL6 levels were reduced compared with FD. Pharmacokinetics (PK) of the active TLR7 agonist drug appeared linear (Cmax, AUC) and time-independent with a short effective $t_{1/2}$ of approximately 5h on average. PK, PD and safety modeling-informed dose decisions.
**Conclusion** CRS was identified as a dose-limiting safety risk, the incidence and severity of CRS appeared dose-dependent, and events were predictable and manageable. SUD reduced the risk of CRS whilst maintaining mode of action relevant PD effects. Combination therapy with a checkpoint inhibitor may be needed to leverage the pro-inflammatory potential of RO7119929 and further increase anti-tumor activity.

**CT097**

**First report of preliminary safety, efficacy, and pharmacokinetics of C-CAR031 (GPC3-specific TGFβRIIDN CAR-T) in patients with advanced HCC.**

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Introduction: Chimeric antigen receptor (CAR) T cells can mediate deep and durable responses in hematologic malignancies, however, achieving success in solid tumors has been so far limited largely by lack of suitable solid tumor-associated antigens and the immunosuppressive tumor microenvironment (TME). GPC3 is a surface antigen overexpressed in hepatocellular cancer (HCC) and virtually absent on healthy tissues. In this first-in-human (FIH) study, we investigated the feasibility, safety and initial anti-HCC efficacy of C-CAR031. C-CAR031 is an autologous, GPC3-directed armored CAR-T with affinity-tuned scFv to enhance the safety profile, and a 4-1BB and CD3ζ signaling domain. The C-CAR031 transgene includes a T2A viral self-cleaving peptide and a dominant negative TGF-β receptor II (TGFβRIIDN). The expression of TGFβRIIDN protects the cells against the immunosuppressive HCC TME and the T2A peptide allows for equimolar expression of the two transgene products.
Methods: This FIH, open-label dose escalation trial employs an accelerated titration plus i3+3 design. Histologically confirmed GPC3+ advanced HCC patients (pts) who failed systemic treatments received a single-dose i.v. infusion of C-CAR031 following standard lymphodepletion. The primary objective was to assess the safety and tolerability. Adverse events (AEs) were graded using CTCAE 5.0, and cytokine release syndrome (CRS) / immune effector cell-associated neurotoxicity syndrome (ICANS) were graded according to ASTCT 2019 criteria.
Results: As of Dec. 31$^{st}$ 2022, 7 pts received two dose levels (DL1, n=1; DL2, n=6) of C-CAR031. The median number of prior lines of therapies was 4 (range 1-6). The median follow-up was 77 (40-213) days. Six pts with ≥28 days' follow-up were eligible for safety evaluation. The only ≥Gr3 non-hematologic product-related AE observed was transient Gr3 AST elevation in two pts. Five of 6 pts experienced Gr1/2 CRS, with median time to onset and duration of 3 (range 2-7) and 4 (4-6) days. No DLT or ICANS was observed. Of the 5 pts evaluable for preliminary efficacy, 4 pts had unconfirmed PR, which are currently pending confirmation. AFP was also stabilized or reduced in all 4 patients with uPR. All 5 pts had reduction in tumor burden, with a median change of -31.2% (range -3.4- -60.6%) / -41.4% (-3.4- -56.6%)

per RECIST1.1 / mRECIST. C-CAR031showed a robust cellular kinetic profile. In DL2, the median Tmax, Cmax and AUC0-28Day were 15 days, 772,014 copies/µg gDNA and 7,747,054 days*copies/µg gDNA, respectively. CAR-T cells were detectable in blood of all pts in the last follow-up.
Conclusions: In this FIH study, C-CAR031 is well tolerated and shows promising anti- tumor activity. Enrollment is ongoing to confirm initial results.

**CT098**

**A first-in-human phase 1 study of LY3410738, a covalent inhibitor of mutant IDH, in advanced IDH-mutant cholangiocarcinoma and other solid tumors.**

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**Background:** Isocitrate dehydrogenase 1/2 (IDH1/2) is mutated in a subset of cholangiocarcinoma (CCA), gliomas, and other solid tumors. LY3410738 is a potent, selective, covalent, dual inhibitor of IDH1/2 mutations (IDH1/2m). LY3410738 binds covalently at a novel binding site, enabling continued potency in preclinical models in the setting of second site IDH resistance mutations. We present initial results from the first-in-human phase 1 study of oral LY3410738 in patients (pts) with IDH1/2m CCA, and IDH1m glioma or other solid tumors.
**Methods:** Dose escalation (3+3 design) evaluated LY3410738 monotherapy in advanced IDHm CCA and other solid tumors (NCT04521686). Key objectives included determining the RP2D, safety, PK, PD (inhibition of plasma D-2-HG), and preliminary antitumor activity.
**Results:** As of 28 July 2022, 80 pts including 42 with CCA (33 IDH1m, 9 IDH2m), 27 with glioma

(IDH1m), and 11 other tumor types (IDH1m) received LY3410738 dosed at 25-600 mg QD or 300 mg BID. Pts were median 52 years of age (range, 23-80) with a median of 2 prior therapies (range, 1-7). 19% of CCA pts had received prior IDH1 inhibitor. Median time on treatment was 3.7 months (range, 0.1-19). No DLTs or treatment related deaths were observed; the MTD was not reached. Treatment emergent adverse events (TEAEs) ≥15% included nausea (35%), vomiting (21%), and decreased appetite (19%) and were mostly grade 1-2. Most frequent grade ≥3 TEAEs >2% were anemia (4%), cholangitis (3%), headache (3%), decreased lymphocyte count (3%), and hyponatremia (3%). LY3410738 exposure was dose proportional. In pts with IDH1m cancers, LY3410738 achieved sustained D-2-HG inhibition at all dose levels, including in pts who received prior IDH1 inhibitor. In pts with IDH2m cancers, a higher dose (≥150 mg daily dose) was required for D-2-HG inhibition. Among the 42 pts with R/R CCA, the best response included 1 PR and 22 SD. Of the 22 glioma pts with contrast enhancing tumors, best response included 3 PR and 9 SD.
**Conclusions:** LY3410738 demonstrated a favorable safety profile with potent and sustained D-2-HG inhibition in pts with IDH1/2m advanced solid tumors. Consistent with the expectations for IDH inhibitor monotherapy in this setting, CCA and glioma pts exhibited prolonged stable disease. RP2D evaluation is ongoing. Updated data on LY3410738 monotherapy will be presented at the meeting.

**CT099**

**First-in-human study of ALF501, polypeptide PSMA-targeted chimeric antigen receptor engineered natural killer cells) for castration-resistant prostate cancer.**

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Abstract Background: The mortality of castration-resistant prostate cancer (CRPC) is high due to lack of an effective treatment. Chimeric antigen receptor (CAR) based therapy is a promising immunotherapeutic strategy. In preclinical models, we established ALF501, a novel CAR-NK cells with both a high affinity for PSMA and a clinically significant tumoricidal effect on CRPC. Here we report the results from the first-in-human case of ALF501 for CRPC.
Methods: In this study, ALF501was dosed intravenous for a total of three courses, with an interval of 3 months, each infusion of $5\times10^8$ cells, and 3 consecutive days of infusion as a course of treatment. Observation continued until progression or unacceptable toxicity for up to 1 years. Primary objectives were safety and tolerability; secondary objectives included preliminary antitumor activity.
Results: An 80-year-old man who underwent laparoscopic radical prostatectomy in February 2018 and developed a relapse in April 2021. Starting with July 16 2021, he received three courses of ALF501. No liver and kidney toxicity was observed, and the safety profile was good. Progression-free survival was 12months as of April 23, 2022, during which PSA decreased by 97.5% from baseline. Imaging follow-up showed that the tumor was stable, and chest CT showed that the original pleural effusion and pericardial effusion disappeared, and the high-density shadow previously considered osteogenic bone metastasis was reduced overall.
Conclusions: ALF501 is safe and feasible for the treatment of CRPC, and has preliminary anti-tumor efficacy. This is a case study, and it is necessary to expand the patient sample size in the future to confirm whether there is a difference in efficacy for different patients.

**CT100**

**A new therapeutic cancer vaccine inducing multifunctional immunity, artificial adjuvant vector cells.**

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Yamasaki,[1] Tomonori Iyoda,[1] Jun Jun Shinga,[1] Michihiro Hidaka,[4] Masanori Nojima,[2] Fumitaka Nagamura,[2] Arinobu Tojo[5]. [1]RIKEN, Yokohama, Japan; [2]The Institute of Medical Science, The University of Tokyo (IMSUT), Tokyo, Japan; [3]Tokyo Medical and Dental University, Ichikawa, Japan; [4]National Hospital Organization Kumamoto Medical Center, Clinical laboratory, Kumamoto, Japan; [5]Tokyo Medical and Dental University, Tokyo, Japan.

**Background:** Cancer immunotherapy is an effective way of battling cancer, and is based on artificial stimulation of the immune system to attack cancer cells. Although several strategies show successful results, it remains to prevent the subsequent cancer escape from the immune surveillance. The further success depends on a variety of functional effector cell types involved and their sustenance following activation. For this purpose, simultaneous induction of innate and adaptive immunity should be one of ideal strategies. We focus on DCs, which play a pivotal role in determining the quality and magnitude of innate and adaptive immunity. To effectively utilize the DC *in situ*, we have developed a system using allogeneic cells as artificial adjuvant vector cells (aAVCs), comprised of a CD1d-NKT ligand complex on the cell surface and containing tumor antigen inside of the cells. This approach induces adjuvant effects by combining NKT cell activation with delivery of tumor antigen to DCs *in vivo*. In addition to linking innate and adaptive immunity, aAVC therapy can lead to efficient trafficking of specific anti-tumor $CD8^+$ T cells to the tumor site and also the formation of long-term memory T cells. This novel design of aAVC therapy is a platform and allows for a replacement of any cancer antigens as the therapeutic package. After completion of the regulatory science consultation for discussing the pharmaceutical quality and the design of a clinical study with PMDA in Japan, we developed the WT1-expressing aAVC (aAVC-WT1) for the clinical application.
**Methods:** This first-in-human, open label, single-center trial involves a bifurcated 3+3 design with aAVC-WT1 dose escalations ($1 \times 10^6$, $1 \times 10^7$, and $1 \times 10^8$ per body). The three patients in each cohort had not received any chemotherapy during more than 2 weeks before the therapy and had received aAVC-WT1 intravenously twice in a 4-week interval. Nine of the ten enrolled patients with relapsed and refractory AML underwent complete analysis per-protocol set and were evaluated in our phase I dose-escalation trial of aAVC-WT1.
**Results:** No dose-limiting toxicities were observed, whereas activation of NKT and/or NK cells was observed in all patients. Five patients experienced objective leukemic regression, which correlated with WT1-specific T-cell responses. Paired single-cell RNA and TCR sequencing demonstrated effector $CD8^+$ T cell clones in the bone marrow. Some bone marrow $CD8^+$ T cells underwent transition from pre-existing precursor exhausted T cells to functional T cells or emerged as newly activated T cells, some of which were maintained long-term. These demonstrate the feasibility and safety of aAVC-WT1 therapy and the capacity of this platform to activate both innate and adaptive immunity in humans.
**Conclusion**: aAVC-WT1 therapy showed an acceptable safety profile at doses tested and encouraging signs of clinical response. These data support further development of aAVC-WT1 for patients with AML.
Clinical trial registry number: UMIN 000028083.

**CT101**

**Phase I study of the T-cell receptor-like antibody Hu8F4 in patients with advanced hematologic malignancies.**

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**Background:** Despite recent advances in the treatment of AML, most approaches are rarely curative and most patients (pts) succumb to relapsed disease. The effectiveness of stem cell transplant and associated graft vs. leukemia effect implies an important role for immune-based therapy in producing long lasting remissions. Traditional approaches using immunotherapy have failed to establish a suitable surface target or treatment paradigm that is effective in myeloid malignancies. Hu8F4 is a humanized T-cell receptor-like monoclonal antibody that binds to the conformational epitope of PR1 bound to HLA-A2, which is highly, differentially expressed on the surface of AML compared to normal progenitors.
**Methods:** We conducted a first in human, phase I dose escalation trial of Hu8F4 in pts with myeloid malignances. Pts with R/R AML, MDS, CMML, and myeloid blast phase of CML with adequate organ function and PS ≤ 2 were eligible. Pts were treated on 7 escalating dose levels, ranging from 0.01 mg/kg to 10 mg/kg IV on D1 & 15. Initial dose levels required 1 pt per dose (0.01, 0.03, 0.1, 0.3, 1), followed by 3 pts per dose (3, 10).
**Results:** 10 pts with R/R AML have been enrolled, with a median age of 65 years (range, 23-77), including 6 females (60%). Pts had received a median of 4 (1-4) prior therapies; 5 pts (50%) had a PS of 2. At enrollment, the median WBC was 1.9 (0.1 - 18.4), median BM blasts were 32% (8 - 76); 9 (90%) pts had complex karyotype and 3 (30%) had a *TP53* mutation. All pts had > 98% surface expression of PR1 on the myeloid blasts. Hu8F4 Cmax ranged up to 160,000 ng/mL with t1/2 of 48 hours and clearance of 2.61 hr*ng/mL at the highest dose. Weak anti-drug antibodies were observed after week 4 in 2 of 3 pts treated at 3 mg/kg. With a median follow up of 3.5 months (1.1 - 9.9), pts have received a median of 1 (1-4) cycle of therapy. Two pts had decline in BM blasts and 4 had stable disease. Routine peripheral blood testing revealed sharp decline in peripheral blasts immediately after infusion of Hu8F4 on D1 and 15 with associated elevation in serum LDH in some pts and a rise in normal granulocytes, consistent with on-tumor effects. The pharmacokinetic parameters and transient blast reduction indicated a possible sink effect mediated by high levels of circulating blasts. SAEs documented on study were mostly disease-related and included infections, cytopenias, hemoptysis, pneumonia, and GI bleeding. Treatment related AEs were temporally related to the infusion included hypotension (Grade 2: N=2), rigors (Grade 2: N=2; Grade 1: N=1). All infusion reactions were observed at dose levels of 3 and 10 mg/kg, but were transient, and managed with steroids and antihistamines. All pts proceeded with their next dose without further issues. No cytokine release syndrome or neurologic toxicity was observed. Correlative studies support antibody dependent cellular cytotoxicity and phagocytosis as important mechanisms of anit-AML activity.
**Conclusion:**Hu8F4 was well tolerated with no dose-limiting toxicities observed at the maximum planned dose. On-target peripheral blast reduction temporally related to infusion suggests biological activity. Real-time pharmacokinetic data on study indicate a possible sink effect that may be overcome by a more frequent dosing strategy.

**CT102**

**Interim results of a first-in-human, dose escalation and expansion study of PLB-1004, an irreversible inhibitor of exon 20 insertion mutations in patients with non-small cell lung cancer.**

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Background: PLB1004, a novel mono-anilino-pyrimidine small molecule inhibitor of EGFR, potently and irreversibly targets exon 20 insertion mutations with IC50 values ranging from 25.67-316.6 nM. The molecule also potently targets classical EGFR mutations ExDel19, L858R and T790M with a high degree of selectivity over wild-type EGFR.

Methods: The study is a multi-center, open-label, dose escalation and expansion study conducted entirely in China, to assess the safety, tolerability, pharmacokinetics, and anti-tumor effect of PLB1004, administered orally once per day, in patients with advanced non-small cell lung cancer. The primary objective of the study is to assess the safety profile of PLB1004 and determine the RP2D of the molecule.
Results: Dose escalation ranged from a starting dose of 10 mg QD to a top dose of 480 mg QD in 11 cohorts of patients. Dose expansion is ongoing at two dose levels, 320 mg QD and 400 mg QD. At the cutoff date for this abstract, July 31, 2022, a total of 65 patients (32 in escalation and 33 in expansion) had received treatment with PLB1004. The median age of the patients is 58 years old (range 31 to 77). Most patients are women (60%) with adenocarcinoma (95%) and good performance status (ECOG 0-1 in 90%). Prior therapy for NSCLC included platinum-based chemotherapy in 54% of patients and TKI therapy in 58%. Of note 58% of patients had intra-cranial metastases at baseline. The most frequent treatment related adverse events included diarrhea in 75% of patients (18% Grade 3), rash in 60% of patients (11% Grade 3), mouth ulceration in 43% of patients (1.5% Grade 3), elevated serum creatinine in 43% of patients (0% Grade 3) and elevated aspartate aminotransferase in 41% of patients (3% Grade 3). The criteria for DLT were not reported at any dose level and thus an MTD was not determined during cycle 1 of drug administration. Beyond Cycle 1, at the highest dose levels, frequent dose interruptions and reductions due to toxicity were observed, and further dose escalation was not attempted above 480 mg QD. A more complete summary of safety data will be presented at the meeting. Across all dose groups, a total of 38 subjects had EGFR Ex20ins mutations, including 29 at doses ≥ 160 mg QD, among whom 26 completed at least 1 tumor assessment. In these 26 patients the confirmed response rate was 57.7% (15/26) and the disease control rate (DCR) was 100% (26/26). Duration of response exceeded 6 months in 40% of responders.
Conclusion: In the ongoing Phase 1 study, PLB1004 appears to be safe and well-tolerated with promising anti-tumor activity in patients with NSCLC harboring EGFR exon 20 insertion mutations.

**CT103**

**A phase 1 first-in-human study of MEM-288 oncolytic virus in solid tumors including non-small cell lung cancer (NSCLC): impact on tumor and systemic T cell immunity.**

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Background: MEM-288 is a conditionally-replicative oncolytic adenovirus expressing human IFNβ and a recombinant membrane-stable form of CD40L (MEM40). Preclinical studies show MEM-288 induces robust dendritic cell-mediated systemic T cell responses capable of inhibiting abscopal tumor growth as monotherapy and synergizes with immune checkpoint inhibitors (ICI).
Methods: Safety, antitumor and immunologic activity of MEM-288 are being evaluated in this Phase 1, multicenter, open-label trial (NCT05076760). Pts with select solid tumors including NSCLC (a) refractory to standard therapy and (b) with a tumor lesion deemed feasible for biopsy and MEM-288 intratumoral injection are eligible. The primary objective is to determine a recommended phase 2 dose of MEM-288 monotherapy across 3 dose levels (DL1-3) spanning 1e10 to 1e11 viral particles by intratumoral injection once every 3 weeks, for up to 6 injections, using a BOIN design. Secondary objectives include efficacy assessment (including response rate of injected and non-injected tumors assessed separately). Tumor biopsies immediately prior to the 1st and 2nd injections, and peripheral blood at serial timepoints, are used to explore biomarkers and anti-tumor immune responses.
Results: As of Jan 2023, the study is ongoing with the DL3 high-dose cohort completing accrual. 11 pts (10 NSCLC and 1 pancreatic) enrolled and received ≥1 dose of MEM-288 (n=3 DL1, n=5 DL2, n=3 DL3). No dose limiting toxicities have been reported to date and no pts have discontinued treatment due to toxicity. Treatment-related adverse events observed in >1 pt include grade 1 injection site reaction

(45%) and chills (18%). Of 7 pts evaluable for response, 3 had shrinkage of injected tumor (range -40 to -54%), and multiple pts also had stabilization or shrinkage of distal non-injected lesions with best RECIST response in uninjected tumors as 2 SD and 5 PD. After a single MEM-288 injection, biopsies show decreased tumor cell percentage concomitant with substantial increases in overall CD8+ T cells, increased T clonotype diversity in both tumor and peripheral blood, and increased TCF1+ stem-like CD8+ T cells that are strongly associated with response to ICIs. Plasma cytokine analysis showed increases in IFNγ and of IFN-inducible cytokines and chemokines, supportive of stimulation of systemic response after MEM-288. Of note, a pt with strong stimulation of tumor and systemic T cell immunity after MEM-288 had a subsequent CR (ongoing >6 months) to docetaxel + ramucirumab following prior treatment failure with platinum doublet + ICI received before MEM-288.
Conclusions: Preliminary safety, antitumor and immune response data are encouraging. Updated results and immune data will be presented. An expansion arm is planned with combination MEM-288 and anti-PD1 antibody in pts with advanced NSCLC refractory to ICI.

**CT104**

**MC1R imaging and histology in the targeted imaging of melanoma for alpha-particle radiotherapy (TIMAR1) trial.**

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Purpose: Melanocortin 1 receptor (MC1R) has been shown in animal models to be a target for effective radionuclide therapy. This first-in-human clinical trial studied the safety and biodistribution of two novel MC1R-targeted imaging tracers designed to support the development of MC1R-targeted alpha particle therapy through image-based identification of MC1R and radiation dosimetry. Imaging was compared to MC1R expression by immunohistochemistry (IHC) of subjects' melanoma tumors.
Methods: Subjects with stage IV melanoma were randomized to receive either [$^{203}$Pb]VMT01 SPECT/CT imaging followed by [$^{68}$Ga]VMT02 PET/CT imaging approximately 1 month later, or vice versa in a cross-over design during their normal melanoma treatment course. Patient selection included a positive [$^{18}$F]FDG PET/CT within 30 days of imaging tracer injection. 555 - 925 mBq of [$^{203}$Pb]VMT01 was injected IV with imaging performed at 1, 4, and 24 hours. 74 - 277 mBq [$^{68}$Ga]VMT02 was injected IV and imaging performed dynamically up to 1 hour, and at 2 and 3 hours. Due to an interruption in drug supply, only 3 subjects received [$^{203}$Pb]VMT01 imaging. Key FDG-avid melanoma lesions were marked and presented to three experienced Radiologists serving as blinded reviewers. The blinded reviewers compared [$^{18}$F]FDG PET/CT to experimental imaging and scored scans and key lesions on multiple metrics. Imaging positivity was defined as tumor uptake and retention of tracer in ≥ 1 melanoma tumors above background liver activity. Melanoma biopsy tissue was obtained and MC1R IHC performed as available.
Results: 7 subjects were enrolled; 6 were imaged and 5 had available pathology. 3 of 6 imaged subjects had MC1R positive tumors *via* experimental imaging tracers. [$^{68}$Ga]VMT02 PET/CT at 3 hours led to the best tumor to background ratio, and [$^{203}$Pb]VMT01 SPECT/CT showed tumor retention at 24 hours. 4 of 5 subjects had positive MC1R staining by IHC. Of the 3 subjects with negative imaging, 2 had positive staining by IHC. Of the 3 subjects with positive imaging, 2 had available biopsies and all stained positive for MC1R by IHC.
Conclusion: A subgroup of advanced melanoma patients were identified to express MC1R using new MC1R-targeted imaging agents. Full concordance between imaging and IHC of positive scans, but not with negative scans, suggests that IHC is a more sensitive biomarker detection method. Additional testing is needed to establish frequency of positive MC1R imaging in stage IV melanoma and its correlation to histological findings and treatment outcomes. Preferential tumor retention provides proof-of-concept that the MC1R-targeting therapy trial for [$^{212}$Pb]VMT01 alpha-emitting therapy will deliver localized

radiation to high MC1R expressing tumors as screened by [$^{203}$Pb]VMT01 or [$^{68}$Ga]VMT02. Imaging quality is sufficient for subject selection and dosimetry but may exclude subjects with MC1R receptor expression only detectable by IHC.

**CT105**

**Preliminary results from the Phase I part of a first-in-human Phase I/II study of HH2853, an EZH1/2 inhibitor, in patients with relapsed/refractory non-Hodgkin lymphomas or advanced solid tumors.**

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**Background:** The dysregulation of polycomb repressive complex 2 (PRC2) promotes tumorigenesis and progression. Two therapeutic agents targeting enhancer of zeste homolog (EZH) 2 or EZH1/2, the catalytic subunits of PRC2, have been approved in several cancer types. HH2853 is a novel selective EZH1/2 dual inhibitor, which has demonstrated superior anti-tumor efficacy to tazemetostat (EZH2 inhibitor approved by FDA) in various preclinical models.
**Methods:** This is a first-in-human, open-label, multi-center, phase (Ph) I/II study of HH2853 in patients (pts) with relapsed/refractory (r/r) non-Hodgkin lymphomas (NHLs) or advanced solid tumors. HH2853 was administered orally twice daily (BID) on a continuous 28-day treatment cycle. Ph I consist of two parts: dose escalation part adopting accelerated titration followed by a Bayesian optimal interval design and dose extension part. Dose limiting toxicity (DLT) was evaluated during the 1$^{st}$ cycle in dose escalation. Safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD) and preliminary anti-tumor activity of HH2853 were explored in this Ph I study.
**Results:** As of Oct 19, 2022, a total of 57 pts, including 50 pts with solid tumors and 7 pts with r/r follicular lymphoma (FL), were enrolled from 12 sites in China and the US. Twenty-five (43.9%) pts received ≥3 lines of prior systemic therapies. Six dose levels(50 mg, 100 mg, 200 mg, 400 mg, 600 mg and 800 mg)were evaluated. DLTs were observed in 2 of 8 DLT evaluable pts at 800 mg: one grade 3 platelet count decreased and one grade 3 diarrhea. A conclusive maximal tolerated dose was not reached. The most common treatment-related adverse events (TRAE) were diarrhea (45.6%), blood bilirubin increased (35.1%), white blood cell count (WBC) decreased (26.3%), platelet count decreased (26.3%), rash (24.6%) and anemia (22.8%). The most common ≥grade 3 TRAEs included anemia (8.8%), platelet count decreased (7.0%), WBC decreased (5.3%) and diarrhea (5.3%). TRAEs leading to dose interruption or reduction were reported in 17.5% and 8.8% pts respectively. No TRAE led to dose discontinuation or death. PK data indicated dose-related increase in exposure from 50 to 600 mg. PD data showed a significant inhibition (maximum reached >90%) of H3K27me3 in granulocytes and monocytes at 400-800 mg. Tumor responses were observed in 7 pts from 200 to 800 mg in multiple tumor types, including complete response in 1 patient with FL, partial response in 3 pts with epithelioid sarcoma, 2 pts with FL and 1 patient with malignant rhabdoid tumor of pancreas.
**Conclusions:** This first-in-human study of HH2853 showed a manageable safety profile and promising anti-tumor activity in multiple tumor types, supporting further exploration in NHLs and solid tumors after recommended Ph II dose is determined.Clinical trial information: NCT04390737

**CT107**

**Phase Ib Study of Pembrolizumab in Combination with Intratumoral Injection of Clostridium**

**novyi-NT in Patients with Advanced Solid Tumors.**

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BACKGROUND: Intra-tumoral Clostridium novyi-NT (non-toxic) is an attenuated strain of C. novyi lacking alpha toxin replication within hypoxic tumor regions, causing tumor cell lysis and inflammation. Immunotherapy (IO) augments anticancer activity with intratumoral agents in prior preclinical and clinical studies. This phase 1b dose escalation study assessed safety and potential synergistic effects of pembrolizumab and C. novyi-NT in advanced solid tumors. METHODS: We enrolled patients with percutaneous injectable, solid tumors to receive single intratumoral injection of C. novyi-NT administered on Day 8 across 4 dose cohorts (3x10^4 to 100 x10^4 spores, 3+3 design) with pembrolizumab 200mg IV Q3weekly starting on Day 0 up to 24 months. Primary objectives: Safety, tolerability and maximum tolerated dose (MTD) of combination. Secondary objectives: anti-tumor activity of combination in the injected tumor lesion and overall response by iRECIST 1.1. RESULTS: At data cutoff on October 24, 2022, 16 patients were enrolled and evaluable for toxicity and efficacy. It was well tolerated across all cohorts without dose limiting toxicities with median number of 5 cycles administered (1-34). 10 patients (63%) experienced progression; 1 withdrew consent; 1 completed therapy; 1 came off trial due to toxicity (immune related dermatitis) and 3 patients remain on trial. Median age was 62.5 years (40-71) while all patients (100%) had performance status of 1. 8 (50%) received prior IO and 7 (44%) had > 4 lines of prior therapies. Confirmed overall objective response rate (ORR) was 25% in 4 patients [non-keratinizing undifferentiated nasopharyngeal squamous carcinoma (NPC), human papilloma virus positive squamous cell carcinoma of base of tongue, vulvar melanoma and chordoma] with 3 partial responses (PR) and 1 complete response (CR). Among responders, median duration of response was 8.18 months and 1/4 (25%) had prior IO. NPC patient completed 24 months on trial to completion with best response at PR (-87%) while vulvar melanoma patient remains on trial with best response at CR (-100%). Median injected tumor size was 2.65cm (1-11) with confirmed ORR in injected lesions at 19% with 2 PRs and 1 CR. Most prevalent grade 1 and 2 adverse events were injection site reaction (25%), pyrexia (19%), pruritus (13%), leukopenia (13%) and anemia (13%). No grade 3 or 4 treatment related adverse events noted. Signs and symptoms of C. novyi-NT germination (infection) including fever, injection site pain, erythema, swelling, tenderness, and in some cases, ulceration, spontaneous drainage, tissue sloughing, bleeding, and malodor were observed in 5 patients. CONCLUSIONS: Intratumoral C. novyi-NT with pembrolizumab demonstrates clinical activity with favorable tolerability in patients regardless of tumor histology. This study is ongoing to define the recommended phase 2 dose (NCT03435952).

**CT108**

**First-in-patient study of the GDF-15 inhibitor ponsegromab in patients with cancer and cachexia: Safety, tolerability, and exploratory measures of efficacy.**

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**Background:** Cachexia is common in patients with advanced cancer and has been associated with elevated serum growth/differentiation factor 15 (GDF-15) concentrations. This first-in-patient, phase 1b, study assessed the use of ponsegromab, a monoclonal antibody against GDF-15, in participants with advanced cancer and cachexia.
**Methods:** Adult participants (n = 10) with cachexia, advanced cancer (non-small cell lung, colorectal, or pancreatic), and elevated serum concentrations of GDF-15 received open-label subcutaneous ponsegromab every three weeks (Q3W) for 12 weeks in addition to standard of care anti-cancer treatment. Study endpoints included assessment of ponsegromab safety, tolerability, and pharmacokinetics. Serum GDF-15 concentrations and exploratory measures of efficacy were also assessed.
**Results:** No treatment-related adverse events or injection site reactions were reported. No adverse trends in clinical laboratory tests, vital signs, or electrocardiogram parameters attributable to ponsegromab dosing were evident. Ninety-two adverse events deemed unrelated to treatment were reported; most were mild (Grade 1 = 58.7%) or moderate (Grade 2 = 28.3%) in severity. All participants were negative for anti-drug antibodies at baseline (n = 10) and after receiving 5 doses (Q3W) of ponsegromab (n = 9). Mean unbound ponsegromab $C_{trough}$ ranged from 4.041-4383 ng/mL between Days 22-106. An elevated GDF-15 concentration was required for inclusion in the study. Following initiation of study treatment, median unbound GDF-15 concentration was reduced to below the lower limit of quantification (0.0424 ng/mL) on day 1 and remained suppressed until week 15 (3 weeks after final dose). Increases in body weight were observed at all time points during the treatment (weeks 3, 6, 9, and 12) and follow-up (weeks 15, 18, and 24) periods. The mean (SD) body weight at baseline was 70.49 (16.97) kg. An LS mean (SE) increase of 4.63 kg (1.98) was observed at week 12 (end of treatment); representing an increase of approximately 6.5% relative to baseline. Improvements in actigraphy-based assessments of physical activity and in quality of life, including appetite, as assessed by Functional Assessment of Anorexia-Cachexia Therapy (FAACT) total and subscale scores, were also observed during the course of ponsegromab treatment.
**Conclusions**: In participants with advanced cancer, cachexia, and elevated baseline GDF-15, ponsegromab was well tolerated and suppressed serum GDF-15 concentrations to below the median concentration seen in healthy subjects. Preliminary evidence of efficacy, including a mean observed weight gain of approximately 6.5% at 12 weeks, supports continued development of ponsegromab for the treatment of cancer cachexia. Funded by Pfizer. **ClinicalTrials.gov:** NCT04299048

**CT109**

**First-in-class first-in-human phase 1 trial and translational study of the mono(ADP-ribose) polymerase-7 (PARP7) inhibitor RBN-2397 in patients with selected advanced solid tumors.**

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**Background:** PARP7 is a stress-induced monoART that suppresses the cellular type I interferon (IFN)

response following cytosolic nucleic acid sensing. RBN-2397 is a first-in-class PARP7 inhibitor that induces IFN and an adaptive immune response. The tumor-intrinsic immunomodulatory mechanism of RBN-2397 and preliminary antitumor activity in patients (pts) was demonstrated during dose escalation (Falchook, ASCO 2021; Kuplast-Barr, AACR 2022).

**Methods:** Pts with solid tumors were treated with RBN-2397 at the RP2D of 200 mg BID in 3 expansion cohorts: squamous cell carcinoma of the lung (SCCL), head and neck squamous cell carcinoma (HNSCC), and hormone receptor-positive breast cancer (HR+ BC). Objectives of the expansion phase included safety, pharmacokinetics, pharmacodynamics, and antitumor activity.

**Results:** As of 2 July 2022, 31 pts have been treated: SCCL (n=13), HNSCC (n=10), and HR+ BC (n=8). RBN-2397-related AEs (all grades >10%) included dysgeusia (42%, n=13), nausea (26%, n=8), fatigue (23%, n=7), with Grade 3 events of nausea and pleural infection (each n=1) and ALT/AST increase (n=2), and no Grade 4 events. No significant chronic toxicities were observed. The disease control rate in response-evaluable pts was 44% in SCCL (stable disease [SD] in 4/9 pts), 71% in HNSCC (RECIST partial response [PR] for 12+ months in 1/7; SD in 4/7), and 29% in HR+ BC (SD in 2/7). Biomarker analyses confirmed *PARP7* mRNA expression in all baseline biopsies, with H-scores higher in tumor cells than in stromal cells (n=26, H-score range 66-256, P<0.0001). Four of 17 evaluable pts showed *PARP7* focal copy number gains, and 2 had copy number gains that tracked with chromosome 3 copy number. One pt with HPV-negative HNSCC (nonsmoker; paranasal sinus primary) has an ongoing durable RECIST PR (−41%) for 12+ months. Tumor analysis of this responder confirmed *PARP7* expression at baseline and *SMARCB1* deletion, and demonstrated an excluded immune phenotype by IHC and MIBI-SCOPE. Analyses of paired tumor biopsies confirmed induction of adaptive immunity with ≥2-fold increases in CD8+ T cells and/or granzyme B expression in 10 (63%) of 16 pts across tumor types. Increases in immune checkpoint expression (PD-1 and LAG3 on T cells; PD-L1 on tumor cells) from 30% to 150% were observed in 3 (60%) of 5 pt tumor samples evaluated using MIBI-SCOPE, indicating the potential to prime tumors for immune checkpoint inhibitor therapy. These changes were independent of PARP7 copy number or *PARP7* mRNA expression level at baseline.
**Conclusions:** RBN-2397 was well tolerated at biologically active drug exposures, with preliminary antitumor activity observed. Paired tumor biopsy translational studies demonstrated the immunomodulatory mechanism of RBN-2397 and support the ongoing trial of RBN-2397 in combination with pembrolizumab (NCT05127590).

**CT110**

**Safety and anti-tumor activity of a novel Treg depleter RG6292, as a single agent and in combination with atezolizumab in patients with solid tumors.**

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Background
RG6292 is the first anti-human CD25 antibody developed to specifically deplete human Tregs while preserving IL-2R STAT5 signaling and Teff activity.
Methods
Patients with advanced/metastatic solid tumors and without standard treatment options were enrolled in dose escalation studies and received RG6292 i.v. Q3W as monotherapy (S1: NCT04158583) or in combination with atezolizumab 1200 mg Q3W (S2: NCT04642365) until disease progression or unacceptable toxicities to determine the maximum tolerated dose (MTD) and/or optimal biological dose, safety and preliminary clinical activity. DLT window was 4 weeks and dose increments were determined by a Bayesian-based continuous reassessment method (CRM) with overdose control. Adverse events (AEs) were graded by NCI CTCAE v5.0. Tumors were assessed by RECIST 1.1 every 8 weeks in the first year and then every 12 weeks.
Results
As of 27th May, 2022, 76 patients have been treated with RG6292 monotherapy (dose ranging from 0.3 - 165 mg). Six DLTs were reported, including rash, papular rash, rash macular-papular, AST elevation and ALT elevation. MTD is 165 mg. Seventy-five patients (99%) experienced at least one AE. The most common AEs were pruritus (32%), rash (29%) and fatigue (29%). Thirty-five patients (46%) experienced grade ≥ 3 AEs and 2 patients discontinued from study treatment due to AEs. Fourteen patients (18%) experienced grade ≥ 3 TRAEs. No AEs led to a fatal outcome. Median treatment duration was 43 days. Twenty-three patients have SD as best overall response. Forty-eight patients have been treated with RG6292 (dose ranging from 0.3 - 160 mg) in combination with atezolizumab. Two DLTs were reported, including immune system disorder and maculopapular rash. MTD has not been reached. Forty-seven patients (98%) experienced at least one AE. The most common AEs reported were pruritus (44%), rash (33%) and fatigue (33%). Twenty patients (42%) experienced grade ≥3 AEs and 2 patients discontinued from study treatment due to AEs. Five patients (10%) experienced grade ≥3 TRAEs. No AEs led to a fatal outcome. Median treatment duration was 54 days. Pruritus and rash were expected AEs and easily managed withtopical steroids or short course, low dose systemic steroids. Two patients have PR and 19 patients have SD as best overall response.
Conclusion
RG6292 is well tolerated and has a manageable safety profile as a single agent and in combination with atezolizumab. The preliminary safety and clinical activity from dose-escalation warrant further investigation of RG6292.

**CT113**

**Safety and efficacy of Tumor Treating Fields (TTFields) combined with bevacizumab and systemic chemotherapy in recurrent GBM.**

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Background: The treatment of recurrent glioblastoma faces many challenges. The development of new therapeutic approaches and the exploration of biomarkers of therapeutic effectiveness will provide a basis for better tumor control and individualized diagnosis and treatment. TTFields therapy, bevacizumab and second-line chemotherapy are the alternative treatment methods for recurrent gliomas. Especially, the

introduction of TTFields has prolonged the tumor control of patients. Clinical studies have shown that bevacizumab combined with chemotherapy can improve the prognosis of patients. In this study, the randomized controlled principle was used to compare TTFields plus second-line chemotherapy and combined chemotherapy on the basis of both, and to explore the safety and effectiveness of combined treatment for patients with recurrent glioblastoma.
Objectives: 1. To evaluate the safety and efficacy of TTFields, bevacizumab, and second-line chemotherapy in the treatment of relapsed GBM. 2. To evaluate the effects of TTFields, bevacizumab and their combination on the permeability of the blood-brain barrier. 3. To explore the correlation between serum VEGF, plasma MMP-2, MMP-3 and other factors and the efficacy of combination therapy.
Methods: A total of 40 patients who met the inclusion criteria were planned to be recruited and randomly assigned to two experimental groups, with 20 patients in each group. On the basis of second-line chemotherapy, patients in each group were treated with TTFields alone, TTFields combined with BEV, respectively. The 6-month survival rate was used as the primary outcome, and the median OS and median PFS were used as the secondary outcomes. The effectiveness and safety of each regimen was evaluated and compared. Krans, CBV, CBF, MTT and other imaging indicators were used to evaluate vascular permeability and blood flow. To compare the differences between BEV alone and BEV combined with TTFields, and to evaluate the efficacy prediction of these hematologic molecules in the combined regimen. Moreover, imaging and hematological indicators were combined to construct a prognostic model that could predict the efficacy of the combined regimen.
Key Issues to be Resolved: 1. To evaluate the comprehensive effect of TTFields combined with BEV treatment on blood-brain barrier permeability and blood perfusion. 2. A prognostic prediction model will be established by combining imaging features and hematological indicators. 3. Preliminarily determine the direction of optimization of the combined treatment program.

**CT114**

**Data from first-in-human study of EXS21546, an A2A receptor antagonist, now progressing into phase 1 in RCC/NSCLC.**

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Background: EXS21546 (discovered in collaboration with Evotec) is a clinical stage A2AR selective antagonist with high potential in cancer immunotherapy as a combination agent. A completed healthy volunteer (HV) first-in-human (FIH) study is now being followed by the IGNITE Phase 1/2 trial in patients, which includes the retrospective study of an adenosine-specific patient enrichment biomarker strategy.
Methods: A 3-part Phase 1 FIH study in 60 HV was completed (NCT04727138). Parts 1 and 2 were double-blind, placebo-controlled single ascending dose (SAD) and multiple ascending dose (MAD) studies investigating EXS21546 safety, tolerability, PK and PD. Part 3 was a 3-period, open label, randomized, sequential study evaluating bioavailability of oral EXS21546 as a granule in capsule vs reference powder suspension. The ongoing Phase 1/2 IGNITE trial will study EXS21546 in patients with immunotherapy relapsed or refractory RCC or NSCLC directly in combination with a PD-1 inhibitor. This combination aims to leverage the properties of A2AR antagonism in the tumor microenvironment, to drive the immune system through PD-1 inhibition. IGNITE will also provide clinical data to support the validation of our adenosine signature to identify patients with adenosine rich tumor microenvironments who may benefit from treatment (Alt et al, 2022). Data will be continually reassessed to determine the recommended Phase 2 dose. A dose escalation phase will be followed by an expansion phase.
Results: PK/PD modeling of Phase 1 study results, along with the EXS21546 safety profile, allowed

identification of a potential therapeutic starting dose. PK results aligned with design specifications, based upon predictive preclinical modeling, and support twice-daily (BID) dosing for continuous A2AR antagonism. EXS21546 induced dose-dependent inhibition of CREB phosphorylation in CD8-positive cells, with the PD profile mirroring plasma exposure. Inhibition of A2AR signaling was sustained over the BID dosing period, demonstrating a level of lasting target engagement. PoP PK/PD simulations informed the starting dose and dosing regimen for the Phase 1/2 trial.
EXS21546 was well-tolerated with no CNS adverse events reported in the SAD at all doses and in the MAD at 150 mg BID. The majority of adverse events were considered mild and unrelated to EXS21546, with the exception of one Grade 3 Serious Adverse Event of elevated ALT/AST.
Conclusions: Safety and tolerability of our A2AR antagonist EXS21546 were confirmed in a HV study, allowing selection of a starting dose for the ongoing IGNITE Phase 1/2 study. IGNITE trial design was based on extensive simulations to enable the most efficient continuous reassessment method settings, and will allow further verification of the patient enrichment biomarker strategy.

**CT115**

**Treatment of skin tumors with intratumoral interleukin 12 gene electrotransfer in the head and neck region: A phase I clinical trial.**

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Immunotherapies with monoclonal antibodies (checkpoint inhibitors) are currently being intensively researched and have led to excellent response in many cases in various tumors. Another potential approach to immunotherapy is the targeted intratumoral administration of interleukin 12 (IL -12), a cytokine with proven anti-tumor activity. Due to its immunomodulatory effect, it can be used as an immunostimulatory component for in situ vaccination of local ablative therapies. We have developed a phIL12 plasmid without antibiotic resistance markers using a transgene for the human IL -12 p70 protein. The plasmid can be introduced intratumorally by gene electrotransfer. In gene electrotransfer, electrical pulses are applied to the tumor to deliver the plasmid DNA into the cells. Based on nonclinical studies of safety, pharmacokinetics, pharmacodynamics, tolerability, and immunogenicity, a phase I clinical trial of phIL12 gene electrotransfer was conducted (ISRCTN15479959, ClinicalTrials NCT05077033). The primary objective of the study is to evaluate the safety and tolerability of phIL12 gene electrotransfer in the treatment of basal cell carcinoma in patients with operable tumors of the head and neck. The study, an exploratory, dose-escalating Phase I study, will enroll 9 patients in three dose-escalating cohorts. Treatment consists of plasmid injection followed by application of electrical pulses to the tumors. Patients are monitored for 30 days, with tumors removed if a complete response is not achieved. To date, two cohorts of patients have completed the study. No adverse effects or treatment-related toxicities have been observed, and treatment has been well tolerated by patients. The results of this study will provide the basis for the use of phIL12 gene electrotransfer as adjuvant therapy to local ablative therapies to enhance their local effect and elicit a systemic response.

**CT116**

**First-in-human study of AZD8853, an anti-growth and differentiation factor 15 (GDF15) antibody, in patients (pts) with advanced/metastatic solid tumors.**

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**Background:** The cytokine GDF15 is overexpressed in solid malignant tumors such as colorectal, lung and urothelial cancer, where it modulates T cells, dendritic cells (DCs) and myeloid-derived cells, driving the tumor microenvironment toward an immunosuppressive, tumor-promoting state. AZD8853 is a humanized immunoglobulin G1 monoclonal antibody that binds to, and neutralizes, GDF15. Anti-GDF15 treatment increased T cell proliferation and DC activation, leading to an antitumor immune response in preclinical studies of anti-PD-L1 resistant models. In vitro and in vivo preclinical data support the potential antitumor activity of AZD8853 in pts with selected advanced/metastatic cancers.
**Methods:** This Phase I/IIa, first-in-human, open-label study (NCT05397171) assesses the safety, pharmacokinetics (PK), pharmacodynamics (PD) and preliminary efficacy of AZD8853 in pts with histologically or cytologically confirmed locally advanced, unresectable or metastatic mismatch repair-proficient colorectal cancer (pMMR-CRC), non-small-cell lung cancer (NSCLC) and urothelial carcinoma (UC). Up to 165 pts will be enrolled in 3 parts: Part A, dose escalation; Part B, pharmacodynamics expansion; and Part C, efficacy expansion. All pts receive AZD8853 IV. Eligible pts are ≥18 years old with ≥1 measurable target lesion per RECIST v1.1, ECOG PS of 0/1, life expectancy ≥12 weeks and adequate organ and bone marrow function. Pts with NSCLC must have had ≥1 prior line of systemic treatment in the advanced/metastatic setting, and no sensitizing *EGFR* or *ALK* aberrations. Pts with pMMR-CRC must have had ≥2 prior treatments in the advanced/metastatic setting. Pts with UC must have had ≥1 prior treatment in the advanced/metastatic setting including platinum-containing therapy and/or a PD-(L)1-inhibitor. Pts with Grade ≥2 unresolved toxicities from prior therapy, symptomatic CNS metastases or leptomeningeal disease, or prespecified active/ongoing infections are excluded. The primary objective is safety, including dose-limiting toxicities, adverse events (AEs), serious AEs and AEs leading to AZD8853 discontinuation. The secondary objectives include assessment of efficacy (objective response rate, disease control rate, duration of response, percentage change from baseline in target lesion size, change from baseline in circulating tumor DNA, and progression-free and overall survival), PK and immunogenicity. Changes in GDF15 serum levels are measured in Parts A and B. Tumoral CD8+ T cell infiltration is measured in a subset of pts from Part B using PET/CT imaging and IHC of paired biopsies. The study is currently recruiting at centers in the USA and Canada with additional sites planned in the UK, France and Spain.

**CT117**

**Synergism between inhibitors of the EGFR-RAS-RAF-MEK pathway and the WNT pathway.**

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**Background** Inhibitors of the EGFR-RAS-RAF-MEK (MAPK) pathway have been successfully developed to treat cancers. However, many patients do not benefit from these inhibitors due to primary or acquired resistance. In colorectal cancer (CRC), resistance to inhibitors of the MAPK pathway is especially prominent, reflected by low response and short survival. Reactivation of MAPK signaling is a common mechanism of acquired resistance in CRC. WNT pathway is hyperactivated in the majority of CRC patients due to genetic alterations of several genes involved in WNT signaling, e.g., *APC, CTNNB1, RSPO* and *RNF43*. Cooperation between RAS-RAF and WNT signaling drives the carcinogenesis of CRC. Concomitant blockade of WNT signaling has been shown to impede the emergence of BRAF

inhibitor-resistant clones, thus offering the possibility to overcome acquired resistance.
**Methods** To prove the concept that simultaneous inhibition of the WNT pathway and the MAPK pathway reduces primary or acquired resistance to CRC treatment, we have tested combinations of various inhibitors of both pathways in CRC models, including *BRAF*$^{V600E}$;*RSPO*3 PDX models, *KRAS*$^{G12C}$;*RSPO*3 cell lines and *APC*-mutated cell lines with or without *KRAS* mutations, including *KRAS*$^{G12C}$;*APC* CRC cell lines. We tested MAPK pathway inhibitors, such as cetuximab, selumetinib and cobimetinib (MEK1/2 inhibitors), encorafenib (BRAF$^{V600E}$ inhibitor), and AMG510/sotorasib (KRAS$^{G12C}$ inhibitor). Inhibitors of the WNT pathway included a porcupine inhibitor, CGX1321, that blocks the secretion of WNT ligands, currently in clinical development, and a tankyrase inhibitor, CGX11071, that stabilizes AXIN-1/2 and degrades β-catenin. Synergistic effects between inhibitors of the MAPK pathway and the WNT pathway were observed in CRC models *in vitro* and *in vivo*. Notably, when CGX1321 was added to encorafenib and cetuximab in CRC PDX models with *BRAF*$^{V600E}$ and *RSPO* fusions, a decrease in tumor size (>50%) occurred in two weeks, compared with tumor progression with encorafenib and cetuximab or CGX1321 treatment alone. Notable is the synergy between tankyrase inhibitor CGX11071 and KRAS$^{G12C}$ inhibitor AMG510/sotorasib in CRC cell lines with *KRAS*$^{G12C}$ and *RSPO* fusion (or *APC* mutations). We launched a phase 1b clinical trial to study the efficacy of CGX1321 in combination with encorafenib and cetuximab in CRC patients with *RSPO* fusions and *BRAF*$^{V600E}$ mutations. (NCT02675946). Primary endpoint included safety and RP2D. Secondary endpoints included PK, overall response rate, CR and PR rate, and duration of response by RECIST 1.1 and irRECIST 1.1. The clinical protocol and available data of CGX1321 in combination with encorafenib and cetuximab in CRC patients with *RSPO* fusions and B*RAF*$^{V600E}$ mutations will be discussed.

**CT118**

**A phase I/II trial of a CXCR1/2 inhibitor in combination with anti-PD-1 for circulating tumor DNA (ctDNA) positive & refractory*RAS*-mutated microsatellite stable (MSS) colorectal cancer.**

Benny Johnson,[1] Cara Haymaker,[1] Van K. Morris,[1] Arvind Dasari,[1] Victoria Serpas Higbie,[1] John Paul Shen,[1] Christine Parseghian,[1] Maria Pia Morelli,[1] Ryan Huey,[1] Michael S. Lee,[1] Jason Willis,[1] Kanwal P. Raghav,[1] Ying Yuan,[1] John Zebala,[2] Ronald A. DePinho,[1] Scott Kopetz,[1] Michael Overman[1]. [1]University of Texas MD Anderson Cancer Center, Houston, TX; [2]Syntrix Pharmaceuticals, Auburn, WA.

Background: Preclinical CRC models reveal *KRAS* mutations activate the CXCR2 axis promoting an immunosuppressive tumor microenvironment (TME). This occurs via KRAS repression of interferon regulatory factor 2 (IRF2) resulting in upregulation of CXCL3 chemokines, which bind CXCR2 and recruit myeloid-derived suppressor cells (*MDSC; Liao et al, Cancer Cell 2019*). MDSCs also accelerate metastases by 'priming' the premetastatic niche via promoting egress of tumors cells into the circulation, inhibiting killing by immune cells and promoting extravasation into the tissues (*Veglia et al. Nature 2021*). SX-682 is a novel oral small-molecule inhibitor of CXCR1/2 chemokine receptors involved in MDSC-recruitment to TME. This proof-of-concept study investigates whether a CXCR1/2 antagonist in combination with anti-PD-1 therapy can overcome de novo immunotherapy resistance in MSS *RAS* mutated mCRC and eradicate micrometastatic disease in ctDNA positive CRC post definitive therapy.
Methods: STOPTRAFFIC-1 is a phase I/II, open-label, dose-escalation/dose expansion study of SX-682 plus nivolumab for patients (pts) with *RAS* mutated MSS mCRC or ctDNA positive CRC. Key eligibility criteria - Arm A: pts with MSS mCRC with progression or intolerance to 2 prior lines of therapy, ECOG 0/1; Arm B: ctDNA positive post completion of definitive therapy (adjuvant chemotherapy for stage III or metastasectomy for stage IV CRC). Pts will receive SX-682 (5 dose levels: 25 mg, 50 mg, 100mg, 200mg, or 400 mg PO twice daily) in an 8-week cycle with intravenous nivolumab (480 mg) on days 1 and 29. Adverse events per CTCAE v5.0. RECIST assessments every 8 weeks. In dose escalation, pts enter a 3-week monotherapy safety run-in of SX-682 followed by 3-week combination with nivolumab for a six-week dose-limiting toxicity (DLT) period. Cohorts 1-4 have been completed without DLT.

Enrollment to Cohort 5 is completed with expansion arms to open February 2023. Dose expansion is a Simon's optimal two-stage design for Arm A. Efficacy will be assessed in the first 15 pts with a requirement of 2 responses in order to enroll 14 additional pts (N=29). Pre- and on-treatment tissue biopsies will be collected. Arm B will enroll 15 pts with positive ctDNA post definitive therapy. Pts will be screened with CLIA certified ctDNA test for minimal residual disease (MRD) and if positive, enrolled for treatment for up to 6 months (m). Pts will undergo radiographic evaluation at 3 and 6m and then every 3-6m for at least 3 years. The primary objectives are to determine safety profile, recommended phase 2 dose, clinical activity & 6m ctDNA clearance rate in CRC pts with MRD following 6m of SX-682 + Nivolumab on Arm B. Translational analyses include correlations of clinical outcomes with genomic and immune biomarkers from paired tissue and plasma samples. Clinical trial information: NCT04599140.

**CT119**

**A first-in-human phase 1 study of LOXO-435, a potent, highly isoform-selective FGFR3 inhibitor in advanced solid tumors with *FGFR3* alterations (trial in progress).**

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**Background:** Alterations in the fibroblast growth factor receptor 3 (FGFR3) have been identified as oncogenic drivers in several solid tumor malignancies, including urothelial carcinoma (UC). Available pan-FGFR inhibitors target all 4 isoforms of FGFR (FGFR1 - 4) and consequently their efficacy can be limited by off-target toxicity (i.e. inhibition of other non-altered FGFR isoforms) including gastrointestinal, oral mucositis, and cutaneous/nail disorders, as well as FGFR1-mediated hyperphosphatemia. Moreover, clinical studies of pan-FGFR inhibitors have reported the development of drug resistance through acquired gatekeeper mutations (e.g. V555M and V565F) at the time of disease progression. LOXO-435 is a potent, highly isoform-selective FGFR3 inhibitor that is designed to preserve activity in the setting of gatekeeper resistance mutations. Preclinically, LOXO-435 has demonstrated potent *in vitro* and *in vivo* activity as a selective inhibitor of both wild-type and oncogenically activated *FGFR3*, including *FGFR3* fusions, point mutations, and acquired gatekeeper resistance mutations without evidence of FGFR1-mediated hyperphosphatemia.

**Methods:** LOXO-FG3-22001 is a multicenter, open-label, first-in-human phase 1a/b study of LOXO-435 in patients (pts) with *FGFR3*-altered advanced solid tumors, including metastatic UC (mUC) (NCT05614739). Eligible pts (≥ 18 years) must have an advanced or metastatic solid tumor with an *FGFR3* alteration detected in tumor or ctDNA, ECOG PS of 0-1, and have received all standard therapy or have no known available options to provide benefit. The study will be conducted in 2 phases: phase 1a dose escalation and phase 1b dose expansion. Dose escalation (cohort A) will begin as a single-patient accelerated design, allowing intra-patient dose escalation, followed by the modified toxicity probability interval-2 method. Additional pts will be allowed to backfill to previously cleared dose levels that demonstrate therapeutically relevant exposures or direct evidence of clinical activity. Dose escalation will assess safety, PK/PD, and antitumor activity of LOXO-435 as monotherapy to determine the recommended phase 2 dose (RP2D). Dose expansion will include 4 cohorts of pts with prespecified activating *FGFR3* alterations. Cohort B will enroll pts with mUC and include 2 cohorts to evaluate LOXO-435 as monotherapy (B1, B2) and 1 cohort to evaluate LOXO-435 in combination with pembrolizumab (B3). Cohort C will enroll pts with non-UC advanced solid tumors and will evaluate LOXO-435 as monotherapy (C1). Pts in cohort B1 must have progressed on or were intolerant to a prior FGFR inhibitor, and pts in cohorts B2, B3, and C1 must be FGFR inhibitor treatment naive. Dose expansion will evaluate the safety, PK/PD, and antitumor activity (per RECIST v1.1) of LOXO-435 at the RP2D.

**CT120**

**The randomized phase 1/2 LITESPARK-024 study of belzutifan with or without palbociclib in patients with advanced renal cell carcinoma (RCC).**

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Background: First-line treatment with immunotherapy alone or in combination with antiangiogenic agents is a standard of care for advanced RCC. Many patients (pts) develop resistance to first-line treatment and effective second-line and beyond options are needed. The von Hippel-Lindau (*VHL*) gene is inactivated in approximately 90% of RCC cases, which results in the constitutive activation of hypoxia-inducible factor 2α (HIF-2α), a key oncogenic driver in RCC. The first-in-class HIF-2α inhibitor belzutifan has demonstrated promising antitumor activity with manageable safety in previously treated pts with advanced RCC. The cyclin-dependent kinase (CDK) pathway has also been implicated in RCC and is associated with poor clinical outcomes. In RCC cell lines, the CDK 4/6 inhibitor palbociclib inhibited cell growth. The addition of CDK 4/6 inhibition had synergistic antiproliferative effects with HIF-2α inhibition in HIF-2α-dependent *VHL* -/- RCC cell lines. Palbociclib could therefore potentially enhance the efficacy of belzutifan as combination therapy for previously treated pts with advanced RCC.
Methods: The two-part, open-label, multicenter, phase 1/2 randomized LITESPARK-024 study (NCT05468697) is intended to establish the recommended phase 2 dose (RP2D) of belzutifan + palbociclib in combination with a modified toxicity probability interval design (Part 1), followed by a direct comparison of belzutifan monotherapy to the combination with respect to safety and efficacy in pts with advanced RCC (Part 2). Pts with histologically confirmed unresectable stage IV RCC with a clear cell component, whose disease progressed on or after having received at least 2 systemic treatments (both an anti-PD-1/PD-L1 monoclonal antibody and a VEGF receptor-targeted tyrosine kinase inhibitor, in sequence or in combination), have measurable disease per RECIST v1.1 by blinded independent central review, and have KPS score of ≥70% will be enrolled. Up to 30 pts will be enrolled into 3 dose groups in Part 1 and will receive belzutifan 120 mg once daily + palbociclib (75, 100, or 125 mg) daily for 21 consecutive days followed by 7 days off. In part 2, approximately 150 pts will be randomly assigned 2:1

to receive belzutifan 120 mg once daily + palbociclib RP2D (21 consecutive days/7 days off) or belzutifan 120 mg once daily. Pts will be stratified by IMDC risk (0 vs 1-2 vs 3-6) and sarcomatoid histology (yes vs no) at randomization in part 2. The primary end point for part 1 is to assess dose-limiting toxicities and adverse events to determine the RP2D of belzutifan + palbociclib. The primary end point for part 2 is ORR per RECIST v1.1 by investigator assessment. Secondary end points for part 2 are CBR, DOR, PFS, OS, and safety and tolerability. This abstract was accepted and previously presented at the 2023 ASCO Genitourinary Cancers Symposium. 

**CT121**

**ARX517, an anti-PSMA ADC targeting mCRPC resistant or refractory to standard therapies: A phase 1 dose escalation and dose expansion study (ARX517-2011, NCT04662580).**

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Background: Targeted therapies against prostate-specific membrane antigen (PSMA) have exhibited promising antitumor activity in prostate cancer. With the FDA's approval of (177) Lu-PSMA-617, PSMA has been validated as a target to treat metastatic castration-resistant prostate cancer (mCRPC). ARX517 is an antibody-drug conjugates (ADC) composed of a fully humanized anti-PSMA mAb site-specifically conjugated to AS269, a potent tubulin inhibitor, yielding a drug-to-antibody (DAR) ratio of 2. After binding to PSMA on the surface of cancer cells, ARX517 is internalized and delivers a cytotoxic payload which inhibits tubulin polymerization and induces cellular apoptosis. ARX517's site-specific linkage, stable conjugation chemistry, and non-cleavable linker exhibit a homogenous drug-antibody-ratio, mAb-like biophysical properties, and exceptional stability. Therefore, ARX517 promotes highly specific tumor cell apoptosis with minimal off-target bystander activity.
Trial Design: The Phase 1 study's dose escalation and dose expansion evaluates the safety, pharmacokinetics, and preliminary evidence of anti-tumor activity of ARX517 in adults with mCRPC. Patients must have mCRPC which is resistant or refractory to standard therapies, including androgen receptor signaling inhibitors, and show disease progression by Prostate Cancer Working Group 3 (PCWG3) criteria. All patients must have adequate organ function and brain metastases must be radiographically stable. Ascending dose levels of ARX517 administered as a single agent will use an i3+3 design in the dose escalation phase. During the dose expansion phase, ARX517 will be administered at the recommended phase 2 dose (RP2D) or maximum tolerated dose (MTD). Exploratory pharmacodynamic assessment includes PSMA-PET/CT imaging. The total number of subjects is dependent on the number of ascending doses and cohort size before dose expansion.
Status: ARX517-2011 began enrolling patients in July 2021. With no dose limiting toxicities through dose level 6 (2.0 mg/kg Q3W), dose escalation remains ongoing. As of January 2023, the APEX-01 study is currently enrolling in cohort 7 (2.4 mg/kg) of dose-escalation. Dose expansion will start after RP2D or MTD.

**CT122**

**A phase 1/2 open-label, multicenter, dose escalation and expansion study of AVB-001, an intraperitoneally administered, cell-generated, human IL-2 immunotherapy in patients with platinum-resistant, high-grade, serous adenocarcinoma of the ovary, primary peritoneum, or**

**fallopian tube.**

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Background: The potential of cytokines as cancer therapeutics has been limited by short half-life and severe adverse effects associated with high systemic exposure when delivered intravenously. Many strategies are being explored to overcome these limitations. A locoregional delivery approach to achieve high sustained local concentrations in the tumor microenvironment with minimal systemic exposure could widen the therapeutic window. Early experience with free recombinant human IL2 (rhIL-2) given intraperitoneally (IP) showed meaningful clinical activity in relapsed ovarian cancer. Still, the cumbersome delivery procedure requiring indwelling catheters and need for high volume IP infusions leading to frequent complications and poor patient compliance limited the utility of this approach. To overcome these shortcomings, we developed a clinically translatable localized delivery LOCOcyte™ platform composed of polymer encapsulated allogeneic epithelial cells engineered to produce immune effector molecules for local delivery with temporal regulation. The first product, AVB-001, produces native hIL-2, for the treatment of ovarian cancer and other peritoneal malignancies. IP AVB-001 inhibited tumor growth and improved survival in an *in vivo* ID8 ovarian cancer murine model. Sustained IL-2 production with well tolerated high IP concentrations were achieved, with >100x differential concentration vs. systemic blood levels in both mice and non-human primates. Strong local and systemic immune activating effects, optimized T cell profile and immune memory were observed without concomitant increase of T regs. The first in human study of AVB-001 in patients with advanced ovarian cancer (NCT05538624) is described here.
Methods: Part 1 dose escalation exploring 4 ascending dose levels (capsules target production of 0.6, 1.2, 2.4, and 3.6 ug hIL2/kg/d) with a Bayesian Optimal Interval 3+3 design, in patients with recurrent high grade serous adenocarcinoma of the ovary, primary peritoneum, or fallopian tube. A minimum of 12 and up to 24 patients will be enrolled to receive one dose of AVB-001 administered IP. The primary objective is to evaluate safety (Incidence and severity of adverse events per NCI CTCAE v5.0), tolerability, and feasibility of delivering AVB-001 IP, and establish the RP2D. Secondary objectives include assessment of antitumor activity (RECIST v1.1, iRECIST), pharmacokinetics, and pharmacodynamic correlates of immune activation. Part 2 will enroll 20 patients at the RP2D with efficacy as the primary objective. This multicenter study will be conducted at 5 sites, currently one site is open. The first patient was dosed in December 2022. Recruitment in dose level 1 cohort continues.

**CT123**

**Trial in progress: First-in-human immunotherapy-trio for advanced head and neck squamous cell carcinoma.**

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**Background:** Glucocorticoid-Induced Tumor Necrosis Factor Receptor-related protein (GITR) is a co-stimulatory pathway that when triggered has potent effects on T-cell memory, proliferation and anti-tumor activity. Preclinical models identified significant synergy between anti-GITR agonist therapy and cancer vaccines to generate stronger tumor specific CD8 T cell responses. DPV-001 is an “off-the-shelf” multivalent autophagosome vaccine generated by in vitro manipulation of the autophagy pathway in human cancer cell lines. The vaccine delivers short-lived proteins (SLiPs) and defective ribosomal products (DRiPs) which are likely the dominant epitopes directly presented by MHC class I of tumor

cells; but because of proteosomal degradation, are normally unavailable for cross-presentation, hence the delivery via vaccine. We hypothesize that addition of aGITR to DPV-001 vaccine will augment expansion of reactive CD4 and CD8 T cells, attenuate contraction of this response, and improve the therapeutic effect of treatment, and will result in the development of a coordinated T and B cell response to some of the same proteins, detectable using a cutting-edge seromics approach, as a window to TCR target identification for immunodynamic tracking of induced anti-cancer responses at an advanced level.
**Methods:** Patient recruitment began in August 2022, for this first-in-human immunotherapy-trio study of DPV-001, with sequenced checkpoint inhibition (aPD-1 mAb; retifanlimab), with or without aGITR agonist mAb (INCAGN-1949), in recurrent or metastatic HNSCC (NCT04470024). Patient population to include HPV-positive or HPV-negative, ECOG 0-2, with therapy continued until confirmed progression (RECIST 1.1), up to 2 years. Primary objective is safety, DLT ≤ 33%, with secondary efficacy objectives of ORR (PR+CR) and 2 year OS. Initial safety lead-in (n = 3+3 per arm), will be followed by phase Ib expansion of one/both arms if immunologically promising, 28 patients per arm, futility if <4/15 responses.

| | |
|---|---|
| Study Drugs | Cyclophosphamide 300mg/m2 IV, priming Day (-2) onlyVaccine (DPV-001)- Day 1 intranodal US bilateral inguinal- Days 8,15 intradermal, then q2wks to week 22- Thereafter q4wks until progression, up to 2 yearsaPD-1 (retifanlimab) 500mg IV q4wks, start Day 15 (Arms 1 & 2)aGITR (INCAGN01876) 300mg IV q2wks, start Day 1 (Arm 2 only) |
| Response (RECIST 1.1) | CT weeks 8 and 12, then q3mos |
| Immunologic Monitoring | PBL and sera are collected regularly and PBL are evaluated by flow cytometry. Biopsies obtained at baseline, Day 15 and Day 57, analyzed by mIF and 10x scRNA-Seq. Sera analyzed by phage immunoprecipitation (PhIP) sequencing for reactivity against the human proteome. Immune monitoring modifications that allow for improved characterization of immune cell subsets will be presented. |

**CT124**

**A first-in-clinic phase 1 study of GSK3745417 STING agonist in relapsed/refractory acute myeloid leukemia and high-risk myelodysplastic syndrome.**

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Background: Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are hematologic malignancies arising from immature myeloid progenitor cells in bone marrow. Depending on age and genetic disposition, as many as 40% to 60% of patients may have disease that is refractory to primary treatment, and of those who achieve initial complete response, more than 40% will experience relapse. In the relapsed/refractory setting, median survival is limited and therefore represents an unmet medical need. Stimulator of interferon genes (STING) is the key adaptor molecule in the cGAS-STING-TBK1 pathway, which mediates the sensing of cytosolic DNA, and its activation generates type I interferons (IFNα and

IFNβ) and pro-inflammatory cytokines instigating T-cell-dependent antitumor immunity. In addition to their immune stimulatory activity, STING agonists have demonstrated direct cytotoxic activity against AML cells in preclinical studies. STING is expressed at a higher level in AML cells than in other tumor types (The Cancer Genome Atlas, National Cancer Institute), which may lead to the observed cytotoxicity upon STING activation (Gulen MF, et al. *Nat Commun* 2017.8:427). The high level of STING expression combined with preclinical evidence of antitumor activity makes AML a suitable indication in which to establish proof of mechanism and evaluate the clinical activity of GSK3745417, a novel STING agonist that has so far only been studied in solid tumors.
Methods: This phase 1 study (NCT05424380) will evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, immunogenicity, and cytotoxicity of GSK3745417 administered as an intravenous infusion. Eligible patients will be 18 to 75 years of age with a diagnosis of relapsed or refractory AML (World Health Organization criteria) or high-/very high-risk MDS by (Revised International Prognostic Scoring System) that has relapsed after or been refractory to prior therapy with a hypomethylating agent. Patients are currently being enrolled in part 1 dose escalation, which incorporates intrapatient dose escalation within cohorts, to determine a cohort-level maximum tolerated dose. Dosing in part 1 will include 3 initial induction cycles of GSK3745417 followed by a maintenance schedule. Part 2 involves an expansion cohort receiving the recommended induction regimen from part 1, followed by
Montesinos, et al. AACR 2023maintenance dose escalation. Approximately 72 patients will be enrolled: approximately 22 patients in part 1 and 50 patients in part 2. Peripheral blood and bone marrow sampling will allow response assessment and biomarker analysis to understand the mechanism of GSK3745417 activity in AML and MDS.

**CT125**

**A Phase 1 study to evaluate the safety and tolerability of a combination autologous CD19 CAR T cell therapy (SYNCAR-001) and orthogonal IL-2 (STK-009) in subjects with relapsed or refractory CD19 expressing hematologic malignancies (NCT05665062).**

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**Background:** Chimeric antigen receptor T cell therapy (CAR T) has demonstrated remarkable clinical efficacy in hematological malignancies. However, compromised T cell effector function, proliferation, and persistence can limit CAR T from reaching their full curative potential. Interleukin-2 (IL-2) is a potent stimulator of T cells, however therapeutic use of IL-2 is limited by systemic toxicity due its pleiotropy. Therefore, to provide a selective IL-2 signal to engineered T cells, we have developed a human orthogonal ligand/receptor system consisting of a half-life extended pegylated IL-2 mutein (STK-009) and a mutated IL-2 Receptor Beta (hoRb) that responds to STK-009 but not wild type IL-2. SYNCAR-001 is an autologous CAR T co-expressing a CD19 CAR and hoRb on the T cell surface. In mouse models of refractory lymphoma, STK-009 treatment led to expansion and activation of SYNCAR-001 cells with a maintenance of stem cell memory and effector T cell phenotypes. When added to SYNCAR-001, STK-009 increased complete response rate and durable responses in a dose dependent manner. In non-human primate studies, STK-009 alone demonstrated no significant biological activity in IL-2 sensitive populations (T cells or NK cells) and was tolerable without toxicity.
**Methods:** This is a first-in-human, open-label, dose escalation study of combination SYNCAR-001 + STK-009 in adults with relapsed or refractory (r/r) CD19+ hematologic malignancies**.** The objectives of this study are to evaluate the safety, preliminary efficacy, pharmacokinetics, immunogenicity, and pharmacodynamics of SYNCAR-001 + STK-009. Dose escalation follows a standard 3+3 design with

STK-009 being escalated while SYNCAR-001 is held at a single fixed dose. A dose extension will enroll a cohort of patients treated at a selected dose level and indication based on dose escalation findings. SYNCAR-001 is dosed intravenously (IV) once at Day 0 and STK-009 is dosed subcutaneously (SC) over the course of the study. Participants receive a single safety lead-in dose of STK-009 prior to lymphodepletion and subsequent SYNCAR-001 infusion. After SYNCAR-001 initiation, STK-009 is dosed SC weekly for 12 weeks and then monthly for 3 months. Eligible participants include individuals with r/r chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and selected r/r B-cell non-Hodgkin lymphoma (NHL subtypes of large B cell, mantle cell, and indolent lymphoma). Prior CD19 CAR use is excluded. The primary endpoint of safety includes outcomes such as adverse events and dose-limiting toxicities. Secondary endpoints include assessments of response, pharmacokinetics, and immunogenicity. Exploratory endpoints include assessment of immune cell populations, and relevant gene/protein expression, as well as persistence, phenotype, and functionality of SYNCAR-001 in the peripheral blood and/or bone marrow in response to STK-009.

**CT126**

**An open-label, first-in-human study of BAY2927088 in patients with advanced non-small cell lung cancer (NSCLC) harboring an EGFR and/or HER2 mutation.**

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**Background:** Despite targeted agents recently approved for treating patients with advanced NSCLC harboring EGFR exon 20 insertion (ex20ins) mutations, there is still a high unmet need for more effective agents with good tolerability. BAY2927088 is an oral, reversible, highly potent TKI targeting EGFR and HER2 driver mutations with high selectivity for ex20ins vs wild-type EGFR. In addition, BAY2927088 retains potent antiproliferative activity in the presence of EGFR C797S acquired resistance (AR) mutation due to its non-covalent binding mode. In preclinical studies, BAY2927088 demonstrated strong dose-dependent *in vivo* tumor growth inhibition in NSCLC EGFR ex20ins mutant patient-derived xenograft models and models carrying the AR mutation C797S (Siegel et al, ENA 2022). The strong potency and high selectivity of BAY2927088 for mutant vs wild-type EGFR offers the prospect of an improved therapeutic window in the clinical setting vs available EGFR TKIs.
**Methods:** 21607 is an open-label, multicenter, first-in-human Phase I study of BAY2927088 in patients with advanced NSCLC harboring EGFR or HER2 mutations. The study comprises dose escalation, backfill, and dose expansion. The dose escalation is enrolling patients with EGFR or HER2 driver mutations and follows a modified continual reassessment method, allowing for concurrent backfill to previously cleared dose levels that demonstrated therapeutically relevant exposures or direct evidence of

clinical activity in patients harboring ex20ins or EGFR C797S mutations. Dose-limiting toxicities are evaluated for 21 days. The primary objective of dose escalation is to determine BAY2927088 safety, tolerability, PK, and maximum tolerated dose. Dose expansion will enroll patients based on specific EGFR/HER2 mutations and previous treatment, including those with ex20ins mutation either naïve or pretreated with ex20ins-targeted agents and those with AR EGFR C797S mutation. Key objectives of dose expansion are to further characterize BAY2927088 safety, tolerability, and to characterize PK of the optimal dose(s) selected during dose escalation and backfill. Key secondary objectives include determining antitumor activity based on overall response rate per RECIST v1.1 and the recommended Phase II dose of BAY2927088. Eligible patients must have measurable disease per RECIST v1.1, have a documented EGFR or HER2 mutation in tumor tissue or plasma, have disease progression after treatment with ≥1 systemic therapy for advanced disease, be appropriate candidates for experimental therapy, be aged ≥18 years, have ECOG PS of 0 or 1, and have adequate organ function. Key exclusion criteria include presence of serious cardiac conditions, interstitial lung disease, active CNS metastasis, or leptomeningeal disease. The trial is currently enrolling patients in dose-escalation and backfill parts (NCT05099172).

**CT127**

**A phase 1 study to assess BDTX-1535, an oral EGFR inhibitor, in patients with glioblastoma or non-small cell lung cancer.**

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**Background:** The epidermal growth factor receptor (EGFR) is a potent oncogene commonly altered in many cancers, including glioblastoma (GBM) and non-small cell lung cancer (NSCLC). EGFR tyrosine kinase activity driven by common EGFR mutations can be inhibited by small molecules, however, resistance to available agents may be driven by mutations in the EGFR active kinase site or other regions. BDTX-1535 is an orally available, highly potent, selective, irreversible inhibitor of EGFR mutations, including extracellular variants and amplifications commonly expressed in GBM and inhibits the uncommon EGFR mutations found in NSCLC, including the C797S mutation acquired following 3rd generation EGFR inhibitor therapy. Preclinical data demonstrated the ability of BDTX-1535 to cross the blood-brain barrier and produce sustained inhibition of EGFR signaling. Preclinical studies suggest that BDTX-1535 has potential to be clinically active in suppressing tumor growth in patients with GBM and NSCLC with or without CNS metastases, including a potential survival benefit.
**Methods:** BDTX-1535-101 (NCT05256290) is Phase 1, open-label, multicenter study to assess the safety, tolerability, PK, CNS penetrance, and preliminary antitumor activity of BDTX-1535 in recurrent GBM (rGBM) or locally advanced or metastatic NSCLC with or without CNS disease. The Monotherapy Dose Escalation portion will evaluate BDTX-1535 in patients with either rGBM expressing EGFR alterations or locally advanced/metastatic NSCLC harboring sensitizing EGFR mutations with or without CNS disease. Patients with rGBM must have previously received available standard therapy of surgical resection followed by chemoradiotherapy and/or temozolomide (TMZ). Eligible NSCLC patients must have EGFR mutated NSCLC that has progressed following standard of care EGFR inhibitor therapy. Once a provisional recommended Phase 2 dose (RP2D) has been established, BDTX-1535 monotherapy will be explored in the following Dose Expansion cohorts to further evaluate safety, PK, and preliminary

assessment of efficacy: 1) rGBM with confirmed EGFR alterations, 2) NSCLC with uncommon EGFR mutations following EGFR inhibitor therapy; 3) NSCLC with acquired EGFR resistance mutation following a 3rd generation EGFR inhibitor in 1L setting. NSCLC patients may enroll with or without CNS metastases and must not be known to express excluded resistance mutations such as EGFR T790M or MET. BDTX-1535 will also be studied in combination with TMZ to assess safety, tolerability, and a recommended combination dose for the treatment of patients with rGBM harboring EGFR mutations or variants. Enrollment was initiated in 2022 and dose escalation is ongoing. Dose Expansion cohorts are expected to open in 2023. For additional information, please contact BDTX_1535_101_Study@bdtx.com

**CT128**

**Phase 1 study of HSK29116, a Bruton tyrosine kinase (BTK) proteolysis-targeting chimera (PROTAC) agent, in patients with relapsed or refractory B-cell malignancies.**

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BACKGROUND: BTK, a non-receptor kinase in the B-cell receptor signaling pathway, plays an essential role in B-cell development and differentiation. BTK is critical for malignant B-cell survival. Oral BTK inhibitors (BTKis), standard treatments for patients (pts) with B-cell malignancies, prevent proliferation and induce apoptosis of B cells. Currently approved BTKis covalently bind to BTK residue C481, irreversibly inhibiting phosphorylation of downstream kinases and blocking B-cell activation. Because these agents are often given continuously, their use may lead to clinical resistance and unacceptable toxicities. Indeed, acquired resistance to covalent BTKis is common and caused by the expansion of clones with a cysteine-to-serine mutation at residue 481.
A novel strategy being developed to overcome BTKi resistance, is PROTAC-induced degradation of BTK. PROTACs are small molecules with one domain that binds the target protein linked to another domain that binds to ubiquitin E3 ligase, resulting in ubiquitin-dependent proteasome degradation of the target protein. HSK29116 is a small BTK PROTAC molecule that is currently undergoing clinical investigation for the treatment of B-cell malignancies. In preclinical studies, HSK29116 overcame drug resistance caused by the C481S BTK mutation. Also, HSK29116 has demonstrated inhibition of B-cell proliferation without measurable loss of other BTK-family kinase activities. This kinase selectivity may limit some of the off-target toxicities seen with currently available BTKis.
METHODS: This first-in-human, multicenter, open-label, phase 1 study (NCT04861779) began recruiting in 2021 to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics of oral HSK29116. Eligible pts are ≥18 years old with diagnosis of a B-cell malignancy that has relapsed or become refractory to standard therapy (≥2 prior systemic therapies, which may have included a BTKi) and for which no other treatments known to provide clinical benefit exist. Pts must have an Eastern Cooperative Oncology Group performance status ≤2 and life expectancy >3 months. Phase 1a includes dose escalation to identify dose-limiting toxicities and establish the maximum tolerated dose and/or recommended dose of HSK29116 for a phase 1b dose expansion, initiating in the US. Approximately 36 pts will be enrolled in phase 1a and 90 pts in phase 1b (≥40 pts must have previously received covalent BTKis). The primary endpoint is safety. Secondary endpoints include PK and investigator-assessed overall response rate, duration of response, time to response, and progression-free survival. Exploratory analyses will evaluate the relationship between HSK29116 anti-tumor activity and *BTK* gene mutations, as well as BTK protein degradation effects of HSK29116 . Descriptive statistics will be used to summarize all data.

**CT129**

**Trial in progress: ATHENA-1 - a phase 1, open-label, first-in-human study to assess safety and tolerability of REGN5837 in combination with odronextamab in patients with relapsed/refractory aggressive B-cell non-Hodgkin lymphomas.**

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Background: Many patients with relapsed/refractory (R/R) aggressive B-cell non-Hodgkin lymphoma (B-NHL) are unable to tolerate, access, or benefit from intensive chemo-therapeutic approaches or cellular therapies and will invariably relapse; therefore, novel approaches are urgently required. Odronextamab (REGN1979) is a hinge-stabilized, human CD20×CD3 IgG4-based bispecific antibody that elicits T-cell-mediated cytotoxicity of malignant B cells. In a Phase 1 study, odronextamab monotherapy showed a manageable safety profile with encouraging preliminary activity in heavily pre-treated patients with R/R B-NHL (Bannerji R, et al. *Lancet Haematol.* 2022;9(5):e327-39). REGN5837 is a hinge-stabilized, human CD28×CD22 IgG4-based bispecific antibody that provides a co-stimulatory signal (signal 2). When combined with odronextamab (signal 1), REGN5837 improved anti-tumor efficacy and survival in in vivo diffuse large B-cell lymphoma tumor models via enhanced T-cell expansion. We hypothesize that combining REGN5837 with odronextamab may deepen and extend anti-tumor activity in patients with aggressive lymphoma.

Methods**:** ATHENA-1 (NCT05685173) is a Phase 1, open-label, first-in-human study of REGN5837 in combination with odronextamab in patients with R/R aggressive B-NHL. During induction, odronextamab and REGN5837 will be administered weekly over 21-day cycles. To mitigate potential CRS events, odronextamab will be introduced with step-up dosing as a monotherapy, followed by introduction of REGN5837 on C2 D15 with step-up dosing. Maintenance will consist of 28-day cycles (odronextamab and REGN5837 administration on D1, 15). Patients who achieve a sustained complete response (≥9 months) will have study drug(s) administration changed to once every 4 weeks. Patients must be aged ≥18 years, have Eastern Cooperative Oncology Group performance status ≤1, with adequate organ function, and have CD20+ aggressive B-NHL that progressed after ≥2 lines of systemic therapy containing at least an anti-CD20 antibody and an alkylating agent, with or without prior chimeric antigen receptor T-cell therapy. Exclusion criteria include prior allogeneic stem cell transplant, organ transplant, or CD20xCD3 bispecific antibodies, or mantle cell lymphoma or central nervous system lymphoma. Primary endpoints are incidence of dose-limiting toxicities and the incidence and severity of treatment-emergent adverse events. Secondary endpoints include pharmacokinetics of odronextamab and REGN5837, anti-drug antibody incidence, objective response rate, complete response rate, duration of response, progression-free survival, and overall survival. Enrolment is planned to open in early 2023.

**CT130**

**An open label, dose escalation, phase 1 study of AT101, a novel CD19-directed CAR-T cell therapy targeting a membrane-proximal epitope of CD19, in patients with relapsed or refractory B cell non-Hodgkin lymphoma.**

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Medicine, Ulsan, Republic of Korea; [5]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea.

Background and Preliminary Data: All the FDA-approved CD19 CAR-T cell therapies are based on an antigen-binding domain (scFv) based on the FMC63 antibody which binds to the membrane-distal region of CD19 to an epitope encoded by exons 3 and 4 (Klesmith JR, Biochemistry, 2019; Zhang Z, JITC, 2020). While these CART19 products are very effective in the clinic, the majority of patients still do not respond or eventually relapse due to several mechanisms of resistance, including T cell dysfunction and epitope CD19-negative escape. Novel strategies to enhance the activity of CART cells and reduce escape are critically needed. We recently demonstrated that modifications of the binding region of the CAR (scFv) (*Singh N., Nat Med, 2021*) can drastically change the interaction between the CAR T cell and the cancer cells, potentially improving the anti-tumor effect. To this goal, we developed a novel anti-CD19 antibody clone (1218) that binds to a membrane-proximal epitope of CD19 (exon 2 region K59-K63) thereby not competing with FMC63. We developed a novel CART19, called AT101, using a humanized 1218 scFv along with 4-1BB costimulatory and CD3zeta domain in a lentiviral backbone. In preclinical models, AT101 showed more potent *in vitro* cytotoxicity against CD19-positive B lymphoma cells in a long-term killing assay and in a B-ALL (NALM6) *in vivo* model as compared to the control of FMC63 based CAR-T cells. In addition, differently than FMC63-based CART, AT101 could target tumor cells expressing point mutations of CD19 that are associated with relapse post-CART19 (FMC63) (*Zhang Z, JITC, 2020*) and leukemic blasts aberrantly expressing FMC63 CAR19 on their surface (*Ruella M, Nat Med, 2018*). Based on the preclinical efficacy and safety, a phase 1 clinical trial testing autologous AT101 was started for patients with relapsed and refractory B-cell non-Hodgkin lymphoma.
Trial Design and Methods: This open-label, multi-center, first-in-human Phase 1 study will assess the safety and feasibility of AT101 in patients with relapsed or refractory B cell non-Hodgkin lymphoma. Key eligibility criteria include patients aged ≥19 years of age with histologically confirmed relapsed or refractory aggressive B-cell non-Hodgkin lymphoma. In this phase 1 trial, patients (n=3 per dose level; up to n=18 in total) are treated with AT101 in 3 dose-escalation cohorts based on a standard 3 + 3 design. CART doses are 2.0 x $10^5$, 1.0 x $10^6$, or 5.0 x $10^6$ CAR+T cells/kg. The primary objective is to determine the safety, the maximum tolerated dose (MTD), and the recommended phase 2 dose (RP2D) of AT101 in participants following lymphodepletion with cyclophosphamide and fludarabine (250 mg/$m^2$ and 25 mg/$m^2$). The secondary objective is to evaluate the preliminary efficacy assessments (overall response rate (ORR), duration of response (DOR), progression-free survival (PFS), overall survival (OS), event-free survival (EFS), and pharmacokinetics of AT101. Exploratory objectives include assessment of CD19 expression and cytokines in the blood. Patients will be followed for safety for at least 60 months post AT101 infusion. Clinical trial registry number: NCT05338931. As of January 11, 2023, AT101 has been infused to six patients in cohort 1 and three patients in cohort 2. Detailed results will be presented at the meeting.

**CT131**

**Initial preclinical and clinical experience of autologous engineered monocytes in T cell lymphoma patients.**

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For many patients with T cell lymphomas (TCL), sustained clinical benefit with standard treatment options is elusive. Myeloid cells, such as monocytes, dendritic cells and macrophages, readily accumulate in tumors, whereby they support tumor progression. The ability to harness the capability of myeloid cells to penetrate into tumors, and to subsequently program them to activate and elicit broad anti-tumor

immunity has the potential to transform cancer therapies. With this goal we developed the first engineered monocyte cell product, by manipulating autologous monocytes to express a novel chimeric antigen receptor (CAR). This CAR contains a tumor recognition domain fused to a CD8 hinge domain, Fcγ and PI3K intracellular signaling domains. In addition to imparting tumor specificity, the Fcγ and PI3K signaling domains promote phagocytosis, cytokine production and antigen presentation upon activation. In a rodent model of melanoma ($gp75^+$ B16/F10-OVA), Ly6C+ monocytes engineered with this receptor were able to phagocytose tumor cells and cross present antigen *in vitro. In vivo* infusion of engineered monocytes was associated with significant suppression of tumor growth. FACS analysis of tumor-infiltrates demonstrated that engineered monocytes preferentially infiltrated tumors and differentiated into antigen presenting cells. Adoptive T cell transfer of CFSE-labeled OT-I $CD8^+$ T cells confirmed that treatment was also associated with increased accumulation of tumor specific T cells in tumor and spleen. Based on these promising data, MT-101 is being assessed in humans in the Phase 1, open-label, first-in-human trial in patients with refractory or relapsed T cell lymphoma, IMAGINE trial (NCT05138458). The primary objective is the assessment of safety and tolerability at Day 28, following 3 weekly cycles of 2 infusions. Secondary objectives include assessment of correlative markers of response, pharmacokinetics, and efficacy. In the first 3 subjects, MT-101 has been well-tolerated, with no evidence of cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), or infusion reactions. Examination of biomarkers by CyTOF in one subject shows changes in circulating leukocytes, including B cells. In this subject, survival has been greater than 10 months, while the median overall survival of patients with R/R PTCL is 5.5 months. Continued patient enrollment and data collection is ongoing to confirm these observations.

**CT132**

**Trial in progress: A phase 1/2 study of ASP1570, a novel inhibitor of DGKζ, in participants with locally advanced or metastatic solid tumors who have progressed on, or are ineligible for, all available standard therapies.**

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**Background:** Checkpoint inhibitors and have been transformative in the treatment practices of oncology. However, only a subset of all patients in most cancer types effectively respond to these therapies and acquired resistance is common, causing some patients who initially respond to experience disease progression. Thus, there is a significant opportunity for immunotherapy expansion in cancer treatment. Diacylglycerol kinase (DGK) is a large family of mammalian isoenzymes that catalyzes the conversion of diacylglycerol (DAG) to phosphatidic acid. Even when PD-1 is blocked by anti-PD-1 antibodies, there may be partial inactivation of T cells by DGK. ASP1570 is a novel inhibitor against DGKζ and has the potential to enhance DAG downstream signaling which can activate T cells regardless of PD-1 signaling and lead to tumor killing. ASP1570 restored T-cell functions suppressed by multiple immunosuppressive signals and induced tumor growth inhibition in mice models of MC38 (anti-PD-1 sensitive) and B16-F1 (tumor-infiltrating lymphocyte [TIL] poor, anti-PD-1 insensitive). Taken together, ASP1570 treatment as a single agent and/or in combination with anti-PD-1 therapy for locally advanced or metastatic solid tumors may provide clinical benefit.
**Methods:** This is a phase 1/2, open-label, multicenter, multiple-dose, dose-escalation/expansion study of

ASP1570 in participants with locally advanced or metastatic solid tumors. The study will enroll approximately 168 participants into 2 phases. Part 1 consists of dose escalation cohorts of 3-12 patients receiving oral administration of ASP1570 in 21-day cycles; 8 total cohorts are planned with doses of 10, 25, 50, 75, 100, 150, and 200 mg administered daily with the final cohort receiving 100 mg administered twice daily. Part 2 consists of ~20 participants per cohort in a two-stage evaluation of safety and efficacy at the recommended phase 2 dose and cohort expansion of an additional ~20 participants each based on expansion criteria. Cohorts are designated for NSCLC and melanoma but may be expanded based on response in other tumor types. Primary endpoints will assess safety and tolerability through dose-limiting toxicities, adverse events, changes in laboratory tests, electrocardiogram results, and vitals. Secondary endpoints will assess efficacy through overall response rate, duration of response, and disease control rate per iRECIST and RECIST v1.1, pharmacokinetics, and influence on TILs. Study periods will consist of screening, treatment with ASP1570 oral dosing in 21-day cycles, end of treatment, follow-up (safety: 45 days after last dose and every 9 weeks thereafter and survival follow-up every 12 weeks), and end of study. The study is actively recruiting and has enrolled 13 participants as of January 2023.

**CT133**

**Trial in progress: a multicenter phase 1/1b dose escalation study of WTX-124 as a monotherapy and in combination with pembrolizumab in patients with selected advanced or metastatic solid tumors.**

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Background: High-dose recombinant human IL-2 (HD IL-2) is an FDA-approved therapy that produces durable complete remissions in a subset of patients with advanced or metastatic cutaneous melanoma and renal cell carcinoma. However, the clinical benefit of HD IL-2 is counterbalanced by life-threatening toxicities such as capillary leak syndrome. WTX-124 is an engineered cytokine prodrug composed of a wild-type IL-2 fused via proprietary cleavable linkers to an inactivation domain and to a half-life extension domain. In the circulation and in normal tissues, WTX-124 is designed to remain inactive and not bind to high- ($\alpha\beta\gamma$) and intermediate-affinity ($\beta\gamma$) IL-2 receptors. In contrast, proteolytic activation of WTX-124 in the tumor microenvironment (TME) is expected to liberate the IL-2 cytokine to stimulate antitumor immune responses. Preclinical data show that WTX-124 is activated by a range of dissociated human tumors *in vitro* but not by normal human cells or by patient serum. Moreover, WTX-124 demonstrates potent antitumor activity in multiple murine syngeneic tumor models with substantial expansion and activation of tumor-infiltrating lymphocytes. In the MC38 model, WTX-124 has a large therapeutic window of >19-fold compared to ~3-fold for recombinant human IL-2.
Methods: This first-in-human, open-label phase 1/1b trial is investigating the safety, tolerability, pharmacokinetics, pharmacodynamics, immunogenicity, and antitumor activity of WTX-124 administered as a monotherapy or in combination with the anti-PD-1 antibody pembrolizumab to patients with advanced or metastatic solid tumors (NCT05479812). All patients must have ≥ 1 measurable lesion per RECIST 1.1 and a tumor type for which immune checkpoint inhibitor (ICI) therapy is indicated. Dose escalation uses a modified toxicity probability interval-2 study design with WTX-124 administered intravenously (IV) every two weeks in 28-day cycles. If the safety of WTX-124 as a monotherapy is established in the first three cohorts, the study will then initiate dose escalation of WTX-124 in combination with pembrolizumab 400mg IV every six weeks. Dose expansion is anticipated to include four arms that will enroll patients with either advanced or metastatic cutaneous melanoma or renal cell carcinoma to receive WTX-124 monotherapy or combination therapy (N=20 patients per arm). Patients

with melanoma assigned to the WTX-124/pembrolizumab combination arm may be naïve to all prior systemic therapy for advanced disease, but all other patients in expansion must have previously received a standard of care ICI regimen. Pre- and on-treatment tumor biopsies, archival tumor tissue, and blood samples will be used to investigate multiple exploratory biomarkers. As of January 12, 2023, WTX-124 monotherapy dose escalation is proceeding.

**CT134**

**Non-viral mesothelin-targeted CAR-T cells armored with IFNg-induced secretion of PD-1 nanobody in treatment of malignant mesothelioma in phase I clinical trial.**

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Background: Malignant pleural/peritoneal mesothelioma (MPM) is a rare, aggressive cancer with poor prognosis and high mortality of 65%-70% for pleural and 30% for peritoneal MPM. Patients who fail the standard therapy often survive less than 1 year, so it is urgent to develop new effective therapies for MPM patients. Chimeric antigen receptor (CAR)-T cells have been applied in MPM, but the efficacy was still limited due to immunosuppressive tumor microenvironment (TME). To overcome these obstacles, we developed armored CAR-T cells with nanobody targeting mesothelin (MSLN) and IFN-γ-activated secretion of PD-1 nanobody in a non-viral transposon system, named as BZDS1901. Preclinical studies have demonstrated cytotoxicity of the BZDS1901 in NCI-H226 lung/mesothelioma xenograft mouse model. To verify the safety and efficacy of BZDS1901, we conducted a single-arm, open label, dose-escalating clinical trials (NCT04503980, 05089266, 03615313) in solid tumors.
Methods: Eligible patients were those who failed prior standard therapies with MSLN expression (≥50%) and PD-L1 positive in tumor specimen and voluntarily signed the informed consent. After apheresis and lymphodepletion with cyclophosphamide and fludarabine. BZDS1901 was administered intravenously in dose cohorts ($1\times10^6$-$2\times10^7$/kg) and the second infusion was given if no disease progression. After infusion, safety was evaluated during 28 days by the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0, efficacy was assessed by RECIST 1.1 or mRECIST with CT scan. Blood CAR copies were measured by qPCR, PD-1 nanobodies and cytokines by Meso Scale Discovery method, and T cell subtypes by flow cytometry. Patients' progression-free survival (PFS) and overall survival (OS) were measured from the day of infusion to progression or death.
Results: From July 20, 2020 to December 31, 2022, 11 MPM patients were enrolled and completed the assessment, while most patients received two infusions. BZDS1901 was safe, demonstrated by 54% grade 3 and 15% grade adverse events (AEs) that were hematological side effects due to lymphodepletion and reversible with supportive care. No on-target, off-tumor toxicity and dose-limiting toxicity were observed. All patients showed expansion of CAR-T cells and increased PD-1 nanobodies in circulation. CAR-T Cmax (cp/μg) copies number was 20062, and continually detectable in blood over 4 months. PD-1 Cmax (pg/ml) was 82841, and continually detectable in blood for up to 9 months. IFN- γ and IL-6 also increased at day 4 or Day 7. All patients obtained objective tumor response, one with complete response, six with partial response, and four with stable disease. The total objective response rate was 63.64%. All enrolled patients are still alive, and mPFS and mOS are not reached. The longest PFS was up to 26 months. Median follow-up was four months.
Conclusions: PD-1 nanobody secreted and MSLN targeting CAR-T cells have demonstrated promising

efficacy on MPM patients. Besides CAR-T direct tumor killing activity, secreted PD-1 nanobodies may provide additional clinical benefit by invigorating CAR-T from PD-L1 inhibition, activating TILs and relieving local immunosuppression.

**CT135**

**A pilot clinical study of orotecan (oral irinotecan HCl, VAL-413) in patients with recurrent pediatric solid tumors.**

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Background: Intravenous irinotecan hydrochloride (IRN-IV) is approved for the treatment of adult colorectal cancer. IRN-IV is also used off-label to treat a range of adult and pediatric tumors including recurrent Ewing sarcoma, rhabdomyosarcoma, neuroblastoma, hepatoblastoma, Wilms tumor, gynecologic cancers, lung cancer and medulloblastoma. Previously, a regimen of IRN-IV administered as a 60-min i.v. infusion daily for 5 days in combination with other agents such as temozolomide has been recommended use in treating children with solid tumors (Blaney. ClinCanRes, 2001). Protracted administration schedule of intravenous irinotecan is inconvenient for patients, so oral regimens utilizing IRN-IV have been developed (Wagner. ClinSarcRes, 2015). Unfortunately, the palatability of the intravenous preparation is poor, leading to reduced compliance especially in younger pediatric patients. Development of an advanced formulation to improve tolerability and patient compliance is an important unmet need. VAL-413 is a novel formulation developed to improve palatability of oral irinotecan.
Methods: Eligibility: Up to 20 patients ≥ 1 year of age or ≤ 30 years of age with recurrent pediatric solid tumors and adequate bone marrow, renal and liver function, for whom irinotecan therapy is a treatment option will be enrolled.
Trial Design: Two different dose levels of VAL-413, 90mg/m2/day or 110mg/m2/day will be studied in combination with fixed-dose temozolomide using a standard 3 + 3 phase I design. In the event the starting dose of 90 mg/m2/day is not tolerable due to toxicity, a lower dose of 75 mg/m2/day may be implemented.
Treatment: During the first cycle of treatment, each patient will receive 4 daily doses of VAL-413 and one daily dose of the intravenous preparation of irinotecan taken orally (IRN-IVPO). During all subsequent cycles, only VAL-413 will be given with temozolomide in 5-day courses administered every 21 days, as tolerated.
Outcome Measures: Toxicity is assessed by NCI CCTCAEv5; tumor response is assessed by RECIST 1.1. A palatability survey instrument will assess palatability of VAL-413 vs. IRN-IVPO; comparative intrapatient pharmacokinetics of irinotecan and its metabolites will be assessed. This trial is ongoing (CT.gov: NCT04337177), with no DLT observed to date.

**CT136**

**An open-label phase I/IIa study to evaluate the safety and efficacy of CCS1477 as monotherapy and in combination in patients with advanced hematological malignancies.**

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**Background**
Inobrodib (CCS1477) is a first in class potent, selective, and orally bioavailable inhibitor of the bromodomains of p300 and CBP, two closely related histone acetyl transferases with oncogenic roles in hematological malignancies. In pre-clinical studies inobrodib potently inhibited cell proliferation in acute myeloid leukemia (AML), multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) cell lines. Inobrodib demonstrated dose-dependent efficacy in corresponding xenograft models, eliciting tumor regression at the highest doses. These effects were accompanied by significant reductions in expression of *MYC*, *MYB* and *IRF4*. Low doses of inobrodib also demonstrated good efficacy when added to standard-of-care treatments, providing a strong rationale for combination treatment in patients. Inobrodib represents a novel and differentiated approach and offers a potential new therapeutic option for patients who have relapsed or are refractory to current standard of care therapies in AML, higher risk MDS, MM or NHL.
**Study Design and Methods**
NCT04068597 is an adaptive multi-arm/multi-stage trial allowing exploration of p300/CBP inhibition as monotherapy or in combination with different agents across multiple indications including AML/higher risk MDS, MM, and NHL.
The trial was initiated as a dose escalation rolling 6 design separately in AML/MDS and MM/NHL to ensure tolerability is explored separately across groups of indications, with the possibility to expand in monotherapy across different indications. Key inclusion criteria include patients with confirmed relapsed or refractory disease, with patients having received standard therapy (typically at least two prior lines of therapy). Response criteria are assessed using standard methodologies for the different indications. Blood and tumor samples are collected for exploratory biomarker analysis to understand mechanisms of response to treatment or disease progression. The recommended phase 2 dose/schedule has been determined, and monotherapy expansion arms are currently open in selected indications.
Cohorts investigating dose escalation with standard of care agents pomalidomide/dexamethasone in MM as well azacytidine with or without venetoclax in AML or MDS are currently enrolling, with the possibility of expansion for promising combinations. An additional ‘rescue’ cohort for MM patient who failed inobrodib monotherapy will be used to determine whether addition of pomalidomide with or without dexamethasone may reverse resistance to inobrodib. This cohort will also explore tolerability and clinical activity of steroid-sparing combination regimen.

**CT137**
**ENLIGHTen-01: a phase 1 study of fluorescein-specific (FITC-E2)-car T cells in combination with folate-fluorescein (UB-TT170) for osteosarcoma.**
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Background: Outcomes for relapsed osteosarcoma (OS) remain poor and there are no systemic therapies that have been shown to provide a survival benefit. Chimeric Antigen Receptor (CAR) T cell immunotherapy involves the adoptive transfer of T-lymphocytes that have been engineered to recognize tumor-specific antigens, resulting in targeted lysis of malignant cells. Folate receptors (FR) are membrane-bound surface proteins that bind folates with high affinity. FRs are overexpressed in OS and have very limited expression in normal tissue. UB-TT170 (Umoja Biopharma) is a small molecule bispecific “adapter” consisting of folate conjugated to fluorescein (FL). It penetrates tumors in minutes and is retained for long periods of time due to high affinity for the FR, while unbound UB-TT170 rapidly clears from the blood and from FR- tissues. We hypothesize that administration of fixed dosing anti-FL(FITC-E2) CAR T cells followed by escalating doses of UB-TT170 will deliver personalized

immunotherapy to OS patients. In addition, this approach offers an attractive safety mechanism since administration of NaFL should reverse CAR T cell reactivity.
Methods: We designed a Phase I study for young adult (15-30 yr) patients with recurrent/refractory OS to examine the safety and feasibility of administering autologous, peripheral blood-derived T cells that have been genetically modified to express a 2nd generation FL-specific CAR in combination with intra-subject dose escalation of UB-TT170. The primary objective is to identify a recommended dose escalation sequence of UB-TT170 to move forward in clinical development. A 3+3 design will be used to investigate 3 possible dosing sequences. The trial opened in July 2022. As of January 5, 2023, 5 subjects have enrolled and one subject has been treated on dose regimen 1. T cell products have been successfully manufactured for all enrolled subjects. The trial remains open to enrollment. Clinical trial registration numbers: NCT05312411

**CT139**

**The STELLAR trial: A phase II/III study of high-dose intermittent sunitinib in patients with recurrent glioblastoma.**

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**Background:** Treatment options for patients with recurrent glioblastoma (rGBM) are limited. The lack of effectiveness from oral multi-targeted tyrosine kinase inhibitors, like sunitinib, might be due to a restricted blood-brain barrier penetration. We previously found that standard daily dosed sunitinib resulted in relatively low tumor drug concentrations in patients with GBM (PMID: 35165100). Based on these results, we here tested whether an alternatively scheduled, high-dose intermittent sunitinib treatment strategy with the aim to increase tumor drug concentrations, would improve the outcome of patients with rGBM compared to standard therapy with lomustine. Preliminary efficacy and safety results of the trial are presented.
**Methods:** The STELLAR-trial is a randomized multicenter phase II/III clinical study for patients with rGBM (NCT03025893). Patients were randomized 1:1 to high-dose intermittent sunitinib (300mg once every week (Q1W) in part 1 or 700mg once every two weeks (Q2W) in part 2) or to standard therapy with lomustine (110mg/m2 once every 6 weeks). Adult patients with de novo or secondary glioblastoma with first recurrence after a maximum of one line of chemotherapy and ≥ 3 months since completion of radiotherapy were eligible. The primary endpoint of the trial was progression-free survival (PFS). Secondary endpoints included overall survival (OS), six-month progression-free survival (PFS6) and tolerability of the treatment. A pre-planned interim analysis for futility was performed after inclusion of 25% of patients in both part 1 and part 2. **Results:** At the pre-planned interim analysis, after inclusion of 26 and 29 patients in part 1 and 2, respectively, the trial was terminated for futility. The median PFS (mPFS) for sunitinib 300mg Q1W was 1.5 months (95% CI 1.4 - 1.7) as compared to 1.5 months (95% CI 1.4 - 1.6) for lomustine (HR 1.24, 95% CI 0.55 - 2.79; p=0.60). For sunitinib dosed at 700mg Q2W, the mPFS was 1.4 months (95% CI 1.3 - 1.5) as compared to 1.5 months (95% CI 1.3 - 1.7) for lomustine (HR 0.96, 95% CI 0.44 - 2.10; p=0.92). The median OS (mOS) for sunitinib 300 mg Q1W was 6.5 months (95% CI 4.5 - 8.5) compared to 4.7 (95% CI 3.3 - 6.0) for lomustine (log rank p=0.83). For sunitinib 700mg Q2W the mOS was 4.7 months (95% CI 3.8 - 5.6) compared to 6.8 months (95% CI 1.7 - 12.0) for lomustine (log rank p=0.65). The percentage of patients that were progression free at 6 months was 8% for both sunitinib groups and 15% for the lomustine group. In general, both treatment strategies with either high-dose intermittent sunitinib or lomustine were tolerated well with maximal grade 3 toxicities in respectively 8% and 15% of patients.

**Conclusion:** Alternative high-dose intermittent sunitinib treatment failed to improve the outcome of patients with rGBM when compared to standard lomustine therapy. Since lomustine remains a poor treatment strategy in rGBM, novel, more effective therapies are urgently needed.

**CT141**

**Combination of hormone therapy, GnRH agonist, and immunotherapy enhance immune activation in premenopausal ER+/HER2- metastatic breast cancer patients: Results of biomarker analysis from a pilot phase II study.**

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**Introduction:** Immunotherapy has not been proved to be efficacious in ER+/HER2- metastatic breast cancer. Our team has reported an effective combination of exemestane, gonadotropin releasing hormone agonist, and pembrolizumab in premenopausal ER+/HER2- MBC. Here we report the updates of the final results and biomarker associates of the study.
**Methods:** Premenopausal ER+/HER2- MBC patients who failed no more than 2 lines of hormone therapy without chemotherapy for MBC were enrolled in a tertiary medical center in Taiwan (NCT02990845). The primary endpoint was progression-free survival (PFS) rate at 8 months. The secondary endpoints included overall response rate, overall survival (OS), progression-free survival (PFS), and other biomarkers. Pre-treatment (N=12) and post-treatment (N=5) tumor tissue were collected for biomarker analysis, including tumor-infiltrating lymphocyte(TIL), PD-L1 expression by immunohistochemical staining, tumor mutational burden, RNAseq, and IO360 analysis.
**Results:** A total of 15 patients were enrolled in the study with 14 evaluable patients. The progression-free survival rate at 8 months was 64.3%. The median PFS and OS were 10.34 months and 39.56 months respectively. The overall response was 35.7%. TIL, tumor mutational burden, or PD-L1 expression were not associated with the response to the treatment. In the NanoString IO-360 analysis, increased expression of genes in the immune related signature was noted in the post-treatment samples as compared to the pre-treatment samples. The expression levels of MHC class II and T cell-related genes were upregulated in the post-treatment samples. Most of the immune cell populations are increased after pembrolizumab/ exemestane/ leuprolide treatment. CD56 dim NK cells, Th1 cells, and NK cells are the top 3 populations that are increased after treatment. Using CIBERSORT algorithms to analyze RNAseq data from pretreatment samples, dendritic cells (p=0.039) and NK cells (p=0.082) were associated with the responders versus non-responders in the study.
**Conclusion:** In this final analysis, pembrolizumab, exemestane, and leuprolide remain an effective treatment for premenopausal ER+/HER2- MBC. Pembrolizumab, exemestane, and leuprolide treatment is associated with immune system activation, turning the tumor microenvironment from cold to hot in luminal disease.

**CT142**

**TALAVE: Induction talazoparib (tala) followed by combined tala and avelumab in patients (pts) with advanced breast cancer (ABC).**

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**Background:** PARP inhibitors (PARPi) significantly extend progression-free survival (PFS) compared to chemotherapy in pts with *BRCA1/2* mutated (*BRCA1/2*m) ABC, but responses are not durable. PARPi activate the cGAS-STING pathway leading to increased PD-L1 expression and cytotoxic T-cell recruitment, creating a tumor microenvironment (TME) that may be more vulnerable to immunotherapy. This study was conducted to evaluate the safety, efficacy and effects on the TME of the PARPi tala combined with the PD-L1 inhibitor avelumab in ABC, and to assess the impact of *BRCA1/2* status on clinical outcomes.
**Methods:** TALAVE was an open-label, multi-institutional trial (NCT03964532) for pts with HER2-negative ABC. Pts were enrolled in two cohorts: cohort 1 - *BRCA1/2*m and HER2-negative ABC; cohort 2 - *BRCA1/2* wildtype TNBC. Pts received a 4-week induction of tala (1mg po daily D1-D28), followed by a combination of daily tala and avelumab (800mg IV D1, D15). The primary objective was the safety and tolerability of the combination. Secondary objectives included ORR, OS and PFS. Pts underwent serial biopsies to investigate molecular signatures associated with BRCA status or clinical benefit using multiple omics techniques: RNA profiling by NanoString PanCancer IO 360™ Panel, GeoMx® Digital Spatial Profiler (DSP) Whole Transcriptome Atlas (WTA) and protein spatial analysis by multiplex immunofluorescence (mIF) and Cyclic Immunofluorescence (CyCIF).
**Results:** 12 pts were enrolled in each cohort. In cohort 1, 5 pts had *gBRCA1*, 6 had *gBRCA2* and 1 had *sBRCA2* mutation. The median age was 50 [IQR:43-59.5]; all pts were female, with median of 1 prior therapy for ABC [IQR: 0-2.5]. 42% pts had prior platinum. ORR was 42% (83% in cohort 1; 0% in cohort 2). There were 10 PRs, all in cohort 1. mPFS was 5.1 months (mo) (95% CI: 3.7-7.3 mo); 9.3mo in cohort 1 and 2.9mo in cohort 2. 5 out of 24 pts remain on treatment, all in cohort 1. Treatment related adverse events (TRAEs) included anemia 33%, neutropenia 25% (gr3+ 13%), thrombocytopenia 21% (gr3+ 13%), fatigue 33% and nausea 29%. Other TRAEs gr3+ included dyspnea (4%) and AST elevation (4%). There were no gr5 events. RNA analysis showed that in cohort 1, tala monotherapy disrupted MMEJ, induced antiproliferative effects and expression of genes in the cGAS-STING pathway, including TBK1-mediated IRF3 activation, with downstream induction of T-cell, dendritic cell and cytokine gene expression. These effects were not seen in biopsies post tala monotherapy in cohort 2. mIF analyses demonstrated T-cell and macrophage infiltration in *BRCA1/2*m tumors. Analyses of post-combination biopsies is ongoing.
**Conclusions:** There were no new safety signals of PARPi combined with immunotherapy. Responses were limited to pts with *BRCA1/2*m. RNA and protein analyses indicate cGAS-STING activation and immune cell infiltration in *BRCA1/2m* tumors, validating murine preclinical findings.

## CT143

## Whole cell antigen presenting immune stimulating cells (Bria-IMT) for the treatment of metastatic breast cancer.

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Background: Bria-IMT (SV-BR-1-GM) is an off-the-shelf whole tumor cell therapeutic vaccine engineered to express class I & class II HLAs, secrete GM-CSF, and function as antigen-presenting cells;

with subsequent enhancements improving in-vitro characteristics. By expressing cancer antigens such as HER2/Neu and PRAME, SV-BR-1-GM also serves as the reservoir of cancer antigens to activates patient's anti-tumor immune responses. The safety and efficacy of the ongoing clinical trial of SV-BR-1-GM in combination with a PD1 inhibitor are reported. We also report post-hoc exploratory data for patients with advanced metastatic breast cancer (aMBC) treated with SV-BR-1-GM regimens.
Methods: Ongoing prospective, phase 1-2, with an expansion randomized phase 2 cohort (NCT03328026; 2018-present) using SV-BR-1-GM with a PD1 inhibitor (pembrolizumab or retifanlimab) with cycles every 3 weeks (16 patients dosed to date). SV-BR-1-GM "monotherapy" (NCT03066947; 2013-8), a completed prospective phase 1-2 results are also presented.
Results: A total of 51 patients received any SV-BR-1-GM regimen; 27 in monotherapy (SV) and 24 in combination with a PD-1 inhibitor (CO) currently ongoing: 4 subjects from SV continued on with CO. Median prior regimens = 5; 61% ER or PR+; 6% ER or PR-/HER2+ and 29% triple negative. SV-BR-1-GM was well tolerated. Disease control rate was 40% with SV and 41% with CO in subjects with available assessment(s). Median PFS was 2.7 months with 6 having PFS >150 days (150-226) for SV and 2.6 months with 5 > 150 days (188-308) for CO. Per protocol, 23 patients had anergy testing prior to treatment. Among those with a normal immunologic response (n=13, 56%), disease outcomes included 4 SD and 2 PR (46% CBR) vs anergic patients (n=7, 30%) with 1 SD (14% CBR); 3 patients (13%) with unknown anergy testing had 0% CBR. All 7 anergic patients developed delayed-type hypersensitivity (DTH) injection site responses to SV-BR-1-GM. Subjects with DTH (injection site induration/erythema) had a significant reduction in the risk of progression or death (HR=0.096, 95%CI:0.02-0.50, $p<0.05$). Subjects with a reduction in circulating tumor cells (CTC) also had an improvement in PFS (HR 0.08, p=.0012). Median OS (uncensored) was 11.2 months in CO (n = 8). 7 out of 20 (35%) for SV and 9 out of 22 subjects (41%) for CO with available data, exceeded prior PFS. Among HLA matched subjects 4/7 (57%) and 6/9 (67%), respectively, had an increase in PFS. PFS ratio improved independent of prior number of lines, HER2 or HR status. The greatest improvement in PFS on a SV-BR-1-GM was in a HLA matched subject (>6mo). TEAEs occurred in 20/24 (83%) of subjects in CO including 9/24 (38%) with injection site AEs. There were no AEs of special interest eg cytokine storm. There were no Grade 5 and only 6 grade 4 AEs in CO.
Conclusions: In an ongoing randomized phase 2, anergic patients had worse outcomes. SV-BR-1-GM was able to illicit an immune response regardless. Responses, stable disease and CBR were seen and associated with immune responses including injection site erythema/induration (DTH), CTC changes and tumor grade I/II. Patients with disease control had better quality of life. SV-BR-1-GM may be able to alter an anergic immune system for clinical benefit. Future pivotal trials will be based upon the ongoing confirmatory randomized clinical trial.

**CT144**

**Randomized phase II trial of preoperative fulvestrant with or without enzalutamide for ER+/Her2- breast cancer.**

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Background: Most estrogen receptor alpha positive (ER+) breast cancers (BCs) express androgen receptor (AR) protein, but its function is unclear. High AR relative to ER is associated with endocrine resistance. This randomized phase II trial of neoadjuvant fulvestrant (F) with or without the anti-androgen enzalutamide (E) was designed to determine if the addition of E to F in women with $\geq$T2 ER+/Her2-

primary BC would increase the % patients (pts) with limited residual disease at time of surgery as measured by modified preoperative endocrine predictive index (PEPI).
Methods: 4 months of therapy was given prior to surgery (F 500 mg IM weeks 1, 3, 5, 9, and 13) and pts were randomized to receive E 160 mg po daily for 16 weeks, stratified by node status and T-stage. Tumor biopsies were required at study entry and after 4 weeks on therapy (Wk5). PEPI score at time of surgery was the primary endpoint for efficacy. The minimax two-stage design had 80% power with type I error rate of 0.08. Reverse phase phosphoprotein array (RPPA), IHC for ER/PR/AR/GR/Ki67, and Polaris multiplex immunofluorescence (IF) panel for myeloid lineage immune cells were performed. Average signal levels were compared across arms, PEPI score (0, >0), PEPI by arm, and Ki67 (<10%, ≥10%). Comparisons at both baseline and with treatment were considered. Significance was assessed with empirical Bayes moderated t-statistics. Since exploratory, p-values were not adjusted for multiple testing.
Results: 69 pts consented; 59 evaluable. 95% completed surgery. PEPI=0 observed in 10 (17%). Of 33 pts on FE arm, 21 had T3/T4 tumors, 91% ER+/PR+, median AR expression 80%, Ki67 15%. Of 26 patients on F arm, 19% had T3/T4 tumors, 96% ER+/PR+, median AR expression 85%, Ki67 10%. PEPI=0 was achieved more frequently on the FE arm (8, 24%) than the F arm (2, 8%) (p=0.16). Toxicity was as expected with endocrine therapy. IHC of baseline vs Wk5 biopsies showed decreased ER, PR, and Ki67 levels by Wk5 that remained decreased at time of surgery. AR and GR were significantly decreased only in the FE arm at the time of surgery. RPPA revealed that baseline EGFR (Y1604 and Y992) was higher in tumors with PEPI=0 tumors, whereas the average baseline mTOR activation was higher among pts with PEPI>0. Significant changes detected by RPPA upon treatment included a significant decrease in AR. Myeloid-derived suppressor cells (MDSC) were significantly reduced by treatment only in the FE arm.
Conclusions: The combination FE had manageable side effects. PEPI=0 was achieved more frequently on the FE arm (8/33) than the F arm (2/26). Activated EGFR was higher pretreatment in tumors achieving PEPI=0. mTOR pathway was elevated pretreatment in PEPI>0 tumors (also observed with resistance to FE in ER+/HER2- metastatic disease (SABCS 2021). When comparing pretreatment tumor to Wk5, total AR by RPPA was the most differentially decreased protein in PEPI=0 tumors. AR signaling is known to support immunosuppressive cells and we observed a marked decrease in MDSCs with treatment only on the FE arm.

**CT145**

**Olaparib +/- atezolizumab in patients with BRCA-mutated (BRCAmt) locally advanced unresectable or metastatic (advanced) breast cancer: an open-label, multicenter, randomized phase II trial.**

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Background: Poly-ADP ribose polymerase inhibitors (PARPi) work by impairing DNA damage repair and have demonstrated activity in patients with BRCAmt breast cancer. Their mechanism of action leads to

high mutational burden and increased neoantigens suggesting tumors exposed to PARPi may have increased response to immunotherapy. Preclinical and phase I/II trials have shown evidence of antitumor effect of PARPi + PD-L1 inhibition. In this study, we assessed the efficacy of the PARPi olaparib as monotherapy vs. in combination with PD-L1 inhibitor atezolizumab in patients with BRCAmt advanced breast cancer.
Methods: A multicenter, randomized, open-label, phase II study was conducted. Patients were enrolled at 20 sites in the US. Patients were allowed prior hormone or chemotherapy and randomized to receive olaparib 300 mg twice daily (O) +/- atezolizumab 1200 mg IV every 21 days (O+A) until disease progression or intolerable side effect. Patients with progression on O were allowed to crossover to O+A arm. The primary endpoint was comparison of progression free survival (PFS) between arms. Overall survival (OS) was also assessed. Safety and activity analyses were done in patients who received at least one dose of study treatment. Serial tumor biopsies were collected at baseline, 6 weeks, and progression to evaluate the effect of therapy on tumor infiltrating lymphocyte populations, whole exome DNA sequencing, and correlate with outcome to assess predictive value. Enrollment has completed and the study is ongoing. The trial is registered with ClinicalTrials.gov, NCT02849496.
Results: 78 patients with BRCAmt advanced breast cancer were enrolled between 5/3/2018 and 3/17/2022 and available for analysis. Groups were generally well matched on baseline characteristics. PFS was 7.0 months (95% CI 5.5-11.5) in O arm and 7.67 months (95% CI 5.6-10) in O+A arm (p=0.92). Median OS was 26.5 months (95% CI 19.2- NR) in O arm and 22.4 months (95% CI 16.6-31.3) in O+A arm (p=0.3). In triple negative breast cancer (TNBC) subgroup (n=23), there was no significant difference in PFS (p=0.92) or OS (p=0.6). Treatment was generally well tolerated with expected side effects, with O+A group experiencing more adverse events of all grades. Analysis of tumor samples from serial biopsies will be presented separately.
Conclusion: Olaparib or combination olaparib + atezolizumab resulted in clinically meaningful PFS in both treatment arms. Addition of atezolizumab did not significantly increase PFS. Treatment in both arms was generally well tolerated with expected side effects based on prior drug profiles. This is the first randomized trial to show that the addition of immunotherapy (atezolizumab) to PARPi (olaparib) in patients with advanced BRCAmt breast cancer does not add to therapeutic response but carries with it additional toxicities.

**CT146**

**Trifluridine/tipiracil (FTD/TPI) and oxaliplatin as induction chemotherapy (IC) in resectable esophageal and gastroesophageal junction adenocarcinoma (EGAC): Preliminary results from a phase II study.**

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**Background:** Pre-operative chemoradiation (CRT) followed by surgery for localized EGAC leads to a pathologic complete response (pCR) rate of 20%. Achievement of pCR is associated with improved overall survival (OS). Therefore, several studies have used IC before CRT to improve pCR rates but saw mixed results. We used a novel combination of FTD/TPI and oxaliplatin as IC before the standard CRT in localized EGAC with the primary objective of increasing the pCR rate.
**Methods:** We enrolled patients (pts) in this open-label, single-arm, multicenter, phase II trial between Jan 2020 and Oct 2022. Pts with potentially resectable EGAC, adequate organ function, ECOG performance status of 0 -1, age <76 years, and endoscopic ultrasound (EUS)-determined node-positive disease with any T-stage or T3-T4a with any N stage were eligible. Pts received three cycles of IC with FTD/TPI (35 mg/m² BID, days 1-5 every 14 days) and oxaliplatin (85 mg/m² every 14 days on day 1). Pts then

underwent concurrent CRT (radiation dose of 5040 cGY) with weekly Carboplatin (AUC 2) and Paclitaxel (50 mg/m2) for 6 weeks, followed by surgery. The primary objective was to evaluate the pCR rate. The secondary objectives were 2-year disease-free survival (DFS), 2-year OS, and toxicities. Using a Simon two-stage design, we enrolled 22 evaluable patients in stage 1 with at least 5 pCRs to proceed to stage 2. We collected blood samples at different time points to measure circulating tumor DNA (Ct DNA) as a correlative endpoint. Clinical trial information: NCT04097028.
**Results:** Of the 22 enrolled pts, 19 (86.4%) were male, and 20 (90.9%) were Caucasian. The median age was 61 years, and 12 (54.5%) had a primary disease at the gastroesophageal junction. Twenty (90.9%) pts had T3 disease, and 15 (68.2%) had node-positive disease by EUS. IC led to a 35% or more reduction in SUVmax in 60% of patients before CRT. At the time of data cutoff, 13 (59.1%) had surgery, 1 (4.5%) was awaiting surgery, 5 (22.7%) had progressive disease during the study, and 3 (13.6%) discontinued the study due to adverse events (AEs). Only 2 pts had pCRs, and an additional 4 had near pCRs. Since we could not meet our pre-defined pCR rate in stage 1, the study was closed due to futility. After a median follow-up of 15.8 months, 2-year OS and DFS were 43% and 41%, respectively. Nine (40.9%) had grade 3 or higher AEs. Nausea (59.1%) and fatigue (59.1%) were the most common treatment-related AEs; these were grade 1-2 events. The most common grade 3 or higher events were neutropenia (13.6%) and lymphopenia (9.1%). No febrile neutropenia was noted.
**Conclusion:** IC with FTD/TPI and oxaliplatin before standard CRT failed to improve pCR in resectable EGAC, although it was reasonably well tolerated and showed activity. Ct DNA analysis is ongoing and will hopefully identify a select subgroup of pts who may benefit from this approach.

**CT147**
**A phase II study of neoadjuvant immune checkpoint inhibitor IMC-001 (anti-PD-L1 antibody) in patients with resectable upper gastrointestinal cancers (Neo-Chance Study).**
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Background: Immune checkpoint inhibitors have shown survival benefits in unresectable/metastatic gastric cancer (GC), esophageal cancer (EC), and hepatocellular carcinoma (HCC). Based on scientific rationale for neoadjuvant immunotherapy, we conducted a phase II study of neoadjuvant IMC-001, a novel PD-L1 targeting fully human monoclonal antibody, for resectable GC, EC, and HCC.
Methods: This is a prospective, phase II study of neoadjuvant IMC-001 (20 mg/kg iv every 2 weeks for 2 cycles) across three cohorts of resectable GC, EC, and HCC. The primary endpoint is major pathologic response rate (<10% of viable tumor cells) and secondary endpoints include safety, feasibility, R0 resection rate, tumor response rate, progression-free survival, relapse-free survival, and overall survival. Exploratory endpoints include immune monitoring and biomarker analysis in tumor tissues, peripheral blood, and stool.
Results: From Sep. 2019 to Nov. 2022, 50 patients (pts) were enrolled and treated (safety analysis population), and among them, 48 pts were evaluable for the efficacy analysis (16 HCC; 16 GC; 16 EC); male (81%), median age=62 yrs (range, 39-77), and clinical stage (AJCC 8$^{th}$ ed.) I (56%)/II (33%)/III (10%). All tumors were microsatellite stable and 33% of tumors were PD-L1+. 45 pts (94%) completed 2 cycles of neoadjuvant IMC-001, all pts underwent surgery, and only one pt's surgery was delayed due to immune-related hepatitis. No pts had disease progression during neoadjuvant therapy, and among the 17 pts with measurable lesion(s) by RECIST 1.1, 3 pts (18%) showed partial response (PR) and 14 pts (82%) had stable disease (SD). Among 30 metabolic response-evaluable pts by EORTC criteria, 7 pts (15%) showed metabolic PR, 20 pts (43%) had metabolic SD, and 3 pts (6%) had metabolic PD. Among 2 of 3 pts with metabolic PD, surgical pathology showed increased tumor infiltrating lymphocytes and tumor regression. The median change of the sum of SUVmax was -13% (range, -76% to 59%). All pts underwent R0 resection with pathologic stage of I (75%)/II (13%)/III (13%). Although there was no major

pathologic response, 51% of pts showed various degree of tumor necrosis or fibrosis and 7 pts (15%) had residual viable tumor cells less than 50%. Except for grade 3 AST/ALT elevation (6%), all treatment-related adverse events (TRAEs) were grade 1 or 2. TRAEs occurring in ≥5% of pts were hyperthyroidism (18%), hypothyroidism (14%), fatigue (14%), pruritus (12%), skin rash (8%), infusion-related reaction (6%), and diarrhea (6%).
Conclusions: Neoadjuvant IMC-001 was well tolerated and demonstrated antitumor activity in microsatellite stable, resectable GC, EC, and HCC pts. The updated clinical and biomarker results will be presented.

**CT148**

**Efficacy, safety, and biomarker analysis of combined MEK (Cobimetinib) and PD-L1 (Atezolizumab) inhibition (COTEZO) in advanced small bowel adenocarcinoma (SBA).**

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**Purpose:** Preclinical evidence supporting immunomodulatory effect of MEK inhibition that augments anti-tumor activity of PD-1 inhibitors is a compelling rationale for investigating combined MEK and PD-L1 inhibition in advanced microsatellite-stable (MSS) SBA wherein immune checkpoint inhibitors (ICIs) have little clinical benefit. We conducted a prospective, phase 2 trial evaluating efficacy and safety of COTEZO regimen in treatment refractory SBA.
**Procedures:** Patients aged ≥ 18 years with metastatic MSS SBA, ECOG PS 0 - 2, and disease progression on prior systemic chemotherapy were enrolled and treated with cobimetinib (60 mg orally once daily for days 1 - 21) and atezolizumab (840 mg intravenously every 2 weeks) every 28-day cycle. The primary endpoint was objective response rate (ORR) per Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1. by independent radiology review. Key pre-specified secondary endpoints were safety, disease control rate (DCR), progression-free (PFS) and overall (OS) survival. Pre/on-treatment biopsies were examined for response biomarkers.
**Results:** Between 4/2017 and 7/2020, 20 patients were enrolled. The confirmed ORR per RECISTv1.1 was 10% [2/20; 95%CI: 1.2 - 31.7] (1 complete response and 1 partial response). Seven patients had stable disease for a DCR of 45% (95%CI: 23.1 - 68.5). Median PFS and OS were 2.4 (95% CI 1.3-3.5) and 8.8 (95% CI 5.6 - 12) months, respectively. Grade 3 treatment-emergent adverse events (no grade 4/5) occurred in 7 (35%) patients; most common being elevated CPK (15%) and vomiting (10%). One (5%) patient had grade 3 vomiting, necessitating treatment discontinuation. There appeared to be a marked difference in OS between patients with increased pre-treatment tumor immune infiltration as compared to those lacking immune infiltration amongst patients with RNASeq data available (n=13). Specifically, increased immune infiltration as assessed by computational deconvolution of RNASeq data pointed to increased macrophage and monocyte infiltration as prognostic factors. This was additionally assessed via application of a global “immune score” algorithm, ESTIMATE**.** In order to generalize our findings, we expanded our computational deconvolution analysis to include additional small bowel adenocarcinoma patients who received standard-of-care treatment (n=7). For the combined standard-of-care and experimentally treated patients we saw that those with immune infiltration had significantly better OS (9 months vs greater than 3 years, HR 5.7, Padj = 0.017), suggesting that immune infiltration is a prognostic, rather than predictive biomarker.
**Conclusions:** Although our study did not meet its primary endpoint of ORR, we identified tumor immune infiltration as a prognostic biomarker in SBA, which could select patients with better outcomes on immunotherapy.

**CT149**

**BOLD-100-001 (TRIO039): a phase 1b/2a dose-escalation study of BOLD-100 in combination with FOLFOX chemotherapy in patients with pre-treated advanced colorectal cancer: interim efficacy, safety and tolerability analysis.**

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Background: BOLD-100 is a first-in-class ruthenium-based anticancer agent in Phase 1b /2a clinical development for the treatment of advanced gastrointestinal (GI) cancers in combination with FOLFOX. BOLD-100 demonstrated synergy in established preclinical models in combination with various anticancer therapies, particularly in resistant cell lines. In the first interim analysis of the phase 1b dose-escalation component of the clinical trial, BOLD-100 plus FOLFOX was well-tolerated in patients (pts) with advanced GI solid cancers.

Methods: This is a prospective, Phase 1b dose-escalation (Part A) and Phase 2a dose-expansion (Part B) study of BOLD-100 in combination with FOLFOX for colorectal (CRC), pancreatic (PDAC), gastric (GC) and biliary tract (BTC) cancers. Pts receive BOLD-100 with FOLFOX on day 1 of each 14-day cycle. In Part A, pts were enrolled in a 3+3 design to determine the combination recommended Phase 2 dose (RP2D), with BOLD-100 dose-escalation (420, 500 and 625 mg/m2). Part B comprises 4 cohorts treated at the RP2D of 625 mg/m2 until progressive disease or unacceptable toxicity. The primary objective of Part B is to evaluate the efficacy of BOLD-100 in three clinical endpoints (PFS, OS, and ORR). Bayesian modelling is used to continually reassess these endpoints; the posterior probability of superiority to a historical landmark for each endpoint.

Results: As of 31 Dec 2022, 17 pts with advanced metastatic colorectal cancer median age 62 years were treated. Pts received a median of 4 prior systemic therapies, 14 had received prior FOLFOX and 16 (94%) were enrolled with stage IV disease. Median number of cycles completed was 7 (range 1-12). Median PFS was 4.7 [2.9,8.6] months, median OS 9.8 [5.2,22] months, and ORR 13% [3,36] compared to the historical benchmark of 2.0 months, 7.1 months, and 1.6% respectively for similar patients treated with approved standard of care. Two pts achieved a partial response and 11 pts had stable disease for an overall disease control rate of 87% (13/15 [64%,97%]). This compares favorably to the historical control 44%. 16 pts reported 1 or more treatment-emergent adverse events (AEs), most commonly neutrophil count decreased (n=9, 52.9%), fatigue (n=6, 35.3%), pyrexia (n=4, 23.5%), platelet count decreased (n=4, 23.5%) and decreased appetite (n=4, 23.5%). Most of the AEs were grade (G) 1-2. 12 G3 AEs were observed (mostly neutrophil count decreased [8]).

Conclusion: BOLD-100 plus FOLFOX is an active and well-tolerated treatment regimen in the heavily pre-treated metastatic CRC trial population. There were no new safety signals. PK and PD data are forthcoming. The preliminary mPFS, mOS, ORR and DCR data in this interim analysis demonstrate significant improvement over the currently available approved therapies.

**CT150**

**Result of an open-label phase 2 trial of dual TORC1/TORC2 inhibitor onatasertib (ATG-008) in HBV+ advanced hepatocellular carcinoma (HCC) subjects who have received at least one prior line of systemic therapy (TORCH).**

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**Background**: The mammalian target of rapamycin (mTOR) is a serine/threonine kinase related to the lipid kinases of the phosphoinositide 3-kinase (PI3K) family, which has been confirmed to be closely related to the development of a variety of human cancers. Onatasertib (ATG-008) is a 2nd generation mTOR inhibitor which inactivates both mTORC1 and mTORC2. Our previous clinical investigation (NCT01177397) has demonstrated preliminary evidence of antitumor activity of onatasertib across multiple solid and hematologic malignancies, with encouraging signals of activity in subjects with hepatitis B virus (HBV)+ unresectable, refractory HCC.

**Methods**: Asian patients with advanced HBV+ HCC who had experienced progressive disease after at least 1 line of systemic therapy were enrolled in this study. Onatasertib was administered orally once a day (QD) at 3 dose levels (15 mg, 30 mg and 45 mg) or 20 mg twice daily (BID). The primary endpoints were pharmacokinetics (PK), safety and efficacy (NCT03591965).

**Results**: As of July 11, 2022, 73 patients were enrolled and evaluated. The median age was 52.0 years and the median follow up duration was 26.5 months. The mean number of prior lines was 1.8. 63 patients (86.3%) had at least one Grade 3-4 treatment emergent adverse event (TEAE), and 25 (34.2%) had at least one serious adverse event (SAE) . Among the 73 subjects, 3 achieved confirmed partial response (PR), all in the 45 mg QD cohort. 18 pts were enrolled in the 45 mg QD cohort, in which 11 (61.1%) pts had received at least 2 prior lines of systemic therapy and 15 (83.3%) pts had been exposed to an anti-PD-1/PD-L1 antibody. The overall response rate (ORR) in the 45 mg QD cohort was 16.7%. The median progression-free survival (mPFS) was 3.0 months (95% CI 1.9, 4.6) in the intention to treat (ITT) population and was 5.3 months (95% CI 1.9, 7.6) in the 45mg QD cohort. Median overall survival time (mOS) was not evaluable in the 45 mg QD cohort and the mOS was 13.4 months (95% CI 7.4, 19.8) in the ITT population. Onatasertib demonstrated linear pharmacokinetics between 15 mg QD and 45 mg QD in this Asian population and there was no significant exposure accumulation after multiple dosing, as previously seen in the US population.

**Conclusion:** Onatasertib (ATG-008) showed encouraging single agent antitumor activity in patients with HBV+ advanced HCC after at least 1 prior systemic therapy, notably in the 45 mg QD dose level, in which most patients had been previously exposed to anti-PD-L1/PD-1 therapy. The results indicates that onatasertib has potential efficacy in HBV+ HCC patients who failed prior CPI treatment. Further study may be warranted, particularly in HBV+ HCC patients who have failed prior anti-VEGFR and anti-PDL1/PD-1 therapy.

**CT151**

**A phase II study of optimized individualized adaptive radiation therapy for hepatocellular carcinoma.**

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**Introduction:** Stereotactic body radiation therapy (SBRT) for the treatment of hepatocellular carcinoma (HCC) remains a challenge due to high rates of toxicity in patients with impaired liver function or tumors not amenable to thermal ablation. We performed a single-arm prospective phase II clinical trial utilizing a novel treatment paradigm optimizing the utility of SBRT based on the individual patient's probability for tumor control traded off against the risk of liver injury. We hypothesized that maximizing the utility of treatment would decrease toxicity while achieving the same tumor control rate as standard therapy.
**Methods:** Patients with Child-Pugh (CP) A to B7 disease with tumors >3.5 cm, or CP ≥ B8 with any size tumor were prospectively enrolled on an IRB approved clinical trial to undergo SBRT with baseline dose optimization and mid-treatment response adaptation. Optimization and adaptation were based on the expected utility of treatment, calculated as a weighted average of the probability of 4 combinations of toxicity and efficacy outcomes. These calculations were based on the individual patient's baseline indocyanine green retention at 15 minutes or albumin-bilirubin score (ALBI), CP score, intended dose and fractionation, and mean liver dose with the goal of maximizing the difference between the probability of local control compared to the probability of treatment-related toxicity. Primary endpoints were rate of liver decompensation as measured by ≥2 point change in CP score within 6 months, and lesion-specific local control. Overlap weighting was used to compare patients treated on protocol with patients receiving conventional SBRT at another high-volume cancer center.
**Results:** 56 patients with 80 tumors met inclusion criteria and had a median follow-up of 11.2 months. 44 tumors with a median size of 3.8 cm were treated in CP-A to B7 patients, while 36 tumors with a median size of 2.1 cm were treated in CP ≥ B8 patients. Optimization resulted modification of initial dose for 38% of patients. Sixty-eight percent of patients underwent mid-treatment adaptation with either omission or dose reduction of the final two treatments based on change in expected utility. The 1 year freedom from local progression was 94%. A total of 21% of patients experienced a ≥ 2 point change in CP score within 6 months. Overlap weighted analysis revealed similar local control (HR 0.69, 95% CI [0.25-1.91], p = 0.48), and overall survival (HR 1.45, 95% CI [0.69-3.0], p = 0.33), with decreased toxicity (OR 0.26, 95% CI [0.07 - 0.99], p = 0.048) compared to conventional SBRT.
**Conclusion:** SBRT for HCC patients with large tumors or poor liver function can be optimized via an individualized, utility-based treatment paradigm which may decrease treatment-related toxicity while maintaining tumor control.

**CT152**

**A multicenter Phase II study of savolitinib in patients with MET-amplified gastroesophogeal junction adenocarcinomas or gastric cancer.**

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**Background**: MET gene amplification is associated with poor prognosis in gastric cancer (GC) and gastroesophageal junction adenocarcinomas (GEJ). Savolitinib is a potent and highly selective oral MET tyrosine-kinase inhibitor. Here we reported the preliminary efficacy and safety from a phase 2 trial of savolitinib monotherapy in patients (pts) with MET-amplified advanced or metastatic GC/GEJ. (NCT04923932).
**Methods**: Eligible pts had 2L+ GEJ or GC, with MET amplification and measurable lesions. Pts received savolitinib at 600 mg QD for body weight (BW) ≥50 kg, while 400 mg QD for BW <50 kg in 21-day cycles until disease progression or meeting other criteria for end of treatment. Savolitinib BID regimen has also been additionally explored. The primary endpoint was objective overall response rate (ORR) evaluated by Independent Review Committee (IRC). One interim analysis (IA) was pre-defined at the first 20 QD pts who had at least 2 tumor assessments.
**Results**: As of IA, 20 pts were enrolled for QD regimen. Demographics and clinical outcomes are shown in table 1. The mean relative dose intensity of 93.07%. Median duration of exposure was 2.09 months. Confirmed ORR by IRC was 45%, and reached 50% in 16 patients with MET GCN (high) while only 1 PR was observed in 4 patients with MET GCN (low). Duration of response rate at 4-month was 85.7% with median follow up time of 5.5 months. The most common Gr≥3 TRAE (≥5%) were platelet count decreased, hypersensitivity, anemia, neutropenia and hepatic function abnormal. In all pts, only 1 patient discontinued treatment due to grade 4 liver function abnormal (TRAE) and no patient died due to TRAE.
**Conclusion:** Savolitinib monotherapy had manageable safety and showed promising efficacy in pts with MET-amplified GEJ or GC, particularly in pts with MET high GCN. BID regimen is being investigated to further evaluate the efficacy and safety of savolitinib in pts with MET high GCN.

**Table 1: Pts baseline characteristics and clinical efficacy**

| **Baseline Characteristics** | **ITT in IA (n=20)** |
|---|---|
| Median age (min, max), yearsSex (male / female), nECOG (0/1/2)Median BMI (min, max), (kg/m$^2$)Primary location of tumor | 57.00 (39.5, 76.8)17/33/15/220.8 (14.9, 25.8)16/4205/10/516/4 |

| | | |
|---|---|---|
| (GC/GEJ)Tumor stage (IV)Prior line of therapy (1/2/≥3)MET GCN (high / low) | | |
| **Clinical Efficacy** | **By IRC** | **By Investigator** |
| Confirmed objective response rateDisease control rate4m-DoR rate,% (95% CI) | 45%65%85.7 (33.4, 97.9) | 40%55%71.4 (25.8, 92.0) |

**CT153**

**Pemigatinib in Chinese patients with advanced/metastatic or surgically unresectable cholangiocarcinoma Including FGFR2 fusion or rearrangement: Updated overall survival from an open-label, single-arm, multicenter Phase II study.**

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**Background:** Pemigatinib is a selective FGFR inhibitor that showed effectiveness and tolerability in patients with cholangiocarcinoma (CAA) with FGFR2 fusion or rearrangement. Data from prior data cutoffs (primary: Jan 29th, 2021; initial update: Dec 20th, 2021) showed that patients receiving pemigatinib had durable responses. Confirmed objective response rate (ORR) (60%) met the primary endpoint, median duration of response (DOR) was 8.3 months, median progression-free survival (PFS) was 9.1 months at the initial update (G-M. Shi et al. ASCO 2022). Safety results were also consistent with previously reported data on pemigatinib. Here we report updated overall survival (OS) results.
**Methods:** Patients aged 18 years or older with recurrent or metastatic CCA that failed at least one prior systemic therapy were enrolled. Thirty-one subjects with documented FGFR2 fusion or rearrangement received 13.5 mg pemigatinib. The primary efficacy endpoint was ORR assessed by the independent radiological review committee (IRRC) per RECIST V1.1. And the second endpoints included disease control rate (DCR), DOR, PFS, and OS. Updated OS were evaluated by Cox proportional hazards model and summarized using Kaplan-Meier methods.
**Results:** At data cutoff (December 28th, 2022), a total of 30 patients were assessed (1 participant excluded due to inadequate FGFR2 aberrant frequency). Median age was 56 years (range, 28-68).With a median OS follow-up of 25.6 months (95% CI, 23.0-25.8), the median OS was 23.9 months (95% CI, 15.2-NC), with 16 (53.3%) OS events. Estimated OS rate at 12 month, 18 month and 24 month were 73.3% (95% CI, 53.7%-85.7%), 66.5% (95% CI, 46.7%-80.4%), and 41.4% (95% CI, 22.4%-59.4%), respectively. No new safety signals were observed.
**Conclusions:** These updated results demonstrated encouraging and durable survival benefit of pemigatinib in Chinese patients with recurrent or metastatic cholangiocarcinoma with FGFR2 fusion or rearrangement.
**Clinical trial identification:** NCT04256980.
**Editorial acknowledgment:** Guoming Shi and Xiaoyong Huang contributed equally to this work. Jian Zhou is the corresponding author.

**CT154**

**Combination of cinrebafusp alfa with ramucirumab and paclitaxel is well tolerated and elicits encouraging clinical activity in patients with HER2-positive gastric/gastroesophageal junction (GEJ) adenocarcinoma.**

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**Introduction:** Cinrebafusp alfa is a first-in-class bispecific antibody-Anticalin® fusion protein that targets HER2 and the costimulatory receptor 4-1BB, leading to enhanced activation of T cells in the tumor, while avoiding liver toxicity. In a Phase 1 monotherapy study, cinrebafusp alfa was well tolerated and showed single agent activity in patients with HER2-positive malignancies. Based on pharmacokinetics (PK), pharmacodynamics and clinical efficacy data, a loading dose of 18mg/kg Q2W in cycle 1 followed by 8mg/kg Q2W in subsequent cycles as maintenance dose was chosen for the Phase 2 study.

**Methods:** This Phase 2 study enrolled patients with metastatic gastric/gastroesophageal junction cancer and confirmed HER2-high status (IHC 3+ or IHC 2+ with HER2/neu gene amplification) who had received one or two prior lines of treatment. Patients received cinrebafusp alfa in combination with ramucirumab and paclitaxel. Primary endpoint was objective response rate (ORR), and key secondary endpoints included safety profile, PK, and immunogenicity.

**Summary of data:** 5 patients were enrolled before enrollment ceased for reasons unrelated to safety or efficacy profile. As of the cut-off date (19-Dec-2022), 5 out of 5 patients achieved a partial response (PR) as best overall response with tumor lesion shrinkage ranging between 35% and 66%. Two of the PRs have been confirmed, one PR is unconfirmed, and 2 patients with currently unconfirmed PRs are continuing to receive treatment. In total, 2 of 5 patients discontinued treatment due to disease progression after 140 and 113 days (patient discontinued cinrebafusp alfa after 42 days but continued on ramucirumab and paclitaxel); 3 patients remain on treatment. Median duration of response was 3.8 months at time of data cut-off. The most frequent treatment emergent adverse events (TEAEs) included Grade 1/2 fatigue (4 patients) and Grade 1-3 diarrhea (4 patients). Infusion related reactions (Grade 2/3) were seen in 1 patient, leading eventually to discontinuation of cinrebafusp alfa. With regards to prior treatment history, all patients received trastuzumab and chemotherapy. 2 patients also received trastuzumab deruxtecan while 4 patients previously received anti-PD1 therapy.

**Conclusions:** The combination of cinrebafusp alfa with ramucirumab and paclitaxel was safe and tolerated in the 5 patients treated. Preliminary activity of cinrebafusp alfa in combination with ramucirumab and paclitaxel demonstrated a high response rate indicating that the combination can elicit clinical responses in patients who have progressed on trastuzumab deruxtecan or checkpoint inhibitor regimens.

**CT155**

**Long term results and ctDNA correlatives for CAPOX BETR: A multi-center phase II trial of capecitabine, oxaliplatin, bevacizumab and trastuzumab for previously untreated HER2 positive metastatic gastroesophageal adenocarcinoma.**

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Introduction: Preclinical studies suggest cooperativity between blocking the human epidermal growth factor receptor 2 (HER2) and the vascular endothelial growth factor (VEGF) pathways in gastroesophageal adenocarcinomas (GEAs).
Methods:Patients with previously untreated advanced HER2 positive GEAs were treated with standard of care chemotherapy capecitabine 1,200 mg/m$^2$ days 1-14 and oxaliplatin 130 mg/m$^2$ day 1 (CAPOX) plus trastuzumab 6 mg/kg day 1, once every 3 weeks. Investigational agent bevacizumab (7.5 mg/kg, VEGF mAb) was added on day 1 of each cycle for all patients. The primary endpoint was radiographic objective response rate by RECIST 1.1. ctDNA was extracted and profiled from serially banked plasma samples (every other treatment cycle) using CLIA certified 152-gene next generation sequencing (NGS) panel assay, PredicineCARE$^{TM}$.
Results:Sixty one patients were screened for the study of whom 24 were ineligible. Thirty-seven patients were enrolled, and one withdrew consent prior to starting. Thirty-six patients were evaluable for efficacy and safety. The median follow-up was 23.2 months (IQR: 11.0 - 46.9 months). All enrolled patients are now off study. Radiographic objective response rate was 81%. Median progression-free survival (PFS) and overall survival (OS) were 14.0 months (95% CI, 11.3 -36.4) and 23.2 months (95% CI, 16.6-36.4), respectively. The most common grade 3-4 toxicities were diarrhea, peripheral neuropathy, and hypertension. Baseline ctDNA profiling identified HER2 amplifications in 76.7% of tested cases (23/30). Baseline ctDNA based tumor fraction (TF) was highly prognostic and TF > 50$^{th}$ centile had a statistically significantly worse PFS of 11.3 months (95% CI 5.2-18) vs. 22.7 months (95% CI 18.1-NA), p value = 0.0013, and OS of 15.4 months (95% CI 8.0-27.6) vs. 28.0 months (95% CI 17.8-NA), p value = 0.022. 56.7% of cases (17/30) had alternative MAPK drivers present in pretreatment ctDNA, most commonly amplifications in *EGFR*, *FGFR1*, *MET*, and *KRAS*. Additional MAPK alterations were associated with worse PFS of 12.5 months (95% CI 5.2-NA) vs. 22.7 months (95% CI 8.5-NA), p-value = 0.0067, and OS of 16.5 months (95% CI 8.0-27.6) vs. 32.3 months (95% CI 17.8-NA), p-value = 0.015. 19 cases had plasma profiled at time of clinical resistance of whom 10 showed new oncogenic mutations not detectable in the matched baseline sample. These included mutations in MAPK pathway (*KRAS*, *NRAS*, *BRAF*, *HER2*; n = 1 each) and PI3K pathway (*PTEN*, *PIK3CA*; n = 1 each), suggesting a role in therapeutic resistance.
Conclusions:The combination of CAPOX, trastuzumab and bevacizumab shows striking clinical activity comparable to novel triplet regimens that include PD1 blockade for HER2+ GEAs . Further evaluation of VEGF mAb in combination with chemoimmunotherapy and anti-PD1 regimens is warranted. Diagnostic ctDNA profiling identifies cases with high TF and alternative MAPK drivers who have worse outcomes. Serial measurement of ctDNA may allow early identification of novel genetic resistance mechanisms which can aid attempts at early intervention.

**CT156**

## MCLA-158 (petosemtamab), an IgG1 bispecific antibody targeting EGFR and LGR5, in advanced gastric/esophageal adenocarcinoma (GEA).

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The epidermal growth factor receptor (EGFR) is a known driver of cancer growth and the leucine-rich, repeat-containing, G-protein coupled receptor 5 (LGR5) is a transmembrane receptor expressed on cancer stem cells. Petosemtamab is a human, common light chain, IgG1 bispecific antibody with enhanced ADCC activity targeting EGFR and LGR5, which has shown potent antitumor activity in patient (pt)-derived xenograft models of gastric and esophageal cancer. The dose escalation part of an ongoing phase 1/2 study is completed (JCO 2021:39.3 Sup 62). In the expansion part, petosemtamab is being investigated at the RP2D (1500 mg Q2W, 4-week cycle) in pts with selected advanced solid tumors, including GEA. Primary objective (expansion): investigator-assessed ORR per RECIST 1.1. Secondary objectives: ORR, DOR, PFS (per investigator and central review), OS, and safety and tolerability. Key eligibility criteria: advanced/metastatic GEA, prior exposure to ≥2 lines of standard therapy, ECOG PS 0-1, measurable disease (RECIST 1.1), available baseline tumor biopsy. At the data cutoff date of 24 October 2022, 14 GEA pts were treated at the RP2D. Median age was 63 years (range 40-80), ECOG PS 0/1: 3/11 pts, and 79% of pts were male. Primary tumor locations were esophagus (57%), stomach (36%), and gastroesophageal junction (7%), and all were adenocarcinoma histology. Pts received a median of 3 lines of prior systemic therapy (range 1-4) including platinum-based chemotherapy (100%) and checkpoint inhibitors (14%). 3/14 pts had EGFR overexpression (H score ≥200). A median of 2 treatment cycles (range 1-24) was administered, with 1 pt continuing therapy at the cutoff. Among the 14 pts evaluable for efficacy (≥2 cycles and ≥1 postbaseline scan, or early progression) limited activity was seen, however 1 pt with tumor EGFR protein overexpression and gene copy number amplification (CNA) showed a confirmed sustained PR (67% tumor reduction; response ongoing after 24 cycles) and 3 pts had SD (1 with EGFR overexpression and gene CNA; 2 not evaluable for IHC), with tumor reductions of 2%, 17%, and 40%. Among 78 pts treated at the RP2D (escalation and all expansion cohorts; cutoff 28 November 2022), the most frequent AEs regardless of causality (all grades/G3-4) were rash (33%/0%), hypotension (26%/6%), dyspnea (26%/4%), nausea (26%/1%), dermatitis acneiform (24%/1%), blood magnesium decreased (19%/5%), erythema (19%/0%), diarrhea (19%/0%); IRRs (composite term) were reported in 74%/21% of pts, mostly at the first infusion, and all resolved. 5 pts (6%) discontinued treatment due to IRRs on Day 1. 1 esophageal cancer pt died due to unrelated G5 GI bleeding. Petosemtamab demonstrated promising clinical efficacy among patients with pretreated GEA having EGFR gene amplification and/or overexpression, with a manageable safety profile.

**CT157**

**Combination of nivolumab with standard of care in the management of grade group 5 prostate cancer: Interim analysis of a phase II trial.**

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Survival outcomes of grade group 5 (GG5) prostate cancer (PCa) after standard of care therapy (SOC) remain poor. Evidence suggest that high dose rate brachytherapy (HDR) and androgen depravation therapy (ADT) can serve as immune modulators. We hypothesize that the combination of HDR, ADT, and immune checkpoint inhibitors (ICI) may synergize to improve immune response and disease control. Here we report an interim analysis of a single-center single-arm phase II trial exploring this novel combination therapy with nivolumab for GG5 PCa.

Patients were enrolled from 9/2018 to 4/2021 and required to have GG5 PCa with > 30% positive cores and receive ICI plus SOC regimen, comprising ADT, external beam RT (EBRT), and HDR. ICI (240 mg)

was administered every 2 weeks for 4 doses beginning 4 weeks prior to HDR. HDR consisted of two 1150 cGy implants. EBRT followed HDR and consisted of 4500 cGy in 25 fractions. Total ADT length and elective nodal radiotherapy were per physician discretion. Biopsies were taken at time of diagnosis, HDR, and 1-month post HDR. Biopsy tumor samples underwent genomic profiling. Major pathologic response (MPR) was defined as < 17% positive cores. Wilcoxon test and Area Under the Curve (AUC) Receiver Operating Characteristics (ROC) curves were used for statistical analyses. Toxicity was evaluated according to CTCAE v5.0. The historic control cohort consisted of all patients treated with SOC between 1/2013 to 11/2021 and met all study inclusion/exclusion criteria. Cox regression was used to calculate 2-year (2Y) risk of metastasis with censorship at 24 months (m). Median follow up (mFUP) defined from diagnostic biopsy.
Of 34 patients who received 2 or more doses of ICI, 1 patient (3%) experienced a dose limiting toxicity of autoimmune hepatitis (AH). The incidence of all grade 3 toxicity related to ICI at 3 and 6 months were both 3% (n=1; AH and QT prolongation, respectively). There was no grade 4+ toxicity at 3 m, nor any grade 3+ toxicity at 6 m. Two subjects were withdrawn from study, leaving 32 evaluable for clinical outcomes. mFUP of ICI cohort was 32 m with a 2Y PSA failure rate of 6%. CD8A, STING, ANPEP, and multiple immune signatures were associated with an early MPR ($p < 0.05$) with ICI. The Ricketts_Immunosuppression genomic signature strongly correlated with MPR to ICI (AUC = 0.7, $p = 0.01$). The control cohort consisted of 59 patients with a mFUP of 35 m and a 2Y PSA failure rate of 19%. ICI demonstrated an 88% reduction in 2Y risk of metastasis compared to controls (HR = 0.12, 95% CI, 0.02 - 1.0, $p = 0.05$).
Combination of ICI with SOC for GG5 PCa is safe and associated with a clinically significant improvement in disease control. Key genomic features were identified to predict for early MPR to ICI. Ricketts_Immunosuppression was identified as a potential biomarker for ICI-sensitive tumor subtypes. Final analyses are anticipated in late 2023. Clinical trial information: NCT03543189.

**CT158**

**BrUOG360: A phase Ib/II study of copanlisib combined with rucaparib in patients with metastatic castration-resistant prostate cancer (mCRPC).**

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**Background:** Molecular alterations in genes regulating homologous recombination (HR) confer sensitivity of mCRPC to poly ADP-ribose polymerase inhibitors (PARPi). Increased activation of thePI3K-AKT-mTOR pathway in mCRPC contributes to tumor progression and represents an important therapeutic target. Preclinical studies have shown that PI3K inhibitors (PI3Ki) impair HR and sensitize cancer cells to PARPi even in the absence of HR gene mutations. We hypothesize that dual PI3K and PARP inhibition may improve clinical outcomes of mCRPC. We describe preliminary results of a phase Ib/II study investigating safety of the combination of copanlisib (pan-class I PI3Ki) and rucaparib (PARP-1, -2 and -3 inhibitor).
**Methods:** Enrollment criteria included progressive mCRPC, prior androgen inhibitors (abiraterone, enzalutamide, and/or apalutamide); prior taxane chemotherapy was allowed. HR defects (HRD) was not required for the phase Ib but was an inclusion criterion for the Phase II component of the trial. The phase I followed a standard 3+3 escalation design. Dose level (DL) 1 consisted of rucaparib 400 mg PO BID continuously and copanlisib 45 mg IV days 1, 8 and 15 every 28 days; in DL -1 the copanlisib was reduced to 45 mg days 1 and 15 only. Adverse events (AE) were graded by CTCAE v5.0. The primary aim of the phase I was to establish the MTD and the recommended phase II dose (RP2D) of copanlisib in combination with rucaparib.
**Results:** Thirteen patients were enrolled with a median age of 64 (55-78) and a median baseline PSA of

11.7 ng/mL (0.015-1939.2). Nine patients (69%) received prior chemotherapy (docetaxel [6], cabazitaxel [3]). Seven patients had HRD (54%) *BRCA1* (1), *BRCA2* (4), *CDK12* (1), and *FANCA* (1). Treatment-related AEs included grade 2 (G2) leukopenia (46%), G2/G3 anemia (31%), G2/G3 rash (31%). Two dose-limiting toxicities (DLTs) were observed in 5 patients treated in DL 1: G3 rash and G3 AST/ALT elevation attributed to both drugs. Six patients were treated at DL -1 without DLTs; this was identified as the RP2D. Two additional patients have been treated at this dose in Phase II of the study. By RECIST 1.1 criteria, one patient (8%) achieved a partial response (BRCA1 mutation) and three (23%) achieved stable disease resulting in a clinical benefit rate of 30.7%. Median duration of treatment was 12 weeks. One patient harboring a PALB2 VUS mutation remained on treatment for 21.7 months. Of 13 evaluable patients, there were 2 confirmed PSA responses (15%) (one patient without an HRD and one with a BRCA1 mutation).
**Conclusions:** The combination of rucaparib and copanlisib is well tolerated. The RP2D was rucaparib 400mg BID with copanlisib 45mg (D1, D15; 28-day cycle) with signal of efficacy in patients with and without HRD. Clinical trial information: NCT04253262.

**CT159**

**CYCLONE 1: abemaciclib in men with heavily pretreated metastatic castration-resistant prostate cancer (mCRPC).**

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Background: Treatment options for patients (pts) with heavily pretreated mCRPC are limited. Augmenting endocrine therapy by targeting CDK4/6 with abemaciclib has dramatically improved outcomes in HR+ breast cancer. Similar to ER, AR signaling activates CDK4 & 6 and abemaciclib showed preclinical anti-tumor activity in prostate cancer (PC). This signal seeking single arm Phase 2 study evaluated the safety and clinical activity of abemaciclib monotherapy in pts with heavily pretreated poor prognosis mCRPC who progressed after both a novel hormonal agent (NHA) and two taxanes.
Methods: Eligible pts had progressive mCRPC, measurable disease by RECIST1.1, and previously received ≥1 NHA, docetaxel, and cabazitaxel for metastatic PC. Abemaciclib was administered at 200 mg BID on a continuous 28-day dosing schedule. The primary endpoint was investigator-assessed objective response rate without concurrent bone progression (ORR). A target ORR of ≥12.5% was deemed suitable to support further evaluation of abemaciclib monotherapy in this refractory mCRPC setting. Key secondary endpoints included disease control rate (DCR), time to PSA progression, radiographic progression-free survival (rPFS), overall survival (OS), and safety.
Results: Forty-four pts (median age 68 yrs; 75% ECOG PS 1, median PSA of 96 ng/mL) were enrolled. In total, 46.5% had visceral metastasis, including 27.9% with liver metastases. Median time from development of mCRPC to study entry was 2.1 yrs and the median number of prior systemic regimens for mCRPC was 3. Median number of treatment cycles was 3 (1-13). Five pts (11.8%) had soft tissue

response. The ORR without concurrent bone progression was 6.8%. Eighteen pts (40.9%) had stable disease, of which 6 (13.6%) lasted ≥6 months (mos); DCR was 47.7%. Median rPFS was 2.7 mos (95% CI 1.9, 3.7); 6-mos rPFS rate was 24.9% (95% CI 12.4, 39.5); median time to PSA progression was 6.5 mos. Median OS was 7.6 mos (95% CI 5.6, NE). Any grade (G) TRAEs experienced by ≥50% of pts were diarrhea (79.5%), decreased appetite (52.3%) and fatigue (50%); majority were G1/2. G3 TRAEs in ≥ 5% of pts were neutropenia (22.7%), anemia (6.8%), fatigue (6.8%) and diarrhea (6.8%). There were no G4 or G5 TRAEs. Discontinuation rate due to AE was 13.6%.
Conclusions: Abemaciclib demonstrated modest but objective single-agent clinical activity in pts with very heavily pretreated progressive mCRPC. The safety profile of abemaciclib was consistent with the experience in breast cancer. Although the primary endpoint was not formally met, the single agent activity observed in this late line mCRPC setting validates CDK4/6 as a therapeutic target in advanced PC. The CYCLONE 2 and CYCLONE 3 studies are currently evaluating abemaciclib in combination with abiraterone/prednisone to leverage synergy of combined CDK4/6 and hormonal inhibition in first line mCRPC and mHSPC settings, respectively.

**CT160**

**Phase 1b/2a clinical trial of the oral BET inhibitor PLX2853 as monotherapy for ARID1A mutated gynecologic cancers and in combination with carboplatin for platinum resistant ovarian cance.**

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The Bromodomain and Extra-Terminal (BET) Domain proteins facilitate the development of many human cancers via epigenetic regulation. BET inhibitors may be effective in reversing platinum resistance in ovarian cancer (OC) and may generate synthetic lethality with ARID1A loss. PLX2853 is an orally active, small molecule inhibitor of BET bromodomain-mediated interactions that exhibits low nanomolar potency in blocking all 4 BET family members (BRD2, BRD3, BRD4, and BRDT). PLX2853 development is continuing at Opna Bio as OPN-2853. Clinical experience with PLX2853 monotherapy in subjects with heavily pretreated solid tumors and lymphoma showed signs of activity. The current study (NCT04493619) was designed as a multicenter, open-label trial with two parallel arms: (1) a phase 2a study of PLX2853 *monotherapy* in advanced gynecological malignancies with a known ARID1A mutation and (2) a phase Ib/2a *combination* study of PLX2853 plus carboplatin in platinum resistant OC. The primary objective of the Ib portion of the study was safety and tolerability, with the primary objective of both phase 2a portions being efficacy. In the monotherapy arm, up to 6 patients were treated at 80 mg PLX2853 daily in a safety lead-in, with progression to phase 2a using a Simon 2-stage design if dose limiting toxicities (DLTs) were observed in fewer than 33% subjects. In Stage 1, 6 additional subjects (N=12 total) were planned, with progression to stage 2 if two or more patients responded in stage 1. The combination arm included an escalation phase Ib. Three to six evaluable subjects were planned for each group, with dose escalation pending less than 33% DLT rate. The combination arm defined by the phase 1b portion of the study continued to a planned phase 2a Simon 2 stage design similar to that described for the monotherapy arm. 34 of 37 enrolled patients were evaluable with data from at least 1 post-baseline response (14 monotherapy, 20 combination therapy). Of the 14 evaluable patients on the monotherapy arm, 1 (7.1%) achieved a partial response (PR) with progression-free survival of 278 days, 5 (35.7%) had stable disease (SD) and 8 (57.1%) had progressive disease (PD). Of the 20 evaluable OC patients on the PLX2843 + carboplatin combination, 1 (5.0%) had PR, 9 (45.0%) had SD, and 10 (50%) had PD. This study in a larger cohort of gynecologic cancer patients confirmed the safety profile of the agent and

demonstrated the feasibility of combination with carboplatin. While these results did not meet the pre-specified response criteria, evidence of clinical activity nevertheless highlights the rationale for further exploration of BRD4 inhibitors in patients with ARID1A-mutated gynecologic malignancies, possibly in combination with agents targeting potential feedback mechanisms such as the PI3K pathway, frequently activated in these cancers.

**CT161**

**A phase II basket trial of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART) SWOG S1609: the non-epithelial ovarian tumor cohort.**

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Background: Dual checkpoint inhibition with Anti-PD-1 and anti-CTLA4 checkpoint inhibitors have proven to be effective in several malignancies but their potential role in numerous rare solid cancers is yet to be established. This study presents the first results of ipilimumab and nivolumab in the non-epithelial ovarian tumor cohort (#13) of the SWOG S1609 Dual Anti-CTLA-4 & Anti-PD-1 blockade in Rare Tumors (DART) trial.

Methods: DART is a prospective, open-label, multicenter/multi-cohort phase 2 clinical trial of ipilimumab (1mg/kg intravenously every 6 weeks) plus nivolumab (240mg intravenously every 2 weeks). This cohort included several histologies grouped for statistical analysis. The primary endpoint was objective response rate (ORR) (RECIST v1.1) (confirmed complete (CR) and partial responses (PR)); progression-free survival (PFS), overall survival (OS), stable disease (SD) >6 months, and toxicity are secondary endpoints.

Results: Seventeen evaluable patients (median age, 64 years) were analyzed. The subtypes of non-epithelial ovarian cancer were: granulosa cell (47%, n=8), carcinosarcoma or malignant mixed Mullerian tumor (MMMT; 35%, n=6), one each for Wolffian duct, yolk sac, and Sertoli-Leydig cell. There were 2 responses in the 8 patients with granulosa cell (ORR of 25% in the granulosa cell tumors): 1CR (79% regression [due to lymph node < 1.0cm], 59 month PFS, juvenile type) and 1 PR (79% regression, 51+ month PFS, adult type). 6/8 patients remain alive (PFS 52-1774 days). In contrast, carcinosarcomas showed no responses. One patient with Sertoli-Leydig cell tumor had a 22% response and 341 day PFS. Overall ORR was 12% (2/17), clinical benefit rate (CBR; no progression > 6months) of 29.4%. The median PFS was 3.5 months, median OS was 42.5 months. The most common adverse events were fatigue (52.9%, n=9) and hypothyroidism (35.3%, n=6). Grade 3-4 adverse events occurred in 47.1% of

patients (n=8). There were 3 adverse events (17.6%) that led to discontinuation, of which 2 (11.8%) were grade 3-4. There were no grade 5 adverse events.
Conclusion: Ipilimumab plus nivolumab in non-epithelial ovarian cancer resulted in an ORR of 12% (with 2 of 8 patients with granulosa ovarian tumors showing a durable CR and PR [both, >4 years)) and CBR of 29.4%, with durable responses seen. In contrast there were no responses in the carcinosarcoma group. One patient with Sertoli Leydig cell tumor suggested a possible benefit. Correlative studies to determine response and resistance markers are ongoing. Expanded prospective studies in granulosa tumor but not carcinosarcoma are warranted.

**CT162**

**A phase II basket trial of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART) SWOG S1609: the clear cell ovarian, endometrial, cervical cancer cohorts.**

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Background: Dual checkpoint inhibition with anti-PD-1 and anti-CTLA4 checkpoint inhibitors have proven to be efficacious in numerous malignancies. This study presents the first results of ipilimumab and nivolumab in the clear cell gynecologic cancer cohorts of the SWOG S1609 Dual Anti-CTLA-4 & Anti-PD-1 blockade in Rare Tumors (DART) trial.
Methods: DART is a prospective, open-label, multicenter/multi-cohort phase 2 clinical trial of ipilimumab (1mg/kg IV every 6wk) plus nivolumab (240mg IV every 2wk). The primary endpoint was objective response rate (ORR) (RECIST v1.1) (confirmed CR and PR); progression-free survival (PFS), overall survival (OS), stable disease (SD) >6 months, and toxicity are secondary endpoints.
Results: Evaluable patients were as follows: clear cell ovarian cancer (N=19); clear cell endometrial cancer (N=8); clear cell cervical (N=5) (median ages, 53, 66, and 59 years; cohorts 46, 45, and 42, respectively) In the clear cell ovarian cancer cohort, ORR was 21.1% [CR, 15.8%, n=3; PR, 5.3%, n=1]; clinical benefit rate (CBR) (includes stable disease $\geq$6 months) was 31.6% (6/19 patients). Among three patients with confirmed CR, two patients showed 100% regression (with ongoing response at 36+ months and 37+ months respectively), and the other patient showed 67% regression (due to lymph node < 1.0cm), but eventually progressed after 722 days. One confirmed PR patient achieved 75% regression (ongoing response at 53+ months). Of note, three patients achieved unconfirmed PR; one showed 34% regression (5 months); another, 38% (51.5+ months); and another, 58% regression (5 months). The ORR when including unconfirmed PR is 36.8% (7/19). Median PFS was 3.7 months (95% confidence interval (CI); 1.7-∞). Median OS was 21.7 months (6.4-∞). In the clear cell endometrial cancer cohort, ORR was 0%; CBR, 25% (2/8 patients). Of note, one patient achieved unconfirmed PR with 69% regression (4 months). The ORR when including unconfirmed PR is 12.5% (1/8). Median PFS was 2.0 months (95% confidence interval; 1.8-na). Median OS was 4.3 months (4.2-na). In the clear cell cervical cancer cohort, ORR was 0%; CBR, 20.0% (1/5 patients). Median PFS was 2.2 months (95% CI; 1.9-na). Median OS was 23.6 months (95% CI; 15.3-na). The most common adverse events, in the three cohorts combined, were nausea (37.5%, n=12), fatigue (34.4%, n=11), anorexia (31.2%, n=10), hypothyroidism (31.2%, n=10), and pruritus (28.1%, n=9). Grade 3-4 adverse events were reported in 17 cases (53.1%) with no grade 5 adverse events.
Conclusion: Ipilimumab plus nivolumab in 19 clear cell ovarian cancer patients resulted in an ORR of

21.1% and CBR of 31.6%, with two durable CRs ongoing at 3+ years; CBR was 25% and 20%, respectively, in clear cell endometrial and cervical cohorts, with no objective responses, albeit with only 8 and 5 patients per cohort. Correlative studies to determine response/resistance markers are ongoing. Further prospective studies in rare gynecologic malignancies are warranted.

**CT163**

**A phase II basket trial of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART) SWOG S1609: the small cell carcinoma of the ovary, hypercalcemic type cohort.**

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Background: Dual checkpoint inhibition with Anti-PD-1 and anti-CTLA4 checkpoint inhibitors have shown to be efficacious in many malignancies, but its potential role in various rare solid cancers is yet to be established. Small cell ovarian carcinoma, hypercalcemic type (SCCOHT) is a rare tumor characterized by loss of SMARC 2/4 function and so presents a novel paradigm for the treatment of SWI/SNF pathway deficient tumors (Petar Jelinic et al., 2018; Marc Tischkowitz et al., 2020). This study presents the first results of ipilimumab and nivolumab used in the SCCOHT cohort (#49) of the SWOG S1609 Dual Anti-CTLA-4 & Anti-PD-1 blockade in Rare Tumors (DART) trial.
Methods: DART is a prospective, open-label, multicenter/multi-cohort phase 2 clinical trial of ipilimumab (1mg/kg intravenously every 6 weeks) plus nivolumab (240mg intravenously every 2 weeks). The primary endpoint includes objective response rate (ORR) (RECIST v1.1) (confirmed complete (CR) and partial responses (PR)). Secondary endpoints include progression-free survival (PFS), overall survival (OS), stable disease (SD) >6 months, and toxicity.
Results: Five evaluable patients (median age 30) with SCCOHT were analyzed. Objective response rate was 20% (1 CR with 100% regression). The patient with CR has a duration of response (DoR) and OS of 35+ months. Another patient, showed unconfirmed PR with 81% regression (DoR 4 months), this patient went on to have confirmed iCR (CR confirmed by iRECIST) at around 24 months and has OS of 38+ months. At 12 months, 3 patients remain alive and 1 patient remains progression free; overall median PFS was 1.8 months (1.0-∞); median OS was 24 months (4.5-∞). The most common adverse events were fatigue, nausea, pruritus, dry mouth, maculo-papular rash and aspartate aminotransferase elevation (50%, n=2, respectively). There were two incidents (33.3%) of grade 3-4 adverse events. None of the adverse events led to discontinuation. There were no grade 5 adverse events.
Conclusion: Ipilimumab plus nivolumab in five patients with the ultra-rare small cell ovarian carcinoma (hypercalcemic type) resulted in one CR durable at 35+ months and one unconfirmed PR with 81% regression. This is the first prospective study demonstrating efficacy of nivolumab and ipilimumab in this rare disease. Correlative studies to determine response and resistance markers are ongoing. Expanded prospective studies in small cell ovarian histologies are needed.

**CT164**

**Tisotumab vedotin (TV) in squamous cell carcinoma of head and neck (SCCHN): interim analysis from innovaTV 207.**

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Introduction: TV, a tissue factor (TF)-directed antibody-drug conjugate, is approved under accelerated approval in the US at a dose of 2.0 mg/kg IV administered every 3 weeks (Q3W) for adult patients (pts) with recurrent or metastatic (r/m) cervical cancer who have progressed on or after chemotherapy. TV is also being evaluated in several advanced solid tumors known to express TF, including SCCHN; antitumor activity has been observed at the approved dose level. Previous reports show that modifying the dosing schedule to optimize key PK parameters such as AUC, $C_{max}$, and $C_{trough}$ can lead to further improvement in clinical efficacy. A population PK model based on 399 pts across clinical trials suggests that when TV is administered at 1.7 mg/kg IV on Days 1 and 15 of a 28-day cycle (Q2W), pts are predicted to achieve 24% higher AUC, a higher $C_{trough}$ level, and a lower $C_{max}$, compared with the approved cervical cancer regimen (2.0 mg/kg Q3W). Here, we report the first analysis of TV 1.7mg/kg Q2W for r/m SCCHN that has progressed after prior platinum combination with or without immunotherapy.
Methods: innovaTV 207 (NCT03485209) is an open label phase 2 multi-center study evaluating TV monotherapy or in combination for advanced tumors, including pts with r/m SCCHN. In Part C of the study, eligible pts could have received up to 3 lines of systemic therapy for r/m disease and must have received prior therapy with a platinum-based regimen and a checkpoint inhibitor (CPI), if eligible. TV was administered at 1.7 mg/kg IV Q2W. The primary endpoint was objective response rate (ORR), and secondary endpoints included safety and tolerability.
Results: At data cutoff (28Nov2022), 15 pts with SCCHN were treated. The median number of prior lines for r/m disease was 2. All pts received prior platinum therapy and majority (93%) received a CPI. 67% of pts received cetuximab and 53% received taxanes, for r/m SCCHN. Confirmed ORR was 40% (95% CI: 16.3, 67.7), with 1 complete response and 5 partial responses. The safety profile was generally consistent with that observed across TV monotherapy clinical studies. 13 pts experienced a treatment-related adverse event (TRAE), most commonly asthenia (n=7), peripheral sensory neuropathy (PSN) (n=7), and vomiting (n=5). 4 pts experienced Grade ≥3 TRAEs. 2 pts discontinued treatment due to an AE (PSN and dry eye, n=1 each). 11 pts received 3 or more cycles of treatment (1 cycle = 28 days).
Conclusions: For pts with r/m SCCHN who have progressed after prior platinum combination and immunotherapy, TV may be a promising treatment option. Preliminary data suggest encouraging antitumor activity at a higher exposure with Q2W administration while maintaining an acceptable safety profile. The innovaTV 207 study is still enrolling and different dosing regimens are currently being evaluated across advanced solid tumors.

**CT165**

**Phase II study of nivolumab and ipilimumab for treatment of metastatic/recurrent adenoid cystic carcinoma (ACC) of all anatomic sites of origin and other malignant salivary gland tumors.**

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Introduction: Dual checkpoint inhibitor therapy with nivolumab and ipilimumab has been FDA-approved for a number of different cancers including melanoma, recurrent NSCLC, and hepatocellular carcinoma. However, their role in the treatment of ACC and other salivary gland carcinomas is not well established.
Methods: We performed a Simon's two-stage prospective single-institution phase II clinical trial of nivolumab (240 mg intravenously every 2 weeks for 16 weeks, then 480 mg every 4 weeks) with ipilimumab (1 mg/kg intravenously every 6 weeks), both of which were provided until intolerable toxicity or disease progression. Two cohorts were analyzed: patients with metastatic/recurrent ACC and patients with other salivary gland cancers. The primary endpoint was median progression-free survival (PFS) based on RECIST and immune-related RECIST (irRECIST) criteria; secondary endpoints were overall response rate (ORR), overall survival (OS), and toxicity.
Results: Patients with ACC (n=19) and other salivary gland carcinomas (total n=5; parotid gland adenocarcinoma (n=3), salivary duct carcinoma (n=1), and myoepithelial carcinoma (n=1)) were enrolled between May 2017 and July 2019. Among the patients with ACC, the median OS was 30.0 months (95% confidence interval (CI) 15.3 months to not reached), the median PFS was 8.3 months (95% CI 5.5 - 30.0 months), and the disease control rate (DCR) was 53% (10/19). The ORR in the ACC group was 5% (CR 0%, n=0; confirmed PR 5%, n=1) using both RECIST and irRECIST criteria, with one patient having continued stable disease at the time of trial conclusion. In the salivary gland tumor cohort, the median OS was 10.4 months (95% CI 6.21 months to not reached), the median PFS time was 6.21 months (95% CI 2.83 months to not reached), and the DCR was 40% (2/5). The ORR in this cohort was 0% using both RECIST and irRECIST criteria. Across both cohorts, platelet counts above the joint cohort's median were significantly associated with better OS (p=0.032) and PFS (p=0.046). Additionally, although not significant, there was a trend for improved OS and PFS among patients with neutrophil-to-lymphocyte ratios >5 (OS p=0.42, PFS p=0.25) and above median neutrophil counts (OS p=0.24, PFS p=0.18). Four patients with ACC underwent circulating tumor DNA sequencing, resulting in the identification of two MYB-NFIB translocations, one NOTCH1 Cys1383 frameshift mutation, one MTOR N1760K mutation, and one PDGFRA copy number gain. Common immune-related toxicities include fatigue (54%), lymphocytopenia (46%), and anemia (42%) with lymphocytopenia being the most common grade III/IV adverse event.
Conclusion: In patients with recurrent or metastatic ACC and other salivary gland neoplasms, combination nivolumab with ipilimumab resulted in moderate disease control. Further studies are warranted to validate our findings.

**CT166**

**Similar outcomes regardless of post-randomization treatment with ibrutinib or ibrutinib + venetoclax in the phase 2 CAPTIVATE study of first-line ibrutinib + venetoclax in CLL.**

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***Background:*** CAPTIVATE (NCT02910583) is a multicenter phase 2 study of first-line ibrutinib (Ibr) + venetoclax (Ven) in CLL. In the Minimal Residual Disease (MRD) cohort, pts underwent randomization to subsequent treatment based on MRD status after completing Ibr + Ven (Wierda et al, *J Clin Oncol* 2021). Here, 4-yr follow-up (3-yr post-randomization) outcomes are reported for MRD cohort pts

randomized to continued Ibr or Ibr + Ven.
***Methods:*** 164 pts aged <70 yr with previously untreated CLL received 3 cycles of Ibr then 12 cycles of Ibr + Ven (Ibr 420 mg/d; Ven ramp-up to 400 mg/d); a 13th cycle occurred concurrent with MRD assessment. Pts not meeting criteria for Confirmed undetectable MRD (uMRD Not Confirmed) were randomized to open-label Ibr (n=31) or Ibr + Ven (n=32; 1 additional yr of Ibr + Ven then Ibr alone). Pts with Confirmed uMRD were randomized 1:1 to double-blind placebo or Ibr (n=86).
***Results:*** In uMRD Not Confirmed pts, median time on study was 48 mo; post-randomization median follow-up was 33 mo. Improvements in overall best uMRD rates from pre-randomization were: +3% (to 48%) with Ibr and +25% (to 72%) with Ibr + Ven in peripheral blood, and +10% (to 42%) with Ibr and +38% (to 69%) with Ibr + Ven in bone marrow. uMRD rates over time are in the Table. Improvements in complete response (CR) rates were similar with Ibr (+29%) and Ibr + Ven (+28%). 4-yr progression-free survival (PFS) rates were 93% in both arms; 4-yr overall survival (OS) rates were 97% (Ibr) and 100% (Ibr + Ven) and consistent with Confirmed uMRD arms (Allan et al, ASH 2022). Adverse events (AEs) generally decreased over time post-randomization (Table) except for hypertension. In this 4th yr of follow-up (25-36 mo post-randomization), atrial fibrillation was rare (n=1/arm); no AEs led to drug discontinuation in either arm. With continued Ibr, outcomes in uMRD Not Confirmed pts were similar to those in Confirmed uMRD pts: median time on study, 56 mo; post-randomization median follow-up, 42 mo; CR rate, 81%; 4-yr PFS, 95%; 4-yr OS, 98%.
***Conclusion:*** In pts without Confirmed uMRD after Ibr + Ven, continued Ibr + Ven improved uMRD rates; PFS and OS were similarly high with continued Ibr or Ibr + Ven (≥97%). AE rates generally decreased over time in both arms. Outcomes with continued Ibr were not impacted by pre-randomization Confirmed uMRD status.
**Table.** uMRD rates and prevalence of AEs over time in uMRD Not Confirmed pts

| | **Ibr + Ven then Ibr** | | | **Ibr + Ven then Ibr + Ven** | | |
|---|---|---|---|---|---|---|
| **uMRD rate at selected timepoints in evaluable pts, n/N (%)** | **End of Pre-Rnd** | **12 cycles Post-Rnd** | **36 cycles Post-Rnd** | **End of Pre-Rnd** | **12 cycles Post-Rnd** | **36 cycles Post-Rnd** |
| Peripheral blood | 13/31 (42) | 10/31 (32) | 7/31 (23) | 9/32 (28) | 19/30 (63) | 11/31 (35) |
| Bone marrow | 10/31 (32) | 9/30 (30) | Not done | 10/31 (32) | 18/31 (58) | Not done |
| **Prevalence of AEs of clinical interest, n (%)[a]** | **First 16 cycles, n=31** | **1-12 mo, n=31** | **25-36 mo, n=27** | **First 16 cycles, n=32** | **1-12 mo, n=32** | **25-36 mo, n=25** |
| Grade ≥3 AEs | | | | | | |
| Hypertension | 2 (6) | 1 (3) | 0 | 2 (6) | 0 | 0 |
| Atrial fibrillation | 1 (3) | 0 | 0 | 0 | 1 (3) | 0 |
| Diarrhea | 2 (6) | 0 | 0 | 2 (6) | 1 (3) | 0 |
| Neutropenia | 10 (32) | 2 (6) | 0 | 10 (31) | 2 (6) | 0 |
| Infections/ infestations | 6 (19) | 0 | 2 (7) | 2 (6) | 2 (6) | 1 (4) |
| Hemorrhage | 0 | 0 | 0 | 0 | 1 (3) | 0 |
| Any-grade AEs | | | | | | |
| Hypertension | 5 (16) | 5 (16) | 6 (22) | 5 (16) | 4 (13) | 5 (20) |
| Atrial fibrillation | 3 (10) | 2 (6) | 1 (4) | 0 | 2 (6) | 1 (4) |

[a]AEs were assessed at every visit pre-Rnd and every 3 cycles post-Rnd. Rnd, randomization.

**CT167**

**Efficacy of pirtobrutinib, a highly selective, non-covalent (reversible) BTK inhibitor in Richter transformation: Results from the phase 1/2 BRUIN study.**

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**Background:** Richter transformation (RT) is an aggressive diffuse large B-cell lymphoma that can occur in 10% of patients with chronic lymphocytic leukemia (CLL). RT has no approved therapies, poor prognosis, and an estimated median overall survival (OS) of 3-11 months. Pirtobrutinib is a well-tolerated highly selective, non-covalent (reversible) Bruton tyrosine kinase inhibitor (BTKi) with favorable oral pharmacology and promising efficacy in patients with poor-prognosis B-cell malignancies following prior covalent BTKi therapy. We now report on RT patients from BRUIN.

**Methods:** Patients previously treated for B-cell malignancies were eligible for pirtobrutinib monotherapy in dose escalation/expansion within the phase 1/2 BRUIN study. Key endpoints: investigator-assessed overall response rate (ORR) and duration of response (DoR) per Lugano 2014 criteria, and safety. The response-evaluable cohort consisted of patients with RT who completed either first response assessment or discontinued therapy. The safety cohort consisted of patients who received ≥1 dose of pirtobrutinib monotherapy (n=725). Data cut was 31 January 2022.

**Results:** Of 57 RT patients (median age: 67 years), 91% (n=52) received at least 1 prior RT-directed therapy. Median lines of prior RT therapy were 2: (anti-CD20 antibody [86%, n=49], chemotherapy [79%, n=45], BCL-2 inhibitor [35%, n=20], BTKi [28%, n=16], PI3K inhibitor [12%, n=7], CAR-T [9%, n=5], immunomodulator [5%, n=3], stem cell transplant [4%, n=2], other systemic therapy [33%, n=19]) and median lines of prior CLL were 2: (BTKi [60%, n=34], anti-CD20 antibody [60%, n=34], chemotherapy [47%, n=27], BCL-2 inhibitor [42%, n=24], immunomodulator [9%, n=5], PI3K inhibitor [7%, n=4], stem cell transplant [7%, n=4], CAR-T [2%, n=1], other systemic therapy [11%, n=6]). ORR for patients within phase 2 (n=56, 200mg once/day starting dose) was 54% (95% CI, 39-68): 5 complete responses,

22 partial responses. Median DoR was 8.6 months (95% CI, 1.9-NE, 63% censored) and median OS was 13.1 months (95% CI, 7.1-NE) at a median follow up time of 5.5 months and 9.7 months respectively. Six patients electively discontinued pirtobrutinib to pursue curative-intent therapy (allogeneic transplant, n=6, 3.8 months median time-on-therapy). The most frequent TEAEs were fatigue (26%, n=191), diarrhea (22%, n=160), and contusion (19%, n=138). Of Grade≥3 TEAEs, neutropenia (20%, n=143) was the most frequent and hypertension (3%, n=20), hemorrhage (2%, n=16), and atrial fibrillation/flutter (1%, n=7) were of low grade. Fifteen (2%) patients discontinued due to a treatment-related AE.
**Conclusions:** Pirtobrutinib demonstrated promising preliminary efficacy and was well tolerated for patients who received prior RT-directed chemoimmunotherapy and covalent BTKi. Presented:ASH2022.

**CT169**

**Bioplatin (NTPX-07) as an oral nano platinum in patients of advanced stage malignancy: Outcomes of a phase I clinical trial.**

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**Purpose:** Platinum derivatives are widely used for treating a variety of solid tumours. Besides its direct cytotoxic action, Platinum derivatives play an important role in antitumor immune response, mainly by its inherent capacity to induce immunogenic cell death. Induction of apoptosis using novel therapeutics can be considered a key therapeutic strategy for preventing recurrence and metastasis in cancer patients. Bioplatin is an oral, nano platinum-based compound manufactured using patented innovative processing, based on green technology with an aim of retaining its anti-cancer properties while at the same time reducing its dose limiting toxicities. This compound has demonstrated the property of inducing apoptosis in cancer cells by preventing DNA replication. Since Bioplatin is developed with an aim of arresting disease progression and improving progression-free survival /disease-free survival its administration is expected to be for a longer duration hence its oral dosing and good tolerability are of utmost importance.
**Methods:** The present study, Phase-I multicentric clinical trial (CTRI/2017/06/008778) was conducted. with an aim of determining the maximum tolerated dose, safety, tolerability and effect on Quality-of-life (QoL) Bioplatin was administered orally for 21 consecutive days to advanced-stage cancer patients of solid tumours who were not responding to any conventional anticancer therapy. Dose escalation was carried out to determine the Maximum Tolerated Dose (MTD).
**Results:** A total of 14 patients received Bioplatin at 4 dose levels, 10 mg, 20 mg, 40 mg, and 60 mg Cohort. The mean progression-free survival (PFS) was 40.25 ± 13.47 days. The highest mean PFS of 50.67 ± 6.66 days was observed in the 40 mg Cohort. The mean PFS was 37.67 ± 16.74 days in 60 mg cohort. Analysis of QoLusing FACT-G showed improvement in QoL (FACT-G) in the 60 mg cohort as compared to Day 1 ($p$<0.05). No dose-limiting toxicity was reported.
**Conclusion:** Considering that Bioplatin can be administered orally, its tolerability, and its property to delay tumor growth, we propose that it can be used for the treatment of patients with platinum-sensitive tumours. Bioplatin has the potential as an orally consumable immunotherapy agent for longer duration. It is expected to increase disease-free survival (DFS) post-surgery /chemotherapy or as maintenance therapy with other agents in advanced stages patients of platinum-sensitive tumours for improving Quality of life and increasing survival .The findings of the study warrant further clinical evaluation.

**CT170**

**Initial safety and feasibility results from a first-in-human clinical trial evaluating a novel magnetic hyperthermia approach for the treatment of metastatic solid tumors.**

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A novel approach for cancer therapy was developed, comprising of magnetic multicore iron oxide nanoparticles (SaNPs) and alternating magnetic field (AMF) application. SaNPs are administered IV to the patient and accumulate in tumors through the enhanced permeability and retention (EPR) effect. The patient then undergoes regional AMF application (300kHz) that is converted to thermal energy, inducing sub-ablative hyperthermic cancer cell death. Based on extensive pre-clinical testing, treatment was proven safe and biocompatible in animal models without any associated toxicities. Efficacy studies in a murine metastatic cancer model, demonstrated the ability to reduce the number and size of metastatic lesions.
**Methods:** A First-in-Human open label dose escalation study was designed to evaluate the feasibility and safety of treatment in patients (n=18) with advanced metastatic solid tumors. A secondary objective was to evaluate initial efficacy signs. Eligibility criteria included patients with a life expectancy of at least 30 days with measurable disease according to RECIST 1.1. The patients underwent toxicity analysis and MRI scans without contrast media before (baseline), after treatment, and at the end of the follow-up period of 1 month. Vital signs were monitored before, during, and after treatment. Measurements included ECG, oral and body surface temperatures, blood pressure, heart rate, and oxygen saturation.
**Results:** 3 patients were enrolled in the first cohort that received a single SaNP injection of 10% (first dose level) without AMF. No systemic toxicity was observed. 7 patients were then enrolled and SaNP was administered at the same dose followed by AMF irradiation (first dose level of 40%) of the torso area, conducted for two intervals of 5 min. each. SaNP administration procedures were successful in all cases whereas, 3 patients succeeded to complete the full treatment in accordance to the study protocol. During AMF exposure, the patients were covered with a cooling blanket and temperatures were monitored using fiber-optic sensors. There were no significant temperature deviations beside one patient, where a rise in oral temperature was noted due to the presence of dental implants which heated up. In all patients there were no significant differences in any of the vital signs post-injection nor before or after AMF except for one patient, for whom increased heart rate was observed during irradiation, returning to baseline thereafter. No treatment-related abnormal clinical pathology or urinalysis results were identified. Some fluctuations were observed, attributed to the disease stage. Although tumor response was not expected at the first dose levels, MRI and CT results for one patient showed a reduction in a breast tumor and in another patient, stable disease was reported.
**Conclusion:** SaNP administration either alone or followed by AMF application was not associated with any significant adverse reactions, and no clinical or humoral toxicity was observed at initial doses of 10% SaNP and 40% AMF.

**CT171**

**A first-in-human phase I study of the ATM inhibitor M4076 in patients with advanced solid tumors (DDRiver Solid Tumors 410): Part 1A results.**

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M4076 is a potent and selective oral inhibitor of ataxia-telangiectasia mutated (ATM), a key kinase of the DNA damage response (DDR) involved in double-strand break repair. Preclinically, M4076 when combined with DNA damage-inducing therapy or other DDR inhibitors caused unrestricted cell cycle progression and DNA damage accumulation, resulting in tumor cell death.
Part 1A of this ongoing open-label study (NCT04882917) evaluated the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and maximum tolerated dose (MTD) of M4076 monotherapy in patients (pts) with advanced solid tumors. Dose escalation was guided by safety, along with Bayesian analysis, PK, and PD.
In total, 22 pts received M4076 at 4 different dose levels (100-400 mg once daily [QD]); 21 pts (95.5%) discontinued treatment (progressive disease: 11 [50%], adverse events [AEs]: 5 [22.7%]). Dose-limiting toxicities (DLTs) were reported in 4 pts. Overall, 6 pts had ≥1 treatment-related AE (TRAE) of Grade ≥3 (including one Grade 4 hypersensitivity). The most common TRAEs were rash and anemia (Table 1).
MTD was determined at 300 mg QD. As per preliminary PK across cohorts, M4076 was rapidly absorbed with median $T_{max}$ ~0.5-2 h and mean elimination half-life ($t_{1/2}$) ~2-10 h; exposure increased in a dose-related manner, with minimum accumulation after multiple QD doses. At ≥200 mg QD, unbound steady state plasma concentration exceeded *in vivo* pCHK2 $IC_{90}$ throughout the dosing interval. PD evaluation was based on the modulation of γ-H2AX (a direct ATM target) in *ex vivo* bleomycin stimulated CD45+ lymphocytes, by flow cytometry. Preliminary analyses showed a trend of reduction of γ-H2AX levels from day 1 to day 2, reaching 80-100% of target inhibition on day 2 with the dose range 100-400 mg QD.
The MTD of M4076 was determined, and target exposure and engagement were achieved without significant hematological toxicity. Future investigations on M4076 as combination therapy are planned.

Table 1: Safety overview

| **Dose** (Safety set) | **Patients with TRAEs (Grade ≥3)** | **DLT analysis set** (Patients with ≥80% of the planned dose or DLT per investigator) | **DLT** | **DLT AE terms** |
|---|---|---|---|---|
| 100 mg (n = 2) | No | n = 2 | No | NA |
| 200 mg (n = 7) | n = 1 (decreased lymphocyte count, maculo-papular rash) | n = 5 | Yes (n = 1) | Grade 3 maculo-papular rash (TRAE, required drug withdrawal; resolved) |
| 300 mg (n = 9) | n = 3 (anemia, spontaneous bacterial peritonitis) | n = 7 | Yes (n = 1) | Grade 1 maculo-papular rash; Grade 2 fever (TRAEs, required treatment discontinuation; resolved) |
| 400 mg (n = 4) | n = 2 (nausea, maculo-papular rash, hypersensitivity) | n = 4 | Yes (n = 2) | Grade 3 maculo-papular rash (TRAEs, required dose reduction/interruption and/or concomitant medication; resolved) |

MedDRA version 23.0, NCI-CTCAE version 5.0. Data cutoff: 2 Nov 2022
AE, adverse event; DLT, dose-limiting toxicity; NA, not applicable; TRAE, treatment-related AE

**CT172**

**A phase 1, open-label, dose-escalation study of PRT1419, a selective induced myeloid leukemia cell differentiation protein (MCL-1) inhibitor, in patients (pts) with advanced/metastatic solid tumors.**

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**Introduction:** PRT1419, a selective inhibitor of MCL-1, has demonstrated preclinical activity in solid tumors and hematologic malignancies. We report the results of a phase 1, open-label, multicenter, dose-escalation study of PRT1419 in pts with advanced/metastatic solid tumors (NCT04837677).
**Methods:** Adults with select advanced/metastatic solid tumors were enrolled. Key inclusion criteria were absolute neutrophil count >1.0, platelets >75, hemoglobin >9, and left ventricular ejection fraction ≥50%. Key exclusion criteria were active central nervous system malignancy, significant cardiac disease, and concurrent strong CYP2C8 inhibitors. The study employed a 3+3 dose escalation design. PRT1419 was administered as a once-weekly (QW) infusion. Primary outcome measures were to evaluate dose-limiting toxicities (DLT) and establish the maximum tolerated dose and recommended phase 2 dose (RP2D). Secondary outcome measures included safety, tolerability, pharmacokinetics, pharmacodynamics, and antitumor activity.
**Results:** Pts with melanoma (n=8), sarcoma (n=5), esophageal (n=4), cervical (n=3), head and neck (n=3), non-small cell lung, small cell lung, and triple-negative breast cancer (all n=1) were enrolled. Median age was 59.5 (range, 32-78) years, 61.5% pts were female, and median prior lines of systemic therapy was 3 (range, 1-8). Overall, 26 pts received ≥1 dose of PRT1419 (20 mg/m$^2$ [n=4], 40 mg/m$^2$ [n=4], 80 mg/m$^2$ [n=15], and 120 mg/m$^2$ [n=3]). Most pts (84.6%) discontinued treatment (primary reason: progressive disease, 72.7%). No DLTs, adverse events (AEs) of increased troponin or heart failure, or grade 5 (fatal) AEs were observed. Any grade treatment-related AEs (TRAE) occurred in 80.8% pts; the most common were nausea (50.0%), diarrhea (46.2%), and vomiting (46.2%). Grade 3/4 TRAEs occurred in 11.5% pts; the most common was neutropenia (11.5%), which was dose related. Due to neutropenia observed at doses >80 mg/m$^2$, further dose escalation was not investigated. BAX and caspase 3 activation in peripheral blood monocytes was observed at 80 mg/m$^2$ and 120 mg/m$^2$. Stable disease was the best response observed. Tumor shrinkage was seen in 1 pt with melanoma (10% reduction) and 1 pt with lung cancer (4% reduction) but did not meet the criteria for partial response. At Week 3, mean clearance was 12.6 L/hr and mean half-life was 2.5 hrs; no PRT1419 accumulation was seen with QW dosing.
**Conclusions:** PRT1419 demonstrated acceptable safety and tolerability in pts with advanced/metastatic solid tumors, with primary TRAEs of neutropenia, nausea, diarrhea, and vomiting. No cardiac toxicity was observed. BAX and caspase 3 activation was observed, suggesting potentially successful MCL-1 inhibition. Investigation of PRT1419 in pts with hematologic malignancy in a separate study is ongoing (NCT05107856).

**CT173**

**A phase 1, open-label, multicenter, dose-escalation study of PRT2527, a cyclin-dependent kinase 9 (CDK9) inhibitor, in adult patients (pts) with advanced solid tumors.**

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**Background**: CDK9 is a key regulator of transcription elongation and recent studies suggest selective

CDK9 inhibition may be a promising approach to treat "transcription-addicted" cancers that are dependent on oncogenic drivers with a short half-life. We report phase 1 dose-escalation results from an ongoing open-label study of PRT2527 (NCT05159518), a potent and highly selective CDK9 inhibitor, in pts with advanced solid tumors.
**Methods**: Key inclusion criteria include pts aged ≥18 years; selected sarcomas, castrate-resistant prostate cancer (CRPC), HR+/HER2- breast cancer, non-small cell lung cancer, or *MYC*-amplified solid tumors; received prior therapy; measurable disease (evaluable for CRPC); ECOG PS ≤1; and adequate organ function. Central nervous system disease is allowed if stable and adequately controlled. Pts received a once-weekly (QW; 21-day cycle) infusion of PRT2527 (3, 6, and 12 mg/m$^2$ QW, n=3 pts per dose level using a Bayesian optimal interval dose escalation design) until intolerance, progression, death, or withdrawal. Primary objectives were to evaluate dose-limiting toxicities (DLT), establish the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of PRT2527. Secondary objectives included safety and tolerability, pharmacokinetics, and antitumor activity.
**Results**: At data cutoff (30 September 2022), 9 pts (CRPC, n=5; breast cancer, n=2; sarcoma, n=2) were treated in the first 3 dose levels of dose escalation. Median age was 64 (range, 57-73) years and 56% of pts were male. No DLTs were observed, the MTD was not reached, and evaluation of the RP2D is ongoing. Overall, 89% of pts have discontinued therapy (75% due to progressive disease); no discontinuations or dose reductions due to adverse events (AEs) and no deaths were reported. One pt on 12 mg/m$^2$ QW had a dose interruption due to grade 2 myalgia and arthralgia; both events were considered related to study drug. All-cause, any grade AEs occurred in 89% of pts, most commonly nausea (44%), decreased appetite (33%), diarrhea (33%), and vomiting (33%). All-cause grade 3/4 AEs occurred in 22% of pts, including anemia (11%) and hyperglycemia (11%); both were unrelated to study treatment. One serious AE unrelated to treatment was reported (pneumothorax). Dose-dependent inhibition of CDK9 transcriptional targets was observed. A mean decrease of 88% (SD, 2.4%; n=3) in *MCL1* and *MYC* mRNA was achieved 2 hours post-infusion of the 12 mg/m$^2$ dose; by 4 hours post-infusion, expression levels had rebounded to 50% (SD, 2.7%; n=3; *MYC*) and 78% (SD, 6.4%; n=3; *MCL1*) inhibition.
**Conclusions**: PRT2527 is generally well tolerated in adults with advanced solid tumors. Updated data (including efficacy) will be presented.

**CT174**

**First-in-class anti-CD200R1 antibody 23ME-00610 in patients with advanced solid malignancies: Phase 1 results.**

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Background: CD200R1 was identified as a promising immuno-oncology (IO) target from the 23andMe database. Pleiotropic causal variants with opposing effect on risks for cancer and immune diseases, referred to as an IO signature, were observed in 3 components of the CD200R1 pathway. 23ME-00610 is a first-in-class monoclonal antibody that potently inhibits the CD200R1 immune checkpoint ($K_D$ less than 0.1 nM). The dose escalation portion of the first-in-human, Phase 1/2a study of 23ME-00610 (NCT05199272) has been completed and we are reporting data for the first time.
Methods: Eligible patients were alteast 18 years with histologically diagnosed locally advanced (unresectable), or metastatic carcinoma or sarcoma who have progressed on standard therapies with ECOG 0 or 1. Key exclusion criteria included active autoimmune disease requiring immunosuppressive therapy and Grade 3 or above immune-mediated toxicity related to prior immunotherapy that led to discontinuation. Dose escalation consisted of accelerated titration (2 and 6 mg) followed by a 3+3 design (20, 60, 200, 600 and 1400 mg). Up to 12 participants were included in a PKPD backfill cohort at the

recommended phase 2 dose (RP2D) or a previously evaluated dose level. Participants received 23ME-00610 intravenously every 3 weeks (Q3W) infused over 30 minutes. The primary objectives of Phase 1 were determination of the maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D), safety and tolerability. Key secondary and exploratory objectives included pharmacokinetics (PK), pharmacodynamics (PD) and antitumor activity of 23ME-00610.
Results: Between January 5th, 2022 and November 28, 2022, 21 participants (11/10 male/female; age range: 21-80 years) were enrolled and received ≥ 1 dose of 23ME-00610 across the 2 to 1400 mg cohorts (median duration of exposure: 50 days; range:1-231 days). No dose limiting toxicities, treatment related serious adverse events (TRSAEs) or AEs leading to discontinuation were observed. 14/21 (67%) participants experienced atleast 1 TRAE; the majority were Grade 1 or 2. The most commonly reported TRAEs occurring in atleast 2 participants across all dose levels were headache, fatigue, nausea and pruritis. There was 1 Grade 3 TRAE of increased blood creatinine phosphokinase. Investigator-assessed immune-related AEs including hypothyroidism, pruritis, fatigue and chills were observed at doses 60 mg and above. The PK of 23ME-00610 were dose-proportional at doses 60 mg and above, with a median terminal half-life of ~12 days at 1400 mg. Peripheral saturation of CD200R1 was observed at doses 60 mg and above, as measured by receptor occupancy on T cells and neutrophils, and levels of free soluble CD200R1. Analysis of additional PD data, including cytokines, is ongoing.
Conclusion: 23ME-00610 demonstrated an acceptable safety and tolerability profile, with favorable PK and peripheral CD200R1 saturation. Increased immune-related AEs were observed at higher, pharmacologically relevant dose levels. Based on Phase 1 data, 1400 mg 23ME-00610 Q3W will be evaluated in Phase 2a.

**CT175**

**Safety, tolerability, pharmacokinetics, and antitumor activity of SHR-A1811 in HER2-expressing/mutated advanced solid tumors: A global phase 1, multi-center, first-in-human study.**

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**Background:** SHR-A1811 is an ADC comprised of a humanized anti-HER2 monoclonal antibody (trastuzumab), a cleavable linker, and a DNA topoisomerase I inhibitor payload. Here we assessed SHR-A1811 in HER2-expressing/mutated unresectable, advanced, or metastatic solid tumors.
**Methods:** Pts were eligible if they had HER2 positive breast cancer (BC), HER2 positive gastric/GEJ carcinoma, HER2 low-expressing BC, HER2-expressing/mutated NSCLC, or other HER2-expressing/mutated solid tumors, and were refractory or intolerant to standard therapy. SHR-A1811 at doses of 1.0-8.0 mg/kg was given Q3W (IV). The primary endpoints were DLT, safety, and the RP2D.
**Results:** From Sep 7, 2020 to Sep 28, 2022, 250 pts who had undergone a median of 3 prior treatment lines in the metastatic setting received at least one dose of SHR-A1811 in dose escalation, PK expansion, and indication expansion part. As of data cutoff on Sep 28, 2022, 1 pt experienced DLT. Treatment-related adverse events (TRAEs) were reported in 243 (97.2%) pts. Grade ≥3 TRAEs, serious TRAEs, and treatment-related deaths were reported in 131 (52.4%), 31 (12.4%), and 3 (1.2%) pts, respectively. Interstitial lung disease (AESI) was reported in 8 (3.2%) pts. Exposures of SHR-A1811, total antibody, and the payload were generally proportional to dose from 3.2 to 8.0 mg/kg. ORR was 61.6% (154/250, 95% CI 55.3-67.7) in all pts. Objective responses were observed in pts with HER2 positive BC (88/108, ORR 81.5%, 95% CI 72.9-88.3), HER2-low BC (43/77, ORR 55.8%, 95% CI 44.1-67.2), urothelial carcinoma (7/11), colorectal cancer (3/10), gastric/GEJ carcinoma (5/9), biliary tract cancer (5/8), NSCLC (1/3), endometrial cancer (1/2), and H&N cancer (1/1). Subgroup analyses of ORR are shown in Table 1. The 6-month PFS rate was 73.9% in all pts.
**Conclusions:** SHR-A1811 was well-tolerated and showed promising antitumor activity in heavily pretreated advanced solid tumors.

Table 1. Subgroup analyses of ORR

| No. of prior treatment lines in metastatic setting in all pts (N=250) | | | |
|---|---|---|---|
| | HER2 positive BC (N=108) | HER2-low BC (N=77) | Other tumor types (N=65) |
| ≤3 | 81.8% (45/55) | 58.7% (27/46) | 36.7% (18/49) |
| >3 | 81.1% (43/53) | 51.6% (16/31) | 31.3% (5/16) |
| Prior anti-HER2 therapies in pts with BC (N=185)* | | | |
| | HER2 positive BC (N=108) | HER2-low BC (N=77) | All BC (N=185) |
| Any | 82.2% (88/107, 73.7-89.0) | 68.8% (11/16, 41.3-89.0) | 80.5% (99/123, 72.4-87.1) |
| Trastuzumab | 81.9% (86/105, 73.2-88.7) | 75.0% (9/12, 42.8-94.5) | 81.2% (95/117, 72.9-87.8) |
| Pertuzumab | 83.0% (39/47, 69.2-92.4) | 100% (5/5, 47.8-100) | 84.6% (44/52, 71.9-93.1) |
| Pyrotinib | 86.9% (53/61, 75.8-94.1) | 71.4% (5/7, 29.0-96.3) | 85.3% (58/68, 74.6-92.7) |
| Lapatinib | 80.0% (28/35, 63.1-91.6) | 100% (1/1, 2.5-100) | 80.6% (29/36, 64.0-91.8) |
| T-DM1 | 82.4% (14/17, 56.6-96.2) | 100% (3/3, 29.2-100) | 85.0% (17/20, 62.1-96.8) |
| Other HER2-ADC (except T-DM1)** | 60.0% (9/15, 32.3-83.7) | 50.0% (2/4, 6.8-93.2) | 57.9% (11/19, 33.5-79.8) |
| ORR in pts with tumor types other than BC (N=65) | | | |

| | HER2 IHC3+ or IHC2+/ISH+ (N=36) | HER2 IHC2+/ISH- or IHC1+ or unknown (N=29) | All other tumor types (N=65) |
|---|---|---|---|
| % (n/N) | 38.9% (14/36) | 31.0% (9/29) | 35.4% (23/65) |
| ORR was shown as % (n/N, 95% CI) or % (n/N). *ORR is calculated using the number of subjects previously treated with anti-HER2 cancer therapy in advanced/metastatic setting as denominator; 2-sided 95% CIs are estimated using Clopper-Pearson method. **Includes RC48-ADC, A166, DP303c, MRG002, ARX788, TAA013, DX126-262, PF-06804103, and BAT8001. | | | |

**CT176**

**Pharmacokinetic/pharmacodynamic (PK/PD) relationships of the novel Treg depleter RG6292 in Phase Ia and Ib studies in patients with solid tumors.**

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INTRODUCTION: Activated Tregs in the tumor micro-environment are correlated with poor outcomes, and are considered as key players in tumor immune-escape. So far, Treg depletion has not been successful in patients, either because adequate Treg depletion was not achieved or because Teff cells have either been impacted or depleted as well. RG6292 is the first anti-human CD25 antibody developed to specifically deplete human Tregs while preserving IL-2R STAT5 signaling and Teff activity. It has been optimized for ADCC and selective depletion of cells with high CD25 density (Treg).
MATERIALS and METHODS: Adult patients with advanced solid tumors were given RG6292 i.v. Q3W as monotherapy (S1: NCT04158583) or in combination with atezolizumab 1200 mg Q3W (S2: NCT04642365) in a Ph1 dose escalation study. 76 pts have been treated at dose levels ranging from 0.3 mg to 165 mg in S1 and 48 pts at dose levels ranging from 0.3 mg to 160 mg in S2. In both studies a Bayesian logistic regression model with overdose control guided dose escalation was utilized. Data cutoff was May 27, 2022. Pharmacokinetic and pharmacodynamic analyses were undertaken in peripheral blood and tumor tissue. PK/PD modeling applied to Treg and Teff cells helped characterize and identify the optimal therapeutic window to ensure (1) relevant Treg depletion and (2) limited impact on T effector.
RESULTS: RG6292 has a linear and time independent PK with no ADA detected. A Population PK-PD modeling approach was applied to Treg and Teff cells in the periphery. To predict the RG6292 effects in

the tumor microenvironment (TME), the PK/PD relationships observed and characterized in the periphery for all cell subpopulations were considered the same, a tumor uptake factor of 15% was considered. At steady-state trough, a 70 mg Q3W dose was predicted to lead to 72% of patients with concentration above the Treg (%CD4) EC50 in tumor and 40% of patients with concentration above the Non-Treg (%CD4) EC50 in plasma. RG6292 induced a dose-dependent peripheral and intratumoral Treg depletion in on-treatment biopsies taken 28 days after initiation of treatment. Treatment did not appear to impact the number nor the functionality of intratumoral CD8 T cells nor any evident effect observed on PDL1 expression. In blood, stable levels of all other immune cells were observed after treatment. Moreover, in both studies a marginal increase of IFNg, CXCL10, IL-10, TNF was observed. No consistent gene expression alterations nor immune signatures could be observed when comparing BSL vs OT biopsies in S1.
CONCLUSION: RG6292, consistent with its proposed mechanism of action, induces profound and preferential depletion of Treg cells over CD8+CD25+ in the periphery and in the TME at clinically safe doses between 35-70mg. Further development of RG6292 is currently being explored.

**CT177**

**A phase I, first in human study of CBA-1205, glycoengineered humanized anti-DLK-1 monoclonal antibody in patients with advanced solid tumors.**

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Background: CBA-1205 is a novel humanized anti-delta-like 1 homolog (DLK-1) antibody. It is a glycoengineered antibody by GlymaxX® technology to potentiate antibody-dependent cellular cytotoxicity activity. DLK-1 is a membrane protein with 6 tandem EGF-like motifs in extracellular region. Overexpression of DLK-1 has been reported in variety of cancer types including hepatocellular carcinoma (HCC), suggesting that DLK-1 could be an ideal target for cancer therapy.
Methods: This is a first in human, multicenter, open-label Phase I study. The purpose of part 1 is to evaluate the safety and tolerability in patients with advanced solid tumors who had no standard therapy or were refractory or intolerant to standard therapies and to determine the starting dose for part 2 in HCC patients. The standard 3+3 dose escalation design was utilized in seven cohorts (0.1, 0.3, 1, 3, 10, 20, 30 mg/kg). CBA-1205 was administered at 2-week intervals in a 28-day cycle. If the patient had no Dose Limiting Toxicity (DLT) during 28 days after the first two doses, CBA-1205 administration will be continued for further cycles unless any of the criteria for discontinuation. Serum DLK-1 concentration before the start of CBA-1205 administration was analyzed using the Human Pref-1/DLK1/FA1 DuoSet ELISA (R&D Systems, Inc.). Immunohistochemical analysis of DLK-1 expression in the archival tumor specimen was determined in FFPE tissue sections using mouse anti-hDLK-1 antibody (clone DI-2-20, Chiome Bioscience).
Results: 22 patients with advanced solid tumors were enrolled. CBA-1205 was well tolerated in these patients and no DLT was observed even at the highest dose tested. No ≥ Grade 3 treatment related adverse events was observed throughout the study periods. The serum concentration of CBA-1205 increased dose-proportionally. The exposure to CBA-1205 exceeded the effective concentrations observed in a mouse xenograft model. No serum anti-CBA-1205 antibody was detected. The disease control was seen in 8 patients (38.1%, all with SD). Especially, long SD was observed in 3 patients with invasive thymoma, malignant melanoma, and pancreatic carcinoma, where progression free survival (PFS) was 39, 72(ongoing, at data cutoff), and 41 weeks, respectively. The serum concentration of soluble DLK-1 was detected in 6 out of 22 patients. Patients with SD by the best overall response had a higher rate of detectable serum DLK-1 concentration and a higher mean concentration than patients with PD. No DLK-1 expression in tumor was detected by IHC method, in 14 patients who consented to use the archival tumor specimens.

Conclusions: CBA-1205 was well tolerated without any severe toxicity in patients with advanced solid tumors.Part 2 of this Phase I study with two cohorts (20, 30 mg/kg) in patients with unresectable advanced/recurrent HCC refractory or intolerable to the standard therapy has been initiated.

**CT178**

**GQ1001: A next generation HER2-targeting ADC that exhibits promising early clinical efficacy with excellent tolerance in a multi-center, Phase Ia study.**

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**Background**: GQ1001 is a novel HER2-targeted antibody-drug conjugate (ADC) that was developed using innovative conjugation technologies coined intelligent Ligase-Dependent Conjugation (iLDC), that can significantly improves homogeneity and biostability of ADC. In preclinical studies, GQ1001 showed a robust anti-tumor response in multiple HER2+ models alone or in combination with HER2 TKIs and chemotherapeutics, and excellent pharmacokinetics and safety profiles in rats and monkeys due to low level of payload release. Herein we report the initial results of the ongoing phase Ia study, which aims to investigate the safety, tolerability, pharmacokinetics and antitumor activity of GQ1001 in subjects with HER2+ advanced solid tumors.
**Methods**: In phase Ia dose escalation, a modified 3+3 model was adopted to assess the safety, dose-limiting toxicity (DLT) and maximal tolerable dose (MTD) or dose recommended for dose expansion (DRDE) of GQ1001. GQ1001 was administered intravenously as a monotherapy on Day 1 of 21-day cycles. The starting dose was 1.2 mg/kg, followed by 2.4, 3.6, 4.8, 6.0, 7.2 and 8.4 mg/kg.
**Results**: As of Dec. 28th, 2022, 32 subjects with HER2-positive advanced solid tumors, predominantly in breast (9), gastric or gastro-esophageal junction (9) and salivary gland (4), were enrolled and received GQ1001 treatment. Patients had a median 3 (range, 0-11) prior lines of therapies, and 37.5% of those previously received ≥2 lines of anti-HER2 therapies. Median exposure time of GQ1001 was 18.5 weeks. The longest treatment duration exceeded 370 days. No DLT was observed in all doses, MTD was not reached up to 8.4 mg/kg, the highest dose tested. Treatment-related adverse events (TRAEs) occurred in 24 subjects (75%). The most common TRAEs (>10.0%) were aspartate aminotransferase (AST) increased (37.5%), thrombocytopenia (28.1%), alanine aminotransferase (ALT) increased (25.0%), pyrexia (21.9%), anemia (18.8%), alkaline phosphatase increased (12.5%), vomiting (12.5%) and nausea (12.5%). Grade ≥3 TRAEs occurred in 9 subjects (28.1%), including 5 myelosuppression, 2 abnormal liver function, 1 hypertension and 1 vomiting. There were no drug-related deaths. The pharmacokinetics analysis showed the concentration of GQ1001 and TAb generally peaked rapidly and declined in a roughly biphasic manner. Among 15 evaluable subjects who received ≥ 7.2 mg/kg, 6 cases achieved confirmed partial response, and 3 had stable disease, the median progression-free survival was 4.8 months
**Conclusions:** GQ1001 demonstrates an excellent tolerability and promising antitumor activity in heavily pretreated HER2-positive advanced solid tumors, supporting further evaluation of the safety and efficacy of GQ1001 at DRDE of 8.4 mg/kg in the following phase Ib trial. (NCT04450732; sponsored by GeneQuantum Healthcare (Suzhou) Co., Ltd.)

**CT179**

**First-in-human study of ICT01, an anti-BTN3A activating monoclonal antibody in combination with low dose IL-2 in patients with advanced solid tumors (EVICTION-2 study).**

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Kunzmann,[4] Aude De Gassart,[5] Emmanuel Valentin,[5] Marina Iche,[6] Maelle Mairesse,[5] Patrick Brune,[5] Katrien Lemmens,[5] Aurélien Marabelle,[2] Daniel Olive,[7] Paul Frohna[5]. [1]The Institute for Cancer Research and Royal Marsden, London, United Kingdom; [2]Gustave Roussy, Paris, France; [3]NCT/UCC Early Clinical Trial Unit, Medical Faculty Carl Gustav Carus, Technical University,, Dresden, Germany; [4]University Clinic of Würzburg, Würzburg, Germany; [5]Imcheck Therapeutics, Marseille, France; [6]Ilife Consulting, Paris, France; [7]Centre de recherche en Cancérologie de Marseille, INSERM U1068, CNRS U7258, Aix Marseille Université, Institut Paoli-Calmettes, Marseille, France.

**Background:** ICT01, an anti-BTN3A mAb, selectively activates γ9δ2 T cells resulting in remodeling of the tumor microenvironment by activated γ9δ2 T, CD8 T, and NK cells (EVICTION- NCT04243499; AACR 2022 CT188). Response to ICT01 depends on the baseline number of γ9δ2 T cells with many cancer patients having inadequate numbers due to prior chemotherapy and/or the underlying malignancy. ICT01 plus IL-2 induces selective expansion of γ9δ2 T cells with minimal Treg expansion in NHPs and is currently being tested in the EVICTION-2 Trial, a phase I/IIa, open-label study to characterize the safety, tolerability, pharmacodynamics, and antitumor activity of ICT01 in combination with low dose subcutaneous (LDSC) IL-2 in patients with advanced-stage solid tumors (NCT04243499).
**Methods:** ICT01 (1, 5, 20 or 75 mg, IV Q3W) is given in combination with LDSC IL-2 (Proleukin®, 1 or 2 MIU/$m^2$) on days 1-5 of the first 3 cycles and will be continued alone thereafter. Per dose combination two patients are enrolled for dose escalation based on the BOIN simulations to identify a safe and pharmacodynamically active dose combination(s) for Phase IIa expansion.
**Results:** To date, 9 patients with colorectal (n=6), ovarian (n=2) or pancreatic cancer (n=1) with a mean/median of 6 prior lines of therapy have received at least their first cycle of ICT01 1, 5, 20 or 75 mg + IL-2 1MIU/$m^2$ or ICT01 1mg + IL-2 2MIU/$m^2$. Treatment-related adverse events (TRAE) were mainly infusion related reactions comprising fever and chills grade 1 and 2 in 8/9 patients, not different in severity from ICT01 or IL-2 monotherapy. No dose limiting toxicities were reported. By RECIST, Stable Disease was observed in 2/6 patients with a Week 8 assessment. In all patients, a 2-to-9-fold increase in γ9δ2 T cells was observed for each of the 3 combination cycles, which is accompanied by peripheral activation, mobilization, and proliferation of CD8 T cells, NKs and granulocytes, which is consistent with the expected contribution of IL-2. Increased IFNγ and IL-8 levels (2.4-to-140 fold and 2-to-94 fold respectively) were observed within 24h after each dose of ICT01/IL-2. Flow cytometry of Tregs in frozen PBMCs and IHC of tumor biopsies collected at baseline and on Day 28 are being analyzed to quantify tumor infiltration.
**Conclusions** ICT01 plus LDSC IL-2 safely induced robust γ9δ2 T cell expansion leading to a broad immune response involving CD8 T cells, NKs, and granulocytes at lower doses than observed with ICT01 monotherapy. More patient data are needed to confirm that IL-2 potentiates the antitumor effects of ICT01 and that this leads to better clinical outcomes.

**CT180**

**Activation of innate and adaptive immune response with a clinical stage TLR7 agonist prodrug PRTX007.**

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**Background.** PRTX007 is an orally administered prodrug of PRX034, a toll-like receptor 7 (TLR7) agonist that elicits an unprecedented pattern of systemic immune induction in non-human primates and in humans. Notably, there is no evidence of NF-Kβ-mediated systemic inflammation at drug levels that stimulate IFN-responsive pathways and corresponding downstream cellular processes, including activation of $CD8^+$ T cells and NK cells. We have previously reported interim results from a Phase 1

single ascending dose (SAD) and multiple ascending dose (MAD) study of PRTX007. PRTX007 demonstrated a favorable safety profile with no serious adverse events (SAEs) and a lack of the adverse events (AEs) historically associated with TLR7 agonists. Robust systemic immune induction was achieved, without counter-regulation or evidence of systemic inflammation, in participants receiving PRTX007 in the 300 - 500 mg MAD cohorts. This report describes new results from the 750 mg MAD cohort, the highest MAD dose level in this now-completed study.
**Methods.** Phase 1, single-center, prospective, randomized, double-blind, placebo-controlled study of 9 SAD and 4 MAD cohorts of PRTX007 administered QOD orally to healthy adult participants. Primary objectives were safety and tolerability. Secondary objectives were evaluation of pharmacokinetics (PK) and pharmacodynamics. In total, 104 of the 130 participants in this study received study drug.
**Results.** AEs were limited to non-dose-related mild headache, minor dose-related increases in ALT (<2X ULN) that rapidly resolved, and mild-moderate fever that were transiently observed in 1 of 8 participants. The PKs of PRTX007 and PRX034 are well-behaved with exposure increasing proportionally to PRTX007 dose without accumulation upon repeated dosing (mean PRX034 AUC (hr*ng/ml) on D1: 13,432; D13: 13,796). Coordinated expression of interferon-stimulated genes and genes known to be related to TLR7 agonism were observed in immune cells in blood in all participants. Increases in IL-1RA, MCP-1 and IP10 in plasma were also observed. IL-6, TNFα and IL- 1β remained essentially unchanged from pretreatment levels. The proportion of $CD8^+$ T cells and NK cells, with significant expression of the proliferation marker Ki67 and activation marker CD38, increased markedly from pretreatment to end of dosing in all participants. $Ki67^+$ expressing $CD8^+$ T cells increased from 1.1% to 9.8% and $Ki67^+$ expressing NK cells increased from 3.6% to 10.3% during the two-week period of drug administration. $CD38^+$ $CD8^+$ T cells increased from 11.8% to 21.9% and $CD38^+$ NK cells increased from 59.4% to 80.0% during the corresponding period.
**Conclusion.** PRTX007 demonstrated a favorable safety profile in all SAD and MAD cohorts. In the 750 mg MAD cohort, activation of the innate and adaptive immune response including important effector cell populations were observed without systemic increases in proinflammatory factors.

**CT181**
**A first-in-human (FIH) phase 1 study of SHR-A1921, a TROP-2 targeted antibody-drug conjugate (ADC), in patients with advanced solid tumors.**
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Background: SHR-A1921 is a novel ADC composed of a humanized anti-trophoblast cell-surface antigen 2 (TROP-2) IgG1 monoclonal antibody attached to a DNA topoisomerase I inhibitor via a tetrapeptide-based cleavable linker. Herein, we present the preliminary clinical results of the FIH study of SHR-A1921.
Methods: This is a multi-center, open-label, phase 1 trial (Clinicaltrials.gov, NCT05154604) consisted of dose-escalation, dose-expansion and efficacy-expansion phases. Patients with advanced solid tumors who had failed standard therapy were enrolled. In the dose-escalation phase, SHR-A1921 was planned to be administered from 1.5 mg/kg to 12.0 mg/kg (Q3W, iv) in an i3+3 design, with accelerated titration used for the starting dose; in the dose-expansion phase, additional patients (up to 20-30 per dose level) were enrolled to 2-3 selected tolerable dose levels. The primary objectives were to evaluate the safety and

tolerability of SHR-A1921 and to determine the maximum tolerated dose (MTD) and/or recommended phase 2 dose.
Results: As of Oct 21. 2022, 38 enrolled patients were included for analysis: 18 enrolled during dose-escalation (1.5 mg/kg, n=1; 3.0 mg/kg, n=4; 4.0 mg/kg, n=8; 6.0 mg/kg, n=5) and 20 during dose-expansion (3.0 mg/kg). 71.1% (27/38) were driver gene-negative non-small cell lung cancer (NSCLC) patients who had previously received platinum-based chemotherapy and anti-PD-(L)1 antibody. 4 patients reported dose-limiting toxicities, with all being grade 3 stomatitis (6.0 mg/kg, n=3; 4.0 mg/kg, n=1). The MTD was established as 4.0 mg/kg. Across all dose cohorts, the most common treatment-related adverse events (TRAEs; ≥30%) were nausea (71.1%), stomatitis (65.8%), anemia (42.1%), vomiting, decreased appetite, decreased weight, and rash (36.8% each). Grade ≥3 TRAEs occurred in 12 patients (31.6%); of these, the most common was stomatitis (n=7, 18.4%), and all other events were reported in ≤2 patients. No patients discontinued study treatment due to TRAEs. As of cut-off date, 10 patients (NSCLC, n=5; triple-negative breast cancer, n=2; ampullary cancer, n=2; ovarian cancer, n=1) had partial response: 4 confirmed and 6 requiring further confirmation. The objective response rate was 33.3% (10/30; 95% CI 17.3-52.8) and disease control rate was 80.0% (24/30; 95% CI 61.4-92.3) in evaluable patients.
Conclusion: SHR-A1921 showed a manageable safety profile and promising anti-tumor activity in patients with pretreated advanced cancer. The trial is ongoing to assess SHR-A1921 at different dosing frequency and the efficacy in selected cancer types.

**CT182**

**OXC-101 shows favorable safety profile in first in human phase 1 trial in patients with advanced solid cancer.**

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Purpose: Many cancers suffer from dysfunctional redox status. MTH1 protects cancer cells from oxidative DNA damage by preventing incorporation of oxidized nucleotides. OXC-101 (Karonudib) is a dual-action mitotic disruptor and MTH1 inhibitor, preventing exit from mitosis and sanitation of oxidative damage to DNA subunits in cancer cells. MASTIFF, (MTH1, A Phase I, Study on Tumors Inhibition, First in Human, First in Class), is a first- in- human phase 1 study, with the primary aim to assess safety, tolerability, and pharmacokinetics (PK) of OXC-101 in patients (pts) with advanced solid malignant tumors (NCT03036228).
Methods: Phase 1a study with 3+3 design and intra-patient dose escalation. New cohorts were opened following review of safety and PK data by the safety data monitoring board after the first treatment cycle of 4 weeks (w). All patients had progressive stage IV disease with no treatment options in SOC at inclusion. Radiological tumor response evaluation (RECIST 1.1) was performed 8w of the first 24w, 12w thereafter. The first 29 patients (cohort 1-10) obtained an oral solution. From cohort 11 pts obtained 100mg tablets.
Results: 50 subjects (50% female, 50% male) were enrolled at 16 dose levels of OXC-101 at varying posology: 25-900 mg BID, 800-1000mg QD; every second day, once or twice weekly. Mean age was 52 years (range:20-74). 92% of pts had received previous chemotherapy (mean: 5 lines, range:0-34), 18% endocrine therapy and 52% other therapies (incl immunotherapy). OXC-101 was generally well tolerated and with manageable toxicity for dose levels for an average of 121 days (range:2-513 days), and 43/50 subjects discontinued due to disease progression. A total of 20 SAEs were reported: 8 SAEs in 7 subjects

were considered related/possibly related to OXC-101 and included pneumonia (3/8), febrile neutropenia (2/8), ileus (1/8), unspecified infection (1/8) and fever (1/8). There were 14 dose-limiting toxicities (DLTs) reported in 7 subjects. At 510mg every second day (1785mg weekly) 2/5 subjects reported G4 neutropenia; at 500mg BID/1000mg QD, twice per week (2000 mg weekly) 2/9 subjects reported G4 neutropenia; at 900mg BID, once weekly (1800 mg weekly) 3/3 subjects reported decrease in white blood count incl. neutropenia. PK was robust and predictable with good exposure correlation between solution and tablet formulation, with an average half-life of 10 hrs in plasma. 15/49 (31% ) of the subjects showed stable disease at first radiological evaluation. One subject had stable disease during 73 weeks on OXC-101 treatment. IHC and western blot analysis on tumor biopsies confirmed mechanism of action. A dose of 900mg (500mg mane, 400mg nocte) twice a week (total weekly dose 1800mg) was determined as the Recommended Phase 2 Dose (RP2D). This will be further explored in tumor specific cohorts.
Conclusion: OXC-101 has a favorable safety profile with an RP2D of 900mg twice a week.

**CT184**

**Preliminary dose escalation results of ERAS-601 in combination with cetuximab in FLAGSHP-1: a phase I study of ERAS-601, a potent and selective SHP2 inhibitor, in patients with previously treated advanced or metastatic solid tumors.**

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Background: SHP2 is an oncogenic tyrosine phosphatase that transduces receptor tyrosine kinase signaling to the RAS/MAPK pathway via its phosphatase-mediated regulation of guanine nucleotide exchange factors. ERAS-601 is a potent, selective, and orally bioavailable allosteric inhibitor of SHP2. In combination with cetuximab, an antibody that targets epidermal growth factor receptor (EGFR), ERAS-601 has demonstrated robust nonclinical activity in human papillomavirus (HPV)-negative head and neck squamous cell carcinoma (HNSCC) and RAS/RAF wild-type colorectal cancer (CRC) tumors.
Method: FLAGSHP-1 is the first-in-human trial of ERAS-601 administered as monotherapy and in combination with other cancer therapies in patients with advanced or metastatic solid tumors. The primary objectives are to characterize the safety profile, determine the maximum tolerated dose (MTD)/recommended dose (RD), and characterize the pharmacokinetics (PK) profile of ERAS-601 as a monotherapy and in combination with other cancer therapies. Secondary objectives include tolerability and antitumor activity in solid tumors. Presented here are results from the combination dose escalation cohorts in which patients received ERAS-601 twice a day for three weeks followed by a one-week break (BID 3/1) in combination with cetuximab (500mg/m$^2$) administered every 2 weeks (Q2W) on a 28-day cycle.
Results: As of October 31, 2022, a total of 15 patients with previously treated advanced or metastatic solid tumors received ERAS-601 BID 3/1 at the following dose levels: 20 mg BID 3/1 (n=4), 40 mg BID 3/1 (n=8), or 60 mg BID 3/1 (n=3) in combination with cetuximab. Combination therapy MTD was determined to be 40 mg BID 3/1. ERAS-601 Treatment Related Adverse Events (TRAEs) at or below the MTD were all Grade 1 and 2. TRAEs occurring in ≥20% of patients included diarrhea (27%), AST increase (27%), ALT increase (20%), dermatitis acneiform (20%). Grade ≥3 TRAEs included Grade 4 hypokalaemia, Grade 3 diarrhea, platelet count decreased anemia (each 7%); high grade TRAEs were only observed at 60 mg BID 3/1 (above the MTD). Dose limiting toxicities (DLTs) were only observed at

the 60mg BID 3/1 dose levels and included Grade 3 platelet count decreased (n=1) and Grade 4 hypokalemia (n=1). Pharmacokinetics of ERAS-601 and cetuximab in combination were generally comparable to historical monotherapy PK values, suggesting lack of drug-drug interaction. The evaluation of clinical activity is still ongoing.
Conclusions: ERAS-601 in combination with cetuximab in patients with previously treated advanced or metastatic solid tumors shows promising preliminary safety and tolerability with reversible and manageable TRAEs. Further evaluation in relevant tumor types are ongoing.

**CT185**

**First in human dose-escalation trial with the c-MET targeting antibody-drug conjugate BYON3521.**

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Background: BYON3521 is a novel c-MET targeting antibody-drug conjugate (ADC) with a cleavable linker-duocarmycin (vc-*seco*-DUBA) payload that causes irreversible alkylation of DNA in tumor cells. BYON3521 has demonstrated potent and selective killing of c-MET expressing tumor cells in preclinical models, even at low c-MET expression levels. In addition, *in vitro* studies showed that the active toxin can passively permeate the cell membrane and kill neighboring, c-MET negative cells as a bystander effect.
Methods: Patients with previously treated progressive locally advanced or metastatic solid tumors, c-MET positive membrane staining by immunohistochemistry and/or *MET*-amplified by dual *in situ* hybridization and/or known activating *MET*-mutation (excluding exon14m), ECOG performance status 0-1 and adequate organ function are eligible for the dose-escalation part of this first-in-human trial (ClinicalTrials.gov identifier: NCT05323045). An adaptive approach using the Continual Reassessment Method of Neuenschwander (N-CRM model) is used to evaluate the safety of BYON3521 and to determine the maximum tolerated dose (MTD) and recommended dose for expansion (RDE). BYON3521 is administered intravenously every three weeks until tumor progression or unacceptable toxicity.
Results: Up to 01 January 2023, 8 patients (1F, 7M, median age 61 yrs) were enrolled. Tumor types were: 4 colorectal cancer, 1 NSCLC, 1 renal cell carcinoma, 1 esophageal cancer and 1 pancreatic cancer. One patient received 0.8 mg/kg, three patients each 1.6 and 3.2 mg/kg and one patient received 4.8 mg/kg. So far, no grade 3 or 4 related adverse events and no dose-limiting toxicities (DLTs) occurred. Most commonly observed related AEs were decreased appetite/weight, fatigue, abdominal pain, back pain, vomiting and chills. Typical ADC associated AEs as keratitis and pneumonitis have not been observed so far. Stable disease was the best response observed in 2 patients at a dose of 3.2 mg/kg. Human PK was in line with predicted preclinical in vivo PK.
Conclusions: To date, BYON3521 is well-tolerated with no DLTs at the investigated dose levels. Patient enrollment is ongoing and updated safety, efficacy and pharmacokinetic data will be presented. After the dose-escalation phase the trial will continue with expanded cohorts of patients with specific c-MET expressing cancer types.

**CT188**

**A phase Ib study of sapacitabine (sapa) and olaparib (ola) in patients (pts) with *BRCA1/2*-mutated (*BRCA1/2*m) metastatic breast cancer (MBC).**

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**Background:** PARP inhibitors (PARPi) are superior to chemotherapy in pts with *BRCA1/2*m MBC, with median PFS (mPFS) of 7 months with single-agent ola. Sapa is an oral nucleoside analog; the active metabolite, CNDAC, generates single-strand DNA breaks that are converted to double-strand breaks (DSB) during subsequent replication, resulting in cell death. CNDAC-induced DSB repair is dependent on homologous recombination. Sapa combined with ola may augment DNA damage compared to ola alone in *BRCA1/2*m tumors.
**Methods:** We conducted a phase Ib study of sapa/ola in pts with *BRCA1/2*m HER2-negative MBC who were not previously PARPi-exposed. Primary objective was to determine the RP2D of sapa with ola. Secondary objectives included safety, tolerability and mPFS. RAD51 staining was performed on archival tissue. Serial blood samples were collected for circulating tumor DNA (ctDNA) analysis using low-pass WGS with targeted sequencing via the DDR Discovery Panel (Resolution Bioscience).
**Results:** 10 women (median age 46) were enrolled before the study was terminated for hematologic toxicity. Germline *BRCA1* and *BRCA2* mutations were present in 3 and 7 pts, respectively. 4 pts had received prior platinum. All pts initially received standard dose ola (300 mg twice daily) with 100 mg (n = 3), 150 mg (n = 4), or 200 mg (n = 3) sapa once daily on days 1-5 and 8-12 of an every 28-day cycle. The RP2D was not determined. Hematologic AEs were common, including anemia (80%), neutropenia (80%), and thrombocytopenia (40%), with 1 DLT of G4 neutropenia lasting more than 7 days. The ORR was 50% (95% CI: 18.7% - 81.3%) with mPFS of 9.7 months (95% CI: 8.02 - NA). 3 pts had clinical benefit of 15+ months, exceeding expectations with olaparib alone, including 2 with PR, and 2 who remained on trial for 36+ months. 3 patients had confirmed PRs of short duration. Of the remaining patients, one withdrew consent early and the other 3 had non-CR/non-PD or SD of 4, 7 and 9 months. At progression, 2 pts had evidence of BRCA reversion mutation associated with an MMEJ signature; ctDNA also demonstrated *MRE11* and *PTEN* amplifications in 1 of these pts, suggesting multiple resistance mechanisms. 3 pts had no evidence of reversion but had acquired putative non-reversion mechanisms of resistance, including changes suggesting enhanced MRN complex function, *FANCD2* and *ATR* amplifications and *PIK3CA* mutation. The majority of tumors (6/10) were RAD51-negative. Other tumors with RAD51 foci demonstrated replication stress evidenced by high levels of pKAP1 and pRPA, suggesting benefit associated with sapa.
**Conclusions:** Sapa/ola produces high rates of hematologic toxicity. However, the ORR of 50%, mPFS of 9.7 months, and durability of 3+ years in 2 pts suggest possible combinatorial benefit. Further exploration with different sapa schedules or with substitution of a PARP1-selective inhibitor to decrease hematologic toxicity is warranted.

**CT189**

**A phase I study of BAT1006, a novel anti-HER2 antibody, in patients with locally advanced/metastatic solid tumors.**

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Purpose: BAT1006 is a HER2 extracellular domain II-targeted monoclonal antibody with enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) effect aiming for Her2-positive locally advanced/metastatic solid tumors. Previous studies have shown that de-fucosylated antibody binds to FcγRIIIa with increased affinity and can thus trigger FcγRIIIa-mediated effector functions more efficiently than native antibody. BAT1006 was expressed in an FUT8-knockout CHO cell line, resulting in antibody completely devoid of fucose modification. With approximate 5-fold higher level of ADCC effect compared to Pertuzumab, BAT1006 also exhibited potent anti-tumor activity in HER2 positive Calu-3 xenograft mice model. The ongoing phase I, open-label, first-in-human study is evaluating the safety, pharmacokinetics (PK), immunogenicity and preliminary efficacy of BAT1006 to determine the Maximum-Tolerated Dose (MTD) and/or recommended phase II dose (RP2D).
Methods: Patients with locally advanced/metastatic solid tumors received escalating doses of BAT1006 (3, 6, 10, 15 mg/kg), administered intravenously (IV) once every three weeks. Patients must have had documented HER2 positivity defined as 3+ by validated immunohistochemistry or amplified on in situ hybridization (ISH). Assessments include archival tumor molecular status, PK, and efficacy by Response Evaluation Criteria in Solid Tumors (RECIST).
Results: Fifteen patients [with 2-9 prior lines of therapy] have been treated with BAT1006. All patients had metastatic breast cancer and had received previous HER2-targeted therapies including Trastuzumab, Pertuzumab, T-DM1 and Pyrotinib. No treatment related ≥ Grade 3 AEs have been observed. Of 9 patients at 10mg/kg and 15mg/kg, 1 in 3 patients had CR at 10mg/kg, 2 in 6 patients had PR at 15mg/kg.
Conclusion: BAT1006 was well tolerated and showed promising anti-tumor activity in patients with heavily pre-treated HER2-positive cancers. Based on the safety and efficacy results, one additional dose level (20 mg/kg) will be added to the escalation phase and combination therapy with other HER2-based therapy will also be initiated.

**CT190**

**Oncolytic virus TG6002 safety and activity after intrahepatic artery administration in patients with liver-dominant metastatic colorectal cancer.**

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The TG6002.03 trial is a dose-escalation phase 1 clinical trial of TG6002 infusion via the hepatic artery in patients with liver-dominant colorectal cancer metastases. TG6002 is an engineered Copenhagen strain oncolytic Vaccinia virus, deleted of thymidine kinase and ribonucleotide reductase to enhance tumor selective viral replication and expressing FCU1, an enzyme converting the non-cytotoxic prodrug 5-fluorocytosine (5-FC) into the chemotherapeutic compound 5-fluorouracil (5-FU). In this trial, patients with advanced unresectable liver-dominant metastatic colorectal cancer who had failed previous oxaliplatin and irinotecan-based chemotherapy were treated with up to 2 cycles of TG6002 infusion 6 weeks apart via the hepatic artery on day 1 combined with oral 5-FC on days 5 to 14 (where day 1 = TG6002 infusion). TG6002 infusion was performed over 30 minutes via selective catheterization of the hepatic artery proper. 5-FC oral dosing was 50mg/kg x4 daily. Blood was sampled for TG6002 pharmacokinetics and 5-FC and 5-FU measurements. Sampling of liver metastases was performed at screening and on day 4 or day 8 for virus detection and 5-FC and 5-FU quantification. In total, 15 patients (median age 61 years, range 37-78) were treated in 1 UK centre and 2 centres in France and received a dose of TG6002 of $1x10^6$ (n=3), $1x10^7$ (n=3), $1x10^8$ (n=3), or $1x10^9$ pfu (n=6). Fourteen of the 15 patients received a single cycle of treatment, including one patient who did not received 5-FC, and one patient received two cycles. TG6002 was transiently detected in plasma following administration, suggesting a strong tissue selectivity for viral replication. In the highest dose cohort, a virus rebound was observed on

day 8, concordant with replication time of the virus. In serum samples, 5-FU was present on day 8 in all patients with a high variability ranging from 0.8 to 1072 ng/mL and was measurable over several days after initiation of therapy. Seven of the 9 patients evaluable showed the biodistribution of the virus in liver lesions by PCR testing on day 4 or day 8. Translational blood samples showed evidence for T-cell activation and immune checkpoint receptor-ligand expression. At $1x10^9$ pfu, there was evidence for T-cell proliferation and activation against tumour-associated antigens by ELISpot and for immunogenic cell death. In terms of safety, a total of 34 TG6002-related adverse events were reported, of which 32 were grade 1-2 and 2 were grade 3. The maximum tolerated dose was not reached, and a single dose-limiting toxicity was observed consisting of a myocardial infarction in a context of recent Covid-19 infection in a 78-year-old patient. These results indicate that TG6002 infused via the hepatic artery in combination with oral 5-FC was well tolerated, effectively localized and replicated in the tumor tissues, expressed its therapeutic payload and showed anti-tumoral immunological activity.

**CT192**

**Phase 1, open-label, single-dose study to evaluate the effect of hepatic impairment on the pharmacokinetics and safety of futibatinib in adult subjects.**

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Background: Futibatinib, an irreversible FGFR1-4 inhibitor, is indicated for the treatment of adult patients with previously treated, unresectable, locally advanced or metastatic intrahepatic cholangiocarcinoma harboring *FGFR2* gene fusions or other rearrangements. As the primary elimination pathway for futibatinib is hepatic metabolism, we conducted a phase 1 study to evaluate the effect of hepatic impairment (HI) on futibatinib pharmacokinetics (PK) and safety in healthy adults.
Methods: A single oral dose of 20 mg futibatinib was administered to adult subjects with mild (Child-Pugh score, 5-6), moderate (7-9), or severe (10-15) HI. Control healthy subjects were matched to each HI cohort according to age, body mass index, and sex. Intensive PK samples were collected up to 72 hours post-dose. Exposure measures ($AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, and $C_{max}$) in subjects with HI were compared with matching control cohorts and with the overall healthy-control cohort. Relationships between plasma PK and HI were examined graphically via scatter/regression plots of PK parameters versus baseline Child-Pugh score, bilirubin, albumin, international normalized ratio, and aspartate aminotransferase.
Results: Overall, 38 subjects were enrolled (mild HI, n = 8; moderate HI, n = 8; severe HI, n = 6; healthy controls, n = 16). Following the administration of futibatinib, no trend was observed between the severity of HI and the extent of futibatinib exposure increase. Compared with matched controls, $AUC_{0\text{-}inf}$ increased by 21%, 20% and 18%, and $C_{max}$ by 43%, 15%, and 10% in subjects with mild, moderate, and severe HI, respectively. Changes in exposure were not considered clinically relevant as geometric mean ratios were within 80-125% bioequivalence limits, except for $C_{max}$ in subjects with mild HI (43%). Futibatinib PK parameters and HI measures did not appear to be associated based on visual inspection or statistical evaluation of regression plots (p-values all > 0.05). No subjects discontinued from the study due to treatment-emergent adverse events (TEAEs). Overall, two (12.5%) subjects in the healthy-control cohort reported one Grade 1 TEAE each (dyspepsia and headache) and two (25.0 %) subjects in the mild HI cohort each reported one Grade 1 TEAE (toothache and headache). All TEAEs were considered related to treatment. No subjects with moderate/severe HI reported TEAEs.
Conclusions: No clinically meaningful differences in the systemic plasma exposure of futibatinib were observed based on the severity of HI. Single oral doses of futibatinib were well tolerated among subjects with varying degrees of HI and matched healthy adult subjects in this study. The data suggest that dose adjustment may not be necessary in patients with HI receiving futibatinib 20 mg QD for its approved

indication.

**CT193**

**Final results from a phase I study to evaluate the safety and efficacy of a telomerase-specific oncolytic adenovirus (OBP-301) with pembrolizumab in patients with advanced solid tumors (EPOC1505).**

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**Background**: PD-1 blockade showed promising efficacy for broad type of cancer patients (pts), though objective response rates are very limited. In addition to the specific killing of cancer cells via oncolytic adenovirus, these agents prompt the immune system to stimulate an antitumor immune response. OBP-301 is an oncolytic adenovirus in which gene is modified to be able to selectively replicate in cancer cells by introducing hTERT promotor. Further antitumor effect might be expected with an active activation of two different antitumor immunity by OBP-301 in combination with pembrolizumab. Therefore, we conducted phase I study to evaluate the safety and efficacy of OBP-301 with pembrolizumab.
**Methods**: The major eligibility criteria are pts with advanced or metastatic solid tumor not responded to or intolerant of standard chemotherapies, and with possibility of intratumoral injection. Phase Ia part was designed to determine the recommended dose in a "3+3" cohort-based dose escalation design of OBP-301 ($1x10^{10}$VP on cohort 1, $1x10^{11}$VP on cohort 2 and $1x10^{12}$VP on cohort 3) with pembrolizumab (200mg/body q3w). OBP-301 is administered at day1, 15, and 29 by intratumoral injection and pembrolizumab is administered at day 8 and thereafter every 3 weeks. Primary endpoint is DLT. Secondary endpoints are response rate, progression free survival, and incidence of adverse event. Phase Ib part was designated to evaluate the safety and efficacy of the recommended dose OBP-301 selected in phase Ia part in combination with pembrolizumab. Biomarker study was planned to use paired samples of both tumor biopsy and blood. Clinical trial information: NCT03172819.
**Results:** A total 22 pts (phase Ia part: 11 pts, phase Ib part: 11 pts) were enrolled in the study from Dec 2017 to Feb 2021. Median age was 65. Among the pts, 18 had esophageal squamous cell carcinoma, 2 had gastric cancer, one EGJ cancer, and one had colon cancer. No DLT was observed and the recommended dose for phase Ib part was $1x10^{12}$VP (cohort 3). Eighteen pts was injected to primary site, and 5 pts injected to metastatic site (Lymph node:1, Liver:3). Common grade 3 or 4 adverse events were fever (4.5%), and abnormal hepatic function (4.5%). Objective tumour responses were documented in 2(9.1%) of 22 pts. Tumor samples were subjected to multiplex immunohistochemistry (17 pts) and showed that tumor-infiltrating PD-1+CD8+ T cells prior to the treatment were significantly higher in 5 responders who experienced PR or >6 months SD, compared with 12 non-responders (P=0.0365).
**Conclusion:** The combination of OBP-301 with pembrolizumab was well tolerated and showed limited response. The result of biomarker analyses using paired samples of both tumor biopsy and blood will be presented. Tumor-infiltrating PD-1+CD8+ T cells could be a biomarker for this combinatory therapy.

**CT194**

**ETCTN 10388: a first in human phase I trial of triapine and lutetium Lu 177 DOTATATE in well-differentiated somatostatin receptor-positive gastroenteropancreatic neuroendocrine tumors (GEP-NETs).**

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Background: Radiation is a potent inducer of DNA double-strand breaks, and ribonucleotide reductase (RNR) is the rate-limiting enzyme in the synthesis and repair of DNA, making RNR-targeted therapy a rationale therapeutic strategy for radiosensitization. ETCTN 10388 (NCT04234568) evaluated safety and efficacy of the combination of lutetium 177 DOTATATE, a beta-emitting radionuclide in combination with triapine, a ribonucleotide reductase (RNR) inhibitor.
Method: This study was a multicenter phase 1 dose escalation trial [using the Bayesian optimal interval design (BOIN)] of triapine in combination with fixed dose lutetium Lu 177 DOTATATE for well-differentiated somatostatin receptor-positive gastroenteropancreatic neuroendocrine tumor (GEP-NETs) after the failure of at least one line of prior systemic cancer treatment with an expansion cohort at the recommended phase 2 dose (RP2D). Oral triapine (100mg, 150mg, 200mg) was administered once daily on days 1-14 and Lu-177 DOTATATE [200 mCi] intravenously on day 1 of every 56-day cycle. A total of 4 cycles were administered. Response and adverse effects were assessed per RECIST and CTCAE 5.0, respectively. Exploratory correlative studies included tumor somatic and germline mutation testing, RNA sequencing, pharmacokinetics, deoxynucleosides and circulating cell free DNA analysis. Primary endpoints were safety and RP2D.
Results: Overall, 31 patients were enrolled between 6 sites, 15 in the dose escalation phase and 16 in the dose expansion phase. Adverse events (AE) were assessed in all 31 patients per CTCAE 5.0. One DLT in dose level 1, seven DLTs in dose level 2, and one grade 5 DLT in dose level 3 were observed. The RP2D of the combination is triapine 150 mg QD (dose level 2) on days 1-14 in combination with Lu-177 DOTATATE on day 1 of every 56-day cycle. Detailed safety and adverse event data will be presented at the meeting. There were 28 patients evaluable for efficacy, of which 6 (21%) achieved a partial response. At 12 months, 6 patients had progressed, while 22 (86%) remained progression free. Median PFS has not been reached. PK data were available for 12 patients enrolled in the dose escalation cohort. The geometric mean (SD) $AUC_{0\text{-}inf}$ was 1159 (1.22) µg/L•h for the 100mg dose level and 1862 (1.76) µg/L•h for the 150 mg dose level, suggesting that exposure increased with dose, and inter-patient variability was as expected for an oral agent.
Conclusion: The combination of triapine and Lu-177 DOTATATE was safe with preliminary efficacy signals, which will be further evaluated in ETCTN 10558, a randomized phase 2 study that is comparing the effectiveness of triapine and Lu-177 DOTATATE to Lu-177 DOTATATE alone.

**CT195**

**Interim safety results from SEPION/AIO-PAK-0118: A multicenter, Phase I/II study of sequential epigenetic and immune targeting in combination with nab-paclitaxel/gemcitabine in patients with advanced pancreatic ductal adenocarcinoma.**

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Background: Pancreatic ductal adenocarcinoma (PDAC) remains a hard-to-treat cancer entity with limited

therapeutic options. We aim to enhance the anti-tumor and pro-immunogenic effect of standard-of-care nab-paclitaxel/gemcitabine (GnP) by concomitant epigenetic targeting followed by immune-modulating maintenance therapy in patients (pts) with treatment-naive metastatic PDAC. This phase 1/2, open-label, multicenter, dose-escalation/dose-expansion study evaluates the safety and tolerability of azacitidine (AZA) and romidepsin (ROM) in combination with GnP and the potential of immune-modulating consolidation with durvalumab and lenalidomide after three cycles of either GnP or GnP plus epigenetic targeting. Here we report interim results of the dose escalation.
Methods: In part 1A, dose escalation of three regimen ROM/GnP (arm A), AZA/GnP (arm B) and ROM/AZA/GnP (arm C) for determination of the recommended dose for expansion (RDE) is performed with a 3 + 3 dose escalation scheme using fixed dose levels. For dose expansion (part 1B), one of the treatment arms (Arm C over B over A) is continued using a Simon two-stage design to a maximum of 35 patients. The efficacy of the sequential programmed death-ligand (PD-L)1 blockade in combination with low-dose lenalidomide is evaluated in study part 2 (consolidation part) for pts with controlled disease (DCR) after 3 cycles of part 1 therapy. Evaluation of the consolidation concept in part 2 requires a sufficient DCR based on the statistical considerations. Thus, in addition to pts in part 1A and 1B, pts treated with GnP alone are additionally recruited (standard arm), so that at least 41 pts with controlled disease after 3 cycles of therapy are available for part 2. Key eligibility includes metastatic PDAC (stage IV), ECOG 0-1 and no prior chemotherapy or radiotherapy therapy for stage IV PDAC. Primary objectives include safety and tolerability of AZA and/or ROM in combination with GnP followed by sequential immune targeting with PD-L1 blockade in combination with low-dose lenalidomide.
Results: In October 2022, 76 pts were enrolled, of which 67 pts were treated with at least one dose of study medication. The median age in the treated population was 62.8 years (range: 33 - 83), 32 (47.8 %) were female, 80.6 %/13.4 % were included with ECOG 0/1. In part 1A, an RDE of 2mg/m² for ROM (N=3, dose level L-1) and 30mg/m² for AZA (N=6, dose level L1) was established, respectively. The combined administration of AZA (30 mg/m²), ROM (2 mg/m²) and GnP (arm C) led to severe DLTs, namely neutropenia, non-hematologic toxicities (> grade2) and ROM related non-hematologic toxicities (≥ grade2) in all three patients. Given the best safety profile, Arm B was chosen for the expansion part 1B.
Conclusions: ROM in combination with GnP showed considerable toxicity at dose level L1 as did the combination of ROM (dose level L-1) and AZA (dose level L1) with GnP. The combination of 30 $mg/m^2$ AZA with GnP was feasible and chosen for further expansion. Dose expansion (part 1B) and sequential immunotherapy consolidation (part 2) are ongoing.
Clinical trial information: NCT04257448. Research Sponsor: GWT-TUD GmbH.

**CT196**

**A phase I clinical trial of oncolytic adenovirus mediated suicide and interleukin-12 gene therapy in patients with recurrent prostate cancer.**

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**Introduction:** Men with locally recurrent prostate cancer, after definitive radiotherapy, have few therapeutic options. Oncolytic adenovirus-mediated cytotoxic gene therapy is an investigational cancer therapy. Delivery of the suicide gene to the tumor is by direct intratumoral or systemic injection of a viral vector containing the suicide gene. Our approach incorporates yeast cytosine deaminase (yCD) and herpes simplex virus thymidine kinase (HSV-1 TK), to confer sensitivity to 5-fluorocytosine (5-FC) and Valganciclovir (vGCV), respectively. The pro-drugs are converted into active drugs that inhibit DNA damage repair. Here we report the safety of oncolytic adenovirus-mediated suicide gene therapy that incorporates an interleukin-12 (IL12) gene for treatment of recurrent prostate cancer.
**Methods:** In this phase I study, a replication-competent adenovirus (Ad5-yCD/*mut*$TK_{SR39}$rep-hIL-12)

expressing yCD/mut$TK_{SR39}$ (yeast cytidine deaminase/mutant S39R HSV-1 thymidine kinase) and human IL-12 (IL12) was injected into tumors of 15 subjects with recurrent prostate cancer (T1c-T2) at escalating doses ($1 \times 10^{10}$, $3 \times 10^{10}$, $1 \times 10^{11}$, $3 \times 10^{11}$, or $1 \times 10^{12}$ viral particles). Subjects received 5-FC and vGCV for 7 days. The study endpoint was toxicity through day 30. Experimental endpoints included measurements of serum IL12, interferon gamma (IFNγ), and CXCL10 to assess immune system activation. Peripheral blood mononuclear cells (PBMC) and proliferation markers were analyzed by flow cytometry.
**Results and Conclusions:** Fifteen patients received Ad5-yCD/*mut*$TK_{SR39}$rep-hIL-12 and oral 5-FC and vGCV. Approximately 92% of the 115 adverse events observed were grade 1/2 requiring no medical intervention. Ad5-yCD/*mut*$TK_{SR39}$rep-hIL-12 DNA was detected in the blood of only two patients. Elevated serum IL12, IFNγ, and CXCL10 levels were detected in 57%, 93%, and 79% of subjects, respectively. Serum cytokines demonstrated viral dose dependency, especially apparent in the highest-dose cohorts. Analysis of immune cell populations indicated activation after Ad5-yCD/*mut*$TK_{SR39}$rep-hIL-12 administration in cohort 5. The study did not detect a significant difference in the PSA doubling time (PSADT) between pre and post treatment by paired Wilcoxon rank test (p=0.17). There was no correlation between adenoviral dose and PSADT in each cohort separately or pooled (cohorts1-3 and cohorts 4-5). The study maximum tolerated dose (MTD) was not reached indicating $10^{12}$ viral particles was safe. This trial confirmed that replication-competent Ad5-IL-12 adenovirus (Ad5-yCD/*mut*$TK_{SR39}$*rep*-hIL-12) was well tolerated when administered locally to prostate tumors.

**CT197**
**Profiling of tumor infiltrating T cells in malignant pleural/peritoneal mesothelioma (MPM) and ovarian cancer patients as part of a Phase 1 clinical trial of gavo-cel (TC-210).**
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Gavocabtagene autoleucel (gavo-cel; TC-210) is a T cell receptor fusion construct (TRuC) cell therapy directed against mesothelin. As part of the phase 1 dose-escalation clinical trial (NCT03907852), patients with advanced solid tumors expressing mesothelin were administered a single infusion of gavo-cel. Thirty-two patients with late-stage disease (23 MPM, 8 ovarian and 1 cholangiocarcinoma) received gavo-cel at doses ranging from $5x10^7$ - $5x10^8$ TRuC cells/$m^2$. The disease control rate (defined as achieving a response by RECISTv1.1 criteria or having stable disease [SD] for at least 12 weeks post-infusion) was 77%. Dose level 3 ($1x10^8$ TRuC cells/$m^2$ following lymphodepletion with cyclophosphamide and fludarabine) was selected as the Recommended Phase 2 Dose (RP2D) for the ongoing phase 2 portion of the trial. Spatial distribution of immune cells, particularly T cells, in the tumor and stromal regions of tumor biopsies has been shown to identify patients that may respond to immunotherapies. To understand the tumor immune contexture, we profiled the T cell compartment in biopsies from five patients collected prior to and eight weeks post treatment using multiplexed immunofluorescence. We observed that the MPM patient with a partial response (PR) had a greater number of T cells at baseline in tumor biopsies when compared with the other SD patients. Notably, this patient with a PR, had a 3.6-fold increase in CD3+CD8+ T cell infiltrates into the tumor bed. In contrast, 2 SD patients with MPM had a minimal increase of T cells in the tumor compartment at 8 weeks post infusion. The density of TILs 8 weeks post infusion was much lower in the SD patients compared to the PR subject, indicating a potentially insufficient number of infiltrating T cells. Interestingly, 2/2 SD patients with ovarian cancer had a decrease in CD3+CD8+ T cells infiltrating into the tumor, consistent with an immune exclusion phenotype. The PR patient with MPM had minimal increase in PD-L1 and CD155 (a ligand for the inhibitory receptor, TIGIT) from negligible levels at baseline, whereas both SD patients with MPM had marked increases in PD-L1 and CD155, indicating potential resistance mechanisms. In summary, this preliminary data suggests although CD8+ TILs are increased following treatment with gavo-cel, the tumor regions in patients with SD show evidence of immune surveillance

escape via PD-L1 and CD155 upregulation, thus acting as putative mechanisms of resistance. However, as seen in the PR patient with MPM there appears to be a critical number of TILs associating with response. We are currently evaluating the tumor immune contexture in phase 2, focusing on differences across tumor types with regards to TIL content, T cell influx, and correlations with response. We will also explore additional markers such as PD-1 and TIGIT, to profile the exhaustion state of immune cells and their spatial relationship with infiltrating gavo-cel T cells.

**CT198**

**Immunomodulatory effects of the ATR inhibitor ceralasertib in a window of opportunity biomarker trial in patients with head and neck squamous cell carcinoma.**

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Head and neck squamous cell carcinoma (HNSCC) is frequently locally advanced, but has a high risk of recurrence after initial treatment. Immune checkpoint blockade with PD-1/PD-L1 inhibitors has revolutionized treatment in the recurrent setting, but resistance to these therapies frequently occurs. Combination of checkpoint inhibitors with the ATR inhibitor ceralasertib could provide an exciting opportunity, with encouraging clinical activity reported in melanoma and non-small cell lung cancer (Kwon *et al.* 2022; Kim *et al.* 2022; Besse *et al.* OA15.05 IASLC 2022 WCLC). This phase 1b trial provided a unique opportunity to understand the immunomodulatory impact of DNA Damage Response (DDR) inhibitors in a clinical setting.
NCT03022409 is an open-label, randomized window of opportunity trial where patients with HNSCC were treated with either the PARP inhibitor olaparib (300mg BID) or ceralasertib (160mg BID) for 9-21 days, prior to definitive surgery (or on-treatment biopsy). 21 patients were randomized; n=12 to ceralasertib and n=9 to olaparib, and ceralasertib data will be presented here. Translational endpoints on frozen and fixed tumor biopsies included spatial pharmacokinetics, pharmacodynamic biomarkers, multiplexed fluorescence of tumor infiltrating immune cells and gene expression panels. Blood assessments included cytokine detection, immune cell phenotyping and gene expression. Primary endpoint analysis utilized a bespoke prognostic immune-focused gene signature and secondary endpoint an IHC immunoscore, both aimed to measure if tumors would shift from a ‘cold’ to ‘hot/active’ immune state.We observed an ‘on-drug’ selective suppression of proliferating Ki67+ T-cells (but not total T-cells) in the tumor microenvironment and periphery, followed by a repopulation and median rebound of 63.8% and 187.5% above baseline levels for peripheral helper (CD4+Ki67+; n=8) and cytotoxic (CD8+Ki67+; n=7) T-cells respectively, when ceralasertib dose stopped. IL-12 plasma cytokine levels dropped on ceralasertib treatment and returned to baseline levels ‘off-drug’ in 8/10 patients. Type-I interferon (IFN1) gene expression associated signatures were also upregulated in PBMCs, suggestive of an immune priming effect. 0/2 and 2/4 patients on ceralasertib met their primary and secondary endpoints respectively, however, interpretation is limited due to small numbers of evaluable patients. 1 patient had a grade 3 serious adverse event of chest pain in the ceralasertib arm. There were no unexpected safety findings for either drug and adverse events were generally low grade.The translational data has generated new insights into the immunomodulatory effect of ceralasertib. Further evaluation of the combination of ceralasertib with immune checkpoint blockade is warranted to explore this novel immune mechanism of action.

**CT199**

**Rescuing response to ICI by blocking the type-2 immune axis in patients with NSCLC progressing on immunotherapy: A phase 1b/2 trial of dupilumab administered with checkpoint blockade.**

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Type-2 cytokines are hypothesized to promote an immune-permissive milieu for cancer to grow. Through scRNAseq and CITEseq on human non-small cell lung cancer (NSCLC) and the $kras^{G12D}p53^{-/-}$ lung cancer model we previously described a tumor-enriched dendritic cell program of concomitant immunosuppression and activation which we termed the "mregDC," that was also notable for a strong Th2 immune signature. We subsequently blocked the canonical Th2 cytokine IL-4 *in vivo* in tumor-bearing mice, and found that this significantly decreased lung tumor burden, which was recapitulated in multiple other tumor models. Furthermore, this effect synergized with PD-L1 blockade. Based on this data, we designed a clinical trial to assess if the addition of dupilumab, an anti-IL-4Ra antibody widely used for treatment of atopic diseases, may rescue responses to checkpoint blockade (NCT05013450).
In this Phase 1b/2 trial, up to 21 patients with NSCLC that have progressed on prior anti-PD-(L)1 therapy will be enrolled. Patients continue PD-(L)1 blockade while receiving three doses of dupilumab, administered every three weeks, with initial radiographic assessment of response at 9 weeks. Patients without progression of disease then continue anti-PD-(L)1 alone. The primary endpoint of Phase 1b is safety and tolerability, while the primary endpoint of Phase 2, inclusive of patients from Phase 1b, is efficacy as per RECIST. Patients undergo pre- and on-treatment biopsies, pre-treatment stool collection for microbiome analysis, as well as blood collection at 6 timepoints for PBMCs, plasma and ctDNA. Here we report the Phase 1b portion that has completed accrual in which 6 patients were enrolled in a Phase 1b run-in to confirm safety and tolerability.
There were no adverse events attributable to study treatment during Phase 1b in any of the 6 patients treated. Serum proteomic analysis of this cohort revealed that dupilumab treatment induced a profound increase in proinflammatory/tumoricidal immune effector molecules, and reversed a systemic Th2 cytokine signature. Furthermore, mass cytometry of circulating immune populations showed an expansion of cytotoxic lymphocyte populations and a reduction in immunosuppressive myeloid cells. The fourth patient treated on trial who had progressive disease after 9 cycles of consolidation checkpoint blockade following concurrent chemoradiation for squamous NSCLC was enrolled, and had a partial response at 9 weeks, with deepening of the PR at 25 weeks. This patient's on-treatment biopsy showed a major increase in CD8 T cell tumor infiltration. Histological analysis using spatial transcriptomics and multiplexed imaging from these patients is ongoing, and will be reported at the conference.
Based on this promising signal in the initial lead-in, the trial will proceed through Simon's Two Stage design and enroll a full 21 patients as part of the Phase 2 expansion cohort. This clinical trial is funded entirely through philanthropy support through the Tisch Cancer Institute.

**CT200**

**Updated PFS & OS from the phase Ib study of TQB2450 alone/with Anlotinib in previously treated advanced non-small cell lung cancer.**

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Hospital, Tianjin, China.

**Background:** At the interim analysis (data cutoff Oct 14, 2021) of this study (NCT03910127), TQB2450 plus anlotinb group significantly improved mPFS versus TQB2450 alone in chemotherapy treated patients with advanced NSCLC without driver gene alterations (6.9 months vs 2.7 months). We report an updated PFS, OS and safety results for this study.
**Method:** This is a multicenter, randomized, double-blind, phase Ib study. Adult patients with histologically or cytologically-confirmed stage IIIB or IV NSCLC who had wild-type EGFR/ALK and at least one line of prior systemic therapy or being intolerable of chemotherapy were randomized 1:1:1 to receive TQB2450 1200 mg plus placebo, or anlotinib 10 or 12 mg. The primary end point was PFS and secondary endpoints included safety and OS.
**Results:** Between July. 2019 and March. 2021, 101 pts were enrolled. 33 patients were randomly assigned to TQB2450 plus placebo and 68 patients to TQB2450 plus anlotinib. As of 19 September 2022, The median follow-up was 26.3 months. The median PFS (7.3 months, 95% CI 5.3-11.0) for TQB2450 plus anlotinib was significantly longer than that for TQB2450 plus placebo group (2.8 months, 95% CI 1.4-4.7) (HR 0.39, 95% CI 0.23-0.64; *P*=0.0001) (**Table**). In patients with PD-L1 ≥ 1%, the mPFS was 17.9 months (95% CI 5.8-31.1) in TQB2450 plus anlotinib 12 mg, which was numerically higher than that in TQB2450 plus anlotinib 10 mg. The median OS of patients with PD-L1 ≥ 1% for TQB2450 plus anlotinib 12 mg was numerically higher than that in TQB2450 plus anlotinib 10 mg (32.2 months vs 21.8 months). Grade 3 or higher TRAEs occurred in 50.0% of the patients in TQB2450 plus anlotinb and in 12.1% of those in TQB2450 plus placebo.

| | TQB2450 | TQB2450+Anlotinib 10mg | TQB2450+Anlotinib 12mg | TQB2450+Anlotinib |
|---|---|---|---|---|
| **mPFS** | | | | |
| n | 33 | 34 | 34 | 68 |
| Censor, n (%) | 7 (21.2) | 9 (26.5) | 8 (23.5) | 17 (25.0) |
| Median (95%CI) | 2.8 (1.4-4.7) | 7.0 (4.5-14.5) | 8.7 (4.1-11.4) | 7.3 (5.3-11.0) |
| HR (95%CI) | | 0.37 (0.21-0.66) | 0.40 (0.22-0.70) | 0.39 (0.23-0.64) |
| P value | 0.0006 | | | 0.0001 |
| **mPFS (PD-L1≥1%)** | | | | |
| n | 19 | 19 | 19 | 38 |
| Censor, n (%) | 3 (15.8) | 6 (31.6) | 7 (36.8) | 13 (34.2) |
| Median (95%CI) | 2.1 (1.3-7.2) | 7.0 (2.1-19.4) | 17.9 (5.8-31.1) | 8.6 (5.3-22.8) |
| HR (95%CI) | | 0.35 (0.16-0.77) | 0.29 (0.13-0.64) | 0.32 (0.16-0.63) |
| P value | 0.0023 | | | 0.0006 |
| **mOS** | | | | |
| n | 33 | 34 | 34 | 68 |
| Censor, n (%) | 12 (36.4) | 14 (41.2) | 12 (35.3) | 26 (38.2) |
| Median (95%CI) | 15.3 (6.6-20.5) | 20.6 (8.6-25.8) | 14.7 (10.0-32.2) | 17.9 (10.5-23.2) |
| HR (95%CI) | | 0.78 (0.42-1.44) | 0.84 (0.46-1.53) | 0.81 (0.48-1.37) |
| P value | 0.7157 | | | 0.4329 |

| **mOS (PD-L1≥1%)** | | | | |
|---|---|---|---|---|
| n | 19 | 19 | 19 | 38 |
| Censor, n (%) | 5 (26.3) | 7 (36.8) | 8 (42.1) | 15 (39.5) |
| Median (95%CI) | 15.7 (7.8-21.0) | 21.8 (7.1-NR) | 32.2 (14.2-33.6) | 21.8 (14.2-33.6) |
| HR (95%CI) | | 0.72 (0.33-1.58) | 0.63 (0.28-1.40) | 0.67 (0.34-1.32) |
| P value | 0.4900 | | | 0.2479 |

**Conclusions:** In this updated analysis with longer follow-up, TQB2450 combined with anlotinib continues to demonstrate overall efficacy and manageable safety profiles in chemotherapy treated patients with advanced NSCLC without driver gene mutations.

**CT201**

**HS-10365, a highly potent and selective RET tyrosine kinase inhibitor, demonstrates robust activity in RET fusion positive NSCLC patients.**

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**Background:** Activating RET alterations drive oncogenic signaling in lung, thyroid, and other solid tumors.Until recently, only two RET tyrosine kinase inhibitor (TKI), BLU-667 and LOXO-292, had received approval for advanced NSCLC by FDA and NMPA. And it still had an unmet clinical need in this therapy area.HS-10365 is a highly potent and selective tyrosine kinase inhibitor, and the preclinical studies have indicated its favorable safety and antitumor activity in RET-altered tumor models. Here, we conducted a phase I study to assess the safety, tolerability, pharmacokinetics (PK), and anti-tumor activity of HS-10365 in RET-altered solid tumors.
**Methods:** This study (NCT05207787) recruited patients (pts) with RET-altered advanced solid tumors, including RET fusion-positive (+) NSCLC, RET-mutated medullary thyroid carcinoma and so on. Pts were dosed orally in 21-day cycles. A rule-based accelerated titration combined with 3+3 dose-escalation scheme was used to determine the MTD as the primary endpoint. Secondary endpoints contained safety, PK parameters, ORR and DCR assessed by RECIST V1.1.
**Results:** As of Dec.15$^{th}$ 2022, 31 RET fusion+ NSCLC pts with RET TKI-naïve were received HS-10365 at 6 doses (40 mg QD to 200 mg BID), including 25 previously received platinum-based chemotherapy pts and 6 treatment-naïve pts. Among all fusion variants, 15 pts had KIF5B, 14 pts had CCDC6, and 2 pts were others. Dose limiting toxicity occurred only in one pt. at 200 mg BID (grade 3 hypertension). The MTD was not been defined, and the 160mg BID was the potentially recommended phase II dose. The common (≥25%) TRAEs were AST increase, bilirubin increase, ALT increase, WBC decrease, PLT decrease, neutrophil decrease, serum creatinine increase, prolonged QT interval, hypoalbuminemia and anemia. No pts discontinued treatment owing to AEs. Efficacy data was available for 30 RET fusion+ NSCLC pts with 24 pretreated pts and 6 treatment naïve pts. The ORR was 70.0% (21/30, 95% CI 50.6%-85.3%), with 66.7% (16/24) in pretreated pts and 83.3% (5/6) in treatment naïve pts. Furthermore, the DCR was 96.7% (29/30, 95% CI 82.8%-99.9%), with 95.8% (23/24) in pretreated pts and 100% (6/6) in treatment naïve pts. The longest response time was more than 48 weeks. Meanwhile, 25 of 31 pts remained on treatment and responses were ongoing. Plasma exposure of HS-10365 increased proportionally following single dose and multiple doses. The mean plasma half-life of HS-10365 was 5~9 hours.

**Conclusions:** HS-10365 showed a manageable safety profile and favorable PK properties. The promising antitumor activity with expectable response time was observed in RET fusion+ NSCLC pts, no matter with or without previous treatments.

**CT202**

**Xevinapant plus avelumab in patients (pts) with advanced or metastatic non-small cell lung cancer (NSCLC): Phase 1b dose-expansion results and exploratory biomarker analyses.**

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**BACKGROUND:** Combining anti-PD-1/L1 antibodies and agents that restore cancer cell susceptibility to apoptosis may enhance antitumor activity. We report results from a phase 1b dose-expansion cohort of xevinapant, a first-in-class, oral, small-molecule IAP (inhibitor of apoptosis protein) inhibitor that restores cancer cell sensitivity to apoptosis, and avelumab (anti-PD-L1) in pts with advanced NSCLC.
**METHODS:** The recommended phase 2 dose (RP2D; 28-day cycles of xevinapant 200 mg/day [days 1-10 and 15-24] + avelumab 10 mg/kg [days 1 and 15]) was previously established during the dose-escalation part of this phase 1, open-label study. In this dose-expansion cohort, pts with advanced NSCLC who progressed on first-line (1L) platinum-based chemotherapy (CTx) or anti-PD-1/L1 ± platinum-based CTx received xevinapant (at RP2D) + avelumab for 13 cycles. The primary endpoint was objective response rate (ORR) per RECIST 1.1. Secondary endpoints included duration of response (DoR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), safety, and pharmacokinetics (PK).
**RESULTS:** 38 pts were treated: most had squamous cell carcinoma (45%) or adenocarcinoma (42%) of the lung, 11% had prior anti-PD-L1 therapy, 71% were men, and median age was 62 years (range, 35-75). 1 pt completed 13 cycles, and 37 permanently discontinued treatment; most common reasons were progressive disease (PD; 70%) and adverse events (AEs; 27%). ORR was 10.5% (95% CI 2.9-24.8). Best overall response (BOR): 4 pts had a confirmed partial response (PR), 19 had stable disease, and 15 had PD. Median DoR in responders was 15.9 months (95% CI 3.5-29.7); DCR was 60.5% (95% CI 43.4-76.0). Median PFS was 3.5 months (95% CI 1.9-5.1); median OS was 9.4 months (95% CI 6.7-16.2). Most pts (n=37; 97.4%) had treatment-emergent AEs (TEAEs); 21 (55.3%) had grade ≥3. Most common TEAEs were decreased appetite (n=13; 34.2%) and ALT increase (n=11; 28.9%). Nine pts died due to TEAEs; none were considered treatment-related. Xevinapant and avelumab PK were comparable to monotherapy at the same doses. In exploratory biomarker analyses, plasma IL-10 levels increased during the study treatment period. In blood, activated CD4 T cells and Tregs increased during cycle 1, and activated CD8 T cells increased during the treatment period; however, these did not correlate with antitumor activity. In tumor samples, low Ki67 expression was associated with BOR of PR (n=4), and increases in macrophages, Tregs, Th1 cells, and dendritic cells were associated with disease control.
**CONCLUSION:** Xevinapant + avelumab had tolerable safety in pts with advanced NSCLC who

progressed on 1L platinum-based CTx or anti-PD-1/L1 ± platinum-based CTx; however, the study did not meet its primary endpoint as antitumor activity was comparable to historic data for avelumab second-line monotherapy.

**CT203**

**Updated data from the Phase I Beamion Lung 1 trial of the HER2 tyrosine kinase inhibitor (TKI), BI 1810631, as monotherapy in patients (pts) with advanced/metastatic solid tumors with HER2 aberrations.**

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Background: There is an unmet need for effective TKIs against HER2 mutations in solid tumors, particularly in NSCLC. BI 1810631 is a HER2-selective TKI that covalently binds to both wild-type and mutated HER2 receptors, including exon 20 insertions, whilst sparing EGFR signaling. This ongoing Phase Ia/Ib, open-label, non-randomized study aims to determine the safety, MTD, PK, pharmacodynamics and preliminary efficacy of BI 1810631 in pts with HER2 aberration-positive solid tumors (NCT04886804). Here, we present interim results of Phase Ia.
Materials and Methods: In Phase Ia, pts with HER2 aberration-positive (overexpression, gene amplification, somatic mutation, or gene rearrangements) advanced/unresectable/metastatic solid tumors refractory/unsuitable for standard therapy were enrolled. Pts received escalating doses of BI 1810631 BID (starting dose: 15 mg) or BI 1810631 QD (starting dose: 60 mg).
Phase Ib will initially include 30 pts with advanced HER2 tyrosine kinase domain mutation-positive, pre-treated NSCLC. Additional cohorts may be included in the future. Primary endpoints: MTD based on number of DLTs; number of pts with DLTs (Phase Ia); objective response (Phase Ib). Secondary endpoints: number of pts with DLTs throughout entire treatment period and PK parameters (Phase Ia/Ib); duration of response, disease control, duration of disease control, and PFS (Phase Ib).
Results: As of 21 December 2022, 34 pts had been treated in the US, The Netherlands, Japan, and China. Pts had NSCLC (n = 21), colorectal cancer (n = 3), or other tumors (n = 10). Most pts had a pathological *HER2* mutation (n = 25). Pts received BI 1810631 15, 30, 60, 100, 150 mg BID (n = 3/3/4/4/3) or 60, 120, 180, 240 mg QD (n = 5/4/5/3). Median number of cycles was 4 (range 1-14). Treatment is ongoing in 23 pts. To date, three DLTs have been observed (grade 2 edema [60 mg BID], grade 3 anemia [60 mg QD], grade 3 elevated ALT [180 mg QD]). The MTD has not been reached with either schedule. Treatment-related adverse events (TRAEs) were reported in 23 pts (68%). The most common TRAEs were diarrhea (n = 9), anemia (n = 5), increased alkaline phosphatase, increased creatinine, increased ALT, hypoalbuminemia (all n = 4), hypocalcemia, elevated AST, dry skin, increased GGT (all n = 3). Three pts had grade 3 TRAEs (anemia/increased GGT [n = 1], increased ALT [n = 2]). In 29 pts evaluable for response the ORR (regardless of confirmation) was 34% (n = 10, all PRs; NSCLC: n = 8; esophagus, cholangiocarcinoma: n = 1). The DCR was 90%. In 19 NSCLC pts evaluable for response, the ORR was 42% and the DCR was 95%. Updated data will be presented at the meeting.
Conclusions: These preliminary data indicate that BI 1810631 is well tolerated and shows encouraging anti-tumor activity in pts with HER2 aberration-positive solid tumors. Recruitment into Phase Ia is ongoing.

**CT204**

**Safety, tolerability, pharmacokinetics, and efficacy of SHR-A1811, an antibody-drug conjugate, in patients with advanced *HER2*-mutant non-small cell lung cancer (NSCLC): a multicenter, open-label, phase 1/2 study.**

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Introduction: *HER2*-mutant NSCLC is associated with poor prognosis. SHR-A1811 is a novel antibody-drug conjugate (ADC) consisting of a humanized HER2-directed monoclonal antibody, cleavable tetrapeptide-based linker, and DNA topoisomerase I inhibitor. Here, we report data from the phase 1 portion of a phase 1/2 study with SHR-A1811 in patients with *HER2*-mutant NSCLC (NCT04818333).
Methods: In this single-arm, dose escalation and expansion phase 1 portion, patients with advanced activating *HER2*-mutant NSCLC who had failed platinum-based chemotherapy in the advanced or metastatic setting or could not tolerate chemotherapy were enrolled. SHR-A1811 was assessed at doses of 3.2, 4.8, 5.6, 6.4, and 8.0 mg/kg intravenously once every 21-day cycle. Dose escalation and determination of maximum tolerated dose (MTD) was guided by Bayesian logistic regression model with overdose control. Dose selected for expansion was determined based on data from dose-escalation phase. The primary endpoints were safety, MTD and recommended phase 2 dose (RP2D).
Results: At data cutoff (Nov 8, 2022), 50 patients were enrolled. All were stage IV with a median of 3 (range 1-8) prior systemic treatments, including HER2-targeted TKI (66%), immune checkpoint inhibitors (68%), and anti-angiogenic drugs (78%). 96% had a HER2 kinase domain mutation. One patient in 8.0 mg/kg dose cohort had dose-limiting toxicities (grade 4 febrile neutropenia and grade 4 thrombocytopenia). The 4.8 mg/kg dose cohort was expanded to 38 patients. The median follow-up duration was 5.6 months (95% CI 4.2-7.0). 62% of patients remained on treatment. Overall, objective response rate was 40.0% (95% CI 26.4-54.8); median duration of response was 8.3 months (95% CI 5.4-13.7); disease control rate was 86.0% (95% CI 73.3-94.2); median progression-free survival was 10.8 months (95% CI 6.7-15.0). All patients had treatment-related adverse events (TRAEs). 42% of patients experienced grade ≥3 TRAEs, with the most common ones being decreased neutrophil count (30%), decreased white blood cell count (20%), anemia (16%), and thrombocytopenia (12%). Nine patients (18%) had serious AEs deemed related to SHR-A1811. Treatment discontinuation due to AEs was reported in two patients. One death was reported to be treatment related (interstitial lung disease). After single dosing, dose exposure ($C_{max}$ and $AUC_{last}$) of SHR-A1811 increased in a dose-proportional manner. PK parameters of SHR-A1811 and total antibody were similar at all dose levels, with low plasma exposure of free topoisomerase I inhibitor observed.
Conclusion: SHR-A1811 showed tolerable safety profile and durable antitumor activity in heavily pretreated patients with *HER2*-mutant NSCLC. Dose expansion at 4.8 mg/kg and 5.6 mg/kg is ongoing to establish the RP2D.

**CT205**

**Safety and preliminary activity of naptumomab estafenatox (NAP) and durvalumab in patients with advanced or metastatic solid tumors: interim results from a phase 1b trial.**

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Background: NAP is a chimeric protein composed of a superantigen (SAg) and a Fab targeting the common tumor antigen 5T4. Its therapeutic effect is associated with activation, expansion and tumor infiltration of SAg-binding specific T cells. Durvalumab is a human monoclonal antibody that blocks programmed death ligand 1 (PD-L1).Initial data are reported from the dose escalation and MTD expansion parts of a phase 1b trial (NCT03983954), evaluating the safety and activity of NAP in combination with durvalumab in patients (pts) with advanced or metastatic solid tumors.
Methods: The primary objective was evaluating safety, tolerability, MTD/ RP2D of NAP in combination with durvalumab. Secondary objectives included efficacy and duration of response based on RECIST/iRECIST. Serum biomarkers were collected. Dose-escalation was used to determine the MTD/RP2D. The MTD was the highest dose at which less than one-third of evaluable patients experienced a dose-limiting toxicity. Patients were treated with NAP at 2, 5, 10, 15 and 20 mcg/kg and durvalumab at a flat dose (1120 mg) in 21 days cycles using 3+3 design. Obinutuzumab pretreatment was employed to inhibit the formation of anti-drug antibodies (ADAs) to NAP. Additional patients were enrolled at MTD; RP2D was determined based on the MTD, pharmacokinetics and biomarkers.
Results: 59 pts were enrolled, median age was 62 yrs (34-88), 56% female, ECOG 0 in 47% and 1 in 53%. 69% had pancreatic, ovarian or TN breast cancers. Pts received a median of 3 prior lines (0-6) and 7 pts (12%) received prior CPIs. AEs reported in 57 pts (97%) and included grade 1-2 infusion related reactions (IRRs) in 85%. 95% of IRRs occurred in cycles 1-2 and were rapidly reversible. Grade 3 IRRs occurred in 10%. Transient grade 1-2 elevations in liver enzymes occurred in 25% of pts. Treatment was discontinued due to toxicities in 4 pts (7%), at doses above RP2D. 23 patients were treated at the RP2D (10mcg/kg). IRRs were reported in 21 pts (91%), grade 3 IRRs occurred in 4 pts, all limited to cycle 1. No pts discontinued treatment due to toxicities at the RP2D. 2 patients had CR (cervical who progressed on prior CPI and pancreatic cancer, 5 m and 35+ m respectively), 2 pts had PR (HCC who progressed on prior CPI and peritoneal mesothelioma, 24+ m and 13 m). Four pts (7%) had SD, with a median duration of 15 months (5-24+).
Conclusions: NAP RP2D was reached at 10 mcg/kg. Antitumor activity with significant response duration was observed. Based on the favorable safety profile and prolonged responses, including CRs, in patients where response from a single agent CPI may not be expected, further evaluation of this combination is warranted including patients who have received prior CPI therapy.

**CT206**

**A phase I study of binimetinib, a MEK inhibitor, in combination with pembrolizumab in patients with advanced non-small cell lung cancer (NSCLC).**

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Introduction: MEK inhibition combined with PD-1 axis inhibition may achieve a greater clinical response

than either inhibitor alone due to increased T-cell infiltration of tumours. We combined binimetinib with pembrolizumab in patients with stage IV advanced non-small cell lung cancer (NSCLC) and PD-L1 tumor proportion score>=50%.
Methods: A 3 + 3 dose escalation design was used. Binimetinib at a dose level 1 (DL1; 45 mg) or dose level -1 (DL-1; 30 mg) twice daily orally continuously was given with pembrolizumab 200 mg IV q 21 days. The primary objective was to define the recommended phase II dose (RP2D) of the combination. Secondary outcomes included safety of the combination and response (RECIST 1.1) with a planned Phase Ib expansion in patients with RAS/RAF/MEK dysregulated tumours via next generation gene sequencing. Genomic markers are being explored in tissue and plasma.
Results: Eleven patients (3 DL1, 8 DL-1) were enrolled with the following mutations: 7 with KRAS (3 G12C, 2 G12D, 1 G12V, 1 G12A), 2 BRAF (1 G409A, 1 V600E) and 1 STK11 which acts as a tumor suppressor gene encoding for LKB1. Two of 3 patients at DL1 experienced dose limiting toxicity (DLT) including grade 3 elevated amylase, grade 3 diarrhea, grade 4 elevated lipase and severe grade 2 rash requiring dose reduction. Of 8 patients treated at DL-1, 1 progressed in cycle 1, another was noncompliant with treatment. Of the remaining 6 patients, 1 experienced DLT with grade 3 rash with inability to administer binimetinib for >75% of cycle 1. The most common toxicities across all cycles (n=11 patients) were: rash (82%), diarrhea (36%) and pruritis (36%). Nine patients were evaluated for response with partial response in 3 (33%), stable disease in 4 (44%) and progressive disease in 2 (22%). All 3 responding patients had RAS or RAF alterations ($KRAS^{G12C}$, $KRAS^{G12V}$, $BRAF^{V600E}$) while the patient with STK11 mutant disease had early disease progression.
Conclusion: The RP2D of the combination in patients with advanced NSCLC is binimetinib 30 mg BID plus pembrolizumab 200 mg IV q21 days. Updated data from cohort expansion including response and molecular correlates of treatment response and resistance will be presented.

**CT207**

**Phase I trial of vesicular stomatitis virus expressing human interferon beta and tyrosinase related protein 1 (VSV-IFNb-TYRP1) in metastatic ocular melanoma.**

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Oncologic viruses (OV) can induce tumor death through both direct oncolysis and immune-mediated destruction. Our study investigates the use of a novel OV with a Vesicular Stomatitis Virus (VSV) vector modified to express interferon-beta (IFN-b) and Tyrosinase Related Protein 1 (TYRP1) (VSV-IFNb-TYRP1). VSV is an attractive vector since humans are not naturally infected with VSV; thus, pre-existing immunity is minimal. IFN-b leads to increased T-cell responses and tumor cell apoptosis, while TYRP1, a melanocyte differentiation antigen, increases immunogenicity of the therapy. We conducted a Phase 1 clinical trial with a 3+3 design in patients with metastatic uveal melanoma. VSV-IFNb-TYRP1 was injected into a liver metastasis under image guidance, then administered on the same day as a single intravenous (IV) infusion. IV doses started at dose level (DL) 1 $1x10^{10}$ $TCID_{50}$, then escalated to DL2 $3x10^{10}$, DL3 $1x10^{11}$, and DL4 $3x10^{11}$. The dose delivered intratumorally (IT) varied based on the IV dose. The primary endpoints were safety and maximum tolerated dose (MTD). Efficacy was a secondary endpoint. Correlative studies focused on understanding viral pharmacokinetics (PK) and immunological responses induced by VSV-IFNb-TYRP1 therapy. Twelve patients with previously treated metastatic uveal melanoma were enrolled. Median follow up was 19.1 months. Four DLs were evaluated with one patient at DL4 experiencing dose limiting toxicities (DLTs), including decreased platelet count (grade 3), increased aspartate aminotransferase (AST), and cytokine release syndrome (CRS). Non-DLTs at DL4 were fatigue, fever, CRS, hematological toxicities (decreased platelets and lymphocytes), and AST

elevation. Four patients had stable disease (SD) and 8 had progressive disease (PD) as their best response. ELIspot data show that three of the four patients with response to TYRP1 also had a response to gp100, suggesting possible epitope spreading. These four patients received immune checkpoint inhibitor treatment as the next line of therapy, which led to clinical benefit for two patients. Our study evaluated VSV-IFNb-TYRP1 administered via IT and IV routes in a previously treated population of metastatic uveal melanoma patients and found no major safety events. Although there were no clear objective responses to VSV-IFNb-TYRP1, dose-dependent immunogenicity to melanoma antigens was seen. While OV response alone is not sufficient for clinical benefit, the evidence of epitope spreading is encouraging. Future studies of VSV-IFNb-TYRP1 will evaluate combinations with other therapies.

**CT208**

**Tebotelimab, a PD-1/LAG-3 bispecific antibody, in patients with untreated, unresectable, recurrent or metastatic, mucosal melanoma: An open-label, single-arm, Phase 1 study.**

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Background: Immune checkpoint inhibitors (CPIs) targeting PD-(L)1 have become a standard of care for untreated, advanced melanoma, but demonstrated limited efficacy in mucosal melanoma. Tebotelimab, also known as MGD013, is a PD-1/LAG-3 bispecific tetravalent DART® molecule with synergistic antitumor activity shown in preclinical studies. We conducted an open-label, single-arm, multi-cohort phase 1 study (NCT04653038) to assess the efficacy and safety of tebotelimab in melanoma patients (pts) including those with CPI-naïve mucosal melanoma.
Methods: The CPI-naïve cohort of this study enrolled pts with unresectable, recurrent or metastatic, mucosal or acral melanoma who had received no systemic therapy. Tebotelimab 600 mg was administered intravenously once every two weeks. The primary endpoint was overall response rate (ORR) assessed by independent radiologic review committee (IRC) per RECIST v1.1 in the efficacy analysis set consisting of pts who received ≥1 dose of tebotelimab. A *post-hoc* sensitivity analysis was conducted in the IRC-response evaluable set consisting of pts with IRC-assessed target lesions in the efficacy analysis set who received ≥1 post-baseline tumor assessment by IRC or died within 13 weeks after first dose. Results are reported for mucosal melanoma.
Results: At data cut-off (January 19, 2022), 25 pts with mucosal melanoma were enrolled (median age, 61 years; male, 40%; ECOG 1, 40%; TNM Stage IV, 92%; metastatic, 80%). LAG-3 expression level was ≥1% in seven (28%), <1% in 15 (60%), and unknown in three (12%). PD-L1 expression was positive (CPS≥1) in three (12%), negative (CPS<1) in 19 (76%), and unknown in three (12%). All pts received ≥1 dose of tebotelimab. In the efficacy analysis set (n=25), three, three, and four pts achieved complete response (CR), partial response (PR), and stable disease (SD), respectively, leading to a confirmed ORR of 24% (95% confidence interval [CI], 9-45), with median duration of response (DOR) not reached, and a disease control rate (DCR) of 40% (95% CI, 21-61). In the IRC-response evaluable set (n=20), three, three, and four pts achieved CR, PR, and SD, respectively, leading to a confirmed ORR of 30% (95% CI,

12-54), with median DOR not reached, and a DCR of 50% (95% CI, 27-73). Immune-related treatment-emergent adverse events occurred in 11 (44%) pts, most commonly, hypothyroidism (20%), hyperthyroidism (16%), and white blood cell count decreased (12%). Grade ≥3 and serious treatment-related adverse events (TRAEs) were reported in three (12%) and four (16%) pts, respectively. TRAEs led to treatment discontinuation and death each in one (4%).
Conclusions: Tebotelimab demonstrated preliminary but promising antitumor activity and a tolerable safety profile in pts with untreated, unresectable, recurrent or metastatic, mucosal melanoma.

**CT212**

**A ctDNA-directed, multi-center phase II study of molecular response adaptive immuno-chemotherapy in patients with non-small cell lung cancer: Analysis of Stage 1 of CCTG BR.36.**

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**Introduction:** Analyses of circulating tumor DNA (ctDNA) have shown promise in capturing tumor burden dynamics during immune checkpoint blockade (ICB), with the potential to allow patients with primary resistance to be rapidly identified and redirected to alternative therapies.
**Methods:** BR.36 is an international multicenter, open label, biomarker-directed, phase II trial of molecular response-adaptive immuno-chemotherapy for patients with treatment-naïve non-small cell lung cancer-NSCLC (NCT04093167). The trial consists of two stages; stage 1 was a single-arm study to validate the ctDNA approach, with stage 2 focusing on ctDNA guided treatment selection. Key eligibility criteria included *EGFR* and *ALK* mutation negative, ICB and chemotherapy-naïve metastatic NSCLC with PD-L1 expression ≥1%. Primary objectives were to ascertain ctDNA molecular response, determine its timing and concordance with radiologic RECIST/iRECIST response. Secondary objectives included the evaluation of time to ctDNA molecular response and its correlation with progression-free (PFS) and overall survival (OS). Liquid biopsy analyses utilized a validated tumor-agnostic white blood cell (WBC) DNA-informed 33-gene panel approach. Ultra-sensitive error-correction ctDNA next-generation sequencing (NGS) was performed at baseline/cycle 1 (C1D1), cycle 2 (C2D1) and cycle 3 (C3D1) of pembrolizumab (200mg IV q3 weeks). The maximum variant allele fraction (maxVAF) of tumor-derived variants was tracked from the pre-treatment to on-therapy timepoints.
**Results:** Activated October 17, 2019, stage 1 completed accrual of 50 patients on April 5, 2022; data lock date was September 20, 2022. Most patients were ever-smokers (98%), had no prior systemic therapy (92%), stage IV NSCLC (98%), adenocarcinoma (76%) with PD-L1 tumor proportion score of ≥ 50% (96%); cohort consisted of 82% white, 52% female, 56% age>65 and 76% ECOG PS 1 participants. Median follow-up was 13.5 months (range 2.5-23.0). Best overall response rate (ORR) by RECIST was 32% with a median duration of 10.1 months. Matched WBC DNA analyses allowed for effective removal of germline and clonal hematopoiesis variants, followed by evaluation of ctDNA response. MaxVAF clearance signified molecular response (mR), while maxVAF persistence indicated molecular disease progression (mPD). Among 35 evaluable patients (77.8%), 15 were classified in the mR category, with an evaluable mR rate of 43% (90% CI: 0.29-0.58). The median time to molecular response was 2.1 months (90% CI: 1.5-2.6). The sensitivity of ctDNA molecular response for RECIST and iRECIST response was

82% (90% CI: 52%-97%) and 83% (90% CI: 56%-97%), with a specificity of 75% (90% CI: 56.5%-88.5%) and 78% (90% CI: 60%-91%) respectively. Patients with mR attained longer PFS (5.03 vs 2.6 months,) and OS (not reached vs 7.23 months); ctDNA response also provided prognostic information for patients with RECIST stable disease.
**Conclusions:** ctDNA molecular responses were concordant with radiologic response assessments, but importantly better predicted PFS and OS for patients with stable disease or better. These findings are now incorporated into the interventional second stage of the trial.

**CT213**
**Biomarker analysis from Phase 1/2 study of tusamitamab ravtansine (SAR408701) in patients with advanced non-small cell lung cancer (NSCLC).**
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**Background** Tusamitamab ravtansine is an antibody-drug conjugate of a humanized carcinoembryonic antigen (CEA)-related cell adhesion molecule 5 (CEACAM5)-specific monoclonal antibody linked to DM4. A Phase 1/2 study (NCT02187848) showed tusamitamab ravtansine antitumor activity in pretreated patients (pts) with advanced nonsquamous NSCLC and high CEACAM5 expression. Here, we explore biomarker associations with tumor CEACAM5 expression by immunohistochemistry (IHC), and whether biomarkers predict objective response rate (ORR).
**Methods** We assessed CEACAM5 expression by IHC, RNA sequencing, and whole exome sequencing (WES) on latest archival tumor samples; and circulating CEACAM5 (cCEACAM5) and CEA (cCEA). We enrolled 2 cohorts of pts with IHC CEACAM5 membrane expression at ≥2+ intensity: in ≥50% of tumor cells (high expressors, HEs, n = 64); and in ≥1% to <50% of tumor cells (moderate expressors, MEs, n = 28). Pts received tusamitamab ravtansine 100 mg/m$^2$ IV every 2 weeks.
**Results** cCEA and cCEACAM5 were strongly associated (Spearman rho, 0.9), with weak associations between IHC CEACAM5 and cCEA or cCEACAM5 (Spearman rho, 0.3 and 0.4, respectively). Higher levels of CEACAM5 mRNA were observed in CEACAM5 HEs vs MEs ($P$=0.0027). *EGFR* and *KRAS* genetic alterations by WES were present in 44.8% and 65.5% of CEACAM5 HEs, respectively, and 21.4% and 78.6% of CEACAM5 MEs, respectively. Confirmed partial responses were seen in 13/64 HEs (ORR 20.3%) and 2/28 MEs (ORR 7.1%). In CEACAM5 HEs with available baseline (BL) cCEA data, 25/62 (40.3%) had a cCEA level ≥100 µg/L, with a median value of 71.6 µg/L (range 1-8809); corresponding values in CEACAM5 MEs were 7/28 (25.0%) and 12.4 µg/L (range 0.5-684). In response evaluable CEACAM5 HEs with available BL cCEA data (n = 61), ORR was 10/24 (41.7%) in pts with high cCEA (≥100 µg/L) and 3/37 (8.1%) in pts with low cCEA (<100 µg/L); corresponding ORRs in CEACAM5 MEs were 0/7 and 2/21 (9.5%).
**Conclusions** In CEACAM5 HEs, high cCEA was associated with numerically greater ORR vs low cCEA (41.7% vs 8.1%). Associations were also observed between: cCEA and cCEACAM5; IHC CEACAM5, cCEA, and cCEACAM5; and IHC CEACAM5 and CEACAM5 tumor mRNA levels, but not between IHC CEACAM5 and actionable oncogenic drivers.
**Clinical Trials Registration:** ClinicalTrials.gov NCT02187848

**CT214**
**Preliminary efficacy of telisotuzumab vedotin (Teliso-V) treatment in the 2L/3L setting in *MET* gene amplified (*MET* Amp), c-Met protein overexpressing (c-Met OE), non-squamous, non-small cell lung cancer (NSQ NSCLC): Retrospective analysis of LUMINOSITY.**
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**Background**: Teliso-V is a first-in-class c-Met-directed antibody-drug conjugate comprising the monoclonal antibody telisotuzumab, a cleavable valine-citrulline linker, and the potent microtubule polymerization inhibitor monomethyl auristatin E. In a Phase 2 study (LUMINOSITY, NCT03539536), Teliso-V has shown an acceptable safety profile and encouraging efficacy in c-Met OE NSQ NSCLC. In NSCLC, while c-Met OE is more common than *MET* Amp, occurring in 25% of patients (pts) versus 1-5%, respectively, ~90% of *MET* Amp tumors are c-Met OE. We performed a retrospective analysis of LUMINOSITY to characterize the efficacy of Teliso-V in previously treated pts with *MET* Amp, c-Met OE NSQ NSCLC.
**Methods**: Pts had locally advanced/metastatic NSQ NSCLC, ≤2 prior lines of systemic therapy, ≤1 line of chemotherapy, *MET* Amp by fluorescence in situ hybridization (FISH), and tumors that were c-Met OE by central immunohistochemistry (IHC). *MET* Amp was defined as a ratio of ≥1.8 *MET* gene copy number (GCN) to chromosome 7 (CEP7) CN by FISH with levels of amplification defined as high, ≥5; intermediate (int), >2.2 - <5; or low, ≥1.8 - ≤2.2. *MET* GCN by FISH was classified as high, ≥10; int, 5 - <10; or low, <5. c-Met OE was defined as ≥25% of tumor cells at 3+ intensity by IHC; patients were classified as MET IHC 3/25 (25 - 49% tumor cells at 3+) or MET IHC 3/50 (≥50% tumor cells at 3+). Teliso-V was dosed at 1.9 mg/kg IV Q2W.
**Results**: As of 27 May 2021, 10 pts with *MET* Amp were treated with Teliso-V. The median age was 69 (48-75), 9 pts were male, 5 were White, 9 had a history of smoking (current or former), and 9 had an ECOG score of 0-1. The median *MET*/*CEP7* ratio was 3.3 (1.84-12.17) with 4, 1, and 5 pts having high, int, and low levels of amplification, respectively. The median *MET* GCN was 10.98 (6.95-65.00) with 6, 3, and 1 pts having high, int, and low GCN, respectively. 7 pts were MET IHC 3/50 and 3 were MET IHC 3/25. Pts with *MET* Amp treated with Teliso-V monotherapy had an ORR of 80% (95% CI [44.4, 97.5]; n=8; all partial response) by independent central review, and a disease control rate of 90%. The median duration of response was 6.9 months. Among the 8 responders, 62.5% (n=5/8) are still event-free and being followed up for disease assessment. Teliso-V monotherapy resulted in median progression-free survival (PFS) of 8.0 months (95% CI [1.3, -]; n=10), compared with 5.7 months (95% CI [2.6, 9.8]) on the last prior line of systemic cancer therapy (n=10).
**Conclusions**: Teliso-V demonstrated a promising ORR in previously treated pts with *MET* Amp NSQ NSCLC with c-Met OE and improved PFS when compared to last prior systemic cancer therapy. These preliminary data support the ongoing Phase 2 trial of Teliso-V monotherapy in pts with previously untreated *MET* Amp NSCLC (TeliMET NSCLC-02; NCT05513703), which is currently enrolling.

**CT215**

**Efficacy and safety of adjuvant (adj) atezolizumab (atezo) from the Phase 2 LCMC3 study.**

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**Background** LCMC3 (NCT02927301), an open-label, single-arm Phase 2 study to investigate

neoadjuvant (neoadj) and adj atezo (anti-PD-L1) in patients (pts) with early-stage non-small cell lung cancer (NSCLC), met its primary endpoint with a 20% major pathological response (MPR) rate after neoadj atezo. Here we report efficacy and safety from the adj phase.

**Methods** Eligible pts aged ≥18 y had resectable stage IB-IIIA or select IIIB NSCLC and ECOG PS of 0 or 1. Pts received neoadj atezo 1200 mg IV for ≤2 cycles (Days 1 and 22) followed by surgery (Day 40±10). Pts deemed to have clinical benefit were offered the option to receive adj atezo every 3 weeks for up to 12 months. The primary endpoint was MPR rate (≤10% viable tumor cells at surgery) in pts without *EGFR/ALK* mutations. Exploratory endpoints included DFS and OS. Safety was assessed during the adj phase.

**Results** Data cutoff was Oct 21, 2022. The primary efficacy population (PEP) was 137 pts without *EGFR/ALK* alterations who had surgery and MPR assessment. In the PEP, 53 pts (39%) received adj atezo and 84 (61%) did not. The 3-y DFS rate was 83% with adj atezo vs 64% without (HR, 0.43; 95% CI: 0.21, 0.90; *P*=0.025). 3-y OS and results by MPR status are provided in the table. In the adj atezo safety population (n=57), treatment-related (TR) AEs after adj atezo occurred in 55 pts (97%; Gr 3/4, 40%), including 11 TRAEs (19%; Gr 3/4, 16%) leading to discontinuation of adj atezo. No Gr 5 TRAEs were seen in the adj phase. Multivariate analysis also showed a trend toward better DFS for pts receiving adj atezo vs those with no adj atezo (HR, 0.52; 95% CI: 0.22, 1.21; *P*=0.126).

**Conclusions** This exploratory analysis revealed that LCMC3 pts with resectable stage IB-IIIA or select IIIB NSCLC who received adj atezo had improved DFS and showed a trend toward improved OS vs pts who did not receive adj atezo. Furthermore, the non-MPR subgroup had the same trend toward improved DFS and OS with adj atezo vs pts who did not receive adj atezo. Adj atezo was well tolerated, with no new safety concerns.

Table

| | **Adj atezo[a] n=53** | **No adj atezo[b] n=84** | | |
|---|---|---|---|---|
| **Tx patterns** | | | | |
| Adj atezo + adj chemo, n (%) | 17 (32) | 0 | | |
| Adj atezo, n (%) | 36 (68) | 0 | | |
| Adj chemo, n (%) | 0 | 36 (43) | | |
| None, n (%) | 0 | 48 (57) | | |
| **Tx cycles** | | | | |
| Adj atezo, n, median (range) | 53, 16 (1-18) | 0 | | |
| Adj chemo, n, median (range) | 16, 3 (0-4.2) | 35, 3 (0-4.1) | | |
| | | | **HR (95% CI)** | ***P*** |
| **PEP (N=137)** | | | | |
| 3-y DFS, % | 83 | 64 | 0.43 (0.21, 0.90) | 0.025 |
| 3-y OS, % | 89 | 77 | 0.48 (0.19, 1.21) | 0.118 |
| **MPR (n=29)** | n=22 | n=7 | | |
| 3-y DFS, % | 86 | 86 | 0.93 (0.10, 8.92) | 0.948 |
| **Non-MPR (n=108)** | n=31 | n=77 | | |
| 3-y DFS, % | 80 | 62 | 0.48 (0.20, 1.12) | 0.088 |
| 3-y OS, % | 87 | 75 | 0.49 (0.17, 1.46) | 0.202 |
| **Multivariable Cox regression (n=114)[c]** | | | | |
| MPR vs non-MPR | | | 0.37 (0.11, 1.31) | 0.125 |

| | | |
|---|---|---|
| Adj atezo vs no adj atezo | 0.52 (0.22, 1.21) | 0.126 |
| Adj chemo vs no adj chemo | 0.57 (0.27, 1.19) | 0.133 |
| Stage I/II vs III | 0.71 (0.35, 1.43) | 0.338 |
| PD-L1 tumor proportion score ≥1% vs <1% | 1.09 (0.54, 2.21) | 0.805 |
| Tx, treatment. [a]Received adj atezo or adj atezo + adj chemo. [b]Received adj chemo or no adj treatment. [c]Overall likelihood ratio test *P*=0.097 for the model. | | |

**CT216**

**Response to first-line (1L) pembrolizumab (pembro) + chemotherapy (chemo) in non-small cell lung cancer (NSCLC) by blood tumor mutational burden (bTMB): the phase 2 KEYNOTE-782 trial.**

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Background: 1L pembro + platinum-based chemo has shown clinical activity in metastatic NSCLC regardless of tissue tumor mutational burden (tTMB) status. bTMB assessed using circulating tumor DNA in plasma may be a surrogate for tTMB. The single-arm, phase 2 KEYNOTE-782 study (NCT03664024) evaluated the correlation of bTMB with efficacy of 1L pembro + chemo in nonsquamous NSCLC.
Methods: Eligible patients (pts) had histologically or cytologically confirmed stage IV nonsquamous NSCLC with measurable disease per RECIST v1.1 and were not eligible for EGFR-, BRAF-, ROS1-, or ALK-directed therapy. Pts were not previously treated for advanced or metastatic disease, had an ECOG PS of 0 or 1, and had an evaluable biopsy sample for biomarker analysis. All pts received intravenous pembro 200 mg Q3W + platinum chemo doublet Q3W (pemetrexed 500 mg/m$^2$ + 4 cycles of carboplatin AUC 5 mg/mL/min or cisplatin 75 mg/m$^2$). The primary objective was to evaluate the association of baseline bTMB with ORR per RECIST v1.1 by investigator assessment. Secondary objectives were to determine safety and the association of baseline bTMB with PFS per RECIST v1.1 by investigator assessment and OS. A study-specific next-generation sequencing-based assay using a 1.9 Mb/654-gene cancer panel, which includes specific lung cancer-associated gene targets, was used to measure bTMB (continuous scale) in cell-free DNA extracted from baseline plasma samples. Paired white blood cell DNA sequencing was also performed to eliminate potential clonal hematopoiesis-derived somatic mutations. Database cutoff: November 5, 2021.
Results: 117 pts were enrolled; median age was 64.0 years (range, 37-85), and the majority were male (60.7%), had an ECOG PS of 1 (69.2%), and no brain metastases at baseline (92.3%). Median time from

first dose to data cutoff was 19.3 months (range, 1.0-35.5). ORR was 40.2% (95% CI, 31.2-49.6; 6 CRs, 41 PRs), median PFS was 7.2 months (95% CI, 5.6-9.8), and median OS was 18.1 months (95% CI, 13.5-25.6). Treatment-related adverse events (TRAEs) occurred in 113 pts (96.6%) and grade 3-5 TRAEs occurred in 56 (47.9%). Eight pts (6.8%) died due to a TRAE (febrile neutropenia and pneumonitis [n = 2 each], and septic shock, pulmonary sepsis, general physical health deterioration, and neutropenia [n = 1 each]). bTMB data were available for 101 pts. The area under the receiver operating curve for bTMB as a continuous variable to discriminate response was 0.47 (95% CI, 0.36-0.59). The posterior probabilities for a positive association of bTMB with PFS and OS were 16.8% and 7.8%, respectively.
Conclusions: Baseline bTMB showed no evidence of an association with ORR, PFS, or OS in pts with nonsquamous NSCLC treated with 1L pembro + chemo. These findings indicate no clinical utility of bTMB in this patient population and treatment setting. No new safety signals were observed.

**CT217**

**3-year update of neoadjuvant atezolizumab + chemotherapy in patients with resectable non-small cell lung cancer.**

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Introduction: Neoadjuvant chemoimmunotherapy is promising in improving outcomes for patients with resectable lung cancer in the phase III setting, though data are immature at this time for overall survival (OS). Here, we present outcomes after 3 years of follow-up from the first reported study of neoadjuvant immunotherapy + chemotherapy in this population.
Methods: This open-label, multi-center single-arm investigator-initiated phase II study was conducted at three hospitals in the USA. Neoadjuvant atezolizumab, carboplatin, and nab-paclitaxel were administered for up to 4 cycles prior to surgical resection. The primary endpoint of major pathologic response (MPR) has been previously reported; here we report mature disease-free survival (DFS) and OS as well as clinical characteristics of patients with recurrent brain metastases (BM) with integrated data from tumor genomics, gene expression, and quantitative immunofluorescent (QIF) measurement of immune markers.
Results: Of 30 enrolled patients, 29 were taken to the operating room with 26 completing successful R0 resection. 17 patients experienced MPR, of whom 10 had pathological complete response (pCR). The median follow-up time was 39.5 months (95% CI: 30.9-49.4). Median OS was 55.8 months (95% CI: 43.6-NA); median DFS was 34.5 months (95% CI: 18.4-NA). Landmark OS at 12, 24, and 36 months were 97%, 80%, and 77%, respectively; DFS: 80%, 60%, and 49%. Radiographic and pathologic measures of response, as well as baseline PD-L1 levels, did not statistically significantly associate with DFS or OS. The strongest trends were observed with pCR (OS: HR 0.22, 95% CI 0.03-1.83; DFS: HR 0.45, 95% CI 0.12-1.65). Percent decrease of radiographic response correlated inversely with percent pathological regression (Pearson correlation -0.44, 95% CI: -0.71—-0.08). Of 14 patients evaluable for site of recurrence, 6 developed BM. 5/6 patients with BM died, with median time to recurrence of 12.4 months (95% CI: 9.9-32.6) and time to death of 45.3 months (95% CI: 22.7-55.8). 3/6 patients who developed BM experienced MPR (2 achieved pCR). Radiographic response and PD-L1 level did not significantly associate with development of BM. Patients whose tumors had mutations in *STK11* and *KEAP1* did not experience statistically worse DFS/OS, though incidence of BM trended higher. Reduced copy number of *STK11* and *KEAP1*, which both reside on chromosome 19p, was observed in about 1/3 of tumors. Deletion of either gene was significantly associated with worse pathologic response, lower levels of PD-L1+ cells by QIF, and BM.
Conclusions: Mature OS data reveal sustained clinical benefit of neoadjuvant chemoimmunotherapy with atezolizumab. Predictivity of radiographic and pathologic surrogates for survival was limited. Further

study is warranted to establish the associations between *STK11* and *KEAP1* genomic alterations and key clinical outcomes in early-stage NSCLC.

**CT218**

**A phase II trial of niraparib plus dostarlimab in relapsed small cell lung cancer and other high-grade neuroendocrine carcinomas.**

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Objectives: Small cell lung cancer (SCLC) and high-grade neuroendocrine carcinomas (NECs) share many clinical features, including limited response to immune checkpoint inhibitors (ICIs). However, preclinical data suggest synergistic response with combined ICI and poly (ADP-ribose) polymerase inhibitors (PARPi) in SCLC models. This single-institution, phase II trial (NCT04701307) assessed the efficacy of a combination of the anti-PD-1 monoclonal antibody, dostarlimab, with the selective PARPi, niraparib, in patients with relapsed SCLC or NECs.
Methods: Eligible patients with SCLC (Cohort 1) or NECs (Cohort 2) had received at least one prior line of therapy and were treated with niraparib 300 mg (or 200 mg if <77kg or platelets < 150,000/μL) PO daily, and dostarlimab 500 mg IV q21d (1000mg IV q42d starting with Cycle 5). Co-primary endpoints were objective response rate (ORR) by RECISTv1.1 and 6-month progression free-survival (PFS6). Using a Bayesian Optimal Phase 2 (BOP2) design, interim futility analyses were planned after enrolling up to 15 SCLC and 9 NEC patients (NEC), respectively. Adverse events (AE) were monitored continuously. Patients underwent biopsies, if feasible, at Cycle 1 and Cycle 3, as well as longitudinal plasma collection for translational studies.
Results: From Feb 2021 to Aug 2021, 14 and 9 patients enrolled with SCLC and NEC, respectively. NECs included tumors from gynecological, head/neck, and gastrointestinal sites. In Cohort 1 (SCLC), 12 of 14 patients were evaluable by RECISTv1.1, including 1 complete response (CR), while 4 patients not achieving partial response (PR) had minor responses (-2%, -14%, -19%, and -24% from baseline). Only 1 patient in this cohort achieved PFS6, though 2 additional patients progressed between 5 and 6 months. In Cohort 2 (NEC), 6 of 9 patients were evaluable by RECISTv1.1 and no CR/PRs were observed. One patient did experience durable minor response (-21%), but no patients achieved PFS6. Dose-limiting, niraparib-related hematological AEs, typically thrombocytopenia, occurred in 12 of 23 patients. Non-hematological, treatment-related serious AEs occurred in only 1 patient (Grade 3 inflammatory arthritis), while 1 additional patient experienced brief dose-interruption due to Grade 2 pneumonitis. Preliminary translational data, including bulk and single-cell RNAseq, indicate molecular features, such as transcriptional subtype, are shared across SCLC and NECs and may predict response to this combination.
Conclusions: In heavily pre-treated patients across SCLC and NEC cohorts, combined niraparib and dostarlimab failed to exceed interim futility criteria. However, multiple SCLC patients experienced durable disease control, including one patient with sustained CR and associated translational studies point to potential biomarker-driven approaches in the future.
Trial supported by GlaxoSmithKline.

**CT219**

**Efficacy and safety of Low dose radiotherapy (LDRT) concurrent Atezolizumab (Atezo) plus chemotherapy as first line (1L) therapy for ES-SCLC: Primary analysis of Phase II MATCH study.**

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**Background:** The IMpower133 represented the current standard of care in the 1L setting for patients (pts) with ES-SCLC (extensive-stage small cell lung cancer). However, there are still unmet needs for ES-SCLC treatment. LDRT could play a key role in the priming effect of immune system by acting as an immune adjuvant and having sensitive cytotoxic activity to SCLC. The interim analysis of MATCH study after stage I showed promising benefit and tolerability of combination of Atezo + chemotherapy + LDRT in pts with ES-SCLC. Here we report the primary efficacy and safety results of this study.
**Methods:** The MATCH study was a single-arm phase II trial conducted in 8 centers across China. Previously untreated ES-SCLC pts with measureable disease per RECIST v1.1 at baseline, age≥18, ECOG 0-1 were eligible. Atezo (1200 mg IV, D1) + Cisplatin (75 mg/m$^2$ IV, D1)/Carboplatin (AUC = 5 IV, D1) +Etoposide (100 mg/m$^2$ IV, D1-D3) were administrated on a 21-day cycle for four cycles. Concurrent LDRT (15 Gy/5f) were conducted from D1-D5 in the first cycle. Then pts received Atezo maintenance until loss of clinical benefit or unacceptable toxicity. The primary endpoint was objective response rate (ORR), defined as the proportion of patients with a complete response or partial response on two consecutive occasions ≥ 4 weeks apart, as determined by the investigator according to RECIST v1.1. The secondary endpoints included disease control rate (DCR), progression-free survival (PFS), overall survival (OS) and safety. A Simon's minimax 2-stage design was adopted.
**Results:** As the cutoff date of 30$^{th}$ Nov. 2022, 56 pts have been enrolled. 49 (87.5%) were males; mean age was 58.9 years with 78.6% pts had ECOG PS of 1. 80.4% pts had smoking history. Most pts were staged T4 (n = 33, 58.9%), N3 (n = 37, 66.1%) and M1(n = 40, 71.4%). Median follow-up was 14.8 months (range: 11.6-17.8 m). The confirmed ORR was 87.5% (95% CI: 75.9%-94.8%), all partial response. DCR was 94.6% (95% CI: 85.1%-98.9%). Median PFS was 6.9 m (95% CI: 5.4-9.3 m). The 6-month and 12-month PFS rate were 56.5% and 27.7%. Median OS was not reached (NR, 95% CI: 13.3m, NR). The 12-month OS rate was 71.9%. The safety profile, analyzed in all 56 pts, was consistent with the previous reports. Neutrophil count decreased (60.7%), white blood cell count decreased (58.9%) and platelet count decreased (23.2%) were the most common grade (G) 3-4 adverse events (AE). G5 AE occurred in 1 pt (pneumonia and pulmonary embolism). 4 pts experienced AEs leading to treatment discontinuation. IrAEs were reported in 21 (37.5%) pts, most common irAEs were hyperthyroidism (5.4%) and rash (5.4%). Radiation pneumonitis (G1) was observed in 1 pt.
**Conclusions:** Adding LDRT to Atezo + chemotherapy shows impressive antitumor activity, potential survival benefit and well tolerability in 1L treatment of ES-SCLC.
**Clinical registration**: NCT04622228.

**CT221**

**Phase II of frontline maintenance rucaparib in combination with nivolumab in ES SCLC.**

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Purpose: Immune checkpoint inhibitor (ICI) maintenance therapy is standard of care for frontline management of extensive stage small cell lung cancer (ES SCLC). However, overall survival benefit of addition of ICI maintenance to frontline ES SCLC treatment is modest and further improvement is needed. We hypothesized that addition of poly (ADP-ribose) polymerase inhibition to ICI maintenance therapy for patients with platinum sensitive ES SCLC could improve antitumor efficacy of ICI.
Methods: A single-arm, investigator-initiated phase II trial (NCT03958045) enrolled patients with platinum sensitive ES SCLC who received frontline maintenance nivolumab 480 mg IV every 4 weeks, and rucaparib, 600 mg PO twice a day after completion of 4-6 cycles of platinum doublet. The primary outcome was median progression free survival. Secondary endpoints included assessment of objective response and adverse effects (AEs) per CTCAE 5.0. Correlative studies included pretreatment and during-treatment immune assays and circulating tumor DNA TP53 mutation status.
Results: A total of 42 patients were consented and 33 met eligibility criteria and were treated. All patients received 4-6 cycles of frontline platinum doublet and had at least a partial response by RECIST at the time of enrollment. In the 33 participants, the most common grade 3 and 4 AEs (at least possibly related) were hypokalemia (3%), hyponatremia (3%) , elevated alanine aminotransferase (3%), neutropenia (3%) and leukocytopenia (3%). No grade 5 AE was noted. The median PFS (mPFS) was 3 months from time of enrollment on frontline maintenance (post platinum doublet). The mPFS was 11 months from cycle 1 of platinum doublet. Overall, 89.8% patients were alive at 12 months and 54.4 % patients were alive at 24 months from the start of platinum doublet. Currently 2 patients are on active treatment and other two have completed study treatment and are on observation with stable disease.
Conclusions: Maintenance rucaparib combined with immune checkpoint inhibition was tolerable and showed promising activity after completion of frontline chemotherapy in platinum sensitive extensive stage small cell lung cancer patients.

**CT222**

**Multimodal mechanism of action of the GSK-3 inhibitor 9-ING-41 (elraglusib) includes an immunomodulatory component: preliminary results from the 1801 phase 1/2 trial.**

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9-ING-41 (elraglusib) is a potent and selective GSK-3 inhibitor that has shown anti-tumor activity in patient-derived xenograft models and phase1/2 clinical studies in patients (pts) with advanced solid tumors. Preclinical studies have demonstrated that elraglusib downregulates PD-1, TIGIT and LAG-3, upregulates expression of MHC class I proteins in tumor cells from “cold tumors”, and shows synergy when combined with PD-1 blockade in mouse xenografts. Clinical activity has been observed both as a single agent and in combination with standard of care chemotherapies in several advanced cancer histologies. Here we present initial ‘omics data from our phase 1/2 study (NCT03678883; ACT1801) spanning >100 pts with advanced cancer evaluating both single agent and chemotherapy combinations. The patients (pts) included in this study received elraglusib as a ≥3rd line therapy for advanced disease. Most pts with melanoma treated with elraglusib monotherapy (8/9; 89%) stayed on study for >2 cycles. One patient with melanoma refractory to checkpoint inhibitors achieved a confirmed and durable CR. Clinical benefit was also observed among pts with colorectal cancer with 4/12 (33.3%) pts treated with monotherapy and 12/15 (80%) that received elraglusib plus irinotecan rechallenge stayed on study >2 cycles reaching median overall survival of 106 and 211 days, respectively. Based on emerging *in*

*vivo* and *in vitro* results demonstrating that elraglusib activates T and NK cells promoting anti-tumor immune responses, we hypothesize immunomodulation by elraglusib may be contributing to anti-tumor immune response in the 1801 trial. We have acquired TCRseq and RNAseq profiles of PBMC samples from seven patients in 1801 during the first two weeks of treatment with elraglusib monotherapy. In these pts, reduced TCR clonality was observed and specific TCR clonotypes expanded after treatment, indicating T cell activation and expansion. These pts also showed changes in PBMC populations during elraglusib therapy as measured by immune deconvolution of PBMC RNAseq. Taken together, these data support a novel, previously unrecognized immunomodulatory mechanism of action for elraglusib and could provide rationale for future clinical development of elraglusib in pts with advanced malignancies. Additional analysis from TCRseq, immune profiling and cytokine analysis from expanded cohort of pts is ongoing.

**CT223**

**Long-term survival follow-up of tebentafusp in previously treated metastatic uveal melanoma (mUM).**

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**Background:** Tebentafusp, a bispecific (gp100 x CD3) ImmTAC, is approved for adult HLA-A*02:01+ patients with unresectable or metastatic uveal melanoma (mUM). In the Ph2 IMCgp100-102 study of pts with previously treated mUM (NCT02570308), tebentafusp demonstrated a median OS of 16.8 months and 1-year OS of 61% after a minimum 2 yrs of follow-up, nearly double the historical observed rates.[1,2] Here we present the final analysis of OS of the Ph2 IMCgp100-102 study.
**Methods:** 127 HLA-A*02:01+ 2L+ pts with mUM were dosed weekly with intravenous tebentafusp following intra-patient dose escalation of 20mcg dose 1, 30mcg dose 2 and 68mcg dose 3+. Primary objective was overall response rate and secondary objectives included safety and OS. OS was estimated by Kaplan-Meier method. Survival for ctDNA evaluable patients was assessed. Analyses are based on a 17 Oct 2022 database lock.
**Results:** With a median follow-up of 46 months, median OS was 16.8 (95% CI: 12.3-21.3) months, consistent with previous reports. Estimated 1-yr, 2-yr, 3-yr and 4-yr OS rates were 61%, 36%, 21% and 11%, respectively. ctDNA clearance by week 9 (n=12) was associated with 100% 1-yr, 73% 2-yr, 45% 3-yr and 23% 4-yr survival. OS for patients with poor prognostic factors compared favorably relative to historical benchmarks (Table 1). Mean and median duration of treatment was 9.9 and 5.6 months. Majority of pts who received a subsequent line of therapy received immunotherapy (48/72; 67%) followed by liver-directed therapy (14/72; 19%). No new safety signals were identified.
**Conclusions:** This study provides the longest follow-up of OS for a soluble TCR therapeutic to date. Tebentafusp continued to show unprecedented survival benefit for 2L+ mUM pts, including the poorest

prognostic groups, with estimated OS rates that remain nearly double the historical rates observed in this population at a median follow-up of ~4 years. Early ctDNA clearance was associated with indication of survival benefit. 1. Carvajal et al. *Nat Med* 2022 2. Sacco et al. *J Immunother Canc* 2021

Table 1. OS in Ph2 IMCgp100-102 versus historical benchmarks

| | **Ph2 IMCgp100-102** | | | **Historical benchmarks**[*,†] | | |
|---|---|---|---|---|---|---|
| **Population** | **1-yr** | **2-yr** | **3-yr** | **1-yr** | **2-yr** | **3-yr** |
| Overall 2L+ | 61% | 36% | 21% | 37% | 15% | 9% |
| *Subgroups* | | | | | | |
| Age ≥ 65 yrs | 51% | 31% | 25% | 38% | 12% | 6% |
| LDH > ULN | 44% | 22% | 11% | 23% | 8% | 3% |
| Baseline largest liver lesion ≥ 3cm | 52% | 26% | 14% | 32% | 11% | 4% |
| * OS benchmark for 2L+ patients calculated from Rantala et al 2019 | | | | | | |
| † OS benchmarks for pt subgroups (all lines of therapy) estimated from digitized OS curves presented in Khoja et al. 2019 | | | | | | |

**CT224**

**mRNA-4157, a personalized cancer vaccine, in combination with pembrolizumab, demonstrates trend for improved recurrence free survival compared to pembrolizumab alone in adjuvant melanoma patients across tumor mutational burden subgroups.**

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Background: The open-label randomized Phase 2 mRNA-4157-P201/Keynote-942 trial met its primary endpoint of prolonged recurrence free survival (RFS) in patients with resected high-risk stage III/IV melanoma. Tumor immunogenicity provides a favorable landscape for inflammatory processes associated with clinical benefit to checkpoint inhibitors (ICI) and tumor mutational burden (TMB) has been shown to be an independent predictor of treatment outcomes in patients treated with ICI therapy. mRNA-4157 is a novel mRNA-based personalized cancer vaccine which encodes up to 34 patient-specific tumor neoantigens. Here we report analyses of baseline biopsies from the trial to explore the novel mechanism of action hypothesized to augment endogenous anti-tumor responses and generate immunity to additional tumor neoantigens.

Methods: Paraffin-embedded formalin-fixed baseline tumor core biopsies underwent whole exome sequencing (WES) and whole transcriptome sequencing. According to the established WES genomic

score for pembrolizumab, the TMB high threshold utilized for analysis was 175/exome (10 mutations/megabase per F1CDx). The distribution of TMB expression in baseline tumor samples across study arms and their association with the primary RFS endpoint was evaluated. The association with RFS of other markers of inflamed tumors, including those established for pembrolizumab (e.g. gene expression profile (GEP) and PD-L1 expression) was also assessed.
Results: The RFS benefit of mRNA-4157 and pembrolizumab combination compared to pembrolizumab monotherapy observed in the intention-to-treat population was maintained with a similar treatment effect magnitude across both high (HR = 0.65; 95% CI: 0.3, 1.5) and low (HR = 0.59; 95% CI: 0.3,1.4) TMB subpopulations. In line with observations from historical data for pembrolizumab, improved RFS was observed in high TMB compared to low TMB patient subgroups in the pembrolizumab monotherapy arm. The trend for increased RFS benefit in the high TMB subpopulation was maintained in the mRNA-4157 and pembrolizumab study arm. Additional subgroup analyses (e.g. GEP and PD-L1) were assessed and will be discussed.
Conclusions: Our results indicate that mRNA-4157 demonstrates improvements in RFS irrespective of TMB status when administered in combination with pembrolizumab compared to pembrolizumab monotherapy in patients with resected high-risk cutaneous melanoma. The novel mechanism of action of mRNA-4157 may both deepen the activity of pembrolizumab and broaden the population of patients that can benefit from immune therapy. The association between TMB and mRNA-4157 treatment effect will be further explored in upcoming planned studies.

**CT225**

**Surufatinib plus toripalimab for first-line treatment of advanced non-small cell lung cancer (NSCLC) with PD-L1 positive expression: A multicenter, single-arm phase 2 study.**

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**Background:** Surufatinib (S, a small-molecule inhibitor of VEGFR 1-3, FGFR1 and CSF-1R) plus toripalimab (T, an anti-PD-1 antibody) showed encouraging antitumor activity in solid tumors (Cao YS, 2022). Programmed death ligand 1 (PD-L1) expression is the established biomarker for 1L immune checkpoint inhibitors therapy in advanced NSCLC. We conducted an open-label, multi-cohort, single-arm phase 2 study to evaluate the safety and efficacy of S+T in patients (pts) with advanced solid tumors. Here, we reported the results of advanced NSCLC with PD-L1 positive expression cohort.
**Methods:** Eligible pts had histologically confirmed advanced NSCLC with no prior systemic chemotherapy, PD-L1 positive (defined as PD-L1 TPS expression ≥1% [sp263]), and without EGFR, ALK or ROS1 genetic alteration if non-sq-NSCLC. Enrolled pts received 21-day cycles of S (250 mg orally QD) plus T (240 mg IV, Q3W) until disease progression or intolerable toxicity or the maximum duration of treatment with toripalimab is 24 months. The primary endpoint was objective response rate (ORR) per RECIST 1.1.
**Results:** From July 2020 to September 2021, 55 pts were screened, of whom 23 pts were enrolled and received the treatment of S+T. Median age was 66 years (range: 49-73), 16 (69.6%) were male and 12 (52.2%) had squamous histology. Pts with PD-L1 TPS ≥50% and <50% were 10 and 13 respectively. At the data cutoff (Aug 31, 2022), the median follow-up duration was 13.1 mos. Among 21 pts with at least one post-baseline tumor assessment, the confirmed ORR was 57.1%, the median DOR was 8.31 mos, and

DCR was 100%. Median PFS (mPFS) (95% CI) was 9.63 mos (5.49, -), median OS (mOS) (95% CI) was not reached (10.78, -), 12m-OS rate was 64%. According to PD-L1 level, the confirmed ORR were 66.7% and 50% for pts with PD-L1 TPS ≥50% and <50%, respectively; mPFS were 9.66 (0.69, -) and 6.93 mos (1.64, -), respectively; and the 12m-OS rate were 70% and 62%, respectively. mPFS did not differ with histology: 9.66 mos (5.49, -) for squamous cell carcinoma and 9.63 mos (0.69, -) for adenocarcinoma. All pts experienced ≥1 treatment emergent adverse event (TEAE). The most common Gr ≥3 TEAEs (≥5% pts) were aspartate aminotransferase increased (17.4%), malignant neoplasm progression (17.4%), hypokalemia (13.0%), hepatic function abnormal (13.0%), lymphocyte count decreased (8.7%), hypertension (8.7%) and pneumonitis (8.7%).
**Conclusion:** Surufatinib and toripalimab combination showed a promising antitumor activity in 1L therapy for advanced PD-L1 positive NSCLC with manageable toxicity. This study might represent a potential treatment option for these pts. Clinical trial information: NCT04169672.

**CT226**

**Crizotinib (C) in patients (pts) with solid tumors with *MET* amplification (amp) or mutation (mut): Results from the Targeted Agent and Profiling Utilization Registry (TAPUR) Study.**

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*Background:* TAPUR is a phase II basket study evaluating antitumor activity of commercially available targeted agents in pts with advanced cancers with specific genomic alterations. Results in a cohort of pts with solid tumors and *MET* amp or mut treated with C are reported.
*Methods:* Eligible pts had no standard treatment (tx) options, had measurable disease, ECOG performance status (PS) 0-2, and adequate organ function. Pts with non-small cell lung cancer were excluded. Genomic testing was performed in CLIA-certified, CAP-accredited site selected labs. Pts received C at 250 mg orally BID until disease progression. Low accruing histology-specific cohorts with *MET* amp or mut were collapsed into 1 histology-pooled cohort for analysis. Primary end point was disease control (DC) per investigator, defined as complete or partial response (PR) or stable disease (SD) of at least 16+ weeks (wks) duration (SD16+) per RECIST v1.1. The primary end point was summarized as a proportion and the hypothesized null DC rate of 15% was evaluated by a 1-sided exact binomial test with $\alpha = 0.10$. Other end points were progression-free survival (PFS), overall survival (OS), duration of response and SD, and safety.
*Results:* 31 pts with solid tumors (12 tumor types) and *MET* mut only (n=10), amp only (n=19), or mut and amp (n=1) were enrolled; 1 pt with overexpression was ineligible. 3 additional pts were unevaluable for efficacy. Table shows demographics and outcomes. 2 PR (both esophageal adenocarcinoma with *MET* amp) and 4 SD16+ (2 renal cell carcinoma, 1 with mut, 1 with amp; colorectal with amp; small intestine with amp) were observed for DC rate of 21% (1-sided 90% CI: 12%, 100%) and objective response rate of 7% (95% CI: 1%, 24%). The null DC rate was not rejected. 5 pts had ≥1 grade 3 tx-related adverse or serious adverse event.

*Conclusions:* C did not meet prespecified criteria to declare a signal of activity in pts with solid tumors with *MET* amp or mut.

| Table: Baseline Characteristics (N=31); Efficacy Outcomes (n=28); Toxicity Outcomes (N=31) | |
|---|---|
| Median (Med) age, years (range) | 61 (30, 82) |
| Female, % | 16 (52) |
| ECOG PS, % | |
| 0 | 15 (48) |
| 1 | 12 (39) |
| 2 | 4 (13) |
| Prior systemic regimens, % | |
| 1 | 3 (10) |
| 2 | 5 (16) |
| ≥3 | 23 (74) |
| DC rate, % (OR and SD16+) (1-sided 90% CI) | 21 (12, 100) |
| Objective response rate, % (95% CI) | 7 (1, 24) |
| Med PFS, wks (95% CI) | 8 (8, 13) |
| Med OS, wks (95% CI) | 37 (26, 68) |
| Duration of response, wks (n=2) | 14 and 20 |
| Med duration SD, wks (n=4) | 27 (26, 28) |
| Number of pts[1] with tx-related grade 3 adverse or any grade serious adverse event | |
| AE[2] | 5 (16) |
| SAE[3] | 3 (10) |
| [1]Pts may have experienced one or more events[2]ALT increase, diarrhea and all SAEs[3]Acute kidney injury, ALT increase, AST increase, blood bilirubin increase, creatinine increase, dehydration, fatigue, GGT increase, hyperkalemia, nausea, sinus bradycardia | |

**CT227**

**Interim results of a phase 1b/2 study of ADG126 (a masked anti-CTLA-4 SAFEbody®) monotherapy and in combination with toripalimab (an anti-PD-1 antibody) in patients (pts) with advanced / metastatic solid tumors.**

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**Background:** ADG126 is an anti-CTLA-4 fully human IgG1 SAFEbody® with a masking peptide blocking the antigen binding site. ADG126 is designed to be preferentially activated in the tumor microenvironment (TME), with the goal of limiting on-target off-tumor toxicities and promoting prolonged exposure to active drug in the TME. Activated ADG126 binds to a unique and conserved epitope of CTLA-4 with species cross-reactivity. Nonclinical studies have demonstrated that activated ADG126 potentiates T cell activation and depletes immunosuppressive Tregs through strong antibody-dependent cellular cytotoxicity (ADCC) specifically in the TME. We present interim results from our ongoing Phase 1b/2 study (ADG126-1001, NCT04645069) on dose escalation and expansion of ADG126 monotherapy as well as dose escalation of ADG126 + toripalimab (Tori).
**Method:** Pts with advanced solid tumors received ADG126 monotherapy (0.1, 0.3, 1, 3, 10, and 20 mg/kg) Q3W IV or ADG126 (6 or 10 mg/kg) + Tori (240mg) Q3W IV. Primary endpoints are safety and tolerability. Secondary endpoints are PK, anti-drug antibody (ADA), as well as ORR, DCR, DOR and PFS per RECIST 1.1.
**Result:** As of Dec. 26, 2022, 30 pts have received ADG126 monotherapy. The median number of treatment cycles was 2 (range: 1-24). The median age was 63.5 (39-84) years. 43% of pts had ≥ 3 prior lines of therapies, and 47% had been previously treated with anti-PD-(L)1 and/or anti-CTLA-4 therapies. ADG126 was well-tolerated with no dose-limiting toxicities (DLTs) observed, nor was the MTD reached. Only Grade (G) 1-2 TRAEs were reported; TRAEs ≥10% included diarrhea (17%), fatigue (17%), pruritus (13%), and rash (10%). Among 27 evaluable pts, DCR = 37%. One pt with ovarian serous carcinoma had a major CA125 response (90% reduction) and her disease was stable with ongoing treatment of ADG126 1 mg/kg at Cycle 24 (~16 months). In addition, 14 pts have received ADG126 + Tori in the dose escalation cohorts. The median age was 60 (36-85) years; 50% had ≥ 3 prior lines of therapies, and 43% received prior anti-PD-1 therapies. Both dose levels were well tolerated with no DLT. TRAEs (> 10%) included diarrhea (21%), fatigue (14%), pruritus (14%), rash (14%) and nausea (14%). After multiple cycles at 6 or 10 mg/kg Q3W, G3 TRAEs were observed in 21% (3/14) pts, including elevated liver function test/ hepatitis, elevated lipase and diarrhea, which are immune-related, and sepsis. No G4/5 TRAEs have been reported. Among 12 evaluable pts, DCR = 58%, including 2 partial responses. Early efficacy signals were observed with continuous tumor shrinkage and stabilization in IO-resistant and cold tumors.
**Conclusion:** The anti-CTLA-4 SAFEbody ADG126 shows favorable safety profiles in monotherapy up to 20 mg/kg and in combination with Tori up to 10 mg/kg. Furthermore, promising anti-tumor activity in heavily pre-treated patients was observed in the dose escalation phase. By enabling higher dose levels in combination with anti-PD-1 therapy, ADG126 may unleash the full therapeutic potential for proven and novel indications.

**CT228**

**Concordance between tissue and circulating tumor DNA (ctDNA) testing for neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions in larotrectinib (laro) clinical trials.**

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University Hospital and Cancer Trials Ireland, Dublin, Ireland; [14]The Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA; [15]The University of Texas MD Anderson Cancer Center, Houston, TX.

**Background:** *NTRK* gene fusions are oncogenic drivers in various tumor types. Laro, a highly selective and central nervous system (CNS)-active tropomyosin receptor kinase (TRK) inhibitor, is approved for the treatment of patients (pts) with TRK fusion cancer. Laro demonstrated an objective response rate (ORR) of 69% in 244 pts with solid (non-primary CNS) tumors (Drilon et al. ASCO 2022). Here, we report on the concordance between baseline local tissue-based testing with central tissue-based and liquid biopsy-based ctDNA testing to identify *NTRK* gene fusions.
**Methods:** Pts with non-primary CNS TRK fusion cancer who underwent independent review committee assessments by July 15, 2019, in three laro clinical trials (NCT02122913, NCT02576431, and NCT02637687) were included. Tumor *NTRK* gene fusion status was determined locally by a variety of methods, and centrally by next-generation sequencing in both tissue (using Illumina TruSight™ Oncology Comprehensive) and ctDNA (using Guardant360® or GuardantOMNI®) at baseline. Data cut-off: July 20, 2021.
**Results:** Of the 164 pts with *NTRK* fusions identified by local testing, 117 had evaluable tissue samples for central testing, and 99 had evaluable liquid biopsy samples. In total, 81.2% (95/117) of tumors had the fusion confirmed by central tissue testing. ctDNA testing detected *NTRK* gene fusions in 33.3% (33/99) of patients. Positive predictive values for each *NTRK* gene fusion are shown in the Table. The ORR for pts with *NTRK* gene fusions determined by local and central testing will be presented.
**Conclusions:** A high proportion of tumors with locally identified *NTRK* gene fusions were confirmed centrally. At present, analysis of ctDNA is significantly less sensitive at detecting *NTRK* gene fusions. A negative ctDNA result requires next-generation sequencing testing of a tissue biopsy. Further research is needed to improve the sensitivity of ctDNA gene fusion detection.

**Table**

| ***NTRK* gene fusion** | **Tissue-based LT** | **Tissue-based CT** | | | | **ctDNA-based CT with LB** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fusion confirmed, n** | **Fusion confirmed, n** | **Fusion not detected, n** | **Unavailable samples, n[†]** | **PPV[‡]: CT vs tissue-based LT, %** | **Fusion confirmed, n** | **Fusion not detected, n** | **Unavailable samples, n[†]** | **PPV[‡]: LB vs tissue-based LT, %** |
| *NTRK1* | 68 | 35 | 14 | 19 | 71.4 | 20 | 34 | 14 | 37.0 |
| *NTRK2* | 4 | 3 | 1 | 0 | 75.0 | 0 | 4 | 0 | 0 |
| *NTRK3* | 92[§] | 57 | 7 | 28 | 89.1 | 13 | 28 | 51 | 31.7 |

[†]Includes no sample available and sample QC failed. [‡]Calculated as a percentage of the number of confirmed fusions over the number of available samples. [§]Includes 9 inferred *NTRK3* fusions. CT, central testing; ctDNA, circulating tumor DNA; LB, liquid biopsy; LT, local testing; *NTRK*, neurotrophic tyrosine receptor kinase; PPV, positive predictive value; QC, quality control.

**CT229**
**CRESTONE: A Phase 2 study of seribantumab in adult patients with neuregulin-1 (NRG1) fusion positive locally advanced or metastatic solid tumors.**

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**Background:** NRG1 fusions represent a rare and potentially actionable oncogenic alteration found in ~0.2% of solid tumors. Seribantumab is a fully human anti-HER3 IgG2 monoclonal antibody that blocks aberrant NRG1 fusion protein binding to HER3 thereby preventing ligand-dependent activation and dimerization resulting in complete inhibition of the PI3K/AKT and MAPK downstream signaling pathways. Seribantumab has shown significant anti-tumor activity in PDX models and in patients (pts) with solid tumors harboring NRG1 fusions.
**Methods:** CRESTONE (NCT04383210) is a phase 2 study of seribantumab in adult pts with solid tumors harboring NRG1 fusions who received at least one prior therapy and were naïve to ERBB-targeted therapy (Cohort 1); pts previously treated with ERBB-targeted therapies (exploratory Cohort 2) and pts with tumors harboring additional molecular alterations (exploratory Cohort 3). Here, we present updated efficacy results for pts in Cohort 1 dosed at 3 g IV QW. Safety data are evaluated for pts enrolled in Cohort 1 and exploratory Cohorts 2 and 3.
**Results:** As of Dec 2, 2022, 51 pts were enrolled across all cohorts with 30 pts in Cohort 1 dosed at 3 g IV QW. Median age was 62 years (range 19-84); median prior lines of therapy was 2 (range 1-7); tumor types included non-small cell lung cancer (NSCLC, n=30), pancreatic adenocarcinoma (PDAC, n=6), biliary tract/cholangiocarcinoma (CCA, n=6), breast (n=4), and others (n=5); 15 different NRG1 fusion partners were identified with CD74 (22%) and SLC3A2 (16%) as the most frequently reported. In the overall safety population, 41 pts (80%) reported at least one treatment-related adverse event (TRAE). The most common TRAEs (occurring in ≥20% of pts) were diarrhea (41%), fatigue (29%), rash (24%), and nausea (22%). Four pts (8%) experienced Gr 3/4 TRAEs; no Gr 5 TRAEs. The safety profile for Cohort 1 was similar to the overall safety population. Among the 30 pts in Cohort 1, 22 were evaluable for investigator assessed (INV) response per RECIST v1.1; 2 pts (9%) had confirmed complete response (CR), 6 pts (27%) had confirmed partial response (PR), and 13 pts (59%) had stable disease (SD) as their best overall response (BOR). The INV objective response rate (ORR, confirmed PR + CR) was 36% and disease control rate (DCR, confirmed PR+CR+SD) was 95%. The overall duration of response ranged from 1.4 to 17.2 months. In pts with NSCLC, the INV-ORR was 39% and DCR was 94%.
**Conclusions:** Seribantumab has an acceptable safety and tolerability profile as a single agent in pts with solid tumors harboring NRG1 fusions. Updated efficacy data indicate seribantumab has robust and durable clinical activity, including CRs, across different tumor types harboring an NRG1 fusion and is a promising treatment option.

**CT231**

**Temsirolimus (T) in patients (pts) with solid tumors with *PTEN* mutation (mut): results from the Targeted Agent and Profiling Utilization Registry (TAPUR) Study.**

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Grantham,[2] Abigail Gregory,[2] Susan Halabi,[14] Richard L. Schilsky[2]. [1]The Angeles Clinic and Research Institute, A Cedars-Sinai Affiliate, Los Angeles, CA; [2]American Society of Clinical Oncology, Alexandria, VA; [3]Trinity Health IHA Hematology/Oncology, Michigan Cancer Research Consortium, Ypsilanti, MI; [4]Cancer Treatment Centers of America - Chicago, part of City of Hope, Zion, IL; [5]University of Nebraska Medical Center, Omaha, NE; [6]Sutter Sacramento Medical Center, Sacramento, CA; [7]Winship Cancer Institute of Emory University, Atlanta, GA; [8]Sylvester Comprehensive Cancer Center, University Of Miami Miller School Of Medicine, Plantation, FL; [9]Cancer Research Consortium of West Michigan, Grand Rapids, MI; [10]Cancer Treatment Centers of America - Atlanta, part of City of Hope, Newnan, GA; [11]Levine Cancer Institute, Atrium Health, Concord, NC; [12]Providence Cancer Institute, Portland, OR; [13]Swedish Cancer Institute, Seattle, WA; [14]Duke University Medical Center, Durham, NC.

Background: TAPUR is a phase II basket study evaluating antitumor activity of commercially available targeted agents in pts with advanced cancers with specific genomic alterations. Results in a cohort of pts with solid tumors with PTEN mut treated with T are reported.
Methods: Eligible pts had no standard treatment (tx) options, measurable disease, ECOG performance status (PS) 0-2, and adequate organ function. Genomic testing was performed in CLIA-certified, CAP-accredited site selected labs. After antihistamine pre-tx, 25 mg T was infused over 30-60 minutes once weekly until disease progression. Low accruing histology-specific cohorts with PTEN mut were collapsed into 1 histology-pooled cohort for analysis. Primary end point was disease control (DC), defined as complete or partial response (PR) or stable disease (SD) of at least 16+ weeks (wks) duration (SD16+) per RECIST v1.1. The primary end point was summarized as a proportion and the hypothesized null DC rate of 15% was evaluated by a 1-sided exact binomial test with alpha 0.10. Other end points were progression-free survival (PFS), overall survival (OS), duration of response (DOR), duration of SD and safety. DOR is defined as time from pt's first documented objective response (OR) until progressive disease (PD). Duration of SD is defined as time from tx start to PD.
Results: 34 pts with solid tumors (10 tumor types; 20/34 pts had prostate, soft tissue sarcoma [STS] or lung) with PTEN mut were enrolled. 6 pts were unevaluable for efficacy, 1 was ineligible. Table shows demographics, outcomes and toxicity. 2 pts obtained PR (prostate and STS) and 5 had SD16+ (lung, head and neck, breast, uterine, and site unspecified) for DC rate of 26% (1-sided 90% CI: 15.1%, 100%) and OR rate of 7% (95% CI: 1%, 24%). The null DC rate was rejected. Median PFS was 10 wks and median OS was 32 wks.
Conclusions: T met prespecified criteria to declare a signal of activity in pts with solid tumors with *PTEN* mut.

| Table: Baseline Characteristics (N=34); Efficacy Outcomes (n=27); Toxicity Outcomes (N=34) | |
|---|---|
| Median (Med) age, years (range) | 65 (42, 84) |
| Female, % | 14 (41) |
| ECOG PS, % | |
| 0 | 13 (38) |
| 1 | 18 (53) |
| 2 | 3 (9) |
| Prior systemic regimens, % | |
| 1 | 3 (9) |
| 2 | 5 (15) |
| ≥3 | 26 (77) |
| DC rate, % (OR and SD16+) (1-sided 90% CI) | 26 (15.1, 100) |
| OR rate, % (95% CI) | 7 (1, 24) |

| | |
|---|---|
| Med PFS, wks (95% CI) | 10 (7, 17) |
| Med OS, wks (95% CI) | 32 (13, 42) |
| DOR, wks (n=2) | 11 and 55 |
| Med duration SD, wks (n=5) | 27 (24, 36) |
| Number of pts[1] with tx-related grade 3-4 AE or SAE | |
| AE[2] | 12 (35) |
| SAE[3] | 6 (18) |
| [1]Pts may have experienced one or more events [2] alkaline phosphatase increase, anemia, chronic kidney disease, diarrhea, fatigue, hyperglycemia, hypokalemia, hypophosphatemia, lung infection, oral pain and all SAEs[3] acute kidney injury, dyspnea, generalized muscle weakness, hypophosphatemia, lung infection, oral mucositis, pericardial effusion | |

**CT232**

**Safety, efficacy, and tolerability of FHND9041 capsules as first-line treatment in patients with EGFR sensitive mutations or second/ third-line treatment in patients with T790M+ advanced non-small cell lung cancer (NSCLC): results from a phase I/II single-arm, multi-center, open-label study.**

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Background: FHND9041 is a third-generation EGFR inhibitor independently developed by Nanjing Chuangte Pharmaceutical Technology Co., Ltd. It irreversibly binds to EGFR protein, and demonstrates strong inhibitory effect on EGFR T790M mutations as well as EGFR sensitive mutations. This is the first single-arm, multi-center, open-label phase I/II study of FHND9041 capsule, which evaluates the safety, tolerability and preliminary efficacy of FHND9041 capsule in initial treatment of NSCLC with EGFR sensitive mutations or treatment of T790M+ advanced NSCLC who progressed after prior EGFR-TKI therapy.
Methods: 124 subjects tested for EGFR mutations and eligible for evaluation were randomized to dose groups of 40 mg, 80 mg (first-line treatment or second/ third-line treatment with T790M+), 120 mg and 180 mg. The primary endpoint is objective response rate (ORR), and key secondary endpoints are progression-free survival (PFS), disease control rate, duration of response, and safety.
Results: The ORR of FHND9041 capsule 80 mg (recommended phase 2 dose, RP2D) is 71.43% in patients newly diagnosed of NSCLC with EGFR sensitive mutations, and 63.64% in patients with T790M+ NSCLC who had progressed after prior EGFR-TKI therapy. No DLT occurred in all dose groups, 40 mg, 80 mg and 120 mg, which demonstrated satisfactory tolerability and safety of FHND9041. Detailed efficacy data of each dose group is shown in the table below.
Conclusion: FHND9041 capsule 80 mg shows significant antitumor activity with favorable efficacy and safety profile in patients for both initial treatment and treatment of T790M+ NSCLC who progressed after prior therapy. For futher research of the capsule, a multicenter, randomized, positive-controlled phase III clinical research is ongoing with recommended dose of 80 mg.

| **Group** | **Dosage(mg)** | **Evaluable Subjects(n)** | **ORR%** |
|---|---|---|---|
| Second/Third-line **T790M+** | 40mg | 3 | 33.33 |
| | **80mg** | 39 | **63.64** |
| | 120mg | 42 | 58.33 |

| | | | |
|---|---|---|---|
| | 180mg | 3 | 100.00 |
| First-line | **80mg** | **37** | **71.43** |

**CT233**

**Initial results of a phase 1b/2 study of ADG126 (a masked anti-CTLA-4 SAFEbody®) in combination with pembrolizumab (an anti-PD-1 antibody) in patients with advanced/metastatic solid tumors.**

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Background: ADG126 is a fully human anti-CTLA-4 IgG1 SAFEbody® that has a cleavable masking peptide at the antigen binding site. This design enables ADG126 to be preferentially activated in the tumor microenvironment (TME) to afford prolonged on-tumor drug exposure and limited off-tumor toxicity. The activated ADG126 binds to a unique and highly conserved epitope of CTLA-4, enabling seamless translational studies. ADG126 is activated efficiently in vitro and in vivo, which primes T cells via partial blockade of CTLA-4 interaction with its ligands. ADG126 depletes immunosuppressive Tregs through strong antibody-dependent cellular cytotoxicity (ADCC) specifically in the TME, and it synergizes with anti-PD-1 antibody to induce potent anti-tumor efficacy. In a Phase 1b/2 study, ADG126 monotherapy demonstrated a favorable safety profile and clinical activity in heavily pre-treated patients (pts). Here we present preliminary results from dose escalation of ADG126 in combination with pembrolizumab (Pembro) in pts with advanced/metastatic solid tumors (NCT05405595).
Method: This is a Phase 1b/2, open-label, multicenter dose escalation and dose expansion study to evaluate the safety, tolerability, PK and preliminary efficacy of ADG126/Pembro combination regimen. Pts received ADG126 (6 or 10 mg/kg, Q3W or Q6W, IV) plus Pembro (200mg, Q3W, IV). Primary endpoints are safety and tolerability. Secondary endpoints are PK, ADA, ORR, DCR, DOR and PFS per RECIST 1.1.
Results: As of Jan 20, 2023, 11 pts have been treated with ADG126/Pembro combination in dose escalation cohorts. The median age was 61 yo (26-75); 82% pts had at least 3 prior therapies, 18% pts received prior IO therapies. A majority of pts (82%) have what are considered immunologically “cold” tumors (MSS CRC, PDAC, ovarian cancer, etc.). ADG126 was dosed at 6 mg/kg Q3W (5 pts), 10 mg/kg Q3W (3 pts) and 10 mg/kg Q6W (3 pts); Pembro was dosed at 200 mg Q3W. Both 6 and 10 mg/kg dose levels on either Q3W or Q6W were well tolerated with no DLT, and no Grade 3 and higher treatment-related adverse events (TRAEs) were reported. Clinical efficacy has been observed in 10 evaluable pts of different tumor types, including 1 confirmed partial response (PR) and 1 stable disease (SD) at 10 mg/kg (5 evaluable pts), and 1 SD at 6 mg/kg (5 evaluable pts). Additional safety and efficacy data with repeat dosing will be presented.
Conclusions: The anti-CTLA-4 SAFEbody ADG126 in combination with Pembro is well-tolerated up to 10 mg/kg Q3W or Q6W in the dose escalation cohorts with repeat dosing. The safety profile of this combination appears to be more favorable than other anti-CTLA-4/ anti-PD-1 combination therapies reported to date, with no ≥ G3 TRAEs in the dose escalation phase. Clinical efficacy, including one confirmed PR, have been observed in multiple tumor types. Taken together, ADG126/Pembro combination may unleash the full therapeutic benefit of anti-CTLA-4/anti-PD-1 combination in advanced cancer pts beyond immune-sensitive tumor types.

**CT234**

**A phase I/II trial investigating safety and efficacy of autologous TAC01-HER2 in relapsed or refractory solid tumors.**

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Background: Despite recent therapeutic developments for patients with advanced, metastatic, unresectable HER2+ solid tumors, significant unmet medical needs still exist, especially in tumors other than breast and gastric. The T cell antigen coupler (TAC) technology is a novel approach to modifying T cells, allowing them to recognize and treat HER2+ solid tumors. The TAC receptor redirects T cells to tumor cells, and upon tumor cell recognition, co-opts the natural T cell receptor (TCR) to yield safer anti-tumor responses versus chimeric antigen receptor (CAR) T cells. In preclinical studies, TAC T cells led to complete tumor clearance in mouse models of human cancer, without any TAC-related toxicities. In the ongoing clinical trial (NCT04727151), TAC01-HER2 treatment of HER2+ solid tumors is hypothesized to result in safe and effective anti-tumor responses. Subjects undergo leukapheresis, bridging therapy (if needed) while their TAC01-HER2 are engineered, lymphodepletion chemotherapy (LDC), and finally TAC01-HER2 infusion.

Trial Design and Early Results: In phase I dose escalation, the primary objective is to evaluate the safety of TAC01-HER2 at increasing doses of 0.3, 0.8, 3, and 8 x $10^6$ cells/kg in HER2+ solid tumors (1+, 2+ or 3+ as identified by immunohistochemistry) in adult subjects who have progressed after ≥2 lines of systemic therapy. Dose limiting toxicities (DLTs) are assessed up to 28 days from cell infusion. In Phase II, dose expansion groups will further evaluate the safety, efficacy, and pharmacokinetics of the optimal TAC01-HER2 dose in HER2+ breast and other solid tumor types. As of 12 Dec 2022, 12 patients have been treated in Cohorts 1-4 with breast, colorectal, gall bladder, gastro-esophageal junction, gastric and ovarian tumors. No DLTs or neurotoxicity events have been reported. All patients treated so far at Cohorts 3 and 4 (3 x $10^6$ and 8 x $10^6$ cells/kg, respectively) experienced Grade ≤2 CRS which were resolved with standard of care treatments. Eight subjects have reported a total of 15 serious adverse events, all unrelated to TAC01-HER2 except for a Grade 1 and a Grade 2 CRS, both resolved with standard of care. Most adverse events were related to LDC or the underlying neoplasm. At Cohort 2, a partial response was observed in a subject with refractory metastatic gastric adenocarcinoma (3+ HER2) at 1$^{st}$ scan, with a 36.5% reduction in measurable disease and clinical benefit was maintained for 6 months. A 71% disease control rate was observed at Cohorts 2 and 3 at 1$^{st}$ scan (0.8 x $10^6$ and 3 x $10^6$ cells/kg, respectively)). Dose escalation of TAC01-HER2 is ongoing, with the first subject treated at Cohort 4. These results in a heavily pre-treated cancer population show manageable safety and promising clinical activity with a novel T cell therapy that may have broad clinical applicability in HER+ cancers.

**CT235**

**A phase I/II clinical study of an oncolytic adenovirus expressing the immunostimulatory transgenes TMZ-CD40L and 4-1BBL in advanced solid malignancies.**

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Sweden; [6]Karolinska Institute, Dept of Clinical Science, Intervention and Technology, Stockholm, Sweden.

There is a need for therapeutics with novel mechanisms-of-action for patients with advanced solid malignancies. In this first completed part of LOKON002, a phase I/II (NCT03225989) clinical study, the safety and clinical response to LOAd703 in combination with standard-of-care (SoC), or conditioning gemcitabine chemotherapy, was investigated. LOAd703 is an oncolytic adenovirus of serotype 5/35 modified to express two immunostimulatory transgenes, trimerized membrane-bound CD40L and 4-1BBL.
In the completed part I (phase I and IIa) of the study, eligible patients with advanced pancreatic-, colorectal-, biliary- and ovarian cancer, were treated with increasing doses of LOAd703 ($5x10^{10}$, $1x10^{11}$ and $5x10^{11}$ viral particles) using a standard 3+3 design. LOAd703 was administered intratumorally (ultrasound-guided) for up to 8 bi-weekly injections. The maximum tolerated dose tested was further evaluated in an extended dose cohort to confirm safety and assess preliminary clinical activity, and to select the best two indications for the part II of the study.
Twenty-eight patients were enrolled in part I presented herein. Fifteen of these received LOAd703 combined with SoC, and thirteen in combination with conditioning gemcitabine. The most frequently reported treatment- or procedure-related adverse events were fever (82% of patients), chills (54%) and fatigue (43%). Four patients developed transient cytokine release syndrome. Twenty-five patients fulfilled the criteria to be evaluable for efficacy, which required receiving at least 3 doses of LOAd703 and results from a CT scan week 9. Partial response (PR) was achieved in two patients with pancreatic cancer treated at the highest LOAd703 dose level in combination with gemcitabine and nab-paclitaxel. One of the PRs was achieved at week 25, and the patient received one additional month of chemotherapy per protocol. This patient remained treatment-free for 17 months before chemotherapy was restarted due to progression, and was still alive at last follow-up 34 months after the study registration date. The other PR was achieved at week 17, two weeks after the last LOAd703 injection; disease progression was evident at week 33 and OS was 21 months. Three patients experienced stable disease for a minimum of 25 weeks. This included a patient with advanced ovarian cancer who showed SD at least until week 40, and had an OS of 40 months. No association between adverse events and response was identified. Anti-adenoviral antibody levels (IgG), which increased in all patients, could not be related to indices of clinical benefit. Based on the safety of LOAd703 at all dose levels studied, as well as evidence of objective clinical activity in patients with advanced pancreatic cancer, further disease-directed studies of intratumoral administration of LOAd703 are warranted.

**CT238**

**Pharmacokinetic analyses and immunophenotyping of patients treated with Nelmastobart (hSTC810), a novel immune checkpoint inhibitor.**

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A Phase I clinical trial is currently underway to test Nelmastobart (hSTC810), an antibody targeting butyrophilin 1A1 (BTN1A1), a novel immune checkpoint protein. This Phase I clinical trial is using a standard 3 + 3 escalation design to (i) explore safety, tolerability, dose-limiting toxicities, and pharmacokinetics, (ii) define a recommended Phase II dose, and (iii) evaluate preliminary efficacy in patients with advanced solid tumors. The current dose range for the clinical trial is 0.3 mg/kg to 15 mg/kg. Currently, all the dose levels have been completed without any dose-limiting toxicity having been observed. Here, we provide an update on the clinical trial data shown previously. First, we provide the results of the pharmacokinetics study of these patients for the selected doses and dosing intervals,

including the peak plasma concentration ($C_{max}$), the time to reach $C_{max}$ ($t_{max}$), and the average plasma concentration of hSTC810 over the dosing interval in steady state ($C_{avg}$). The change in cytokine expression and immune cell concentrations in patient blood samples were also analyzed. Specifically, a panel of cytokines was measured including IFN- γ, IL-2, IL-4, IL-6, IL-10, TNF-α, MCP-1, and TGF- β, while flow cytometry was used to detect a panel of immunological marker proteins including CD3, CD4, CD8, CD19, CD14, CD16, CD56, CD25, CD45. For pharmacokinetic studies, the concentrations of hSTC810 and anti-hSTC810 antibodies were quantified. Immunohistochemistry was performed on patient tumor biopsies that were stained for PD-L1, CD3, CD4, CD8, and BTN1A1 expression. The resultant slides were analyzed for PD-L1 tumor proportional score (TPS), PD-L1 combined positive score (CPS), BTN1A1 scoring, the percentage of CD3+ lymphocytes, CD4+ lymphocytes, CD8+ lymphocytes, tumor-infiltrating lymphocytes, and necrosis.

**CT239**

**XMT-2056: A phase 1 study of a HER2 targeting STING antibody drug conjugate (ADC) in solid tumors expressing HER2.**

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**Background:** The STimulator of INterferon Gene (STING) pathway is a conserved innate immune pathway that is necessary to mount an efficient immune-mediated tumor response in mice (Woo et al, *Immunity*, 2014). Preclinical data show that STING activation induces prolonged anti-tumor activity and generates immune memory. A STING ADC has the potential to overcome the limitations of free, unconjugated STING agonists that are delivered intravenously or intratumorally by reducing the risk of systemic toxicity and increasing accessibility of metastatic sites. Despite recent advancements, most HER2+ patients do not have an approved immunotherapy treatment option. XMT-2056 is a systemically administered Immunosynthen STING agonist antibody drug conjugate (DAR 8) that targets a novel HER2 epitope and locally activates STING signaling in both tumor-resident immune cells and in tumor cells (a “one-two punch”), providing the potential to treat patients with HER2-high or -low tumors as monotherapy or in combination with standard of care HER2 agents. XMT-2056 showed promising anti-tumor activity in multiple preclinical models with varying HER2 expression and enhanced efficacy in combination with anti-HER2 or anti-PD-1 agents (Duvall et al, AACR 2022), warranting further investigation in the clinical setting.
**Methods:** This first-in-human monotherapy trial consists of a dose escalation (DES) and expansion (EXP) portion. In both the DES and EXP, participants must have HER2+ disease as determined by local IHC, ISH, or NGS. The DES cohorts include breast cancer, GEJ, CRC, NSCLC, and other HER2+ cancers and all patients must have progressed on or are intolerant to the appropriate SOC therapy. In the DES, Bayesian optimal interval (BOIN) design will be used to determine the maximum tolerated dose (MTD). The primary objective in DES is to define safety and tolerability, determine the MTD, and provide safety and tolerability characterization towards a recommended phase 2 dose (RP2D). EXP will be initiated in 3 disease-specific cohorts (HER2+ breast; HER2+ GC/GEJ; and HER2+ CRC/NSCLC/Other), with the primary objective of evaluating safety and preliminary efficacy of intravenous XMT-2056 at the RP2D. The trial is open for enrollment NCT05514717.

**CT240**

**On-going phase 1A clinical trial for AG01 an first-in-class anti-progranulin (GP88) monoclonal antibody in patients with advanced malignancies.**

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Progranulin (PGRN/GP88) is an 88 kDa glycoprotein characterized by seven and a half double cysteine rich repeats which is the largest member of the granulin-epithelin protein family. PGRN/GP88 has been demonstrated as a biological driver of tumorigenesis, survival, and drug resistance in several cancers including breast cancer (BC), lung prostate, ovarian and digestive cancers. PGRN/GP88 tissue expression is an independent prognostic factor of recurrence in breast, lung cancers while elevated serum PGRN/GP88 level in metastatic breast, lung, ovarian and prostate cancer patients. Elevated PGRN/GP88 levels are associated with poor outcomes such as progression and shortened survival. An anti-human PGRN/GP88 monoclonal antibody able to inhibit PGRN/GP88 action in vitro and in vivo has been developed, chimerized and expressed in CHO cells. All IND enabling activities including pharmacology, GMP manufacturing, formulation and GLP toxicology studies have been conducted. The IND application has been filed and cleared by the Food and Drug Administration. A first-in-human, first-in-class phase 1 safety and efficacy clinical study of AG01 in patients with solid tumors and advanced disease with special focus on patients with breast, lung and ovarian cancers has been initiated and is on-going at the University of Maryland Greenebaum Cancer Center. The trial is registered as NCT05627960 to clinicaltrials.gov site. Supported by grants R44CA162629 and R44CA224718 from the National Cancer Institute to GS.

**CT241**

**A phase 1, first-in-human (FIH) study of autologous anti-HER2 chimeric antigen receptor macrophage (CAR-M) in participants (pt) with HER2 overexpressing solid tumors.**

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**Background:** Macrophages are abundant in the solid tumor microenvironment (sTME) and can exhibit both pro- and anti-tumor functions. Macrophages can be redirected by CAR expression to phagocytose cancer cells in an antigen-specific manner. CAR-M can reprogram the sTME and present neoantigens to T cells, leading to epitope spreading and anti-tumor immunity. CT-0508 is comprised of autologous monocyte-derived proinflammatory macrophages expressing an anti-HER2 CAR. Pre-clinical studies showed that CT-0508 induced targeted cancer cell phagocytosis while sparing normal cells, decreased tumor burden, prolonged survival, and was safe and effective. Notably, anti-HER2 CAR-M treatment led to activation of the sTME, with infiltration of CD8+ and CD4+ T cells, NK cells, dendritic cells, and increased activated CD8+ tumor infiltrating lymphocytes. In a pre-clinical model of advanced solid tumor resistant to PD1 blockade, mice treated with anti-HER2 CAR-M combined with a PD1 blocking antibody demonstrated improved tumor control, overall survival, and TME activation compared to either treatment alone, indicating synergy and capacity for CAR-M to sensitize solid tumors to checkpoint blockade.
**Methods:** This Phase 1, FIH study is evaluating safety, tolerability, cell manufacturing feasibility, trafficking, TME activation, and preliminary evidence of efficacy of investigational product CT-0508 in 18 pt with locally advanced (unresectable) /metastatic solid tumors overexpressing HER2. Pt previously treated with anti-HER2 therapies are eligible. Filgrastim mobilized autologous CD14+ monocytes are collected by apheresis, followed by manufacturing and cryopreservation. Group 1 pt (n = 9; enrollment complete) received fractionated doses over Days 1, 3, and 5. Group 2 pt (n = 9) receive CT-0508 as a

single infusion on D1. Additional cohorts include: CT-0508 co-administered with pembrolizumab and CT-0508 monotherapy administered intraperitoneally in pt with peritoneal predominant disease. Correlative assessments include pre- and post-treatment biopsies and blood samples for safety (immunogenicity), trafficking (qPCR, RNA in situ hybridization), CT-0508 persistence in blood and tumor, target antigen engagement, TME modulation (single cell RNA sequencing), immune response (TCR sequencing) and others.

**CT242**

**A phase I study of ruxolitinib in combination with abemaciclib for patients with primary or post-polycythemia vera/essential thrombocythemia myelofibrosis.**

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**Introduction:** Patients (pts) with primary and secondary myelofibrosis (MF) have a reduced life expectancy. While several oral Janus Kinase (JAK)-1/2 inhibitors such as ruxolitinib (RUX) have demonstrated improvements in symptom burden and spleen volume reduction compared to physician choice of best available therapy leading to their approval by the US FDA, responses to these agents are generally time-limited. Additionally, these agents are not considered to be disease-modifying and survival in pts after RUX discontinuation is poor with a median survival of 11-14 months. Thus, novel mechanism-based therapies for pts with suboptimal response or progression on RUX are needed.
**Preclinical Rationale:** *JAK2* V617F mutations promote transition from G1 to S-phase of the cell cycle via increased expression of CDC25A in preclinical models and CDC25A expression is upregulated in primary MPN patient samples. Additional cell cycle regulators such as CDK6 and Cyclin D also play a role in MF pathogenesis and drive myeloproliferation. Finally, combination of the CDK4/6 inhibitor ribociclib and RUX demonstrated reduction in spleen and liver size when compared to RUX alone in murine models, suggesting synergy (Rampal et al., CCR 2021).
**Methods:** This multicenter, phase I dose-escalation trial evaluates the safety of RUX + the CDK4/6 inhibitor abemaciclib in pts with primary or secondary MF with intermediate-1/2 or high-risk disease by DIPSS who require treatment and had an inadequate response to RUX. An inadequate response will be defined by (I) palpable splenomegaly ≥5 cm below the left costal margin at study entry AND/OR (II) active MPN symptoms [presence of 1 symptom score ≥5 or 2 symptom scores ≥3 using the screening MPN-SAF TSS. This study follows a conventional "3+3" dose-escalation design, in which pts on a stable dose of RUX (10mg or 15mg BID) with sufficient baseline platelet and absolute neutrophil count will be treated with increasing doses of abemaciclib. Abemaciclib was chosen due to less myelosuppression compared with other CDK4/6 inhibitors. The pre-study dose of ruxolitinib will be maintained to determine the maximum tolerated dose of the combination of RUX+ abemaciclib. The primary endpoint is to determine the safety and tolerability, and to identify the recommended phase II dose of abemaciclib in MF pts on a stable dose of RUX. Secondary endpoints include overall response rate, progression-free and overall survival, and duration of response per the modified International Working Group - Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) and the European Leukemia Net (ELN) 2013 consensus definitions. Correlative studies are planned to identify biomarkers predictive of response to RUX + abemaciclib, mechanisms of resistance (e.g., MAP kinase pathway activation), and any disease-modifying effects of combination therapy.

**CT243**

**Clinical trial simulation to inform dose selection of zilurgisertib, an ALK2 inhibitor, in patients with anemia due to myelofibrosis (MF).**

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Disease-associated anemia is reported in over one-third of patients at MF diagnosis and can be exacerbated by currently available MF therapies. Further, anemia is associated with poor prognosis in MF. Activation of activin receptor-like kinase (ALK)-2 (also known as ACVR1) may contribute to MF-associated anemia via up-regulated levels of hepcidin. Zilurgisertib (INCB000928) is a potent and selective ALK2 inhibitor that has demonstrated dose-dependent improvement in hemoglobin levels in a cancer-induced anemia mouse model, in which maximum hemoglobin increase was observed when zilurgisertib trough concentration covered pSMAD $IC_{50}$ for at least half of the dosing interval. Zilurgisertib is being evaluated as monotherapy or in combination with ruxolitinib in a phase 1/2 dose-escalation and -expansion study in patients with anemia due to MF (NCT04455841). The objective of the current study is to use modeling and simulation, which integrate both preclinical and clinical data, to inform dose finding for the ongoing phase 1/2 trial in MF patients. Population (Pop) PK analyses were conducted using a total of 3089 zilurgisertib plasma concentrations from 161 healthy subjects in 3 completed phase 1 studies: a single ascending dose study (64 subjects, 10-500 mg), a multiple ascending dose study (59 subjects, 50-400 mg QD and 300 mg BID), and a drug-drug interaction study (38 subjects, 100 mg single dose of zilurgisertib alone). A nonlinear mixed-effects modeling approach in Monolix software was used for the analyses. Zilurgisertib PK were well-characterized by a 3-compartment model with nonlinear clearance and inter-occasion variability on absorption rate constant and bioavailability. The diagnostic plots and visual predictive check demonstrated that the model appropriately characterized the zilurgisertib plasma concentrations. Because zilurgisertib PK are similar between MF patients and healthy subjects, as suggested by available PK data in MF patients receiving doses up to 200 mg, the pop PK model developed with data from healthy subjects was used to simulate steady-state exposures in MF patients receiving 200 to 600 mg daily dose. Simulation results showed that daily doses of ≥600 mg would result in >90% of patients achieving exposure similar to the observed exposure associated with maximum efficacy in the mouse model. The conclusions from this clinical trial simulation support the decision to continue dose escalation in MF patients receiving zilurgisertib monotherapy.

**CT244**

**Phase 1/2 study of PRO1184, a novel folate receptor alpha-directed antibody-drug conjugate, in patients with locally advanced and/or metastatic solid tumors.**

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*Background:* PRO1184 is an antibody-drug conjugate (ADC) directed to folate receptor alpha (FRα), a cell surface antigen overexpressed in multiple cancers including ovarian, endometrial, lung, mesothelioma, and breast cancer. PRO1184 consists of a human monoclonal antibody that selectively binds FRα, a novel cleavable hydrophilic linker, and a topoisomerase 1 inhibitor payload, exatecan. Previous studies demonstrated that the hydrophilic linker confers excellent physicochemical properties and pharmacokinetic (PK) profiles across a range of payload mechanisms and is superior to conventional linkers on these critical parameters for ADCs. In addition, exatecan is broadly active in many tumor types, is membrane permeable, and is not a substrate of multidrug resistance efflux pumps. It may thus lend a robust bystander effect and induce deeper or more durable responses in refractory tumors. Preclinical studies further established that PRO1184 exerts potent antitumor activity in mouse xenograft models with

high, moderate, and low FRα expression, consistent with the inherent potency and expected bystander activity of the exatecan payload. PRO1184 is stable in plasma and retains the excellent PK properties and bioactivity of the unconjugated parent antibody. The preliminary safety profile of PRO1184 was more favorable than a benchmarking deruxtecan-based ADC in cynomolgus monkeys. PRO1184 is thus a promising development candidate with a potentially large therapeutic index to benefit a broad population of patients with FRα-expressing solid tumors.
*Methods:* PRO1184-001 is an ongoing, phase 1/2, open-label dose escalation and expansion study. Eligible patients are adults with metastatic or unresectable solid tumors, including ovarian, endometrial, non-small cell lung, breast cancer, or mesothelioma. Patients must have measurable disease per the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, or mRECIST 1.1 for pleural mesothelioma. Patients must also have previously received therapies known to confer clinical benefit unless considered ineligible, refused by the patient, or not available in the region. PRO1184 is given by intravenous infusion on Day 1 of a 21-day cycle and treatment may continue until disease progression, unacceptable toxicity, or other reason for discontinuation. The primary objectives are to evaluate the safety and tolerability of PRO1184 and to identify the maximum tolerated dose, if reached, and recommended phase 2 dose (RP2D). Part A of the study consists of a dose escalation phase and Part B consists of 4 FRα-expressing tumor-specific expansion cohorts treated at the RP2D. PK, immunogenicity, and antitumor activity will also be evaluated. The study is currently enrolling at sites in the US, with future enrollment in China planned (NCT05579366).

**CT245**

**Clinical development of a novel form of interleukin-12 with extended pharmacokinetics.**

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Recombinant interleukins (IL) have had limited clinical success due to inefficient tumor targeting and short pharmacokinetics (PK), requiring frequent dosing that leads to aberrant immunostimulation and toxicity. IL-12 is a promising cancer treatment due to its activation of T and natural killer (NK) lymphocytes to produce interferon (IFN)-γ, yet dosing strategies have failed to provide adequate therapeutic benefit in humans. To address these issues, we developed a novel platform that delivers either mono- or bifunctional immunomodulator(s) linked to a Fully-Human, Albumin Binding scFv domain ($F_H$AB®), which provides enhanced tumor targeting and retention through albumin binding to over-expressed FcRn, GP60, and SPARC, an improved PK profile, a dose-sparing effect that decreases the toxicity risk, and a broader therapeutic index. Excellent tumor growth inhibition was seen using a “cold” immunosuppressive B16F10 melanoma model for comparing the efficacy of IL12-$F_H$AB with rIL-12, resulting in significant increases in activated NK, NKT, Th1, and cytotoxic CD8 T cells.
We are conducting a first-in-human, dose-escalation trial to evaluate the safety and tolerability of SON-1010 (IL12-$F_H$AB) and to determine the maximum tolerated dose (MTD) in patients with advanced solid tumors. The study has a traditional 3+3 design, modified to take advantage of the known tachyphylaxis of IL-12 with the introduction of a desensitizing first dose to allow administration of higher maintenance doses. No dose-limiting toxicities have been encountered in the first 3 dose cohorts and the MTD is at least 300 ng/kg. While adverse events seen in other studies of IL-12 have occurred, they have been transient and tolerable, allowing further dose escalation. Increases in IFN-γ were dose-related, controlled, and prolonged. The levels peaked at 24 to 48 hours and returned to baseline after 2 to 4 weeks. Low levels of IL-10 were induced in a dose-dependent manner. No drug-related increase was seen with IL-1β, IL-6, IL-8, or TNF-α and there was no evidence of cytokine release syndrome at these doses. The preliminary geomean elimination half-life ($T_{½}$) was 122 hrs with first-order kinetics, compared with 12 hrs for SC rhIL-12. The accumulation estimates are within the margin of error and are not likely to be physiologically significant with subcutaneous dosing of SON-1010 every 3 weeks. Eight of 11 patients

had stable disease at the first follow-up CT, 4 of whom were progressing at study entry. Two patients were stable at 4 months and 2 had unconfirmed progressive disease; 1 patient remains stable after 8 months on SON-1010 with evidence of tumor regression.
SON-1010 may have a positive synergistic effect with an immune checkpoint inhibitor (ICI), particularly with 'cold' tumors that over-express SPARC. The next stage of development will be to explore the MTD of SON-1010 in combination with an ICI, then to compare that approach with the standard of care in Phase 2.

**CT246**

**An open-label, single-center, phase 0, microdose study to demonstrate delivery of TTX-MC138-NODAGA-Cu64 to radiographically confirmed metastases in subjects with advanced solid tumors.**

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**Purpose:** Cu-64 labeled TTX-MC138, a microRNA-10b (miR-10b) inhibitor, will be evaluated in a Phase 0 clinical study conducted under an Exploratory IND to evaluate delivery of the molecule to metastatic lesions in subjects with advanced solid tumors by using positron emission tomography-magnetic resonance imaging (PET-MRI).
**Background:** miR10b is a master regulator of the viability of metastatic tumor cells and promotes capacity of tumor cells to migrate and invade surrounding tissue. These findings prompted design of a miR10b-targeted therapeutic consisting of an anti-miR10b antagomir conjugated to ultrasmall iron oxide nanoparticles (MN), termed TTX-MC138. In mouse models of cancer, TTX-MC138 caused durable regressions of established metastases with no systemic toxicity. Clinical development will investigate if TTX-MC138 would accumulate in clinical metastases. TTX-MC138 is radiolabeled with isotope Cu-64 to generate the radiopharmaceutical TTX-MC138-NODAGA-Cu64. PET-MRI will assess pharmacokinetics (PK) of the radiolabeled product in humans and determine uptake in metastatic lesions.
**Methods**: This open-label, single-center, single-arm, phase 0, microdose study in subjects with advanced solid tumors and radiographically confirmed metastases will evaluate delivery of TTX-MC138-NODAGA-Cu64 by using PET-MRI; establish PK of TTX-MC138-NODAGA-Cu64; and assess safety of TTX-MC138-NODAGA-Cu64. Approximately 12 subjects age ≥ 18 years are expected to be enrolled in the study. Eligible subjects will have confirmed diagnosis of at least 1 metastatic solid tumor (that is not amenable to curative therapy unless participation in the study would delay standard therapy), Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and at least 1 target lesion per Response Evaluation Criteria in Solid Tumors version 1.1 (at least 10 mm per MRI from fludeoxyglucose [FDG] PET-MRI). Study duration is up to 46 days and includes a screening visit, dosing period (2 visits), and a follow-up visit. On Day 1, subjects receive 1 microdose of TTX-MC138-NODAGA-Cu64 by intravenous bolus. Days 1 and 2 include whole body PET-MRI imaging, ECG and blood collection. Safety data are collected at all visits. A Data Review Committee may review specific study data, including safety data. The primary endpoint will measure percent injected dose per cubic centimeter (%ID/cc) tissue of TTX-MC138-NODAGA-Cu64 delivered to metastatic lesions. Secondary endpoints may include evaluation of PK of key components of TTX-MC138-NODAGA-Cu64, metabolite analysis, and target engagement. Safety data, including incidence of adverse events, clinical lab data, ECGs, vital signs and concomitant medications, will be summarized. Enrollment is expected to start in 2023.

**CT247**

**A Phase 1/2a, multicenter, open-label, non-randomized first-in-human study to assess the safety, tolerability, pharmacokinetics, and preliminary antitumor activity of DB-1303 in patients with advanced/metastatic solid tumors.**

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**Background:** Epidermal growth factor receptor 2 (EGFR2 or HER2) has shown gene amplification/over-expression in more than 30% of all human cancers, including breast cancer, gastric, colon, salivary gland, bladder, and uterine serous carcinoma, and its overexpression in tumors is associated with poor prognosis. DB-1303 (developed by DUALITYBIO INC.) is an antibody-drug conjugate (ADC) comprised of a humanized anti-HER2 IgG1 monoclonal antibody (BAT0606), covalently linked to a proprietary DNA topoisomerase I inhibitor (P1003) via a cleavable linker containing maleimide tetrapeptide (GGFG), with a drug-to-antibody ratio (DAR) of approximately 8. Preclinical studies demonstrated a favorable safety profile and a potent antitumor activity of DB-1303 compared with approved HER2-ADC. These studies warrant further clinical development of the study drug.
**Methods:** This is a global first-in-human Phase 1/2a study (NCT05150691) to assess the safety, tolerability, and anti-tumor activities of the study drug DB-1303 in patients with pretreated advanced/metastatic solid tumors. Patients should have histologically confirmed HER2-positive or HER2-expressing cancers who failed previously systemic therapies, ECOG 0-1, and adequate organ function. The dose escalation part will evaluate seven ascending dose levels of DB-1303 (accelerated titration design for the first dose level and "3+3" design for the rest dose levels) to determine recommended phase 2 dose (RP2D) or maximum tolerated dose (MTD). The study drug will be administrated iv infusion Q3W, and dose-limiting toxicity (DLT) will be assessed during Cycle 1 (1$^{st}$ 3 weeks). Phase 2a part will initiate after MTD and/or RP2D are determined. Phase 2a part only enrolls the patients with HER2-positive gastric, esophageal, or gastroesophageal junction adenocarcinoma, colorectal cancers, HER2 overexpression and HER2-low endometrial carcinoma, hormone receptor-positive (HR+)/HER2-low breast cancer, HER2-positive breast cancer, and activating HER2-mutated NSCLC. The study treatment of DB-1303 will continue until disease progression, withdrawal of consent, or unacceptable toxicity. Tumor responses will be assessed every 6-9 weeks with RECIST v1.1. The study planned to enroll 253 patients from sites in the United States, Australia, and China (88 patients in Phase I and 165 patients in Phase 2a).

**CT248**

**First in human trial of DB1305 in patients with advanced malignant solid tumors.**

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**Background:** Trophoblast cell-surface antigen 2 (Trop-2) is known as tumor-associated calcium signal transducer 2 (TACSTD2) and plays a role in tumor progression, given its active interplay with several key molecular pathways associated with cancer development and progression. DB-1305 is a Trop-2 antibody-drug conjugate (ADC) composed of a humanized anti-Trop-2 immunoglobulin G1 (IgG1) monoclonal antibody, covalently linked to a proprietary DNA topoisomerase I inhibitor P1021 via a cleavable linker containing maleimide tetrapeptide, with a drug-to-antibody ratio (DAR) of approximately 4. Preclinical *in vivo* models show that DB-1305 demonstrates robust anti-tumor activity in Trop-2-high triple-negative breast cancer (TNBC), Trop-2-low small cell lung cancer (SCLC) and Trop-2-medium colon cancer but not Trop-2-negative SCLC, demonstrating the dependence of Trop-2 expression for the tumor-suppression activity of DB-1305 and appropriate safety profile supports the intended clinical use. This study aims to

evaluate DB-1305 in terms of tolerability and preliminary anti-tumor activity in patients with advanced solid tumors.
**Methods:** This is an open-label, multicenter, multiple-dose, Phase 1/2a study (NCT05438329), including dose escalation Phase 1 and dose-expansion Phase 2a in patients with pretreated advanced solid tumors. Patients should have histologically documented progressed/refractory disease on or after standard systemic anticancer treatments with proven benefits for their disease or without available standard treatments; adequate performance score (ECOG 0-1); adequate organ function and measurable disease as per RECIST v1.1 while without uncontrolled metastatic central nervous system (CNS) involvement and history of interstitial lung diseases. The dose escalation part will evaluate approximately five ascending dose levels of DB-1305 with accelerated titration for the first dose level followed by a "3+3" design for subsequent dose levels to identify the optimal dose, which will enroll up to 70 patients from the United States and China. Upon determining the optimal dose, seven tumor cohorts of 10-40 patients will be included in the dose expansion part, including non-small cell lung cancer, SCLC, hormone receptor-positive breast cancer, and TNBC. DB-1305 will be dosed in both parts until disease progression, clinical deterioration, withdrawal of consent, or unacceptable toxicity. The primary objectives are evaluating safety tolerability and identifying the optimal dose from Phase 1; assessing safety, tolerability, and objective response rate per RECIST v1.1 from Phase 2a. Secondary objectives include the assessment of the pharmacokinetics and immunogenicity of DB-1305. Exploratory objectives include the assessment of pharmacodynamic biomarkers and Exposure-Response correlation. As of 19 Dec 2022, 20 patients have been enrolled in the dose escalation part.

**CT249**
**A study to evaluate the safety and tolerability of the covalent phosphoinositide-3-kinase (PI3K)-alpha Inhibitor, TOS-358, in adult subjects with select solid tumors.**
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Background: TOS-358 is an orally available, highly selective covalent inhibitor of PI3Kα capable of achieving a deep and durable inhibition of PI3K-AKT signaling with virtually no off-target effects. In patient-derived xenograft models, TOS-358 has shown anti-cancer activity in several different tumor types including breast, colorectal, gastric, head and neck, bladder, and esophageal cancer, and its' activity was superior to non-covalent compounds targeting PI3Kα. Administered at the equivalent dose, TOS-358 has shown a good safety profile in 28-day repeat dose toxicity studies in rats and dogs.
Methods: The TOS-358-001 study is a multicenter dose escalation (phase 1) and dose expansion (phase 1b) clinical study evaluating the safety, tolerability, and preliminary efficacy of TOS-358. Eligible subjects are adults with one of protocol-defined tumor types, known PIK3CA mutation or amplification, adequate organ function, ECOG performance status 0 or 1, available archived or fresh tumor tissue for PIK3CA mutation detection and no prior treatment with PI3K, AKT or mTOR inhibitors (except subjects with breast cancer). The phase 1 portion of the study follows a 3+3 dose escalation design and is intended to determine the minimum effective dose (MED) and the maximum tolerated dose (MTD) of TOS-358 when administered orally on a once daily (QD) or a twice daily (BID) schedule, and the recommended phase 2 dose (RP2D). Cohort expansions at the MTD and MED to support the RP2D selection are planned. The phase 1b portion of the study will enroll subjects with tumor PIK3CA mutations or amplifications in several tumor-specific cohorts (colorectal, gastric, non-small cell lung, breast, squamous cell carcinoma of the head and neck, urothelial and select gynecological cancers) to further assess the safety and tolerability of TOS-358 and to evaluate the initial efficacy of the investigational agent. Enrolment to the study commences in US with European sites planned to open later in 2023. Clinical trial information: NCT05683418.

**CT250**

**An open-label, phase 1a/b study of AB248, a CD8+ selective IL-2 mutein fusion protein, alone or in combination with pembrolizumab in patients with advanced solid tumors.**

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Background: High-dose interleukin-2 (HD IL-2) induces durable clinical responses in a subset of patients with melanoma and RCC, but severe toxicity limits its therapeutic utility. Early clinical data from several not-α IL-2Rβγ agonists suggest better tolerability but lower objective response rates compared to historical HD IL-2 data.[1-2] In patients, these not-α IL-2s demonstrate a profound NK cell bias, while retaining activity on Tregs, and exhibiting limited CD8+ T cell expansion.[3-5]AB248 is a CD8+ selective IL-2 that demonstrates more than 500-fold selectivity for CD8+ T cells over other immune cell types. AB248 has demonstrated a highly differentiated preclinical profile, with compelling anti-tumor activity and less toxicity when given alone and in combination with anti-PD1 in multiple murine tumor models. These data suggest that AB248 may have an improved therapeutic index compared to broad-acting IL-2Rβγ agonists by increasing CD8+ T cell expansion and activation, avoiding NK cell-driven toxicity and avoiding Treg-mediated immunosuppression. Here we introduce the first-in-human study which aims to investigate AB248 when administered alone and in combination with pembrolizumab in subjects with advanced solid tumors who failed prior standard of care therapies.
Methods: This open-label phase 1a/b study consisting of a dose escalation and expansion phase aims to investigate the safety, pharmacokinetics (PK), pharmacodynamics (PD), and anti-tumor activity of AB248 alone or in combination with pembrolizumab in subjects with locally advanced/metastatic tumors, including melanoma, renal cell carcinoma, NSCLC and SCCHN, who failed prior therapies, including prior anti-PD(L)1, as well as in first-line SCCHN during the expansion phase. Key eligibility criteria include age ≥18 years, ECOG ≤1, measurable disease per RECIST v1.1, adequate end-organ function, and no autoimmune disease. The dose escalation phase will follow the Bayesian Optimal Interval (BOIN) design and enroll subjects at multiple dose levels and schedules for the AB248 monotherapy and pembrolizumab combination portion. Upon identifying suitable dose and schedule based on the totality of cumulative data including safety, PK, PD and preliminary efficacy, additional subjects will be enrolled in indication specific cohorts in the expansion phase according to the Simon 2-stage design. The primary objectives for this study are to assess the safety and tolerability of AB248 alone or in combination with pembrolizumab. Secondary objectives include assessing the PK, PD changes, immunogenicity, and antitumor activity. Exploratory objectives include evaluating the potential response-predictive and/or associated changes in immune cells, blood, and tissue biomarkers. Tumor assessments will be performed every 6 weeks for the first 30 weeks and every 9 weeks thereafter. Adverse events will be assessed by CTCAE v5.0. The study is currently open for enrollment in the dose escalation phase at multiple sites in the US.

**CT251**

**Phase 1/2 study of HST-1011, an oral CBL-B inhibitor, alone and in combination with anti-PD1 in patients with advanced solid tumors.**

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Anderson Cancer Center, Houston, TX; [4]Florida Cancer Specialists/Sarah Cannon Research Institute, Sarasota, FL; [5]University of Pennsylvania, Philadelphia, PA; [6]HotSpot Therapeutics, Inc, Boston, MA; [7]Memorial Sloan Kettering Cancer Center; Weill Cornell Medical College, New York, NY.

Background: The E3 ligase Casitas B-lineage lymphoma proto-oncogene B (CBL-B) is a master negative regulator of the immune system and thus an attractive target to address suboptimal outcomes to immune checkpoint inhibitors (ICI). CBL-B controls T-cell/NK cell activation and co-stimulatory pathways, including the signaling threshold for T-cell receptor (TCR) activation. CBL-B inhibition uncouples TCR stimulation from the requirement for CD28 co-stimulation while reducing T-cell susceptibility to immunosuppression mediated by PD1, immunosuppressive cytokines, and $T_{reg}$ cells. Accordingly, targeting CBL-B may enable immune activation even in tumors with low antigen levels, low intratumoral inflammation, inadequate co-stimulation and/or active immunosuppression associated with poor response/resistance to existing ICIs. HST-1011 is a novel, potent, selective, orally bioavailable allosteric small molecule CBL-B inhibitor that has been shown to robustly increase anti-tumor immunity *in vitro* and *in vivo*, including in model systems where other ICIs have minimal effect.
Methods: SOLAR1 (NCT05662397) is a first-in-human, multicenter Ph1/2 trial investigating safety and tolerability (primary endpoint), pharmacokinetics (PK), pharmacodynamics (PD), and preliminary efficacy (secondary endpoints) of HST-1011 monotherapy and an HST-1011/PD1 inhibitor combination. HST-1011 will be given orally while the PD1 inhibitor cemiplimab will be administered intravenously. Eligibility: Patients with advanced solid tumors and progression on standard of care diagnosed with a) tumor types for which PD-(L)1 therapies are approved with documented PD-(L)1 refractory/resistant disease, including patients with best response of stable disease for ≥ 6 months while on a PD-(L)1, OR b) selected tumor types in which patients may be naïve to anti-PD(L)1 (platinum-resistant ovarian cancer, metastatic castration-resistant prostate cancer without bony lesions, rectal cancer, anal cancer).S
tudy Design: Ph1, Part A1: HST-1011 monotherapy dose escalation, utilizing a Bayesian optimal interval design (BOIN). Optional dosing cohorts will assess alternative dosing strategies. Ph1, Part A2: HST-1011 monotherapy dose optimization with up to 4 cohorts utilizing dose(s)/schedule(s) deemed safe in Part A1 but focused on histology-specific patient populations to generate additional PK, PD, and efficacy data within a potential Recommended Phase 2 Dose (RP2D) range. Ph1, Part B: HST-1011 dose escalation in combination with cemiplimab, as in Part A1 with a BOIN design and optional dosing cohorts.
Biomarkers: Target engagement and PD will be assessed via a) serial monitoring of cytokines/chemokines and transcriptional profiles; b) peripheral immune cell profiling; and c) in-depth analysis of screening and on-treatment tumor biopsies. The study is open with competitive enrollment.

**CT252**

**A modular, open-label, phase I/II study to evaluate the safety, tolerability, pharmacokinetics and efficacy of EP0031, a next generation selective RET inhibitor, in patients with advanced RET-altered malignancies.**

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Background: Addressing resistance to 1st generation selective RET inhibitors (SRI) is an area of high unmet need, with a number of RET dependent and independent pathways identified[1,2]. The ideal profile of a next generation SRI has been proposed: broad activity against RET fusions/mutations, inhibition of both V804M/L gatekeeper and G810C/S/R solvent front mutations and penetration of the CNS to address brain metastases[1,2]. This trial is evaluating whether EP0031, an orally available next generation SRI, can

address resistance to 1st generation SRIs and improve on their clinical profile.
Methods: This Phase 1/2 study (NCT05443126) is recruiting up to 265 patients with NSCLC, thyroid cancer or other solid tumors with RET aberrations. The 1st part of the study is a dose escalation to investigate safety, tolerability, PK and PD and to define the maximum tolerated dose (MTD) and/or Recommended Phase II Dose (RP2D). Dose escalation is based on a rolling 6 design and is expected to recruit up to 40 patients.
Once an RP2D is established, expansion cohorts of approximately 25 evaluable patients each will further explore the safety and tolerability of EP0031, and provide preliminary efficacy data in selected patient populations with RET-altered tumors:•Four cohorts of patients with NSCLC and medullary thyroid cancer who have progressed on 1st generation SRI therapy and in patients with no prior SRI•Two cohorts of patients with other solid tumors, including differentiated thyroid cancer, who have progressed following 1st generation SRI therapy, and in patients with no prior SRI
Key inclusion criteria are as follows:•Male or female ≥ 18 years of age, with a diagnosis of an advanced solid tumor with documented RET altered malignancy •ECOG Performance Status of 0 or 1 at screening with no deterioration over the previous 2 weeks. For expansion cohorts patients must have a solid tumor measurable by RECIST v1.1, with/without asymptomatic, stable brain metastases
Recruitment was initiated in the US in November 2022 and is expanding to centers across Europe and rest of world. A parallel Phase I/II trial is ongoing in China (A400, NCT05265091, Kelun Biotech).
[1]Annals of Oncology 32(2) S66 (2021)[2]Journal Thoracic Oncol 15(4) 542 (2020)

**CT253**

**Phase 1/2 study of the safety, pharmacokinetics, and preliminary clinical activity of BT7480 in patients with nectin-4 associated advanced malignancies.**

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Background: *Bicycles* are a novel class of synthetic molecules formed by using a central scaffold to constrain short linear peptides into a stabilized bi-cyclic structure, which can then be readily conjugated to other molecules. BT7480 is a trimeric first-in-class *Bicycle* tumor-targeted immune cell agonist (*Bicycle* TICA) comprised of two unique bicyclic peptides: one that binds to Nectin-4 and two identical *Bicycles* that bind and agonize CD137. The resulting multi-specific molecule stimulates an immune response in the presence of both Nectin-4-expressing tumor cells as well as CD137-expressing immune cells. Nectin-4, a cell adhesion molecule overexpressed on the surface of tumor cells and CD137, a costimulatory receptor expressed on immune cells, are co-expressed in a variety of solid tumors including lung, head and neck, breast, ovarian, and bladder. Co-ligation of Nectin-4 and CD137 by BT7480 is hypothesized to induce oligomerization and activation of CD137, resulting in a tumor-localized costimulatory signal. A favorable preclinical profile supported the initiation of this first-in-human study to investigate the safety and efficacy of BT7480 in patients with solid tumors associated with Nectin-4 expression (NCT05163041).
Methods: This is a phase 1/2, open-label, multicenter study to assess safety, clinical activity, and pharmacokinetics (PK) and pharmacodynamics (PD) of BT7480. Patients must have an advanced

malignancy associated with Nectin-4 expression that is not eligible for standard therapy. Additional key criteria include ≥18 years of age, ECOG of 0 or 1, adequate organ function, and no prior therapy with a CD137-targeted agent. Patients are treated at one of the escalating doses (3+3 design) by weekly IV infusion in a 4-week cycle. Primary objectives are to assess safety (phase 1) and clinical activity per RECIST v1.1 (phase 2). Secondary objectives include assessment of PK parameters, measurement of anti-drug antibodies, and evaluation of CD137 target engagement in blood. Key exploratory objectives include evaluating pharmacogenomics, ctDNA, and biomarkers in blood and tumors associated with pharmacological activity, including signals of immune activations and target expression. PK and PD analyses will be performed to support dose escalation decisions and to further the understanding of safety and clinical activity signals as a result of treatment with BT7480. This study is actively recruiting.

**CT254**

**Trial in progress: a first-in-human, phase 1, multicenter dose escalation and dose expansion study of WTX-330 in adult patients with advanced or metastatic solid tumors or non-Hodgkin lymphoma.**

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Background: Interleukin-12 (IL-12) is a potent proinflammatory cytokine with activating effects on both innate and adaptive immune cells. Though recombinant IL-12 (rIL-12) causes tumor regression with antitumor immune memory formation in a range of immunocompetent mouse models, clinical development of rIL-12 was limited by serious toxicities resulting in several patient deaths. WTX-330 is an engineered cytokine prodrug composed of a wild-type IL-12 heterodimer fused via proprietary cleavable linkers to an inactivation domain and to a half-life extension domain. In the circulation and in normal tissues, WTX-330 is designed to remain inactive and not bind to high-affinity IL-12 receptors. In contrast, proteolytic activation of WTX-330 in the tumor microenvironment is expected to liberate the IL-12 cytokine to stimulate antitumor immunity. Preclinical data show that WTX-330 is activated by a range of dissociated human tumors *in vitro* but not by normal human cells or by patient serum. Moreover, a WTX-330 murine surrogate molecule has potent antitumor activity as a monotherapy in multiple syngeneic mouse models, including in those resistant to anti-PD-1 therapy. In the MC38 model, the WTX-330 surrogate has a large therapeutic window of 49-fold compared to 5-fold for recombinant chimeric IL-12.

Methods: This first-in-human, open-label phase 1 trial is investigating the safety, tolerability, pharmacokinetics, pharmacodynamics, immunogenicity, and antitumor activity of WTX-330 administered as a monotherapy to patients with advanced or metastatic solid tumors or non-Hodgkin lymphoma (NCT05678998). All patients must have ≥ 1 measurable lesion per RECIST 1.1 and be refractory to all standard of care therapies for their indication. Dose escalation utilizes a Bayesian logistic regression model with overdose control with WTX-330 administered intravenously every two weeks in 28-day cycles. Dose expansion is anticipated to include two arms (A, B) of 20 patients each. Patients eligible for Arm A will have tumor types for which immune checkpoint inhibitors (ICIs) are indicated but demonstrate either primary or secondary resistance to this therapy. Primary resistance is defined as disease progression or SD < 6 months as the best response after at least 6 weeks of exposure to PD-(L)1 inhibitors. Secondary resistance is defined as disease progression ≥ 6 months after initiation of PD-(L)1 inhibitors in patients who received clinical benefit (i.e., CR, PR, or SD > 6 months). Patients eligible for Arm B will have any solid tumor for which ICI therapy is not indicated, or non-Hodgkin lymphoma. Pre- and on-treatment tumor biopsies, archival tumor tissue, and blood samples will be assayed for a range of exploratory biomarkers. As of January 12, 2023, the trial is opening sites and recruiting patients.

**CT255**

**ELU-FRα-1: a study to evaluate ELU001 in patients with solid tumors that overexpress folate receptor alpha (FRα).**

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Background: ELU001 is a novel, first-in-class, new molecular entity described as a C'Dot Drug Conjugate (CDC). ELU001 consists of ~13 folic acid targeting moieties and a payload of ~22 molecules of the topoisomerase-1 inhibitor, exatecan. Folic acid and exatecan are covalently bound by non-cleavable and cathepsin-B cleavable linkers, respectively, to short polyethylene glycol chains which surround the C'Dot's silica core. CDCs are very small (~6 nm), allowing greater ability to penetrate more efficiently into solid tumors compared to ADCs. CDCs are rapidly eliminated by the kidneys, which is expected to lead to less toxicity than ADCs that have a longer half-life in circulation. ELU001's high avidity is designed to promote binding to FRα on the surface on FRα overexpressing cancer cells with a wide range of antigen expression including high, moderate and low expressing tumor cells. Following antigen binding, ELU001 internalizes into the tumor cell, and traffics to the lysosome where enzymatic cleavage releases the exatecan payload. The first-in-human trial, ELU-FRα-1, is currently recruiting patients that have advanced, recurrent or refractory tumors associated with indications that are known to potentially overexpress FRα and have been shown to be topoisomerase 1 inhibitor-sensitive, and, in the opinion of the Investigator, have no satisfactory therapeutic options available.
Methods: This is a Phase 1 / 2 multicenter, open label clinical trial with two parts: Part 1 Dose Escalation and Part 2 Tumor Group Expansion Cohort(s). Part 1 is a basket trial enrolling patients with cancer types with a high likelihood of having FRα overexpressing tumors, (specifically, ovarian, endometrial, colorectal, gastric, gastroesophageal junction, triple negative breast, or non-small cell lung cancers, or cholangiocarcinoma). Patients are receiving ELU001 weekly (QW) (once a week for 3 weeks, 1 week rest), every other week (Q2W, with no rest between cycles), or every three weeks (Q3W). Analysis of FRα expression will be retrospectively determined. Part 2 will use Simon's Two-Stage design to evaluate 4-6 Expansion Cohorts, comprised of tumor histologies anticipated to have greatest activity to ELU001 treatment. FRα will be prospectively determined. The primary objective for Part 1 is to identify the MTD/RP2D. The primary objective for Part 2 is to determine the ORR. Secondary objectives include DOR, PFS, TFST, PFS2, OS, frequency, severity and tolerability of adverse events, PK, ADA, and FRα expression assessments. Part 1 Dose Escalation will recruit about 25 patients per dose regimen (QW; Q2W; Q3W). The first stage of Part 2 (Dose Expansion) will recruit about 15 patients per tumor group expansion cohort. The study is actively enrolling in the US and currently recruiting in Q2W Cohort 201 and Q3W Cohort A. QW Cohorts 1-3 and Q2W Cohort 101 are complete. Clinical trial information: NCT05001282.

**CT256**

**Modi-1, anti-citrullinated neoepitope vaccine alone and combined with checkpoint inhibitors in patients with head and neck, breast, renal and ovarian carcinoma: protocol for the ModiFY phase I/II basket clinical Ttial.**

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Stressful conditions in the tumor microenvironment induce autophagy in cancer cells as a mechanism to promote their survival. High levels of calcium within autophagosomes activates peptidylarginine deaminase enzymes which convert arginine residues within polypeptides to citrulline and alters proteolytic cleavage. In the presence of inflammation, the MHC-II pathway presents these new citrullinated peptides to CD4 T cells. Modi-1 vaccine comprising three citrullinated, adjuvanted peptides induces and expands a population of activated CD4 T cells. On reaching the tumor site the CD4 T cells release proinflammatory cytokines including, INFγ, which upregulates MHC class II and the same, but endogenous, modified peptides are presented on the tumor cell surface. This likely causes a positive feed-forward loop with killing of tumor cells. The objective of this study is to evaluate the safety, tolerability, cellular immune and tumor response to 2 citrullinated vimentin and one citrullinated enolase peptide each conjugated to the toll-like receptor 1/2 adjuvant Amplivant® ModiFY is an open-label, prospective, multicohort, multicenter, phase I/II basket trial. Eligible patients have unresectable disease in one of the following tumor types: Squamous Cell Carcinoma of the Head and Neck (SCCHN), Triple Negative Breast Cancer, Renal Cell Carcinoma and High Grade Serous Ovarian Carcinoma. Depending on the status of the disease and eligibility for standard of care (SOC) checkpoint inhibitor (CPI) monotherapy, patients will be treated either with Modi-1 alone or Modi-1 +SOC CPI. A randomized neoadjuvant sub-study in patients with SCCHN scheduled to have tumor resection surgery is included in the protocol. These patients are randomized 1:1 to receive either Modi-1 alone or Modi-1+ pembrolizumab. The primary endpoints are the adverse event rate as measured by CTCAE v5.0 in the initial dose escalation cohorts and the strength of the cellular immune response IFNγ enzyme-linked immune absorbent spot (ELISpot) assay in the dose expansion cohorts. Secondary endpoints (RECIST 1.1 and iRECIST), are objective response rate duration of response, progression-free survival, and overall survival. In the SCCHN neoadjuvant cohort, tumor infiltrating lymphocytes in resected tumor tissue will be profiled using scRNAseq and immunohistochemistry. The study is an adaptive trial, comprising 3+3 dose escalation cohorts followed by a Simon 2-stage design in the dose expansion cohorts. The Modi-1 only dose expansion cohort will recruit 16 patients of each of the target tumor types, whilst the Modi-1+CPI will recruit a total of 21 patients. A total of 21/138 patients have been treated to date. ClinicalTrials.gov NCT05329532

**CT257**

**First-in-human phase 1 studies of PTPN2/1 inhibitors ABBV-CLS-484 and ABBV-CLS-579 in locally advanced or metastatic tumors.**

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Background: Protein tyrosine phosphatase non-receptor type 2 and type 1 (PTPN2/1) antagonists inhibit PTPN2/1-mediated negative regulation of immune response pathways. They may promote antitumor activity by increasing function of immune cells such as T cells and dendritic cells, inducing cytokine production, increasing antigen presentation, and amplifying interferon gamma-mediated tumor growth arrest. ABBV-CLS-484 and ABBV-CLS-579 are potent, orally bioavailable, first-in-class PTPN2/1 inhibitors that showed promising antitumor activity and were well tolerated in preclinical models. Preclinical data also indicated that PTPN2/1 inhibitors have improved efficacy when combined with PD-1-targeting agents (eg, pembrolizumab) or vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors (TKIs) in multiple tumor models. Here, we describe 2 first-in-human trials of ABBV-CLS-484 or ABBV-CLS-579 as monotherapy and in combination with pembrolizumab or VEGFR TKI in patients with locally advanced/metastatic tumors.
Methods: These phase 1, open-label, multicenter, non-randomized trials (ABBV-CLS-484: NCT04777994; ABBV-CLS-579: NCT04417465) comprise dose-escalation (ESC) and dose-expansion (EXP) phases. ESC is guided by a Bayesian optimal interval design based on dose-limiting toxicities. ESC data are used to inform evaluation of EXP cohorts in select tumor types including relapsed/refractory (R/R) head and neck squamous cell carcinoma, R/R non-small cell lung cancer, advanced renal cell carcinoma, and microsatellite instability-high tumors. In both ESC and EXP phases, the study drug (ABBV-CLS-484 or ABBV-CLS-579) is administered orally either alone or in combination with pembrolizumab (200 mg intravenously once every 3 weeks) and is slated to also be administered in combination with a VEGFR TKI. Eligible patients have locally advanced/metastatic tumors for which no effective standard therapy exists (or has failed), and Eastern Cooperative Oncology Group performance status ≤2. Patients (≥18 years) must have received ≥1 prior systemic anticancer therapy for the indication being considered, have relapsed or be refractory to ≥1 prior anti-PD-1/PD-L1 therapy (EXP monotherapy and pembrolizumab combination), or have relapsed after ≤1 (ABBV-CLS-484) or ≥1 (ABBV-CLS-579) prior VEGFR TKI therapy (EXP VEGFR TKI combination). Primary objectives are to assess safety, tolerability, pharmacokinetics, and to determine the recommended expansion dose and/or maximum tolerated dose of ABBV-CLS-484 or ABBV-CLS-579, both as monotherapy and in combination. Evaluation of objective response rate (RECIST v1.1) is a primary objective in EXP phase and a secondary objective in ESC phase. Both studies are active, and as of 30 Nov 2022, 30 (ABBV-CLS-484) and 45 (ABBV-CLS-579) patients had been enrolled in ESC phase.

**CT258**

**A phase 1 / 2 study of JK08, an IL-15 antibody fusion protein targeting CTLA-4, in patients with advanced solid tumors.**

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Background: CTLA-4 is a transmembrane receptor that functions as an immune checkpoint and fundamental negative regulator of T cell activation. CTLA-4 is highly expressed on most regulatory T cells (Tregs) but only upregulated in effector T cells following activation. Emerging evidence suggests anti-tumor activity of antibodies targeting CTLA-4 may be modulated by the local presence of NK cells. Interleukin 15 (IL-15) is a pleiotropic cytokine important in both innate and adaptive immunity. The IL-15/IL-15Rα complex can stimulate adjacent cells through the IL-2Rβ/γ complex. This receptor complex is present on many hematopoietic cells; however, IL-2Rβ expression is highest on NK cells and $CD8^+$ T cells. Trans-presentation of IL-15 is believed to be the optimal mode of action for IL-15-induced proliferation and activation of NK cells and $CD8^+$ T cells, two essential mediators of anti-tumor responses. JK08 is a recombinant fusion protein consisting of two functional elements - a fully human

monoclonal antibody directed against CTLA-4 and a protein complex consisting of human IL-15 and the Sushi domain of human IL-15Rα. JK08 is intended to widen the therapeutic window for IL-15 cytokine-mediated cancer therapy and CTLA-4-targeted antibody-mediated cancer therapy. This widening of the therapeutic window can be achieved by local activation and expansion of NK cells at sites of Tregs, and by IL-15 enhancement of the activity and potency of the proximal CTLA-4 antibody, mirroring the endogenous trans-presentation orientation. Preclinical studies demonstrate JK08 can elicit ADCC-mediated killing of CTLA-4-expressing cells and interact with IL-2Rβ to promote robust T cell proliferation independent of IL-15Rα expression, suggesting that JK08 could effectively activate IL-2Rβ/γC-expressing cells preferentially at sites of Tregs, and achieve enhanced anti-tumor responses including through ADCC-mediated depletion of T regulatory cells. *In vivo* studies show JK08 induces robust NK and $CD8^+$ T cell expansion in cynomolgus monkeys and anti-tumor activity in syngeneic murine models. In summary, JK08 treatment may lead to clinical activity in multiple cancer indications by counteracting the contribution of Tregs to cancer progression.
Methods: The Phase 1/2 study of JK08 will enroll patients with advanced relapsed/refractory solid tumors. The study will employ an accelerated 3+3 escalation design to explore the safety, PK, immuno-regulatory activity, and preliminary anti-tumor activity of JK08. Patients will receive treatment with JK08 subcutaneously once weekly until confirmed disease progression or intolerable toxicity. Four tumor specific expansion cohorts will be initiated once dose and schedule are established from dose escalation and include melanoma, breast cancer, colorectal cancer, and a basket of advanced solid tumors. Response will be assessed every 9 weeks per RECIST v1.1.

**CT261**

**A Phase 1, open-label, dose-escalation study of selinexor plus ruxolitinib in patients with treatment-naïve myelofibrosis.**

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**Background** Myelofibrosis (MF) is a myeloproliferative neoplasm that commonly harbors acquired somatic gene mutations in *JAK2, CALR*, or *MPL*. In the Phase 3 COMFORT-1 trial, which enrolled 309 patients (pts) with JAK inhibitor-naïve MF, ruxolitinib (RUX) showed spleen volume reduction of ≥35% (SVR35) and an improvement of 50% or more in the total symptom score (TSS50) in 42% and 46%, respectively compared to placebo. Activity has been shown with the combination of selinexor (SEL) plus RUX in preclinical studies.
**Methods** XPORT-MF-034 is an open-label, Phase 1/2 study (NCT04562389) to evaluate the safety and efficacy of SEL plus RUX in treatment-naïve MF pts. SEL is evaluated at 2 dose levels, 40mg and 60mg once-weekly plus twice daily RUX in 28-day cycles. For nausea, all pts receive prophylaxis with a 5-HT3 antagonist prior to each SEL dose and as needed. Primary endpoints are to determine maximum tolerated dose, recommended Phase 2 dose (RP2D), and safety. Secondary endpoints include spleen and symptom response, and hemoglobin stabilization and improvement. The efficacy population for spleen and symptom evaluable pts included those who had a spleen assessment or at least one symptom score available, respectively, at baseline and the W12 or W24 timepoint.
**Results** As of Oct 21, 2022, 24 pts have received at least one dose of 40mg or 60mg weekly SEL with RUX twice daily as per standard of care. Median age was 64 years old (range 44-77) and 11 pts had primary MF, 6 had post-ET MF, and 7 had post-PV MF. DIPSS risk category was int-1, int-2, and high risk for 7, 11, and 6, respectively. The median daily dose of ruxolitinib received was 20 mg. In efficacy evaluable pts, 63% (12/19) and 92% (11/12) achieved SVR35 at W12 and W24, and 83% (10/12) and 67% (4/6) achieved TSS50 at W12 and W24. Among the 11 pts who had a baseline hemoglobin level <10

g/dL, the median hemoglobin level decreased by 0.6 g/dL from baseline to W12 and increased by 0.8 g/dL from baseline to W24. The most common adverse events (AEs) were nausea (75%), anemia (63%), fatigue (58%), and thrombocytopenia (54%). The most common Grade ≥3 AEs were anemia (38%), thrombocytopenia (21%), neutropenia (17%), and atrial fibrillation (13%). Two pts discontinued treatment due to treatment-related AEs (G3 thrombocytopenia, G3 peripheral neuropathy) and no treatment-related deaths were reported.
**Conclusions** To date, in pts with treatment-naïve MF, the novel combination of SEL and RUX has been reasonably well-tolerated with a generally manageable safety profile and has shown encouraging activity in spleen and symptom responses, in addition to hemoglobin stabilization. Updated safety and efficacy, including symptom data amongst those pts non evaluable for TSS50 at the time of the Oct data cutoff, as well as RP2D, will be available for presentation at AACR 2023.

**CT262**

**Durable responses following anti-TIGIT (BMS-986207) and anti-LAG3 (BMS-980616) in combination with pomalidomide in relapsed myeloma: MMRF MyCheckpoint trial.**

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Background: T cell redirection with agents such as Chimeric Antigen Receptor T cells or bispecific T cell engagers is remarkably effective in relapsed multiple myeloma (MM), however, they are not curative. Translational studies suggest that T cell exhaustion, characterized by upregulation of immune checkpoints LAG-3 and TIGIT, is a potential resistance mechanism to T cell redirection. These findings support the hypothesis that checkpoint inhibition with anti-LAG-3 or anti-TIGIT mAbs may restore anti-myeloma T cell activity. There is an unmet need to assess the feasibility and safety of checkpoint blockade in this malignancy.
Patients and Methods: MyCheckpoint (NCT04150965) is a phase 1/2 platform trial for relapsed MM patients who have received ≥3 lines of therapy, including a proteasome inhibitor, immunomodulatory drug, and anti-CD38 mAb. Primary objectives were safety and tolerability of the investigational agents alone and in combination with pomalidomide (Pom) and dexamethasone (Dex) and overall response rates in each arm. In the phase 1 portion subjects were randomized to Arm B (anti-LAG-3) or Arm C (anti-TIGIT mAb). Subjects received one cycle (C1) of mAb therapy alone, with the addition of Pom and Dex starting with cycle 2 (C2) onward. Bone marrow aspirates and peripheral blood were obtained at screening, C2D1, C3D1, and end of study for correlative analysis.
Results: Fourteen (14) eligible subjects were enrolled (seven subjects in Arm B dose level (DL) 1, one not evaluable; six subjects in Arm C DL1, and 1 subject Arm C DL2 not evaluable). The most common adverse events (AE) were anemia and dyspnea. Grade 3-4 AEs related to therapy in the anti-LAG-3 arm were dyspnea and neutropenia; those in anti-TIGIT arm were neutropenia, thrombocytopenia, and anemia. There was 1 death due to progression of disease unrelated to the study treatment. There was 1 death due to a serious adverse event of pneumonia (unrelated). There was one adverse event of special interest in Arm B DL1 (grade 1 AST elevation). No autoimmune AEs were reported. Best overall responses for Arm B DL1 were 1 very good partial response (VGPR), 1 partial response (PR), 4 stable disease (SD); for Arm C DL1, 1 VGPR, 2 PR, and 3 SD. Eight subjects withdrew from treatment for disease progression, 2 for AE, and 2 for patient decision. Two subjects continue in Arm C DL1 as of December 2022, 1 PR as of C16D1 and 1 VGPR (C23D1).

Conclusion: Anti-LAG-3 and anti-TIGIT mAb alone and in combination with Pom are safe, feasible and clinically active in relapsed MM after Pom and anti-CD38 mAb. These data provide the first evidence of clinical activity of TIGIT and LAG-3 blockade in MM. Translational results utilizing serial analyses with mass cytometry suggest evidence of immune activation following combination therapy.

**CT263**

**A phase Ib dose-expansion study of porustobart, an anti-CTLA-4 heavy chain only monoclonal antibody, in combination with toripalimab in patients with advanced high-grade neuroendocrine neoplasms (NENs).**

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**Background:** Porustobart (HBM4003) is a fully human heavy chain only monoclonal antibody targeting CTLA-4. In addition to blocking the CTLA-4 pathway, porustobart was engineered to deplete Treg cells by enhanced antibody-dependent cellular cytotoxicity (ADCC) that was clinically validated. The prognosis of advanced high-grade NENs is poor without standard second line treatments. 4003.6, a multi-center phase Ib study is evaluating porustobart plus toripalimab (an anti-PD-1 antibody) in advanced high-grade NENs (NCT05167071).
**Methods:** Patients (pts) with pretreated advanced high-grade NENs received porustobart at one of the two dose levels (0.3 mg/kg and 0.45 mg/kg) plus toripalimab 240 mg every three weeks (Q3W). The primary endpoint is objective response rate (ORR) per RECIST 1.1 by investigator.
**Results:** As of 31 Aug 2022, 21 pts had been dosed, including 15 pts with neuroendocrine carcinoma (NEC), 3 pts with grade 3 neuroendocrine tumor (G3-NET) and 3 pts with mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN). 66.7% (14/21) pts received at least 2 prior lines of chemotherapy. Nine tested pts were all microsatellite stable (MSS). Median follow up time was 163 days for 0.3mg/kg dose group and 56 days for 0.45mg/kg dose group.Sixteen pts had post-baseline tumor assessments. The ORR and DCR were 37.5% and 62.5%, respectively. Median duration of response was not reached. For the 11 evaluable pts with NEC, the ORR and DCR were 36.4% and 72.7%, respectively. No significant difference in efficacy was observed between the two dose groups.
Treatment-related adverse events (TRAEs) were reported in 100.0% (21/21) pts, and ≥Grade 3 TRAEs were reported in 33.3% (7/21) pts. In 0.3mg/kg dose group, 75% (6/8) pts received 5-16 cycles of study treatment, and 50% (4/8) pts experienced ≥Grade 3 TRAEs. In 0.45mg/kg dose group, 92.3% (12/13) pts received ≤4 cycles, and 23.1% (3/13) pts experienced ≥Grade 3 TRAEs. Most common (≥20%) TRAEs by pooled term were hepatic function abnormal, hyperthyroidism, rash, leukopenia, anaemia, pyrexia, hypothyroidism, neutrophil count decreased and thrombocytopenia. PK data indicated no potential interaction between porustobart and toripalimab. Increase of T cell proliferation were observed in the peripheral blood of all pts.
**Conclusions:** Porustobart 0.3 mg/kg or 0.45 mg/kg plus toripalimab 240mg Q3W showed promising anti-tumor activity and an acceptable safety profile in pts with advanced high-grade NENs.

**CT264**

**Taking sotorasib with an acidic beverage improves sotorasib exposure for subjects on omeprazole.**

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The solubility of KRASG12C inhibitor sotorasib is pH dependent and coadministration with a proton pump inhibitor (omeprazole) decreased sotorasib maximum concentration (Cmax) by 56.9% and the area under the concentration curve from time zero to the time of the last quantifiable concentration (AUClast) by 42.4% under fasted conditions. This study in healthy volunteers evaluated if taking sotorasib with an acidic beverage such as Coca Cola would alter sotorasib absorption when coadministered with omeprazole and improve sotorasib exposure .Two separate phase 1, open-label, fixed sequence, 2-period studies enrolled at least 14 healthy subjects each. In the first study, each subject received a single 960 mg dose of sotorasib under fasted conditions on day 1, followed by omeprazole 40 mg once daily for 5 days on days 4 to 8, and then received omeprazole 40 mg in combination with sotorasib 960 mg on day 9 with water. For the second phase 1 study all conditions were kept the same except subjects received omeprazole in combination with sotorasib on day 9 with an 8-ounce (240 mL) glass of acidic beverage (Coca-Cola) instead of water. In both studies, intensive pharmacokinetic plasma sampling occurred predose and at 13 timepoints up to 48 hours post-dose. Sotorasib plasma concentrations were measured using a validated high-performance liquid chromatography tandem mass spectrometry method. Non-compartmental analysis was used to calculate pharmacokinetic parameters. Safety and tolerability were monitored throughout the study.Increased sotorasib exposure was observed when sotorasib and omeprazole were taken with an acidic beverage compared to when sotorasib and omeprazole were taken with water for both AUClast (23200 vs 16700 h·ng/mL respectively) and Cmax (4850 ng/mL vs 3110 ng/mL respectively). Compared to the geometric mean ratio of sotorasib and omeprazole taken with water, taking sotorasib and omeprazole with an acidic beverage resulted in an increase of 19.0 percentage-point in AUClast and a 24.6 percentage-point increase in Cmax. Coadministration of sotorasib and omeprazole with Coca Cola was safe and well tolerated.For patients on acid-reducing drugs, sotorasib taken with an acidic beverage is an effective strategy to increase sotorasib exposure and counteract the reduced exposures resulting from the drug-drug interaction effect of acid-reducing drugs.

**CT265**

**Phase 1 dose-escalation study of SGN-TGT monotherapy in patients with advanced malignancies.**

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**Background:** T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) is an inhibitory immune checkpoint receptor expressed on subsets of T-cells and natural killer (NK) cells. TIGIT inhibits T and NK cell function by binding to CD155 and CD112. SEA-TGT is an investigational human, nonfucosylated monoclonal antibody directed against

TIGIT, blocking TIGIT's interaction with CD155 and CD112. SGNTGT-001 (NCT04254107) is a phase 1, open-label, multicenter study of SEA-TGT in patients (pts) with advanced malignancies (solid tumors and lymphomas). In this abstract, we present data from the dose-escalation phase (Part A).
**Methods:** Pts received SEA-TGT ranging from 0.01 to 6.0 mg/kg of body weight intravenously every 3 weeks (Q3W). The primary objectives were to evaluate the safety and tolerability of SEA-TGT and identify the maximum tolerated dose (MTD) or the recommended dose and schedule of SEA-TGT. The optimal biological dose was defined using the Clinical Utility Index. Secondary objectives included the evaluation of antitumor activity, pharmacokinetics (PK) and immunogenicity. Antitumor activity was based on Response Evaluation Criteria in Solid Tumors version 1.1 or the Lugano classification criteria with Lymphoma Response to Immunomodulatory Therapy Criteria.
**Results:** From June 12, 2020, to October 5, 2022, 41 pts were enrolled (2, 4, 5, 11, 12, and 7 pts in the 0.01, 0.1, 0.3, 1, 3, and 6 mg/kg cohorts, respectively). Among the 39 pts who were treated 23 pts had solid tumors and 16 had lymphomas. The median number of prior therapies across all cohorts was 5. Treatment emergent adverse events of any grade were reported in 100% of pts, and treatment-related adverse events (TRAEs) of any grade were reported in 69.2%. TRAEs seen in ≥10% of the pts were infusion-related reaction (38.5%), chills (25.6%), pyrexia (17.9%), fatigue (12.8%), maculopapular rash (12.8%), and rash (10.3%). Seven pts (17.9%) reported TRAEs grade ≥3, and rash (5.1%) was the most frequently reported. No grade 4 or 5 TRAEs were reported. One dose-limiting toxicity, pruritic rash, was observed in 1 pt in the 6 mg/kg cohort, and MTD was not exceeded. Based on the PK and pharmacodynamic data, 1 mg/kg Q3W was selected as the optimal biological dose for the expansion cohorts. One partial response was observed in a pt with gastric cancer, and 2 partial metabolic responses were seen in a pt with Hodgkin's lymphoma and a pt with diffuse large B-cell lymphoma.
**Conclusions:** Overall, clinical data for SEA-TGT from the SGNTGT-001 trial suggest a manageable and tolerable safety profile. Preliminary antitumor activity warrants further clinical evaluation of SEA-TGT. SGNTGT-001 is ongoing and evaluating monotherapy and combination therapies.

**CT266**

**Chinese race and origin have no clinically meaningful effect on tiragolumab and atezolizumab pharmacokinetics and safety in patients with advanced solid tumors.**

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**Background**: Tiragolumab is a fully human IgG1 monoclonal antibody targeting the immune checkpoint TIGIT and is under evaluation in combination with anti-PD-L1 (atezolizumab) therapy. Here we report PK, safety, and preliminary anti-tumor activity from a Phase I open-label study (YP42514, CTR20210219) evaluating tiragolumab + atezolizumab in Chinese patients (pts) with advanced or metastatic solid tumors.
**Methods**: Pts ≥18 years of age with an ECOG performance status of 0 or 1, a life expectancy of ≥12 weeks, adequate hematologic and end organ function who were residents in mainland China were eligible. Pts received tiragolumab 600 mg + atezolizumab 1200 mg IV every 3 weeks. Serial PK sampling was obtained in the first dosing Cycle on Days 1, 2, 8, 15, and 21, followed by sparse peak and trough collection. PK parameters were assessed by noncompartmental analysis and summarized using descriptive statistics. The severity of adverse events (AEs) was assessed using NCI CTCAE v5. Confirmed overall response rate and duration of response (DOR) were determined by investigator. Data cutoff: Feb 10, 2022. To support the dosing approach, findings in the current study were compared with results of a global Phase I study GO30103 (NCT02794571) [Bendell JC, et al. *Cancer Res* 2020;80:(suppl 16; Abst CT302)]

in pts from France, Korea, Spain, the United States, Canada, and Australia receiving the same combination and dosing regimen.
**Results**: Twenty Chinese pts were enrolled and received a median of 5 doses of tiragolumab + atezolizumab. Pts had a median age of 57.5 (range 44-73) years, were mostly male (85%), had a median of ≥2 prior lines of therapy and 30% of pts received prior immunotherapy. Non-small cell lung cancer was the most common tumor type (55%). Exposures in Chinese pts were comparable to the global population, with a geometric mean ratio (GMR) of 1.07 for Cycle 1 tiragolumab area under the concentration-time curve from 0 to 21 days, and a GMR of 0.92 and 0.93 for Cycle 1 peak and trough atezolizumab exposure, respectively. The frequency of treatment-related AEs, all-cause grade 3/4 AEs, grade 3/4 AEs of special interest, and AEs leading to withdrawal from study treatment were generally similar for pts in China (85.0%, 40%, 10%, and 5.0%) and those in the global GO30103 study (71.3%, 41.7%, 5.3%, and 5.6%). Treatment exposure was similar in the YP42514 study vs the respective global GO30103 cohort. Two Chinese pts (10%) had a partial response, their DORs were 3 and 6 months (censored), and seven pts (35%) had stable disease.
**Conclusions**: Tiragolumab + atezolizumab was tolerable with preliminary anti-tumor activity. No meaningful differences in the PK or safety profile of tiragolumab + atezolizumab were seen between Chinese and global populations.

**CT267**

## ENB-003, an ETBR antagonist, in combination with pembrolizumab for advanced solid tumor: The ENBolden trial.

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Programmed cell death 1 (PD-1) inhibitors have minimal effect in ovarian cancer (OC) and pancreatic ductal adenocarcinoma (PDAC). Selective endothelin B receptor (ETBR) blockade stimulates T cell tumor infiltration and synergizes with anti-PD-1 therapy in OC and PDAC mouse models. We are conducting an ongoing Phase Ib, open-label dose escalation study to assess the safety and tolerability of the selective ETBR antagonist ENB-003 (vodudeutentan) in combination with pembrolizumab in metastatic PDAC, OC and other advanced solid tumors (NCT04205227). The primary objectives are safety and tolerability. Secondary objectives include overall survival (OS), progression free survival (PFS), and disease control rate (DCR). Chemotherapy-resistant PDAC and platinum refractory/ platinum resistant OC patients were microsatellite stable (MSS). The ENB-003 plus pembrolizumab combination was well tolerated across 6 dosing cohorts with no increase in toxicity above what is typically observed with pembrolizumab as a single agent. Encouraging preliminary efficacy signals have been observed. The DCR across 21 patients is 43% including 8 patients with stable disease (38%) and 1 (5%) with partial response (PR). The DCR for OC is 100% including 3 patients with stable disease (75%) and one MSS, PDL1 negative platinum refractory patient with a 95% PR (25%) of 12-month duration. In the current and final cohort, 3 PAC patients have been treated. One 4$^{th}$ line PDAC patient has exceeded 6-month survival (33%). We also observed a 7-month arrest of disease progression in a tonsillar SCC patient with innate resistance to anti-PD1. These data suggest that ETBR blockade is well tolerated, may expand the benefit of anti-PD1 in drug resistant solid tumors and warrants further study in subsequent trials.

**CT268**

**Targeting replication stress and chemotherapy resistance with a combination of sacituzumab govitecan and berzosertib: A phase I clinical trial.**

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Despite provocative preclinical results, dose-limiting toxicities have precluded rational combinations of cytotoxic chemotherapies that increase DNA damage with DNA damage response (DDR) inhibitors. We hypothesized that tumor-targeted delivery of cytotoxic chemotherapy might enable tolerable and active combinations with DDR inhibitors. We conducted a phase I clinical trial combining ataxia telangiectasia and Rad3-related (ATR) inhibitor berzosertib with sacituzumab govitecan, a trophoblast cell surface antigen 2 (Trop-2) directed antibody drug conjugate (ADC) that delivers high tumoral concentrations of topoisomerase 1 (TOP1) inhibitor SN-38. Depletion of ATR, the main transducer of replication stress is synthetically lethal with double-strand breaks (DSB) generated by TOP1 inhibitors. Patients with DDR gene-mutated or high replication stress solid tumors were enrolled since such tumors are particularly susceptible to ATR inhibition. Primary end point was identification of the maximum tolerated dose of the combination. Efficacy and pharmacodynamics were secondary end points. Using 3 + 3 dose escalation, sacituzumab govitecan (8-10 mg/m2, days 1, 8) and berzosertib (140-210 mg/m2, days 2, 9) were administered to 12 patients across three dose levels in 21-day cycles. The combination was well tolerated, with improved safety profile over conventional chemotherapy-based combinations, which allowed dose escalation to the highest planned dose level. There were no dose limiting toxicities. Common treatment-related adverse events (TRAE) were neutropenia (41.7%), diarrhea (50%), and fatigue (50%). Grade 3 TRAEs occurred in 58.3% of patients and included neutropenia (25%) and diarrhea (8.3%). There were no instances of febrile neutropenia or clinically significant grade 4 TRAEs. Pharmacodynamic studies showed evidence of ATR inhibition and enhanced DNA DSB in response to the combination. While no tumor responses were seen in three patients with DDR defects including *BRCA1* and *ATM* mutations, two patients with neuroendocrine prostate cancer, a highly aggressive subtype of prostate cancer, showed partial or metabolic responses. A patient with *EGFR*-transformed small cell lung cancer (SCLC) also experienced partial response, together yielding objective responses in 3 of 12 evaluable patients (25%). Ongoing phase II expansion cohorts are evaluating efficacy of sacituzumab govitecan 10mg/m2 and berzosertib 210mg/m2 in patients with SCLC, extra-pulmonary small cell cancers, and DDR-mutated solid tumors. ADC-based delivery of cytotoxic payload represents a new therapeutic paradigm to extend the benefit of DDR inhibitors to target replication stress and chemotherapy resistance, with minimal added toxicities. Clinical trial information: NCT04826341

**CT269**

**A highly sensitive and specific PARylation assay confirms significant and durable target engagement by AZD5305 in patients.**

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**Background** AZD5305 is a selective PARP1 inhibitor (PARPi) generated for improved therapeutic index compared with non-selective PARPi. Measurement of PARylation inhibition has been used to determine the pharmacodynamics (PD) of PARPi in clinical trials, but the assays used were insensitive and non-specific. Here we report on a new, highly sensitive and specific PARylation assay as evaluated in the PETRA trial (NCT04644068).
**Methods** PETRA is a Phase 1/2 clinical study investigating AZD5305 alone or in combination with other anticancer agents in patients (pts) with advanced solid tumors. Peripheral blood mononuclear cells (PBMC) collected at multiple timepoints during treatment (cycle [C] 0 day [D] 1 pre-dose, 1, 4, 24 and 48 hours [hrs] post-dose, and C1D1 pre-dose) were lysed, subjected to an *ex-vivo* PARylation reaction, and quantified with the novel MSD PARylation assay.
**Results** PARylation data were available from 32 patients treated from 20 mg to 140 mg. AZD5305 significantly inhibited PARylation in PBMC with maximum PARylation inhibition occurring around time to plasma concentration maximum (Tmax, 1 to 4 hrs after dosing; Table). At longer timepoints, a majority of patients showed >90% PARylation inhibition up to 48 hrs, 72 hrs, and even 168 hrs. Inhibition >90% for up to 72 hrs was observed in 4/4 evaluable samples at 140 mg, while inhibition >90% for an extended period (up to 168 hrs) was observed in 3/4 samples at 20 mg and 1/2 samples at 60 mg. These data demonstrate that AZD5305 causes significant PARylation inhibition at doses ≥20 mg which is consistent with *in vitro* findings.
**Conclusions** Significant and durable PARylation inhibition was demonstrated with the novel PARylation assay at all doses of AZD5305 in PBMC samples from the majority of pts tested.

Table. Residual PARylation levels by dose in the PETRA study

| | 20 mg | | 60 mg | |
|---|---|---|---|---|
| Time post-dose | 1 hour | 4 hours | 1 hour | 4 hours |
| Patients with >90% PARylation inhibition, n (%) | 9/15 (60.0) | 10/15 (66.7) | 11/13 (84.6) | 10/13 (76.9) |
| Median residual PARylation levels (residual PARylation), % | 5.3 | 5.0 | 4.0 | 3.7 |

**CT270**
**Immunogenicity of PGV_001 neoantigen vaccine in a Phase-I clinical trial, across various types of cancers in adjuvant setting.**
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**Introduction:** Immunotherapies such as checkpoint blockade, have demonstrated remarkable clinical efficacy yet a large percentage of patients do not respond, potentially due to a paucity of pre-existing immune priming against neoantigens. We developed a personalized genome vaccine (PGV_001) platform to generate neoantigen vaccines targeting each patient's unique mutanome. Primary objectives of the study were to determine 1) the safety and tolerability; 2) the feasibility of PGV_001 production and administration; and 3) the immunogenicity of PGV_001. Secondary objectives included immunophenotyping vaccine driven cellular and soluble immune milieu in peripheral blood. We previously reported on the clinical efficacy, and here we report, analysis of vaccine-driven immune

responses in all treated patients.
**Methods:** The study (Trial Registration NCT02721043) enrolled patients with resected malignancies, including Head and neck squamous cell carcinomas, breast cancer and bladder cancer, or, in the case of multiple myeloma treated with autologous stem cell transplant; all patients determined to have a high risk of disease recurrence (>30% over 5 years). Tumor-derived and germline RNA and DNA was sequenced to predict neoantigens utilizing our custom computation pipeline, OpenVax. Approximately 10 peptides were synthesized per patient, and a mixture of these peptides was administered as 10 subcutaneous and intradermal vaccines over 27 weeks in combination with poly-ICLC and helper Tetanus peptide as adjuvants. Immune responses were analyzed utilizing assays including IFN-gamma ELISPOT, antigen specific T cell expansion followed by flow cytometry, etc.
**Results:** In total 148 neoantigen peptides were manufactured for 15 patients. Overall, 136 PGV_001 doses were administered to 13 patients. Vaccine-specific T cell immunity was observed against multiple vaccine neoepitopes in all 13 subjects. Of the peptides administered, 45% of vaccine antigens (57/126) induced *de novo* immunity, starting as early as Week8 and often sustaining past last vaccination. Notably, while the vaccine driven T cell immunity was CD4 T cell dominant, most evaluated subjects also displayed vaccine induced polyfunctional CD8-T cell responses. Additional studies are ongoing to define qualities of reactive T cells, evaluate vaccine-induced humoral responses and probe the circulating inflammatory immune milieu. These will be presented.
**Conclusions:** We have established a platform for generating personalized neoantigen vaccines. 100% of the vaccinated patients developed an immune response specific to the vaccine neoantigens predicted by OpenVax. Subjects who received treatment experienced mild Grade 1 or 2 adverse reactions as per the CTEP v 4.0 NCI CTCAE. This vaccine trial reached the primary endpoint of safety, tolerability, feasibility and immunogenicity. Based on the PGV_001 platform two clinical trials, one in patients with glioblastoma multiforme (NCT03223103) in combination with TT fields and second in patients with urothelial carcinoma (NCT03359239) in combination with Atezolizumab have been performed. Data from these trials is under evaluation.

**CT271**

**Translational analyses of ATR inhibitor M1774 in a Phase I study in patients with solid tumors (DDRiver Solid Tumors 301).**

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**Background:** Ataxia telangiectasia and Rad3-related (ATR) protein kinase plays a critical role in the DNA damage response. M1774, a potent, selective, orally administered ATR inhibitor with antitumor activity in preclinical models, was evaluated as monotherapy in Part A1 of the first-in-human open-label, single-arm study (NCT04170153). M1774 was well-tolerated and pharmacodynamic analyses showed that maximum target engagement was reached from the dose of 130 mg QD. The totality of evidence, including quantitative model-based analyses, suggested the recommended dose for expansion (RDE) as 180 mg QD 2 weeks on / 1 week off.[1] One patient with platinum and PARP inhibitor-resistant $BRCA^{wt}$ ovarian cancer and *ATRX* mutation on local tumor testing achieved an unconfirmed RECIST v1.1 partial response. Retrospective analyses were conducted to explore the molecular portrait and evolution of the underlying disease of patients treated with M1774.
**Methods:** Archival tumor biopsies, baseline and serial on-treatment circulating tumor DNA (ctDNA) samples collected from patients enrolled in the study were analyzed by next generation sequencing.

Somatic putative CHIP mutations and cases with mutation variant allele frequency (VAF) < 0.3% in circulating free DNA at baseline were excluded. Molecular response (MR) was defined as at least 50% reduction of VAF.
**Results:** Molecular data were generated from archival biopsies collected for 33/55 patients and ctDNA samples from 55/55 patients enrolled in the study. High impact mutations were detected in *ARID1A* (N=10), *ATM* (N=5), *ATRX* (N=3), *BRCA1/2* (N=13), and other homologous recombination-related genes (N=5). MRs were observed in 11/34 (32%) patients treated with more than 130 mg QD, none were observed in the 10 patients treated with lower doses. The MRs were enriched in patients with ovarian (3/8, 38%), prostate (4/8, 50%), and breast cancer (1/3, 33%), while less frequent (3/15, 25%) in other indications. *TP53* mutations were significantly associated with MR, independently of tumor type. Complete MRs were achieved for any mutations in *ARID1A* (2/7), *ATRX* (2/5), *DAXX* (1/3), *BRCA1/2* (1/10). A complete MR was seen in a patient with ovarian cancer with prolonged stable disease by RECIST (200 days), and *ARID1A* mutation in ctDNA.
**Conclusions:** M1774 induced MRs in patients treated with doses in the predicted efficacious concentration range. *TP53* alterations were significantly associated with MR. Complete MRs were detected for mutations in the genes *ARID1A*, *ATRX*, *DAXX* that are being tested for participant selection in the ongoing biomarker expansion cohorts of the DDRiver 301 study.
[1]TA Yap, et al. *Ann Oncol.* 2022; 33(suppl_7): S197-S224.

**CT272**

**Pharmacodynamic and immunophenotyping analyses of ATR inhibitor M1774 in a Phase I study in patients with solid tumors (DDRiver Solid Tumors 301).**

Ruth Plummer,[1] Anthony W. Tolcher,[2] Timothy A. Yap,[3] Giuseppe Sessa,[4] Jatinder K. Mukker,[5] Annick Seithel-Keuth,[4] Christine Hicking,[4] Zoltan Szucs,[6] Ioannis Gounaris,[6] Giuseppe Locatelli,[4] Johann S. de Bono[7]. [1]Newcastle University and Northern Centre for Cancer Care, Newcastle Hospitals NHS Trust, Newcastle Upon Tyne, United Kingdom; [2]New Experimental Therapeutics (NEXT), San Antonio, TX; [3]University of Texas MD Anderson Cancer Center, Houston, TX; [4]Merck KGaA, Darmstadt, Germany; [5]EMD Serono, Billerica, MA; [6]Merck Serono Ltd., an affiliate of Merck KGaA, Darmstadt, Germany, Feltham, United Kingdom; [7]Royal Marsden Hospital, Sutton, United Kingdom.

Background: Ataxia telangiectasia and Rad3-related (ATR) protein kinase plays a critical role in the DNA damage response. M1774, a potent, selective, orally administered ATR inhibitor with antitumor activity in preclinical models, was evaluated in Part A1 of an open-label, single-arm study (NCT04170153) for safety, tolerability, maximum tolerated dose, pharmacokinetics (PK) and pharmacodynamics (PD). M1774 monotherapy in patients with advanced solid tumors was well-tolerated and the totality of evidence, including quantitative model-based analyses, suggested the recommended dose for expansion (RDE) as 180 mg QD 2 weeks on / 1 week off.[1] Here, we report findings of the M1774 PD and immunophenotyping analyses.
Methods: M1774 PD was explored by assessing phosphorylation by ATR of CHK1 (p-CHK1) in tumor and of H2AX (γ-H2AX) in serial blood samples, stimulated *ex vivo* with the radiomimetic 4-Nitroquinoline N-oxide or Dimethyl sulfoxide as control. A flow cytometry quantitative assay was used to measure γ-H2AX in the CD45+ lymphocytes fraction. The effect of M1774 on the immunophenotype was explored by flow cytometry. Blood samples were collected at baseline, 3 and 24 hours after first M1774 administration on day 1 of cycle 1 for the γ-H2AX analysis, and on Days 1 and 15 of Cycles 1 and 2 before treatment for immunophenotyping.
Results: Preclinical tumor tissue-blood bridging PD analyses in a mouse model demonstrated that the inhibition of γ-H2AX in lymphocytes highly correlated with inhibition of p-CHK1 in tumor. Clinical data of γ-H2AX levels and immunophenotyping were generated for the blood samples collected from the 55 participants of Part A1 of the study. Exploratory PK-PD analysis using γ-H2AX levels 3 h post-dose on day 1 across the doses predicted target inhibition >80% for doses ≥130 mg, suggesting target engagement.

The levels of ɣ-H2AX at 24 h after first dose intake were variable and mean levels rebounded to baseline value. M1774 treatment did not cause any significant and consistent change in the levels of all explored immune cell subsets at the tested dose levels, including myeloid-derived suppressor cells, T and B lymphocytes, monocytes, and natural killer cells.
Conclusions: PD analyses showed that M1774 efficiently inhibited ATR at the RDE without impacting the immunophenotype.
[1]TA Yap, et al. *Ann Oncol.* 2022; 33(suppl_7): S197-S224.

**CT273**
**Evaluation of the effect of rivoceranib on the pharmacokinetics of cytochrome P450 enzyme substrates in healthy volunteers.**
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Introduction: Rivoceranib is a selective vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor with potent antitumor activity. Rivoceranib is metabolized in the liver mostly by cytochrome P450 (CYP)3A4/5, with minor contributions from CYP2D6, CYP2C9, and CYP2E1. In vitro and in vivo studies suggest rivoceranib may interact with various CYP substrates, including CYP 1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A. The purpose of study is to evaluate the effect of rivoceranib on the pharmacokinetics (PK) of various CYP substrates.
Methods: This study was an open-label, 2-treatment, fixed-sequence drug-drug interaction phase 1 study evaluating the impact of multiple oral doses of 700 mg rivoceranib on the single-dose PK of CYP enzyme substrates administered in a 5+1 probe cocktail (caffeine [CYP1A2], S- and R-warfarin [CYP2C9] + vitamin K, omeprazole [CYP2C19], dextromethorphan [CYP2D6], and midazolam [CYP3A]) in healthy volunteers (N=32). On day 1, volunteers received a single dose of the 5+1 cocktail comprising 200 mg caffeine, 10 mg S- and R-warfarin with 10 mg vitamin K, 40 mg omeprazole, 30 mg dextromethorphan, and 2 mg midazolam. Blood samples were collected predose on day 1 and up to 120 hours post-dose for PK analyses of the substrates in the 5+1 cocktail. On days 6 to 15, volunteers received 700 mg rivoceranib once daily for 10 consecutive days with a single dose of the 5+1 cocktail administered on day 11. Blood samples were collected predose on day 11 and up to 120 hours post cocktail dosing (day 16) for PK analyses. Each dosing was under fasted conditions. There was a washout period of 5 days between Day 1 dosing and the first rivoceranib dose on Day 6; the 2 cocktail dosings were spaced by 10 days.
Results: Rivoceranib reduced caffeine $AUC_{0\text{-}inf}$ by 15%, and did not change caffeine $C_{max}$, indicating a minimal effect of rivoceranib on the PK of CYP1A2 substrates. S-warfarin and R-warfarin $AUC_{0\text{-}inf}$ increased by 68% and 32%, respectively, and $C_{max}$ by 19% and 15%, respectively, when co-administered with rivoceranib, indicating rivoceranib weakly inhibits CYP2C9. Rivoceranib appeared to act as a moderate inhibitor of CYP2C19, increasing omeprazole $AUC_{0\text{-}inf}$ 3.3-fold and increasing $C_{max}$ 2-fold. Dextromethorphan metabolism (CYP2D6) was inhibited, with a 2- to 2.7-fold increase in dextromethorphan exposure. Rivoceranib appeared to moderately inhibit midazolam metabolism by CYP3A4, with 2.4- to 2.8-fold increases in midazolam exposures.
Conclusion: In the analysis, the effect of rivoceranib on the PK of CYP1A2 substrates did not appear to be clinically significant. Rivoceranib may inhibit the metabolism of CYP2C9, CYP2C19, CYP2D6, and CYP3A4 substrates, suggesting that dose adjustment of substrates of these CYP isozymes and/or cautiously monitoring patients' adverse events may be needed when they are co-administered with rivoceranib.

**CT274**
**Individualized APC targeting VB10.NEO cancer vaccines induce broad neoepitope-specific CD8 T**

**cell responses in patients with advanced or metastatic solid tumors: interim results from a phase 1/2a trial.**

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Background: VB N-01 is an open label phase 1/2a basket trial to evaluate safety, feasibility, and immunogenicity of a therapeutic DNA cancer vaccine VB10.NEO in patients with locally advanced or metastatic solid cancers. Each VB10.NEO vaccine contains up to 20 neoepitopes selected by the proprietary AI platform NeoSELECT and is designed to target antigen presenting cells using Nykode' s modular vaccine platform known as Vaccibody™.
Patients and Methods: The trial enrolled patients with locally advanced or metastatic solid cancers (renal cell carcinoma, urothelial cancer, non-small cell lung cancer, squamous cell carcinoma of the head and neck, and melanoma), who did not obtain complete responses on immune checkpoint inhibitor therapy (CPI). Up to 14 VB10.NEO doses (3 mg per dose) were administered i.m. by PharmaJet Stratis® for up to 1 year in combination with standard of care (CPI and/or other anti-cancer therapies). Blood samples and tumor biopsies were collected at baseline and during treatment for evaluation of immune responses.
Results: At cut-off of May 2022, 41 patients had received at least one vaccination with VB10.NEO. The vaccine was safe and well-tolerated with no new or additional toxicity reported beyond that expected for CPIs alone. All patients displayed immune responses to a minimum of 3 neoepitopes (average 53% of vaccine neoepitopes), assessed by *in vitro* stimulation (IVS) interferon-gamma ELISpot. T cell responses were elicited in both high and low tumor mutational burden patients (TMB range 2-69mut/Mb). Multiple vaccinations increased the breadth and magnitude of the immune responses with vaccine-induced T cell responses (*de novo* and/or amplified pre-existing) measured in 95% of eligible patients. IVS intracellular cytokine staining of selected patients demonstrated that the majority of neoepitopes induced polyfunctional CD8 T cells. TCR sequencing of baseline and on-treatment samples (6 patients) showed expansion of T cell clones in both blood and tumor with selected clones being expanded in both compartments.
Conclusions: VB10.NEO was generally well tolerated in patients with various pre-treated and advanced cancers. Assessment of neoepitope-specific T cell reactivity demonstrated VB10.NEO-induced broad and long-lasting T cell responses, and the majority of tested neoepitopes activated polyfunctional CD8 T cells. Pre- and post-vaccination TCRseq analysis of blood and tumor samples demonstrated the presence of blood-expanded clones in the on-treatment tumor sample potentially indicating trafficking of VB10.NEO-expanded T cells to the tumor site.

**CT275**

**Phase 1 clinical update of allogeneic invariant natural killer T cells (iNKTs), agenT-797, alone or in combination with pembrolizumab or nivolumab in patients with advanced solid tumors.**

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MA; [11]Beth Israel Deaconess Medical Center, Boston, MA.

Introduction: AgenT-797 is an allogeneic iNKT cell therapy and represents a novel, scalable, off-the-shelf approach against solid tumors. iNKTs are a unique subset of T cells, that mediate antitumor responses by direct killing, targeting CD1d and other ligands in the tumor microenvironment, and by activating host immune cells. We conducted a clinical trial to investigate agenT-797 activity as single agent and in combination (combo) with PD-1 blockade after prior progression on PD-1 therapy.
Methods: Patients (pts) with relapsed or refractory solid tumors were treated with single IV infusion of agenT-797 (no lymphodepletion) at 4.3 x 10^6 or 1.4 x 10^7 cells/kg, as monotherapy or in combo with pembrolizumab (pembro) or nivolumab (nivo). Dose escalation followed 3+3 scheme. Endpoints included safety, persistence of agenT-797, objective responses, duration of response, progression-free survival, and time to response. Adverse events (AEs) were reported per CTCAE v5.0. Dose limiting toxicities (DLTs) were evaluated. AgenT-797 persistence was assessed by assays utilizing SNPs and cfDNA analysis. Serum biomarkers were measured with MSD V-PLEX cytokine assays. On-treatment tumor biopsy was obtained for multiplex immunofluorescence staining and next generation sequencing.
Results: As of February 5th, 32 pts (median age 62y, range 30-83) were treated with agenT-797 monotherapy (n=26) or combo (n=6) with pembro or nivo. Pts had median 4 lines of prior therapy (range 1-13). Tumor types included pancreatic (6), NSCLC (4), rectal (4), cholangiocarcinoma/biliary duct (4), and other (14)*. Tolerability was favorable, with no cytokine release syndrome, neurotoxicity, and no DLTs. Treatment-related AEs included 16.7% (5) grade 1, 3.3% (1) grade 2, and 3.3% (1) grade 3 (anemia). At data cutoff, agenT-797 monotherapy and combo revealed early clinical activity. Among 29 evaluable pts, a confirmed partial response in MSI-H gastric cancer refractory to PD-1 treated with agenT-797 + nivo (remains ongoing >6 months) and 8 pts had stable disease (SD). Prolonged SD (>3 months) for 3 monotherapy pts. Overall response rate 20% (1/5) for pts treated with agenT-797 + pembro or nivo. AgenT-797 was detected in peripheral blood up to day 8 post infusion. Preliminary data identified transient increase in serum IFNgamma levels day 2 post infusion.
Conclusion: AgenT-797 was well tolerated as monotherapy and in combo with PD-1 (pembro or nivo). Anti-tumor activity in combo with nivo was observed in gastric cancer. Results support the potential of a novel therapeutic strategy employed by agenT-797 to enhance antitumor immunity in PD-1 refractory tumors. Enrollment ongoing and correlative studies will be presented.
*PD-1 refractory NSCLC, pancreatic, rectal, cholangiocarcinoma, cervical, gastric, ocular melanoma, renal, and upper tract urothelial.

**CT276**

**Preliminary analysis of pharmacokinetic (PK) and target engagement biomarkers from a first in human phase 1 study of immunomodulatory aryl hydrocarbon receptor (AhR) inhibitor BAY2416964.**

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Introduction: Many patients do not benefit from immunotherapies targeting immune checkpoints such as

PD(L)-1 due to a variety of resistance mechanisms. The AhR pathway is downstream of the Trp-IDO/TDO-Kyn axis. High levels of immune-suppressive AhR-activating ligands, such as kynurenine derived from IDO1/TDO2-expressing tumors, have been implicated as a potential resistance mechanism and are associated with poor responses to PD-1 therapies. Compared with inhibition of IDO1/TDO2 alone, direct AhR inhibition can block the activation of this transcription factor and may better counteract immune suppression. BAY2416964 is a novel, oral AhR inhibitor currently in a Phase I clinical trial in patients with solid tumors. Preclinically, BAY2416964 can block the activation of AhR by kynurenine and relieve its immune-suppressive effects, thereby restoring anti-tumor T-cell activity, reducing the level of inhibitory myeloid-derived suppressor cells and regulatory T cells, and improving the effectiveness of PD-1 blockade. We have analyzed PK and biomarker data from the ongoing monotherapy dose-escalation study to explore a potential optimal dose and schedule for effective AhR inhibition.
Methods: PK data were intensely sampled from patients in 10 different groups with different dosing regimens (once and twice daily) and various food-intake scenarios. AhR downstream target gene expression after *ex vivo* kynurenic acid stimulation of patients' peripheral blood mononuclear cells (PBMCs) was assessed. A population PK (popPK) model was developed and calibrated to characterize the PK of BAY2416964 across treatment groups. This model accounts for the non-linear relationship between dose and bioavailability as well as the effect of food intake.
Results: The preliminary popPK model identified a non-linear relationship between the BAY2416964 dose and its (relative) bioavailability which was dependent on the respective food condition. The popPK model was able to describe the clinical BAY2416964 plasma exposures from all dose groups, including the effect of food intake. *Ex vivo* analysis of PBMCs showed inhibition of kynurenic acid-induced AhR downstream gene expression (such as CYP1A1, CYP1B1), suggesting effective *in vivo* target engagement in the doses tested.
Conclusion: This modeling-based PK analysis along with target engagement in peripheral blood informed the posology to be tested in the dose-expansion part of the ongoing clinical trial.

**CT278**

***ERBB2* amplification detected in ctDNA as a surrogate for tumor tissue FISH analysis of HER2 status in a phase 1 study with zanidatamab for the treatment of locally advanced or metastatic HER2 expressing cancers.**

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**Background:** HER2-targeted therapies have substantially improved outcomes for patients with HER2-positive breast and gastric cancers. Several other cancers exhibit HER2 expression and/or amplification of its gene (ERBB2), suggesting that HER2-targeted agents may have broader therapeutic utility. Zanidatamab is a humanized, novel bispecific antibody directed against two non-overlapping domains of HER2. The aim of this Phase 1 dose-escalation and expansion study (NCT02892123) was to evaluate the safety and efficacy of zanidatamab across a range of solid tumors. Parallel to drug development, there has been rapid advancements in NGS technologies including the Guardant360 assay that can specifically sequence ctDNA and detect amplifications of the ERBB2 gene, which can lead to overexpression of HER2. FISH, the current gold standard for HER2 amplification detection, is a tissue-based assay that assesses the raw ERBB2 copy number as well as ratio of ERBB2 to a centromeric protein of chromosome 17 where the ERBB2 gene resides. We evaluated concordance of the FISH and Guardant360 assays to detect ERBB2 amplification in plasma samples. Unlike gene copy number in tissue analysis, the observed plasma copy number (pCN) is also a function of the tumor burden and rate of tumor shedding of ctDNA

into the bloodstream.
**Methods:** HER2 status was determined from a fresh tumor biopsy or in archival FFPE tissue samples by IHC and FISH according to ASCO-CAP guidelines from the Phase 1 study with zanidatamab in multiple cancer types (cholangiocarcinoma [21], colorectal carcinoma [27], all other [87]). Plasma samples were collected prior to the first cycle of zanidatamab and on-treatment for testing with Guardant360, 74 gene ctDNA NGS-based assays.
**Results:** A concordance of 82% was observed in ERBB2/HER2 amplifications between the Guardant360 and FISH assays. An exploratory adjustment method based on tumor DNA shedding was developed by Guardant using the maximum mutant allele fraction (maxVAF) as a surrogate for tumor content. Majority of patients experienced a decrease in HER2 pCN post treatment, with 9 PD patients having the least and 21 PR patients the largest changes in ctDNA fraction (maxVAF).
**Conclusion:** These results indicate that ERBB2 amplification detected by the Guardant360 assay could be used as a surrogate for FISH analysis in lieu of invasive surgical procedures.