Therapeutic agents: other

LBA002 Targeting GSPT1 by a novel cereblon E3 ligase modulator for the treatment of Acute Lymphoblastic Leukemia Fatemeh Keramatnia[1], Yunchao Chang[1], Gisele Nishiguchi[1], Jaeki Min[1], Charles Mullighan[1], Marcus Fischer[1], Zoran Rankovic[1], Fatemeh Keramatnia[1]. [1]St. Jude Children's Research Hospital, Memphis, TN.

Acute Lymphoblastic Leukemia (ALL), the most common childhood cancer and the second most common acute leukemia in adults, arises from clonal expansion of undifferentiated lymphoid precursor cells in bone marrow. Although the majority of childhood ALL cases harbor clonal genetic alterations of transcription factor (TF) gene mutations or rearrangements, TF alterations remain difficult therapeutic targets. Small molecule induced protein degradation is a novel strategy that can be applied to currently undruggable targets such as TF and fusion oncoproteins. In this paradigm, small molecule degraders (either PROTAC or Molecular Glue (MG)) redirect the cell's endogenous ubiquitin proteasome system and induce ubiquitination of the target protein or non-native substrate of an E3 ligase (neosubstrate) and its subsequent proteasomal degradation. Recently, the CRBN E3 ligase modulator, CC90009 was reported to show potent antitumor activity in acute myeloid leukemia, leading to the identification of GSPT1 (G1 to S phase transition factor) as a CRBN neosubstrate. These findings suggest the potential of MGs to target unanticipated vulnerabilities in different malignancies. Here, using a structurally diverse and unique set of MGs (Molecular Glue Library (MGL)) with confirmed CRBN binding affinity, we sought to identify novel CRBN modulators through phenotypic and proteomic methods. Our screening of the MGL in a panel of representative acute leukemia cell lines, including the *CRLF2*-rearranged ALL cell line MHH-CALL-4 identified several active MGs with EC50 <5 μM. A lenalidomide competition assay and MHH-CALL-4 CRBN knock down cells confirmed the CRBN dependency of these MGs. Among these compounds, SJ6986, a thalidomide-driven sulfonamide showed potent cytotoxicity in over 10 ALL cell lines tested *in vitro*. TMT-MS proteomic analyses identified GSPT1/2 as the primary targets for this compound with high selectivity. We next tested SJ6986 activity in a panel of patient derived ALL xenografts (PDX) harboring rearrangement of IGH-CRLF2, EPOR, ATF7IP-JAK2 *ex vivo*. All the tested tumors were highly sensitive to SJ6986 with IC50s at nanomolar range. PK analyses in NSG mice indicated rapid absorption and over 80% oral bioavailability for SJ6986. PD studies in an IGH-CRLF2 PDX showed dose-dependent degradation of GSPT1 within 48 hours of treatment. Finally, we examined the antitumor activity of SJ6986 in 6 different PDX representative of high-risk subtypes of ALL including near haploid, low-hypodiploid, CRLF2-rearranged and EPOR-rearranged, *in vivo* for 28 days. SJ6986 was able to dramatically decrease the tumor burden at 1 mg/kg dose in most of the tumor models. Collectively, these results affirm that SJ6986 is a novel CRBN modulator and a potential therapeutic agent by targeting GSPT1 protein with high selectivity and potency for the treatment of ALL.

Therapeutic agents: small-molecule kinase inhibitors

LBA003 Erdafitinib in patients with tumors harboring *FGFR* gene mutations or fusions: Results from the NCI-MATCH ECOG-ACRIN Trial (EAY131) Sub-protocol K2 Alain C Mita[1], Zihan Wei[2], Ingrid A Mayer[3], Heather Cheng[4], Edith P Mitchell[5], John J Wright[6], Percy Ivy[6], Robert J Gray[2], Victoria Wang[2], Lisa M McShane[6], Larry V Rubinstein[6], David R

Patton[6], Mickey Williams[6], Stanley R Hamilton[7], Barbara A Conley[6], Carlos L Arteaga[8], Lyndsay N Harris[9], Peter J O'Dwyer[10], Alice P Chen[6], Keith T Flaherty[11]. [1]Cedars-Sinai Medical Center, Los Angeles, CA, [2]Dana-Farber Cancer Institute, Boston, MA, [3]Vanderbilt University Medical Center/ Vanderbilt-Ingram Cancer Center, Nashville, TN, [4]University of Washington, Seattle, WA, [5]Sidney Kimmel Cancer Center at Jefferson, Philadelphia, PA, [6]National Cancer Institute, Bethesda, MD, [7]City of Hope National Medical Center, Duarte, CA, [8]University of Texas Southwestern Medical Center, Dallas, TX, [9]National Cancer Institute, Rockville, MD, [10]University of Pennsylvania Medical Center, Philadelphia, PA, [11]Massachusetts General Hospital, Boston, MA.

Background: The NCI-MATCH precision medicine trial assigns patients (pts) with solid tumors, lymphoma, or multiple myeloma whose cancers have progressed on prior treatment to a targeted therapy based on genetic alterations identified in pre-treatment biopsies. Arm K2 (EAY131-K2) evaluated the pan-FGFR inhibitor erdafitinib (E) in pts with *FGFR* mutations or fusions.**Patients and methods:** Pts with bladder or urothelial cancers were excluded. Pts received E 8 mg PO daily (28-day cycle) until disease progression or unacceptable toxicity; dose reduction for toxicities was allowed; imaging was performed every 2 cycles. The primary endpoint was objective response rate (ORR); secondary endpoints included progression-free survival (PFS), 6-month PFS, and overall survival (OS).**Results:** A total of 35 pts were enrolled to this arm from 07/2018-07/2019; one was ineligible and one did not receive treatment. Nine distinct tumor histologies were represented, most common being pancreatobiliary (11), CNS (7) and gynecological tumors (5). 73% of pts were female, with median age of 59y (range 26-83y), 70% were Caucasian, and 61% of pts had received at least 3 prior therapies (range 0-22). Alterations in *FGFR1, FGFR2 and FGFR3* were recorded in 6, 18, and 9 evaluable pts, respectively. 18 pt tumors had fusions and 15 had mutations in an *FGFR* gene. The confirmed ORR was 12% (90% CI 4%, 26%), with a median duration of response (DoR) of 7.3 months (mo), range 4.2-11.7 mo. Responses were seen in cholangiocarcinoma (2 pts), Brenner ovarian tumor and adenoid cystic carcinoma (1 pt each). Two (50%) of these 4 tumors harbored *FGFR* fusions and 2 *FGFR* mutations. 13 pts had stable disease (SD). Median PFS was 3.9 mo, and 6-mo PFS was 32.8% (90% CI 21.2%, 50.6%). Median OS was 11.0 mo. Of the 6 pts with intrahepatic cholangiocarcinoma, 2 had PR and 2 SD. The most frequent grade 3 treatment-related AEs were oral mucositis/pain (5 pts), paronychia, electrolyte disorders, and anemia/lymphopenia (2 pts each). There were no treatment-related grade 4-5 toxicities. Toxicities were reversible and manageable with E dose interruptions and/or dose reduction.**Conclusions:** In this pre-treated, mixed histology cohort with tumors harboring *FGFR* somatic alterations, E showed activity with durable responses and disease stabilizations outside of currently approved FDA indications, although the pre-specified criterion that the primary endpoint, ORR, be significantly greater than 16% was not reached. Toxicities were consistent with E safety profile. Responses were observed in tumors harboring *FGFR* fusions as well as in those with mutations of *FGFR*; further correlative analyses are planned.

Therapeutic agents: other

LBA004 Identification of GSPT1-directed molecular glue degrader (MGD) for the treatment of Myc-driven breast cancer Gerald Gavory[1], Bernhard Fasching[1], Debora Bonenfant[1], Amine Sadok[1], Ambika Singh[1], Martin Schillo[1], Vittoria Massafra[1], Anne-Cecile

d'Alessandro[1], John Castle[1], Mahmoud Ghandi[2], Agustin Chicas[2], Frederic Delobel[3], Alexander Flohr[3], Giorgio Ottaviani[3], Thomas Ryckmans[3], Anne-Laure Laine[3], Oliv Eidam[3], Hannah Wang[4], Ilona Bernett[4], Laura Chan[4], Chiara Gorrini[4], Theo Roumiliotis[4], Jyoti Choudhary[4], Yann-Vai LeBihan[4], Marc Cabry[4], Mark Stubbs[4], Rosemary Burke[4], Rob Van Montfort[4], John Caldwell[4], Rajesh Chopra[5], Ian Collins[4], Silvia Buonamici[2]. [1]Monte Rosa Therapeutics AG, Basel, Switzerland, [2]Monte Rosa Therapeutics Inc, Boston, [3]Ridgeline Discovery, Basel, Switzerland, [4]The Institute of Cancer Research, London, United Kingdom, [5]Apple Tree Partners, London, United Kingdom.

The Myc family of transcription factors is a well-established driver of human cancers. However, despite being amongst the most frequently mutated, translocated and overexpressed oncogenes, no therapy directly targeting the Myc family members has been developed to date. Abnormal activation of Myc results in uncontrolled cell growth that is associated with high translational output and ramp up of the protein translational machinery. This creates a dependency to protein translation and in turn represents a potential therapeutic vulnerability for Myc-driven tumors. Based on these considerations, we hypothesized that targeting the translational termination factor GSPT1, a key player of protein synthesis, may constitute a vulnerability for Myc-driven tumors. Using our proprietary Quantitative and Engineered Elimination of Neosubstrates (QuEEN^{TM}) platform we characterized and explored the known G-loop degron in GSPT1 that renders it amenable to cereblon-induced degradation by molecular glue degraders (MGDs). We rationally designed and subsequently screened a proprietary library of cereblon-binding small molecules, including GSPT1-directed MGDs, in human mammary epithelial cells (HMECs) expressing doxycycline-inducible c-Myc. Doxycycline treatment led to sustained c-Myc expression and as a consequence to the induction of key biomarkers of enhanced protein translation, such as phospho 4EBP1 (p4EBP1). We identified MRT-048 as a potent and highly selective GSPT1 degrader and demonstrated its ability to induce cell death in Myc-driven HMEC cells whilst sparing control cells (EC50 0.64 μM vs 30 μM respectively). This confirmed the selective vulnerability of Myc-driven cell growth to GSPT1 degradation. In follow-up studies, we confirmed the correlation between p4EBP1 as biomarker of Myc-activation and sensitivity to MRT-048 in a large panel of breast cancer cell lines. Moreover, MRT-048 treatment of animals xenografted with breast cancer cells induced tumor regression and was associated with complete GSPT1 degradation. Mechanistically, we observed that GSPT1 degradation induced by MRT-048 led to inhibition of genes regulated by Myc and ribosomal stalling at stop codons of several mRNAs. Additionally, polysome profiling of cancer cells treated with MRT-048 was associated with a global reduction of the intensities of the polysome peaks and concomitant increase in the monosome peaks as previously observed in GSPT1 knockdown experiments, suggesting that GSPT1 degradation by our MGD molecules affects both the termination and initiation stages of protein translation. We believe these data support the therapeutic potential of GSPT1-directed MGDs in Myc-driven tumors dependent on protein translation machinery.

Clinical trials

LBA005 Detection of KRAS amplification on extrachromosomal DNA (ecDNA) upon acquired resistance to KRAS^{G12C} inhibitors Ryan J Hansen[1], Steven Horton[2], Julie Wiese[1], BellJohn Bibay[1], E.Lorena Mora-Blanco[1], Nam-Phuong Nguyen[1], Lars D Engstrom[3], David M Briere[3], Andres Calinisan[3], Peter Olson[3], Mark M Awad[4], Andrew J Aguirre[4], Jason

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The recent discovery and clinical validation of KRAS inhibitors (KRASi) has ushered in a new therapeutic approach to directly address the previously undruggable mutant KRAS-driven cancers. Unfortunately, as with other oncogene-directed therapies, acquired resistance to KRASi has been observed that is partially attributed to secondary mutations in *KRAS* and/or mutations or fusions in other mitogen-activated protein kinase (MAPK) signaling genes. Emerging evidence suggests that amplification of oncogenes on acentric extrachromosomal DNA (ecDNA) might constitute an important mechanism of rapid resistance to receptor tyrosine kinase (RTK) and MAPK targeting therapies. Therefore, we investigated whether resistance to KRASi monotherapy is mediated through an ecDNA mechanism. We previously noted focal gene amplification of KRAS and MET in recurrent tumors isolated from patients treated with the selective $KRAS^{G12C}$ inhibitor, adagrasib. Analysis of next-generation sequencing (NGS) data from a patient tumor revealed evidence of circular DNA structures encompassing the KRAS locus. Consistent with this finding, interphase FISH analysis indicated the presence of KRAS amplifications on ecDNA in this adagrasib-treated patient tumor. To further interrogate this biology, we examined a genetically modified CT26 murine tumor model harboring $KRAS^{G12C}$ alteration. Analogous to the clinical observations, both adagrasib as well as the recently approved KRASi sotorasib, induced transient tumor regressions that subsequently resumed growth following several weeks of continuous therapy. Strikingly, whole-genome sequencing data from isolated recurrent tumors revealed focal amplification of $KRAS^{G12C}$ and metaphase FISH confirmed high levels of $KRAS^{G12C}$ on ecDNA as compared to vehicle-treated samples. Further mechanistic studies are underway. Collectively, these observations implicate ecDNA as an important mediator of resistance to KRASi monotherapy and reinforce the critical need for novel therapeutic strategies to address ecDNA oncogene amplification-driven cancers.

New molecular targets

LBA006 Identification of novel, tumor specific pHLA targets recognized by TILs from CPI responders in a high-throughput, high-diversity pHLA library screening platform (3T-TRACE) Hans-Peter Gerber[1], Marvin Gee[1], Leah Sibener[1]. [1]3T Biosciences, South San Francisco, CA.

BackgroundThe ideal cancer target antigen is selectively expressed on neoplastic cells, absent on normal tissues, and contributes to the survival of cancer cells. Recent clinical trial reports identified T cell receptors (TCRs) recognizing intracellular targets presented as peptides in the context of HLA (pHLA) molecules as the critical molecular mediators of deep and long-lasting anti-tumor responses in a few solid tumor patients with complete responses to checkpoint inhibitor (CPI) treatment. The pHLA targets recognized by these TCRs may thus represent the long sought-after, ideal targets for solid tumor targeting with high-potency therapeutic modalities. Here we describe an immune-response guided target identification platform (3T-TRACE) and its utility to identify novel, intracellular targets, and their corresponding T cell receptors (TCRs) for the treatment of solid tumors. We provide an overview of the frequency and expression levels of the most promising, novel cancer testis antigens (CTAs) and tumor associated antigens (TAAs) identified. **Methods**By employing single cell sequencing methods,

we identified TCRs that are uniquely associated with CPI responses and generated recombinant TCRs for screening against yeast display libraries expressing synthetic peptides in the context of the matching HLA complex, reviewed in (Figure 1). We analyzed over 140,000 CD8/CD4+ TILs from over 90 patient tumor biopsies and/or peripheral blood samples, including colorectal, lung, breast, ovarian, melanoma, and renal cancer (Figure 2). High value TCRs were selected based on the following criteria: high TCR clonality, immunophenotype indicative of T cell activation, shared alpha and/or beta TCR chains across patient samples (Public TCRs) and TCRs with anti-tumor activities. Statistical- and machine learning algorithms were developed to predict human peptides from the library selection data. Predicted target peptides were tested in *in vitro* T cell co-cultures for TCR activation. Antigen processing assays or mass spec analysis was employed to validate immunological processing of the epitope to identify bona fide targets.**Results**Among the first 100 targets identified by 3T-TRACE, we found 15 novel cancer testis antigens (CTAs) and 42 tumor associated antigens (TAAs) that were not previously described as targets in CPI treated solid tumor patients (Figure 3).**Conclusions**By combining TCR repertoire profiling with a library-based, antigen identification approach we found a strong enrichment of CTA- and TAA targets recognized by TCRs on TILs from CPI responders (Figure 4). Our findings are consistent with previous reports, demonstrating long-lasting responses induced by epitope spreading and CD8 T cell responses towards self-antigens, including CTAs and TAAs.

Novel assay technology

LBA007 Discovery and characterization of oncogenic KRAS:RAF1 conformational modulators with *in vitro* and *in vivo* MAPK inhibition Elizabeth Donohue Vo[1], Juan Luengo[2], Hong Lin[2], Jerry Chen[2], Ben Reid[1], Brooke McDonough[1], Norman Fultang[2], Jillian Silva[1], Cameron Pitt[1]. [1]Quanta Therapeutics, Inc., South San Francisco, CA, [2]Quanta Therapeutics, Inc., Malvern, PA.

Oncogenic mutations in the RAS family are the most frequently occurring among human cancers. The recent development of KRAS-targeted covalent inhibitors display efficacy in KRAS-mutant tumors; however, this approach is limited to $KRAS^{G12C}$ mutant cancers. Here, we describe a novel drug discovery program to target the full-scope of mutant RAS-driven cancers through allosteric inhibition of the oncogenic KRAS-RAF1 signaling complex. To identify novel allosteric inhibitors, we developed a second harmonic generation (SHG) assay to detect conformational changes in the KRAS-RAF1 membrane-bound complex. Fully processed farnesylated and methylated KRAS4b-G12D protein ($KRAS^{G12D}$-FMe) was complexed with the RAF1 N-terminal RAS-binding domain (RBD) and cysteine rich domain (CRD) and immobilized on a phosphatidylserine-enriched bilayer. The RAF1RBD-CRD protein was rendered SHG-active by chemical conjugation and tethered to the bilayer by $KRAS^{G12D}$-FMe, thus replicating the physiological complex orientation. Ligands that disrupted or altered the orientation of the complex relative to the surface were identified from a curated diversity library of 60,000 chemical compounds based on % SHG signal change relative to baseline. Validated hit compounds were selected for SAR characterization and development wherein newly synthesized molecules were assayed by SHG and inhibition of cellular ERK phosphorylation by HTRF. Quanta (QTX) molecules were confirmed to rapidly inhibit ERK phosphorylation across multiple RAS-mutant cell lines by Western blot. Biochemical cellular target engagement studies reveal inhibition of RAF1:BRAF dimerization downstream of allosteric modulation of

the KRAS:RAF1 signaling complex. Mass spectrometry with a photoactive derivative identified modified residues clustered along the KRAS:RAF1 interface, thus providing structural evidence for this unique mechanism of action. In addition to MAPK inhibition, we observe inhibition of cellular proliferation across mutant RAS cell lines. Cellular growth assays in the presence of QTX molecules combined with the SHP-2 inhibitor, RMC4550, displayed synergistic growth inhibition, providing a rationale combination therapy. Moreover, RAS-mutant cell-line derived xenograft tumors treated with QTX inhibitors elicited significant tumor growth inhibition and dose-dependent reductions in the pharmacodynamic MAPK targets, P-ERK and DUSP6, which is consistent with anti-tumor activity. This research illustrates a promising approach towards the development of mutant RAS-targeted inhibitors with a unique mechanism of action and target engagement.

Therapeutic agents: biological

LBA008 CS5001, a novel ROR1-targeting antibody drug conjugate (ADC) armed with tumor-cleavable β-glucuronide linkers and pyrrolobenzodiazepine (PBD) prodrugs for hematological and solid malignancies Fu Li[1], Yongwang Li[2], Lan Zhang[2], Clarence K. Zhang[1], Hui-Han A. Hu[1], Juan Zhang[2], Ying Pan[1], Jinwon Jung[3], Sang Hoon Lee[3], Hyun-Min Ryu[4], Yun-Hee Park[4], Haixiang Yu[1], Archie N. Tse[1]. [1]CStone Pharmaceuticals, Shanghai, China (Mainland), [2]CStone Pharmaceuticals, Suzhou, China (Mainland), [3]ABL Bio, Seongnam, Korea, Republic of, [4]LegoChem Biosciences, Inc., Daejeon, Korea, Republic of.

ROR1 (receptor tyrosine kinase-like orphan receptor 1) is an oncofetal protein prevalently expressed in a variety of hematological and solid malignancies but largely absent in normal adult tissues, making it an attractive ADC target. CS5001/ABL202/LCB71 is an ADC composed of a human monoclonal antibody targeting ROR1, site-specifically conjugated with a proprietary cleavable β-glucuronide linker to a prodrug of PBD dimer. Both linker and prodrug are selectively cleaved by the lysosomal b-glucuronidase, which is overexpressed in many cancerous cells, to allow tumor-selective release of the DNA-crosslinking PBD dimer. *In vitro* and *in vivo* pharmacology of CS5001 were evaluated and benchmarked against CS5001-BMK1 (an MMAE-based ROR1 ADC). Binding specificity, affinity and internalization were determined by Octet, ELISA and flow cytometry. Cytotoxicity and predictive biomarkers were evaluated in 20 human cancer cell lines with various levels of ROR1. *In vivo* efficacy was studied in Jeko-1 mantle cell lymphoma and MDA-MB-231 triple-negative breast cancer xenografts. CS5001 bound to human ROR1, but not ROR2, with a K_D value of 1.38 nM. CS5001 has cross reactivity against mouse, rat and cynomolgus ROR1 at similar affinities. Upon binding, CS5001 was rapidly internalized by ROR1-expressing cancer cells at 37 ℃. CS5001 demonstrated potent cytotoxicity towards ROR1 high expressing cell lines such as Jeko-1 and MDA-MB-231, with an IC_{50}value of 0.161 and 0.900 nM, whereas the IC_{50} value of CS5001-BMK1 was 10.8 nM and 129 nM, respectively. The growth inhibition activity of CS5001 but not CS5001BMK was significantly correlated with ROR1 density (Pearson correlation= -0.697, $P = 6.4 \times 10^{-4}$). CS5001 potently induced DNA damage in MDA-MB-231 cells, and resulted in increased apoptosis and G_2-M cell cycle arrest in a concentration-dependent manner, indicating a mechanism of cytotoxicity consistent with that of the PBD payload. CS5001 exhibited prominent antitumor activity in both Jeko-1 and MDA-MB-231 xenograft models in a dose-dependent manner. In Jeko-1, complete regression (CR) was observed after a single administration of CS5001 at

1mg/kg (approximate 1/5th the maximum tolerated dose (MTD) in mice), whereas no CR was achieved for CS5001-BMK1 at the highest dose (2.5mg/kg, QWx3). When both CS5001 and CS5001-BMK1 were administrated at 1/20th of their respective MTDs, CS5001 (0.25mg/kg, single dose) produced significantly superior efficacy to CS5001-BMK1 (2.5mg/kg, single dose) with tumor growth inhibition (TGI) of 60% and 38%, respectively (p=0.018). In MDA-MB-231, 106% and 65.7% of TGI were observed for CS5001 at 1mg/kg (single dose) and CS5001-BMK1 at 2.5mg/kg (QWx3) respectively. CS5001 exhibited potent and selective cytotoxicity to a variety of ROR1-expressing cell lines and showed remarkable *in vivo* antitumor activity. ROR1 cytometric density predicts sensitivity to CS5001 *in vitro*. CS5001 is a promising therapeutic candidate for ROR1-expressing hematological and solid malignancies with precision medicine potential.

Therapeutic agents: other

LBA009 Orally available ENPP1 inhibitor, TXN10128, restores STING activation in tumor microenvironment and confers anti-tumor responses in combination with immune checkpoint blockade. Sungjoon Kim[1], Imran Ali[1], Ahran Yu[1], Sun woo Lee[1], Sung young Park[1], Jung hwan Choi[1], Yong-yea Park[1], Chan sun Park[1]. [1]TXINNO Bioscience inc., Yongin, Korea, Republic of.

Background Recently, there are growing needs of immune modulators that can convert cold tumors into hot tumors, which can be utilized for combination treatment with existing immune related therapies. An orally available small molecule that is capable of activating innate immune response can be an ideal candidate to meet those needs. Upon binding to 2’3’-cGAMP, STING activates TBK1-IRF3 signaling cascade in cancer cells as well as host cells and promotes innate immune responses against cancer cells, leading to T cell mediated anti-tumor immunity by facilitating T cell priming and infiltration. Ectonucleotide pyrophosphatase/ phosphodiesterase 1 (ENPP1), a transmembrane protein highly expressed in subset of cancer cells, has been known to hydrolyze 2’3’-cGAMP and negatively regulates the STING activation. Inhibition of ENPP1 can prevent 2’3’-cGAMP degradation in tumor microenvironment (TME) and restore STING activation, leading to innate immune responses. It has been shown that ENPP1 inhibitor can enhance anti-tumor immunity by restoring 2’3’-cGAMP level in TME and can prevent tumor growth when co-treated with immune checkpoint blockade (ICB) as well as with radiation therapy. Methods The activity of ENPP1 inhibitors were measured in enzymatic assay using 2’3’-cGAMP as substrate and in cellular STING activation assay using IRF3-responsive reporter. Also, the effects on innate immune response were estimated in LIN (Lymphocyte INfiltration) assay measuring lymphocyte infiltration and TMED (TME in Dish) assay measuring tumor spheroid growth and immune cell activation. In vivo efficacy of TXN10128 (PO dosing) was evaluated in MC38 syngeneic model in combination with and anti-PD-L1 (IV dosing). Tumor growth was monitored and immune cells in tumor were analyzed. Results TXN10128 inhibited ENPP1 activity with single digit nanomolar potency in enzyme assay and further induced STING activation in cellular assay. In 3D spheroid co-culture condition, TXN10128 enhances lymphocyte infiltration and inhibits the spheroid growth. TXN10128 has a drug-like properties desirable for oral administration in terms of physicochemical properties and pharmacokinetics parameters. Systemic exposure of TXN10128 by PO dosing resulted in synergistic tumor growth inhibition with anti-PD-L1 antibody and improved tumor-infiltrating lymphocytes (TIL) profile

as expected in MC38 syngeneic model. Additional in vivo studies are on-going to expand applicable cancer types to TXN10128 treatment. Conclusions TXN10128 is a potent and selective ENPP1 inhibitor that can exert immune response in 3D co-culture condition, which is consistent with tumor growth inhibition and favorable TIL profile in animal model. Together with promising drug-likeness, these studies demonstrate that TXN10128 is a suitable candidate for clinical investigation as a combination partner with existing immunotherapies.

Cellular responses to therapy

LBA010 Therapeutic stimulation of nucleic acid receptor RIG-I enhances efficacy of kinase inhibitor treatment in oncogene-driven tumors Johannes Brägelmann[1], Carina Lorenz[2], Sven Borchmann[3], Kazuya Nishii[4], Julia Wegner[5], Jenny Ostendorp[6], David Ast[6], Alena Heimsoeth[6], Philipp Lohneis[6], Thomas Zillinger[5], Roland Ullrich[3], Kadoaki Ohashi[4], Martin Schlee[5], Martin Sos[6]. [1]Mildred Scheel School of Oncology, Molecular Pathology, Institute of Pathology, University Hospital of Cologne, 50937 Cologne, Germany, Cologne, Germany, [2]Molecular Pathology, Institute of Pathology, University Hospital of Cologne, 50937 Cologne, Germany, Cologe, Germany, [3]Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, University of Cologne, Cologne, Germany., Cologne, Germany, [4]Department of Hematology, Oncology and Respiratory Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, Okayama, Japan, [5]Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany., Bonn, Germany, [6]Molecular Pathology, Institute of Pathology, University Hospital of Cologne, 50937 Cologne, Germany, Cologne, Germany.

Introduction: A major challenge in the treatment of kinase driven tumors is the inevitable emergence of resistance and the limited efficacy of subsequent treatments including immunotherapy.**Methods:** To gain deeper insights into the adaptive processes of cells surviving kinase inhibition we studied the mechanisms enabling cell persistence despite targeted treatment across various in vitro and in vivo tumor models. This included transcriptomic profiles of EGFR-, BRAF-, HER2- or ALK-driven colon, melanoma and lung cancer cells and of melanoma patients during kinase inhibition. To investigate underlying mechanisms we employed CRISPR/Cas9 and overexpression experiments and stimulation of nucleic acid receptors (NARs) TLR3, cGAS and RIG-I with synthetic agonists to assess combination therapies. Finally, we used humanized tumor xenografts and a syngeneic EGFR-mutant lung cancer model to validate the inflammatory effects of kinase inhibition and the efficacy of adding RIG-I agonist IVT4 to kinase inhibition in vivo.**Results:** Analysis of dynamic transcriptomic changes during kinase inhibition by time-series RNA-seq of oncogene-dependent cells displayed rapid induction of an interferon gene signature and suppression of MYC- and E2F-target genes. This was accompanied by induction of p27 protein levels, cell cycle arrest and an increase of b-galactosidase positive cells, indicating a senescence-associated inflammatory response that occurred in a cell autonomous manner in vitro and in vivo. In vivo, EGFR inhibition also increased T-cells, but anti-PD-1 therapy following EGFR inhibitor pre-treatment did not prevent tumor recurrence. Interestingly, the kinase inhibitor induced interferon signature was independent of caspase activation and of the nucleic acid sensing machinery. Instead, functional experiments indicated that induction of interferon signaling was dependent on inhibition of MAPK signaling and subsequent IRF1 activation, providing a potential mechanism of action. Numerous innate

immune pathway members including NARs MDA-5, TLR3 and RIG-I were upregulated, which we aimed to exploit therapeutically. Evaluation of NAR agonists showed that only stimulation of RIG-I with its agonist IVT4 evoked cytokine secretion and synergistically enhanced cell death in vitro. In humanized xenografts and syngenic immunocompetent mouse models adding IVT4 led to additional tumor shrinkage after EGFR-inhibition and decreased T cell exhaustion. Effects of IVT4 on tumor volumes were lower in immunodeficient xenografts and after NK- or CD8-cell depletion in the syngeneic model indicating that immunological influences in addition to tumor cell intrinsic effects contribute to the efficacy in vivo. **Conclusion:** Taken together, our findings show that kinase inhibition may promote tumor cell-autonomous, senescence-associated inflammatory signaling and may sensitize oncogene-driven tumors to therapeutic intervention using RIG-I agonist IVT4. Our results thus provide a novel treatment strategy and a basis to explore combining immunotherapy with kinase inhibition in the future.

Cell cycle regulators

LBA011 Discovery of combination targets of CDK inhibitors from CRISPR screens
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CRISPR/Cas9 functional genetic screens are a powerful tool to discover drug targets in cancer. While many screens have been performed for identification of intrinsic cancer vulnerabilities, synthetic lethalities of targeted therapies remain largely unexplored, despite the fact that they may provide a potential targets and pathways for potential combination therapies, advance understanding of target biology, and hone patient selection strategies. To discover novel combination targets of Palbociclib, a CDK2 inhibitor, and a CDK2,4,6 inhibitor, we have screened a genome-wide CRISPR library in priority tumors with genetic background of interest, including ER+ breast cancer (HCC1428, MCF7), cyclin E amplified breast (HCC1806) and/or ovarian cancer (OVCAR4), and pancreatic cancer cell lines (MIAPACA2, Hs766T, SU8686). We analyzed the samples by condition and compared using MAGeCK-MLE for gene-level drop out and enrichment to identify common sensitizers and resistors to different CDK inhibitors. Differential genes, complexes, and pathways were assessed, and we have found that genes involved in G1/S transition (CyclinE/CDK2) are top Palbo sensitizers and CDK1/CyclinA, CDC25A/B, MuvB complex (MYBL2 and FOXM1) are major CDK2 sensitizing genes. In addition to sensitizers, we have identified genes with strong signals of resistance to CDK inhibitors. Furthermore, we highlighted major mechanisms influencing response to CDK2 inhibitors; 1) Regulation of DREAM may influence response to CDK inhibitors, 2) Regulation of cell cycle check points mediated by CDC25. Our targeted CRISPR KO screens in different CDKi combinations have identified common sensitizers and mediators of resistance to CDK inhibitors, and highlighted biology worthy of deeper consideration and offers opportunities to consider new directions for therapeutic intervention into the cancer cell cycle

Drug screening

LBA012 Engineered mini-livers for high-throughput tumor organoid screening of prodrugs
Peyton J Tebon[1], Alice Soragni[1]. [1]UCLA, Los Angeles, CA.

Several anti-cancer compounds, including chemotherapy and targeted agents such as ifosfamide, cyclophosphamide, and imatinib, are administered as prodrugs which are metabolized to become pharmacologically active. Prodrugs are particularly difficult to study in vitro or ex vivo as many are activated in the liver by Cytochrome P450 enzymes. Preclinical studies of these compounds are restricted to *in vivo* models, as cancer cells typically do not express the enzymes required to transform prodrugs into their active form. While animal models effectively recapitulate the complexity of systemic drug metabolism and distribution, *in vivo* studies are time-consuming, expensive, and unsuitable for high-throughput drug screening studies. In previous work, we have developed a platform for screening patient-derived tumor organoids that yields results within a week of surgery (Phan et al, *Commun Biol* **2,** 78, 2019; Nguyen and Soragni, *STAR Protoc* **1**, 2, 2020; Al Shihabi et al, *bioRxiv*, 2021). Tumor organoids are promising pre-clinical models as they closely recapitulate features of the parent tumor, including drug responses. Here, we expand the physiological relevance of tumor organoid screenings by including mini-livers to metabolize prodrugs of interest. To retain the ability to perform automated, high-throughput screenings, we engineered a miniature liver insert (MLI). The MLI is a removable system that facilitates the addition of liver organoids to specific wells of 96-well plates in which prodrugs are tested. The MLI is designed to integrate with our existing tumor organoid screening platform (Phan et al, *Commun Biol* **2,** 78, 2019; Nguyen and Soragni, *STAR Protoc* **1**, 2, 2020; Al Shihabi et al, *bioRxiv*, 2021), and allows for simultaneous monitoring of tumor organoids and hepatocytes with brightfield and fluorescent imaging. Hepatocytes seeded in the MLI are viable and maintain their expression of the key Cytochrome P450 enzymes responsible for the metabolism of many clinically used prodrugs. Finally, we demonstrate that incorporating the MLI into prodrug screening experiments sensitizes tumor organoids to treatment with chemotherapeutic and targeted agents. Due to the facile integration with existing screening protocols, the MLI is a simple and effective system for studying anti-cancer prodrugs ex vivo. The MLI is a novel tool that facilitates the co-culture of functional liver organoids with clinically relevant tumor organoid models for drug discovery studies and precision medicine applications.

Genomics, proteomics, and target discovery

LBA013 Phosphoproteomics reveals active drug targets on pathways of resistance and predicts response to midostaurin plus chemotherapy in FLT3 mutant-positive acute myeloid leukemia Luis Veiga Nobre[1], Celia Colomina Basanta[1], Salvatore Federico Pedicona[1], Arran David Dokal[1], Andrea Arruda[2], Ryan Smith[1], Calum Greenhalgh[1], Francesca Patella[1], Pedro Maria Casado-Izquierdo[3], Bela Wrench[4], Jane Theaker[1], Andrew Thompson[1], Mark D. Minden[2], John G. Gribben[4], David James Britton[1], Pedro Rodriguez Cutillas[3]. [1]Kinomica Ltd, Alderley Park, Macclesfield, United Kingdom, [2]Princess Margaret Cancer Centre, Toronto, Canada, [3]Centre for Genomics and Computational Biology, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, [4]Centre for Haemato-oncology, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom.

Background: Midostaurin (mido) is approved for treatment of FLT3 mutant-positive ($FLT3^+$) acute myeloid leukemia (AML). However, FLT3 mutation is not the only determinant of mido sensitivity. Here we report phosphoprotein signatures which predict response to chemotherapy (chemo) plus mido, and identify active drug targets on potential resistance pathways.**Methods:** Samples collected at diagnosis, post-treatment and relapse from $FLT3^+$ patients treated with

chemo+mido were obtained from the Leukemia Tissue Bank at the Princess Margaret Cancer Centre. Peptides and enriched phosphopeptides from bone marrow (BM) and peripheral blood (PB) mononuclear cells were quantified using liquid chromatography-tandem mass spectrometry. Signatures for BM/PB diagnosis samples were analyzed independently and used to train a classification machine learning algorithm to group patients (n=54) based on response to treatment. Additional features (e.g. genetic mutations) were also analyzed. Kaplan-Meier and Log-Rank test methods were used to assess differential survival between patient groups. To investigate pathways potentially driving resistance to chemo+mido, differential protein phosphorylation indexes were identified through comparison of post-treatment or relapse samples to paired diagnosis samples. To account for population heterogeneity, a filter was applied based on frequency of observation. Activated pathways potentially driving resistance were identified with functional enrichment tools and kinase-substrate enrichment analysis. Statistical significance of enrichment were determined using parametric methods and p-values adjusted for multiple testing using the Benjamini-Hochberg method.**Results:** Patients positive for a signature consisting of 26 phosphorylation sites showed a markedly longer survival time than negative patients (PB: 269 vs 76 weeks, Log-Rank p=1.30e-05; BM: 241 vs 56, Log-Rank p=2.13e-09). This signature partially overlapped with an ex-vivo signature of response to mido, described previously by Casado *et al* (Leukemia, 2018). A proteomic signature was also identified, with positive patients showing a longer survival time than negative patients (PB: 330 vs 173 weeks, Log-Rank p=5.0e-04; BM: 460 vs 156, Log-Rank p=5.2e-06). Key, diverging phosphorylation site signatures were identified between patients with refractory disease/early relapse and patients with complete response and no relapse or death within 2 years post-treatment. Pathways with increased activity in post-treatment or relapse specimens were associated with molecular functions such as regulation of cell proliferation, migration, differentiation and anti-apoptosis.**Conclusions:** We identified phosphoproteomic and proteomic signatures that differentiate survival mediated by response to chemo+mido. While the former was more predictive, both may enable further stratification of $FLT3^+$ AML receiving mido treatment. Drug targets on pathways demonstrating increased activity in relapse/refractory cases may play a role in resistance; this will be determined in follow-up inhibitor studies.

Bioinformatics

LBA015 Accelerating clinically-translatable discoveries using a network-and RNA-based precision-oncology framework Alessandro Vasciaveo[1], Min Zou[1], Juan Martín Arriaga[1], Francisca Nunes De Almeida De Almeida[1], Eugene Douglass[1], Michael Shen[1], Andrea Califano[1], Cory Abate-Shen[1]. [1]Columbia University Medical Center, New York, NY.

Despite recent advances, prioritizing therapy at the individual patient level remains challenging. In fact, inter-patient tumor heterogeneity remains one of the major challenges in cancer therapy, making it difficult to optimize available treatments on an individual patient basis. Likewise, the systematic prediction of drug sensitivity *in vivo* is still a major challenge in translational biology, where targeted therapeutics are currently selected based on the presence of either actionable oncogene dependencies or aberrant cellular mechanisms. A further challenge is the limited availability of models that faithfully recapitulate the biology, complexity, and heterogeneity of human tumors, including their interaction with a competent immune system. To address these challenges, we introduce OncoLoop, a highly-generalizable, network-based precision medicine

framework to triangulate between available mouse models, human tumors, and large-scale drug perturbational assays with *in vivo* validation to predict personalized treatment. OncoLoop requires only transcriptomic data (i.e., RNA-seq profiles) and leverages regulatory network analysis to (a) identify cognate models based on conservation of patient-specific Master Regulator (MR) proteins and (b) prioritize drugs based on their ability to invert the activity of MR proteins (MR-inverters), using drug perturbation profiles in cognate cell lines. As proof-of-concept, we applied OncoLoop to prostate cancer using a series of genetically engineered mouse models (GEMMs) that capture a broad range of phenotypes, including metastatic, castration-resistant and neuroendocrine disease. Indeed, ~70% of patients in published cohorts had at least one high-fidelity matched GEMM. Drugs targeting shared Master Regulator dependencies of a patient and its cognate GEMM(s) were predicted using perturbational profiles of >300 drugs in MR-matched cell lines, resulting in an 80% validation rate in GEMM allografts and human xenografts. This network-based approach is highly generalized and can be applied to both cancer and non-cancer-related contexts.

Hormonal agents and therapy

LBA016 Androgen Receptor (AR) N-Terminus-Domain-Binding Small Molecule Degraders for the Treatment of AR Splice Variant-Positive Castration-Resistant Prostate Cancer Ramesh Narayanan[1]. [1]University of Tennessee Health Science Center, Memphis, TN.

Prostate cancer is the second leading cause of cancer-related deaths in men in the United States. Over 30,000 men die of prostate cancer each year in the U.S. and the deaths predominantly occur due to late-stage castration-resistant prostate cancer (CRPC). Androgen receptor (AR) is the primary therapeutic target in castration-sensitive prostate cancer and CRPC, with over 85% of the cases expressing AR. About 30% of CRPC fail to respond to ligand-binding domain (LBD)-binding AR-signaling inhibitors, while the remaining develop resistance after an initial period of response of 12-24 months. Expression of AR splice variants (AR-SVs) that lack LBD has been identified as one of the primary causes for resistance development to AR-signaling inhibitors. AR-SVs also promote aggressive CRPC growth, resulting in shorter overall survival. We discovered novel AR N-terminus domain (NTD)-binding small molecule, GTx-534 that induce degradation of AR and AR-SV proteins, resulting in loss of AR signaling. Surface Plasmon Resonance (SPR), intrinsic fluorescence biophysical studies and thermal stability analysis with purified AR activation function-1 (AF-1) domain in the NTD showed GTX-534 binding. Notably, GTx-534 significantly inhibited AR and AR-V7-positive 22RV1 cell line xenograft in castrated immune compromised rodents with a tumor growth inhibition (TGI) of over 50% ($p<0.005$), while enzalutamide failed to inhibit the 22RV1 tumor growth. Serum PSA concentrations of 22RV1-bearing animals were reduced to background levels by GTx-534 ($p<0.01$), but not by enzalutamide. Genome-wide expression studies demonstrated a distinct mechanism of action for GTx-534 in AR-SV-positive tumors. The results from 22RV1 xenograft studies were validated in LuCaP 86.2 patient-derived xenograft (PDX) that express AR v567es, another AR variant that lacks the LBD widely expressed in CRPC. GTx-534 completely inhibited LuCaP 86.2 tumor growth with a TGI of greater than 90%, while enzalutamide failed to alter the tumor growth. These results provide preclinical proof-of-concept for the efficacy of GTx-534 in AR and AR-SV-positive CRPC. Disclosure. The work presented in here was funded by a Sponsored Research Agreement from Oncternal Therapeutics, Inc. to RN and DDM and by

National Cancer Institute grant R01CA229164 to RN. RN is a consultant to Oncternal Therapeutics Inc. GFK and JBB are employees of Oncternal Therapeutics Inc.

Therapeutic agents: biological

LBA018 Discovery of novel functional TROP2 antibodies for treatment of epithelial cancers Israel Matos[1], Hiba Zahreddine[2], Yahya Ashraf[2], Aditya Pandey[2], Emily Chen[1], Liying Gong[2], Alex Zhou[1], Claire Bonfils[2], Aniel Moya[2], Yun Cui[2], Xiaowei Wang[1], Elijus Undzyz[1], Shugang Yao[2], Jacynthe Toulouse[2], Dominic Hou[2], Gordon Ngan[1], Luis da Cruz[1], David Young[2]. [1]KisoJi Biotechnology Inc., Toronto, ON, Canada, [2]KisoJi Biotechnology Inc., Montreal, QC, Canada.

Trophoblast cell surface antigen-2 (TROP2) is a membrane-bound protein with an extracellular domain highly expressed in a wide range of late-stage epithelial cancers. TROP2 upregulation in solid cancers is associated with increased tumor aggressiveness, metastasis, and an overall decreased survival in large groups of difficult-to-treat cancers, making it an attractive target for cancer therapy. TROP2 positive cancers have been successfully targeted by antibody-drug conjugates (ADC) but not naked functional antibodies. ADC's have associated drug toxicity in healthy tissues, off-target effects, and payload delivery issues. Given ADC dose-limiting side effects, narrow therapeutic windows, and efficacy limitations, there is a pressing need to improve anti-TROP2 therapeutics for patients who are poorly served by current therapies, if at all. Understanding these needs, we applied our proprietary heavy chain-only antibody (HCAb) transgenic mouse platform to the creation of therapeutic anti-TROP2 antibodies. After immunization, anti-TROP2 antibodies were selected using VHH (variable region of HCAbs)-phage-display. Due to their size and shape, VHH antibodies target a broader spectrum of epitopes compared to full size VH/VL antibodies. Further, VHH antibodies are associated with high affinity, stability, and robustness. In this abstract, we describe five novel anti-TROP2 HCAbs without ADC payloads that induce *in vivo* tumor regression in murine xenograft models of human cancers. When tested in an *in vitro* viability assay with the MDA-MB-453 triple negative breast cancer (TNBC) cell line, a novel anti-TROP2 HCAb significantly reduced cancer cell numbers compared to other anti-TROP2 antibodies in the absence of complement or effector cells. In the *in vivo* MDA-MB-453 TNBC established xenograft model, tumor regression was achieved with five different TROP2 HCAbs at 8mg/kg administered once per week, and in the *in vivo* established model of MDA-MB-231 strong anti-tumor efficacy was also achieved. In binding cross-reactivity experiments, anti-TROP2 HCAbs showed similar binding to human and cynomolgus TROP2 and low binding to the mouse or rat orthologs. Anti-TROP2 HCAbs bind to a panel of TROP2-expressing human cancer cell lines, including a wide range of human epithelial tumours, such as breast, colon, lung and pancreatic cancers. Epitope binding by competitive ELISA showed that binding to TROP2 by these HCAbs could not be competed by hRS7 or other anti-TROP2 antibodies demonstrating that they target epitopes different from known TROP2 antibodies. Our preclinical data demonstrates the potent anti-tumor activity on TROP2-positive cancers by novel anti-TROP2 HCAbs without the need for cytotoxic payloads. These results and additional data highlight the feasibility of developing functional anti-TROP2 antibodies for clinical use which avoid the challenges of ADCs.

Protein-protein interaction

LBA019 The immune checkpoint protein BTN1A1 suppresses T cell activation through interactions with Gal9 and PD-1 Ezra M Chung[1], Young-Seung Kim[1], Chunai Wu[1], Andrew H Park[1], Hyunjin Jung[2], Stephen S Yoo[1]. [1]STCube Pharmaceuticals, Gaithersburg, MD, [2]STCube, Seoul, Korea, Republic of.

Butyrophilin (BTN) proteins are members of the B7 immunoglobulin superfamily and exhibit well-characterized immunomodulatory functions in mammals. We have recently identified BTN1A1 as an immune checkpoint protein prominently upregulated in response to acute inflammatory insults. Further *in vitro* and *in vivo* assays have validated BTN1A1 as an immune checkpoint target, particularly for patients refractory to anti-PD-1/PD-L1 antibody treatment. We have also developed a humanized antibody targeting human BTN1A1, hSTC810, which is expected to enter into Phase I clinical trials in the first quarter of 2022. In this study, a cell microarray from Retrogenix (Whaley Bridge, UK) was used to identify binding partner(s) for the extracellular domain of human BTN1A1. Through this screening approach, we found that BTN1A1 binds to galectin-1 (Gal1), galectin-9 (Gal9), and neuropilin 2 (NRP2). These three putative binding partners could specifically bind to wild-type BTN1A1 but not to this protein's unglycosylated (2NQ) form. Of these three targets, immunoprecipitation and Biacore binding assays revealed that Gal9 exhibited the greatest affinity for human BTN1A1, followed by Gal1, with respective K_D values of 22.7 nM and 1.88 μM - an 83-fold difference. Gal9 binding to human BTN1A1 was dependent on BTN1A1 glycosylation status and required the carbohydrate recognition domain (CRD) of Gal9. As Gal9 is a known PD-1-binding protein, the K_D of Gal9 for PD-1 was additionally assessed and found to be 19.7 nM. These results thus predicted the potential formation of BTN1A1/Gal9/PD-1 complexes. Consistent with these predictions, immunoprecipitation assays performed using cells expressing Myc-tagged versions of these three proteins demonstrated the formation of BTN1A1/Gal9, PD-1/Gal9, and BTN1A1/Gal9/PD-1 complexes. CRISPR-mediated BTN1A1 knockout in Jurkat T cells induced both PD-1 expression and T cell activation. BTN1A1 also suppressed T cell receptor (TCR) signaling in Jurkat cells, and the addition of exogenous recombinant Gal9 protein further blunted such BTN1A1-mediated TCR-signaling downregulation. Such downregulation was not observed in PD-1 knockout Jurkat cells. Together with the observation that BTN1A1 does not bind to PD-1 directly, the data suggest that BTN1A1 suppresses T cell activation by interacting with PD-1 through Gal9. As high Gal9 expression levels are correlated with poor prognosis in multiple cancers, our results highlight this BTN1A1-Gal9-PD-1 axis as a novel therapeutic target for immunotherapeutic drug development. (1) Chung EM, Bong YS, Kim YS, Park A, You YO, Sharma A, Lin SH, Lee YJ, Jung H, Yoo SS. BTN1A1: a novel immune checkpoint for cancer immunotherapy beyond the PD-1/PD-L1 axis. Cancer Res 2021;81(13_Suppl): Abstract nr 1643.

In vitro and in vivo models for targets

LBA020 Targeting FGFR2c isoform, a novel therapeutic target with FGFR inhibitor in endometrial cancer Asmerom T Sengal[1], Vanessa Bonazzi[2], Olga Kondrashova[3], Lewis Perrin[4], Naven Chetty[5], Deborah Smith[6], Antonio Gil-Moreno[7], Eva Colas[7], Pamela M Pollock[1]. [1]Queensland University of Technology (QUT)/Translational Research Institute (TRI), Brisbane, QLD, Australia, [2]University of Queensland/Translational Research Institute (TRI), Brisbane, QLD, Australia, [3]QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia, [4]Mater Cancer, Mater Hospital, Brisbane, QLD, Australia, [5]Mater Hospital, Brisbane, QLD, Australia,

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Purpose: Endometrial cancer (EC) is the most frequently diagnosed gynaecological cancer. The majority of women with EC are treated surgically and have a good outcome, however 25-30% of patients presenting with metastases or recurrent disease do not have effective therapies and have <12 months survival. Recent investigations demonstrated radio-chemotherapy has little benefit for women with high-risk EC within the deficient mismatch repair (dMMR) and p53 wild type (p53wt) molecular subtypes. We reported isoform switching from the FGFR2b (epithelial) splice-isoform to the FGFR2c (mesenchymal) splice-isoform in 40% of dMMR and 30% of p53wt ECs. This isoform switching was associated with adverse clinicopathologic markers, shorter recurrence free survival and disease specific survival in the Canadian cohort used to identify and validate the ProMisE molecular risk stratification approach. The objectives of the current study were i) to identify patient derived xenograft (PDX) models with FGFR2c expression and develop PDX derived organoids (PDXOs) for preclinical drug testing, ii) assess the efficacy of the BGJ398 FGFR inhibitor (FGFRi) in FGFR2c expressing models and generate preclinical data that would support an early phase clinical trial in FGFR2c stratified EC patients.**Method**: BaseScope RNA ISH was used to detect FGFR2c expression in patient tumours, PDX models and PDXOs. PDXOs derived from three independent PDX tumours were established from each of five PDX models (3 showing high FGFR2c expression, 2 showing low/no FGFR2c expression). Each PDXO culture was treated with 300nM BGJ398 (pan-FGFRi) or DMSO for 72 h and assessed using a live-dead assay and confocal microscopy. PDXs from three models were engrafted subcutaneously into 8 weeks female NSG mice and when tumours reached 100-150mm^3, mice were randomized (4 mice/arm) and treated with 30mg/Kg BGJ398 or vehicle daily for 21 days. Tumours were measured 3x/week and mice sacrificed when tumours reached 900mm3.**Results and conclusion**: FGFR2c expression was higher in PDXs representing the dMMR and p53wt subtype compared to p53mut subtype and similar expression levels were seen between patient tumours, PDXs and PDXOs. *In vitro* FGFRi with BGJ398 showed significant cell death occurred in PDXOs with high FGFR2c expression ($p< 0.0001$, 2-way ANOVA). These *in vitro* findings were validated in vivo using PDX68 carrying a FGFR2 C383R mutation and PDX52 and PDX59, both showing FGFR2 isoform switching. Significant tumour growth inhibition and a ~2-fold increase in survival was seen in all three models (PDX68, $p<0.0001$ and $p<0.007$; PDX52, $p<0.02$ and $P<0.03$; and PDX59 $P<0.0001$ and $P<0.0006$ respectively). In conclusion, our investigation revealed FGFRi (BGJ398) was effective in EC PDX models representing both mutational activation and isoform switching of FGFR2. As FGFR isoform switching occurs most commonly in the dMMR subtype where immune checkpoint inhibitors (ICIs) are approved, we propose the combination of ICIs and FGFRi may be more effective in women with FGFR2 activation compared to ICIs alone.

Drug resistance and modifiers

LBA021 Immune resistance interrogation study (IRIS): Initial report of next generation sequencing (NGS) results in patients with primary versus acquired resistance to anti-PD1/L1 antibodies Sofia Genta[1], Farnoosh Abbas Aghababazadeh[2], Ming S Tsao[2], Aaron R Hansen[2], Marcus O Butler[2], Albiruni R Razak[2], Philippe L Bedard[3], Ben X Wang[3], Pugh J Trevor[3], Sevan Hakgor[3], Jeffrey Woo[3], Benjamin Haibe-Kains[3], Lillian L Siu[3], Anna Spreafico[3].

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Background: The molecular mechanisms underlying primary versus acquired resistance to anti-PD-1/L1 antibodies have not been comprehensively evaluated across different tumor types.**Methods:** The Immune Resistance Interrogation Study (IRIS; NCT04243720) is a prospective, investigator-initiated study at the Princess Margaret Cancer Centre, aimed to perform extensive multi-omic characterization of solid tumors with primary resistance (disease progression on first imaging; or stable disease <6 months) versus acquired resistance (partial or complete response; or stable disease ≥6 months) to antiPD-1/L1 agents. A one-time fresh tumor biopsy is collected from patients (pts) who have progressed on anti-PD1/L1 antibody-based treatment as their most recent line of therapy; liquid biopsy or archived FFPE tissue is allowed as alternates when fresh biopsy is insufficient. NGS was performed using Foundation One (324 genes) or Foundation Liquid (309 genes) panels. Frequencies of disrupted genes and variants were compared in pts with primary versus acquired resistance using Fisher's exact test. The planned samples size of IRIS is 100 pts.**Results:** Among the first 35 pts enrolled, 22 (63%) had primary resistance and 13 (37%) had acquired resistance. The most common diagnosis was melanoma (17 pts; 49%); followed by head and neck squamous cell carcinoma (13 pts; 37%); endometrial cancer (2 pts; 6%); pleural mesothelioma, gastroesophageal junction and colorectal cancer (1 pt each; 3%). Foundation One was performed in 23 pts (66%), 22 of them (63%) had biopsies with sufficient quality for NGS, and 1 (3%) had the analysis performed using archival FFPE tissue. The remaining 12 pts (34%) had Foundation Liquid testing done using liquid biopsy. Thirty-three pts (94%) had at least one oncogenic alteration. Genes most frequently altered included: *TP53* in 16 patients (46%), *TERT* promoter in 12 pts (34%), *CDKN2A* in 9 pts (26%), *PIK3CA* in 6 pts (17%), and *PTEN* in 5 pts (14%). At the variant level, the most frequent alterations were: *TERT promoter -146C>T* mutation in 6 patients (17%), followed by *TERT promoter -124C>T* mutation in 5 patients (14%), *FGF19*/*FGF4*/*FGF3* and *CCND1* amplifications in 4 pts each (11%), and *MDM2* amplificat*ion, CDK4* amplification and *CDKN2A loss,* detected in 3 pts each (9%). Pts with acquired resistance had a higher frequency of *TP53* mutations (9/13 = 69%) compared to primary resistance pts (7/22 = 32%), p=0.04; however this was not significant when corrected for multiple testing. Interestingly, amplifications in *CDK4, CCND1, FGF 19/FGF 3/FGF 4, MDM2* and mutations in *NF1* were only identified in pts with primary resistant tumors.**Conclusions:** No significant differences in disrupted genes and variants were observed in the current analysis. However, this can be due to the small number of pts analyzed thus far. Accrual to this study is ongoing. A comparison of alterations in oncogenic pathways is planned to further define the genomic landscape of pts with primary versus acquired resistance to anti-PD1/PDL1 blockade.

Immune checkpoints

LBA022 IMM20324, a first-in-class, anti-interleukin-38 monoclonal antibody, rescues myeloid cell activation *in vitro* and induces robust anti-tumor responses *in vivo.*

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Immune checkpoint inhibitors that overcome T cell suppressive mechanisms in tumors have revolutionized the treatment of cancer. However, therapies targeting innate immune cell subsets that orchestrate adaptive immune responses could significantly improve the incidence of clinical response by more effectively activating a multi-lineage response to the tumor. Here, we show that IMM20324, an antibody that binds and neutralizes IL-38, enhances innate immune cell function *in vitro* and inhibits tumor growth *in vivo*. IMM20324 binds human, mouse and cynomolgus IL-38 and inhibits the binding of IL-38 to its putative receptors, IL1RAPL1 and IL-36R. *In vivo*, IMM20324 administration leads to inhibition of tumor growth and improved survival in two different syngeneic tumor models. Importantly, while IMM20324 targets innate immune cells, antibody treatment leads to the induction of immunological memory responses that induce tumor rejection following tumor rechallenge. Together, our data support the hypothesis that IL-38 plays an important role in tumor immunity and that therapies targeting IL-38 could reverse suppressive mechanisms in tumor microenvironment. IMM20324 demonstrated favorable *in vitro* potency and *in vivo* efficacy and therefore represents a promising development candidate with the potential to treat multiple cancers.

Metastasis and invasion

LBA023 Regulation and function of ZEB1 dimerization in lung adenocarcinoma progression and metastasis Mabel Perez-Oquendo[1], Roxsan Manshouri[1], Don L. Gibbons[1].
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Lung cancer is the leading cause of cancer-related death worldwide due to the ability of cancer cells to metastasize. Therefore, it is essential to expand our current knowledge of the biological processes that contribute to metastasis to guide the discovery of novel therapeutic modalities. The Epithelial-to-mesenchymal transition (EMT) is a mechanism for metastasis, which changes polarized epithelial cells into invasive mesenchymal cells. High expression of the Zinc finger E-box binding homeobox 1 (ZEB1) transcription factor is correlated to poor outcomes in cancer, including therapeutic resistance and EMT-mediated metastasis. ZEB1 has a predicted molecular weight of 125kDa; however, multiple groups have reported discrepancies in the observed molecular weight (approximately 190-250kDa). This has been attributed to dimerization mediated by post-translational modifications (PTMs). Therefore, we performed mass spectrometry and identified a novel PTM - K811 acetylation - that may regulate ZEB1 dimerization and function. To define the role of ZEB1 acetylation, we generated ZEB1 acetyl mimetic (K811Q) and deficient (K811R) mutants in the 393P lung adenocarcinoma cell line. We hypothesize that ZEB1 acetylation regulates dimerization and protein stability to promote lung adenocarcinoma progression and metastasis. We determined that the acetyl-deficient mutant (125kDa) exhibits a decreased half-life compared to WT and acetylated ZEB1, suggesting that disruption of acetylation hinders dimerization and protein stability. However, the acetyl-mimetic mutant (250kDa) protects ZEB1 from proteasomal degradation brought by the action of the E3 ubiquitin ligase SIAH1. siRNA-mediated silencing of SIAH1 increased the mRNA and protein levels of ZEB1 WT and both acetylation mutants. Importantly, silencing of SIAH1 markedly increased the half-life of 250kDa ZEB1 WT from 28.03hrs to 44.37hrs and acetyl-mimetic from 19.43hrs to 41.25hrs, as well as 125kDa ZEB1 acetyl-deficient from 9.38hrs to 12.70hrs. Although, we determined that 250kDa ZEB1 is significantly more stable than 125kDa ZEB1, the

increase in the stability by silencing SIAH1 contributes to ZEB1 acetyl-deficient binding at the promoter of its target genes *mir200c-141* and *SEMA3F* by Chromatin Immunoprecipitation. 250kDa ZEB1 WT and acetyl-mimetic promotes lung adenocarcinoma invasion and metastasis compared to ZEB1 acetyl-deficient. We further established that the stability of ZEB1 is integral to promoting these mesenchymal features. Our results suggest that PTMs (acetylation and ubiquitination) regulate ZEB1 to promote its stability and progression of lung adenocarcinoma via EMT. Future studies are focused on further understanding the role of ZEB1 dimerization to recruit co-repressors and subsequently mediate tumorigenesis, invasion and metastasis

Immune response to therapies

LBA024 IL-36γ induces immune infiltration and suppresses tumor growth in a syngeneic mouse model for pancreatic cancer Kuntal Halder[1], Daniel D Von Hoff[1], Haiyong Han[1]. [1]The Translational Genomics Research Institute, Phoenix, AZ.

Pancreatic cancer (PC) is one of the most aggressive and lethal types of cancer. It is the third leading cause of cancer-related mortality in U.S., accounting for 6% of annual death. Immunotherapeutic strategies such as immune checkpoint inhibitors have shown great success in a number of tumor types, but only with limited activity in patients with PC. Pancreatic tumor is characterized by the presence of dense stromal tissue which primarily consists of immune-suppressive fibroblasts. The immune microenvironment remains dysfunctional due to the lack of tumor infiltrating lymphocytes (TILs) and immune stimulatory molecules. Proinflammatory cytokines play a pivotal role in regulating tumor immunogenicity and antitumor immunity. Elucidating the role of proinflammatory host mediators can contribute to the development of new therapies for PC. IL-36γ, a proinflammatory mediator is important for the IL-23/IL-17-dominated inflammation and induces Th1 immune responses. The role of IL-36γ in pancreatic tumor immunity has not been well studied. In this study, we examined the expression and functional regulation of IL-36γ in PC cells and found that IL-36γ cytokine treatment induces immune infiltration and suppresses tumor growth in a murine PC model. Immunohistochemical (IHC) analysis of pancreatic ductal adenocarcinoma (PDAC) specimens showed minimal IL-36γ positive staining, thus indicating very low expression of IL-36γ in PDAC. Intratumoral treatment with recombinant IL-36γ in a syngeneic mouse model of PDAC significantly inhibited tumor growth (57.8% inhibition after two weeks of treatment) without any significant animal weight loss compared to the vehicle control. Flow cytometry analysis showed increased infiltration of lymphocyte subsets ($CD45^+$ and $CD3^+$ cells) in tumors derived from the IL-36γ treated mice. Further studies are ongoing to investigate the effect of IL-36γ treatment on dendritic cells and macrophages in PDAC tumors. Our study establishes the potential role of IL-36γ in promoting antitumor immune responses and lay the foundation for clinical application in PC (This work was supported by TGen's Women Philanthropy Council).

Tumor microenvironment

LBA025 AXL-STAT3 targeting of lung tumor microenvironments Josephine A. Taverna[1]. [1]UT Health San Antonio MD Anderson, San Antonio, TX.

The tumor microenvironment contains multiple cell types, each providing a unique functionality

in support of malignant growth. To prepare a pro-metastatic niche, a tight signaling network may be present to coordinate delivery of co-stimulatory signals among different cell subpopulations. Here, we report that AXL signaling collaborates with STAT3 to create a symbiotic ecosystem that favors partial epithelial-mesenchymal transition of cancer cells, pro-tumorigenic remodeling of fibroblasts, and M2 polarization of macrophages. To facilitate intercellular communication, AXL-regulated IL-11 secreted from cancer cells and fibroblasts binds to surface GP130 on macrophages to activate STAT3 signaling for M2 polarization. Disruption of AXL-STAT3 circuit not only compromises this signaling co-dependency in lung cancer cells implanted in xenograft mice, but also limits their ability to conscript murine fibroblasts, macrophages, and other immune cells to form a shared habitat for tumor growth and invasion. Therefore, targeting AXL-STAT3 network is an attractive strategy to render inoperative tumor microenvironments that exploit this cellular synchronicity for tumor progression.**Significance:** Combined targeting of AXL and STAT3 signaling that facilitates co-dependency between malignant and non-malignant cells in tumor microenvironments is a promising strategy for the treatment of advanced lung adenocarcinomas.

Immune response to therapies

LBA026 Survivin peptides formulated in the DPX delivery platform rather than standard emulsions, elicit a robust, sustained T cell response to survivin in advanced and recurrent ovarian cancer patients. Yogesh Bramhecha[1], Oliver Dorigo[2], Valarmathy Kaliaperumal[3], Heather Torrey[3], Walead Ebrahimizadeh[3], Kelcey Patterson[3], Brennan Dirk[3], Moamen Bydoun[3], Barry Kennedy[3], Aurelio Lobo[3], Genevieve Weir[3], Jeremy Graff[3], Stephan Fiset[3], Olga Hrytsenko[3]. [1]IMV Inc., Dartmouth, NS, Canada, [2]Stanford University, Stanford, CA, [3]IMV inc., Dartmouth, NS, Canada.

Despite improvements in peptide-based tumor antigen discovery, success in eliciting a clinically meaningful T cell response to these peptides has been limited. The choice of antigen delivery platform strongly impacts the quality, quantity, and durability of induced immune responses. DPX is a unique, water-free, lipid-based formulation that can deliver peptides, proteins, and small molecules specifically to antigen presenting cells (APCs), eliciting a targeted, robust, and sustained T cell-based immune response. DPX allows for antigenic peptides to remain at the site of injection without significant diffusion into surrounding tissues, in direct contrast to most emulsion-based delivery methods. Maveropepimut-S (MVP-S; formerly known as DPX-Survivac) is a DPX formulation that incorporates 5 HLA-restricted peptides derived from the anti-apoptotic protein survivin, which is commonly overexpressed in advanced cancers including ovarian cancer. Prior efforts with these same peptides emulsified in Montanide ISA 51 VG provided limited clinical benefit and no objective responses (Lennerz 2014). We now show in pre-clinical models that, when packaged in DPX, these peptides elicit higher avidity, more abundant and cytolytically-active survivin-specific T cells than standard emulsion-based formulations. Early clinical studies with MVP-S in advanced ovarian cancer patients have shown a persistent, survivin-specific T cell response, which in a subset of patients lasted more than 2 years. The recently completed phase 2 study of MVP-S and low-dose cyclophosphamide (CPA) in advanced and recurrent ovarian cancer subjects (DeCidE[1]), provided encouraging clinical benefit across multiple measures (ORR = 26% & DCR = 79% on target lesions, mOS = 19.9 months in evaluable patients). We now show that majority of patients in this study had survivin-

specific T cells on treatment by in vitro tetramer staining (87%) and ex vivo ELISPOT analyses (56%). Importantly, 75% of patients deriving clinical benefit had active survivin-specific T cells by *ex vivo* ELISPOT analyses. Immunophenotyping of the longitudinally collected PBMCs showed evidence of sustained T cell proliferation by tetramer staining across time and did not show increased expression of immunosuppressive markers (e.g., PD-1, Tim3), suggesting that MVP-S induced T cells remain active over time. TCRβ analyses of tumor TCR repertoires further showed the ability of *de novo* elicited survivin-specific T cells to infiltrate on-treatment tumours. Collectively, these preclinical and clinical data show that MVP-S treatment effectively elicits a robust, persistent, survivin-specific T cell response. These data also provide compelling evidence that clinical benefit in DeCidE[1] patients is most evident in those with survivin-specific T cells. Finally, these data further underscore the unique capacity of DPX technology to effectively elicit peptide-specific, T cell based immune responses when conventional formulations do not.

Other

LBA027 Mechanistic insights on the effects of the lead next generation galeterone analog, VNPP433-3β in castration resistant prostate cancer Elizabeth Thomas[1], Retheesh S Thankan[1], Puranik Purushottamachar[1], Vincent C.O. Njar[1]. [1]University of Maryland SOM, Baltimore, MD.

IntroductionMetastatic castration-resistant prostate cancer (mCRPC) remains the second leading cause of cancer-related death in men in the United States and the second most frequently diagnosed cancer among males worldwide. Dysregulated androgen receptor (AR) is associated with nearly all known cases of prostate cancer (PCa) and its expression level has been correlated with poor survival. This study describes the mechanism of action of VNPP433-3β, a next generation galeterone analog (NGGA) in inhibiting PCa.**Methods**PCa cell lines CWR22Rv1 and LNCaP were treated with VNPP433-3β and followed up using cellular thermal shift assay (CETSA), RNA seq, mRNA 5' cap-binding assay, translation and proteasome inhibition assays. Co-immunoprecipitation, molecular docking and fluorescence spectroscopy were used to study the interactions.**Results**VNPP433-3β, the lead next generation galeterone analog (NGGA) directly interacts with AR and inhibits metastatic castration resistant PCa (mCRPC) by enhancing degradation of full-length AR (fAR) and its splice variant AR-V7 in a dose-dependent manner. Moreover, VNPP433-3β drives the proteasomal degradation of fAR and AR-V7 by augmenting the interaction between AR and E3 ligases MDM2 & CHIP but disrupting HSP90 binding to AR. VNPP433-3β also impedes eIF4E phosphorylation by depleting MNK1/2 besides disrupting binding of eIF4E and eIF4G to mRNA 5' cap thereby impeding translational activity of the cancer cells. Finally, the transcriptome analyses by RNA-seq reveal that VNPP433-3β modulates transcription of several genes and activates more than 20 cellular pathways, each of which synergistically contribute to PCa inhibition. Hence, VNPP433-3β obstructs PCa by invoking 1) transcriptional regulation of AR-responsive oncogenes *via* degrading AR, 2) translational regulation by depleting MNK1/2 thereby impeding phosphorylation of eIF4E and subsequent mRNA 5'cap-dependent translation initiation and 3) affecting AR half-life through enhanced proteasomal degradation. **Conclusion**As VNPP433-3β promotes degradation of fAR and AR-V7, concurrently depletes Mnk1/2, prevents eIF4E phosphorylation and modulates

several cellular pathways to halt PCa, it could potentially be developed for clinical trials in mCRPC patients.

Tumor microenvironment

LBA029 Development of computational tools for evaluating differential protein expression relative to spatial oxygen gradients using imaging mass cytometry Mark Zaidi[1], Phoebe Lombard[2], Sheila Mansouri[2], Gelareh Zadeh[2], Trevor D McKee[3], Bradly G Wouters[2]. [1]University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada, [3]HistoWiz, New York, NY.

The solid tumor microenvironment is highly heterogeneous, conferring numerous differences in tumor cell gene expression and phenotype. One predominant driver of this heterogeneity is variable oxygenation. Low oxygen, termed hypoxia, has been shown to increase metastasis, radiation resistance, and lead to an overall poor patient prognosis. Characterizing hypoxia in patients can be used to personalize an adjuvant therapy targeting resistant hypoxic cell populations, which may otherwise survive conventional therapies. Because of the spatial heterogeneity intrinsic to hypoxia, current approaches to measure hypoxia, such as using polarographic oxygen electrodes, may underestimate the severity of hypoxia. Endogenous markers such as CA9 and VEGFA provide some semblance of evaluating hypoxia, however expression of such markers is not always solely driven by oxygenation. Multiple markers in tandem may prove to be a more reliable metric for evaluating hypoxia than individual markers alone. Here, we present an open-source collection of QuPath and Python scripts for analyzing differential protein expression relative to hypoxia gradients for imaging mass cytometry (IMC) data. IMC enables highly multiplexed imaging of tissue sections at the single cell resolution, with no spectral overlap of immunohistochemical markers. This is achieved through substitution of fluorophores with unique isotopes of heavy metals, and a mass spectrometer in place of a conventional fluorescent microscope. Previous work in our lab has identified 17 markers with extensive heterogeneity in clinical glioblastoma multiforme (GBM) patient samples. Pimonidazole, an exogenous hypoxia probe, has been administered to these patients, and is detectable through immunohistochemical techniques such as IMC. Serving as our ground truth for hypoxia, we conduct a differential gene expression analysis across regions of relative hypoxia for all markers in our panel, using a variety of supervised and unsupervised tools available in the open-source digital pathology platform QuPath, and through custom data visualization and statistical analysis tools built in Python. As such, we present an end-to-end image analysis workflow for evaluating hypoxia-regulated differential protein expression of clinical GBM cases imaged through IMC. This method is applicable for evaluating differential gene expression across multiple disease sites, multiplexed imaging modalities, and histomorphological regions of interest. Such computational methods enable high throughput biomarker discovery, while also being used to elucidate novel oxygen-dependent biological pathways.

Therapeutic agents: biological

LBA030 DPX-SurMAGE, a novel dual-targeted immunotherapy for bladder cancer, induces target-specific T cells with a favorable safety profile in preclinical model Valérie Picard[1], Valarmathy Kaliaperumal[2], Fanny Gaignier[1], Marjorie Besançon[1], Yogesh Bramhecha[3],

Olga Hrytsenko[3], Alecia McKay[3], Stephan Fiset[3], Alain Bergeron[1], Yves Fradet[1]. [1]Centre de recherche du CHU de Québec-Université Laval, Québec, QC, Canada, [2]IMV Inc, Dartmouth, NS, Canada, [3]IMV Inc., Dartmouth, NS, Canada.

Thirty to forty percent of high-risk non-muscle invasive bladder cancer (NMIBC) fail standard of care, leaving radical cystectomy as the most recommended option for these patients. Therefore, new efficient and tolerable treatments are required to prevent or delay cystectomy. The DPX platform is a unique, water-free, lipid-based formulation that can deliver peptides, proteins, and small molecules specifically to antigen presenting cells (APCs), eliciting a targeted, robust, and sustained T cell-based immune response. Peptides that are packaged in DPX are specifically taken up by APCs. Maveropepimut-S (MVP-S, formerly known as DPX-Survivac) incorporates 5 HLA-restricted peptides derived from the anti-apoptotic protein survivin and is the first DPX-based immunotherapy. MVP-S demonstrated survivin-specific and sustained T cell response along with clinical activity and limited adverse events in patients with solid and hematologic tumors. Survivin and MAGE-A9 are well characterized tumor-associated antigens (TAAs) frequently overexpressed in bladder tumours. The DPX platform was leveraged to develop a new dual-targeted T cell activating immunotherapy, DPX-SurMAGE, incorporating HLA-A2 restricted peptides of both survivin and MAGE-A9. The feasibility of packaging peptides targeting two different TAAs into the DPX platform was assessed by performing comparative immunogenicity evaluation between 2 MVP-S peptides and DPX-SurMAGE in HLA-A2 transgenic mice. The magnitude of peptide-specific T cell responses against the shared HLA-A2 survivin peptide was similar in animals immunized with either DPX-SurMAGE or MVP-S indicating that the presence of MAGE-A9 peptides did not affect the immunogenicity of the MVP-S-based survivin peptide in the DPX-SurMAGE formulation. Immunogenicity and toxicity of DPX-SurMAGE with and without metronomic cyclophosphamide (mCPA) was further evaluated. IFN-γ ELISPOT assessment demonstrated robust induction of peptide-specific T cell responses to survivin and MAGE-A9 peptide pools. These strong responses were maintained at similar levels in the long-term chronic phase of the assessments both with and without mCPA. Preliminary safety observations including Detailed Clinical Examination (DCE), body weights and organ weights showed no sign of toxicity nor significant changes in mice treated with DPX-SurMAGE with or without mCPA. There were no significant differences in injection site reactions between DPX-SurMAGE groups (with and without mCPA). The local injection site reactions for DPX-SurMAGE groups were comparable to that of DPX-Empty control group. This study demonstrates the unique ability of the DPX delivery platform to deliver multiple antigenic TAA peptides without compromising the immunogenicity of each peptide. Furthermore, the DPX-SurMAGE treatment was well tolerated and was shown to induce robust and sustained target-specific T cell responses against both survivin and MAGE-A9 antigens, supporting the impending phase 1 clinical trial in high risk NMIBC patients.

Radiotherapeutics

LBA032 Pan-cancer analysis of fibroblast activation protein alpha (FAP) expression to guide tumor selection for the peptide-targeted radionuclide therapy FAP-2286 Tanya T. Kwan[1], Minh Nguyen[1], Dirk Zboralski[2], Anne Schumann[2], Anne Bredenbeck[2], Matthias Paschke[2], Christian Haase[2], Aileen Hoehne[2], Ulrich Reineke[2], Christiane Smerling[2], Frank Osterkamp[2], Jim Xiao[1], Andrew D. Simmons[1], Thomas C. Harding[1], Kevin L. Lin[1]. [1]Clovis

Oncology, Inc., Boulder, CO, [2]3B Pharmaceuticals GmbH, Berlin, Germany.

Background: FAP is a membrane-bound protease with limited expression in normal tissues but high expression on cancer-associated fibroblasts abundant in the stroma of most tumors. FAP-2286 is a potent and selective FAP-targeted peptide linked to the chelator DOTA that allows for attachment of radionuclides for therapeutic and imaging applications. Assessing patterns of FAP expression in different tumor types and correlating expression with FAP-2286 uptake can help guide tumor selection for FAP-2286 therapy. Methods: FAP immunohistochemistry (IHC) was performed on FFPE tissue specimens from 16 tumor types using the SP325 antibody. Overall and tumor/stroma-specific H-scores were calculated using Visiopharm and HALO analysis, respectively. Autoradiography with ^{111}In-FAP-2286 was performed on a subset of matched frozen tissue sections. Results: Gene expression analysis across The Cancer Genome Atlas data set revealed elevated FAP mRNA expression in multiple tumor types. A pan-tumor IHC screen confirmed that ≥30% of samples in various indications (eg, sarcoma, pancreatic adenocarcinoma, mesothelioma, head and neck squamous cell carcinoma) had high FAP expression (H-score ≥30). While in most tumor types FAP was predominantly localized to the stroma, FAP expression was also observed in tumor cells, especially in tumors of mesenchymal origin, eg, sarcoma and mesothelioma. High FAP expression was independent of tumor stage or grade and detected in both primary and metastatic samples. Multiple sarcoma and mesothelioma subtypes demonstrated high FAP H-scores, suggesting that FAP expression is not limited to a specific subtype. There was significant correlation between FAP expression observed by IHC and FAP-2286 binding as assessed by autoradiography in matched frozen tissues (Pearson r=0.73; p<0.01). Conclusions: Our IHC screen identified high FAP expression in various tumor types that correlated with in vitro FAP-2286 binding, suggesting that FAP is an attractive target across a broad range of tumor types for peptide-targeted radionuclide therapy. Accordingly, the phase 1/2 LuMIERE clinical trial (NCT04939610) will evaluate FAP-2286 as a therapeutic (^{177}Lu-FAP-2286) and imaging (^{68}Ga-FAP-2286) agent in multiple indications.

Target identification and validation

LBA033 YM155 induces DNA damage and cell death in anaplastic thyroid cancer cells by inhibiting DNA topoisomerase IIα at the ATP binding site Ryan Mackay[1], Paul Weinberger[1], Elahe Mahdavian[2], Qinqin Xu[1]. [1]Louisiana State University Health Sciences Center - Shreveport, Shreveport, LA, [2]Louisiana State University in Shreveport, Shreveport, LA.

Introduction: Anaplastic thyroid cancer (ATC) is among the most aggressive of all human cancers with a median survival of 4.3 months. Currently, there is no effective treatment for most ATC patients - surgery, radiation, and chemotherapy all fail to significantly prolong ATC patient survival. YM155, first identified as a survivin inhibitor, was highlighted in a high-throughput screen performed by the National Cancer Institute, killing anaplastic thyroid cancer cells in vitro and in vivo. However, there was no association between survivin expression and response to YM155 in clinical trials (not including ATC), and YM155 has been mostly abandoned for development despite favorable pharmacokinetic and toxicity profiles. A number of additional mechanisms have been proposed for YM155. The purpose of this study was to investigate the mechanisms underlying YM155-mediated ATC cell death.**Methods**: ATC cell line THJ16T was used as the model for this study. AlamarBlue was used to measure cell viability.

Immunofluorescent detection of phosphorylated histone H2AX (γ-H2AX, p-Ser139) foci was used as a surrogate marker for double-strand DNA breaks. siRNA was used to knockdown topoisomerase 2α (Top2α), and Lipofectamine-3000 was used to overexpress Top2α plasmids. In-vivo complex of enzyme assay was used to measure the association between Top2α and DNA. Modeling software Molecular Operating Environment and the crystal structure of human Top2α bound to AMP-PNP (Protein Data Bank # 1ZXM) were used to estimate binding affinities for ATP and YM155 in the ATP binding site of Top2α. Site-directed mutagenesis was used to introduce mutation Ser148 → Ala into a recombinant human Top2α.**Results**: Previously, we presented gene expression data from ATC patients showing significant overexpression of Top2α compared to benign thyroid samples. ATC cell lines that overexpress Top2α are more sensitive to YM155 and exhibit larger increases in DNA damage with YM155 treatment. In this study, ATC cells grown to be resistant to YM155 show decreased expression of Top2α, and overexpression of Top2α re-sensitizes resistant cells to YM155. Molecular modeling shows favorable binding for YM155 in the Top2α ATP binding site and identifies key amino acids for YM155-Top2α interaction. An engineered Top2α mutant abrogates the effect of YM155 as measured by cell viability and DNA damage assays, confirming the contribution of Top2α to YM155 mechanism of action.**Conclusions**: Our results strongly suggest that Top2α is an important target mediating YM155's anticancer activity in ATC. We propose that Top2α inhibition by YM155 leads to DNA damage and eventual cell death. Given YM155's potent anti-proliferative effects in ATC cells with sparing of benign thyroid cells, along with a plausible mechanism of action, we suggest further evaluation and optimization of YM155 for future clinical application in ATC. In conclusion, our study lays the foundation for targeting the enzymatic activity of Top2α as a novel therapeutic strategy for the treatment of ATC, with YM155 serving as the predicate drug.

Apoptosis, necrosis, and autophagy

LBA034 Necrostatin-1 Protects Ferroptosis by xCT Induction in a RIPK1- and IDO-independent manner Md Abdullah[1], Hanna Yuk[2], Do Hyung Kim[3], Haeseung Lee[4], Seung Jin Lee[1]. [1]Chungnam National University, Daejeon, Korea, Republic of, [2]Chungnam national university, Daejeon, Korea, Republic of, [3]Chungnam National university, Daejeon, Korea, Republic of, [4]Pusan National University, Busan, Korea, Republic of.

Ferroptosis is caused by the iron-mediated accumulation of lipid peroxidation, which is distinct from apoptosis and necroptosis. Necrostatin-1 inhibits receptor-interacting ser-ine/threonine-protein kinase 1 (RIPK1) to initiate necroptosis; it also inhibits indoleamine 2,3-dioxygenase (IDO) to regulate tumor immunity. However, few studies have examined the off-target effect of necrostatin-1 on the ferroptosis pathway. The present study examined wheth-er necrostatin-1 could interrupt ferroptosis induced by system x_c^- inhibitors (sulfasalazine and erastin) and a glutathione peroxidase 4 inhibitor (RSL3) in Huh7 and SK-HEP-1 cells. Necrostatin-1 completely prevented decreases in cell viability induced by sulfasalazine and erastin; it partially blunted decreases in cell viability induced by RSL3. Necrostatin-1, ferrostatin-1, and deferoxamine repressed sulfasalazine-provoked membrane permeabilization, as detected by 7-aminoactinomycin D staining and lipid peroxidation measured using a C11-BODIPY probe. However, other RIPK1 inhibitors (necrostatin-1s and GSK2982772) and an IDO inhibitor (1-methyl-D-tryptophan) did not recover the decrease in cell viability induced by sulfasalazine.

Necrostatin-1 potentiated sulfasalazine-induced expression of xCT, a catalytic subunit of system x_c^- in these cells. These results demonstrated that necrostatin-1 blocked fer-roptosis through a mechanism independent from RIPK1 and IDO inhibition in Huh7 and SK-HEP-1 cells, indicating that its antioxidant activity should be considered when using necrostatin-1 as a RIPK1 inhibitor.

Clinical trials

LBA035 Canakinumab or Pembrolizumab as Monotherapy or in Combination as Neoadjuvant Therapy in Patients With Resectable Non-Small Cell Lung Cancer: CANOPY-N Trial Jay M. Lee[1], Jean-Louis Pujol[2], Pilar Garrido[3], Edward S. Kim[4], Masahiro Tsuboi[5], Rafael Caparica Bitton[6], Jiawei Duan[6], Cecile Blin[7], Alexander Savchenko[6], Tony Mok[8]. [1]University of California, Los Angeles, Los Angeles, CA, [2]Hôpital Arnaud de Villeneuve, Montpellier, France, [3]Hospital Universitario Ramón y Cajal, Madrid, Spain, [4]City of Hope Orange County, Newport Beach, CA, [5]Department of Thoracic Surgery, National Cancer Center Hospital East, Kashiwa, Japan, [6]Novartis Pharmaceuticals Corporation, East Hanover, NJ, [7]Novartis Pharma AG, Basel, Switzerland, [8]Chinese University of Hong Kong, Shatin, China (Mainland).

Background: Complete surgical resection is the standard treatment for patients with stage I-IIIA non-small cell lung cancer (NSCLC). Five-year survival rates in this population range from 19% to 50%, with most patients dying from distant recurrence. As neoadjuvant/adjuvant chemotherapy yields a modest 5% absolute overall survival benefit for these patients with NSCLC, new treatment options are needed. Preliminary data with PD-1 or PD-L1 inhibitors as neoadjuvant therapy have shown major pathologic responses (MPRs) or pathologic complete responses in patients with resectable NSCLC. In the CANTOS study, reduced incidence of NSCLC and decreased lung cancer–related mortality were observed in a dose-dependent manner in patients with atherosclerosis who received canakinumab (interleukin 1β inhibitor) versus those who received placebo. In preclinical NSCLC humanized models, treatment with canakinumab ± anti–PD-1 inhibitor has shown promising activity, and a synergistic effect was observed with this combination, which may enhance the efficacy of PD-1 inhibition by modulating pro-tumor inflammation in the tumor microenvironment. In this context, the CANOPY-N study was designed to evaluate the effect of canakinumab or pembrolizumab as monotherapy or in combination as neoadjuvant treatment for patients with resectable NSCLC.**Methods:**CANOPY-N (NCT03968419) is a Phase II, randomized, open-label study evaluating the effect of canakinumab or pembrolizumab as monotherapy or in combination as neoadjuvant treatment in patients with resectable NSCLC. Patients with histologically confirmed stage IB-IIIA NSCLC (excluding N2 and T4 tumors), no prior systemic therapy, Eastern Cooperative Oncology Group performance status 0 or 1, and eligibility for surgery with a planned resection in approximately 4-6 weeks after first dose of study treatment are eligible to participate. An archival (if obtained up to 6 months before first day of treatment) or new biopsy is required. Approximately 110 patients will be randomized in a 2:2:1 ratio (stratified by histology [squamous/nonsquamous]) to receive a total of 2 doses (200 mg every 3 weeks) of canakinumab alone (n=44) or in combination with pembrolizumab (n=44) or pembrolizumab alone (n=22), with safety follow-up up to 130 days from last study-drug dose. The primary endpoint is MPR rate (≤10% of residual viable tumor cells at time of surgery), and secondary endpoints include overall response rate per

RECIST 1.1, MPR rate based on local assessment, surgical feasibility rates, safety, incidence of antidrug antibodies, pharmacokinetic parameters, and assessment of the relationship between blood- or tissue-based biomarkers and MPR. As of May 27, 2021, there are 44 active study locations.

Biomarkers

LBA036 Whole-exome sequencing of germ cell tumors in childhood Janaina Mello Soares Galvão[1]. [1]Barretos Cancer Hospital, Barretos, Brazil.

The germ cell tumors (GCTs) are a rare group of neoplasms that affect about 3.5% of pediatric patients, that comprise both benign and malignant histologies. They are part of a heterogenous group clinically and pathologically complex, derived from totipotent primordial germ cells. Despite their heterogeneity, GCTs can occur in gonadal and extragonadal sites, presenting different histological subtypes. Pediatric cancers in general are known to have low mutational burdens or 'quiet' genomes relative to adult tumors, with pediatric malignant tumors having 1,000-fold fewer somatic mutations than several adult cancers. Molecular genetic analyses of pediatric GCT revealed distinct biological differences between childhood GCT compared to those of adults tumors, however, most of those studies have been performed in adult patients, and there is not much knowledge about molecular alterations in children. In contrast to many other solid cancers, previous studies show that GCTs have a relatively low mutational burden which comprise embryonic epigenetic state, copy number alterations (CNAs) and single nucleotide mutation or somatic insertion/deletion (SNV/indel). According to those studies, molecular alterations in the *KIT*, *KRAS* and *NRAS* genes and chromosome 12p amplification are frequently found. Therefore, the aim of this study is to perform a genomic profile by whole-exome sequencing (WES) of pediatric patients diagnosed with GCTs at the Barretos Children's Cancer Hospital and compare them with adult GCT database. We reported the WES using Illumina paired-end sequencing strategy (>100 X-fold coverage) of sixteen cases and respective matched normal. Data analysis was performed as follows: Mutect, Pindel and Mutsig for SNVs/Indels; HMMcopy, Nexus Copy Number and Gistic for CNAs; and Signal (Cosmic v3) for mutational signatures. The somatic alterations found were compared with data available in the literature and database on adult GCTs. Our samples represent 10 ovarian GCTs, five testicular GCTs and one mediastinal tumor. Somatic mutations in cancer genomes are caused by multiple mutational processes, which may generate a characteristic mutational signature. Analyses of single base substitution signature (SBSS) showed SBSS39 in 11 samples (68,75%) and the SBSS22 in two ovary samples (12,5%). Copy number alterations were observed on chromosomes 4, 7, 8, 10, 12, 21, and 22, with gain of genes *KRAS*, *CCND2*, *ETV6*, *KDM5A*, *MYC*, and *OLIG2*, and loss of genes *KIT*, *FBXW7*, *PDGFRA*, and *PTEN*. In addition, mutations were observed in following genes *MTOR* (19%), *KIT* (12%), *CTNNB1* (12%), *ATM* (12%), *KRAS* (6%) and *PI3KCA* (6%). Even though there have been limitations in pediatric CGT studies, our analyzes show that the somatic alterations found correspond to the alterations established in adult CGT and in recent pediatric CGT studies. Therefore, additional whole-exome sequencing studies are necessary to identify and confirm gene mutations associated to pediatric GCT.

Drug resistance and modifiers

LBA037 Reposition of the antibiotic nitroxoline as a novel STAT3 inhibitor for drug-resistant urothelial bladder cancer WENFENG LIN[1], Jingkai Sun[1], Takuya Sadahira[1], Motoo Araki[1], Masami Watanabe[1], Yasutomo Nasu[1], Peng Huang[1]. [1]Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.

Background: Repeated cycles of first-line chemotherapy drugs such as doxorubicin (DOX) and cisplatin (CIS) trigger frequent chemoresistance in recurrent urothelial bladder cancer (UBC), posing a threat to the prognosis of patients. Nitroxoline (NTX), an antibiotic to treat urinary tract infections, has been recently repurposed for the treatment of multiple tumors. Here we aimed to investigate whether NTX suppresses the growth of drug-resistant UBC in vitro and in vivo and the underlying mechanism at the molecular level.**Methods:** The drug-resistant cell lines T24/DOX and T24/CIS were established by continual exposure of parental cell line T24 to DOX and CIS, respectively. The drug effects on cell proliferation were measured by XTT assay. Cell cycle distribution was analyzed by PI/RNase staining, while apoptosis was assessed by Hoechst 33342 and Annexin V-FITC/PI staining. The expressions of proteins related to STAT3 signaling, cell cycle, and apoptosis were detected by western blotting. Subcutaneous xenograft models of T24/DOX and T24/CIS were established to determine the in vivo anti-tumor effect of NTX.**Results:** T24/DOX and T24/CIS cells were resistant to DOX and CIS respectively, but they were sensitive to NTX in a time- and dose-dependent manner. Overexpressions of STAT3 and P-glycoprotein (P-gp) were identified in T24/DOX and T24/CIS, which could be reversed by NTX. Furthermore, western blotting showed that NTX downregulated the expressions of phosphorylated STAT3, c-Myc, Cyclin D1, CDK4, CDK6, Bcl-xL, Mcl-1, and Survivin, which were further confirmed by Stattic, a selective STAT3 inhibitor. In vivo, NTX exhibits the significant anti-tumor effect in T24/DOX and T24/CIS tumor-bearing mice. These results suggested that NTX-induced G0/G1 arrest, apoptosis, and P-gp reversal in drug-resistant UBC were mediated by inhibition of STAT3 signaling.**Conclusion:** Our findings repurpose NTX as a novel STAT3 inhibitor to induce P-gp reversal, G0/G1 arrest, and apoptosis in drug-resistant UBC.

Clinical trials

LBA038 KontRASt: A Phase Ib/II, open-label, multi-center, dose-escalation study of JDQ443 in patients with advanced solid tumors harboring the *KRAS G12C* mutation Benjamin Solomon[1], Rebecca S Heist[2], Daniel SW Tan[3], Philippe A Cassier[4], Christophe Dooms[5], Eric Van Cutsem[6], Conor E Steuer[7], Neeltje Steeghs[8], Martin Schuler[9], Anas Gazzah[10], Martin Wermke[11], Enriqueta Felip[12], Herbert HF Loong[13], Maria J De Miguel Luken[14], Ross A Soo[15], Ashley Jaeger[16], Kun Xu[17], Xueying Chen[17], Xiaoming Cui[17], Heather Burks[16], Anna F Farago[16], Toshio Shimizu[18]. [1]Peter MacCallum Cancer Centre, Melbourne, Australia, [2]Massachusetts General Hospital, Boston, MA, [3]National Cancer Centre Singapore, Singapore, Singapore, [4]Centre Léon Bérard, Lyon, France, [5]UZ Leuven University Hospital, Leuven, Belgium, [6]University Hospitals Gasthuisberg, Leuven and KU Leuven, Leuven, Belgium, [7]Winship Cancer Institute of Emory University School of Medicine, Atlanta, GA, [8]The Netherlands Cancer Institute, Amsterdam, Netherlands, [9]West German Cancer Center,

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BackgroundKirsten rat sarcoma virus (KRAS) is a GTPase that regulates cell signaling pathways involved in cell proliferation, survival, and tumorigenesis. Somatic mutations in *KRAS* resulting in a glycine to cysteine substitution at codon 12 (*KRAS G12C*) lead to a shift toward active, GTP-bound KRAS and increased oncogenic signaling. *KRAS G12C* mutations occur in approximately 13% of non-squamous, non-small cell lung cancer (NSCLC) cases, and at lower frequencies in other solid tumor malignancies. JDQ443 (NVP-JDQ443) is a selective, covalent, and orally bioavailable investigational $KRAS^{G12C}$ inhibitor that binds under the switch II loop, and irreversibly traps $KRAS^{G12C}$ in a GDP-bound, inactive state. In preclinical models, JDQ443 potently inhibited $KRAS^{G12C}$ cellular signaling and proliferation in a mutant-selective manner and demonstrated dose-dependent anti-tumor activity. In patients with *KRAS G12C*-mutated solid tumors, JDQ443 may have clinically significant antitumor activity alone and in combination with TNO155, an investigational, SHP2 inhibitor, and in combination with PD-1 blockade.**Methods**This is a Phase Ib/II, open-label, dose-escalation study with four arms: (A) JDQ443 monotherapy; (B) JDQ443 + TNO155; (C) JDQ443 + anti–PD-1; and (D) JDQ443 + TNO155 + anti–PD-1. Each arm has a dose-escalation portion followed by dose expansion at the maximum tolerated dose (MTD) and/or recommended dose (RD). The escalations are conducted in adult patients with advanced *KRAS* G12C-mutated solid tumors who have previously received standard-of-care therapies. Dose escalation is guided by an adaptive Bayesian hierarchical logistic regression model following the escalation with overdose control principle. Expansions are planned for patients with advanced (metastatic or unresectable), *KRAS G12C*-mutated NSCLC who have received prior immune checkpoint inhibitor therapy and platinum-based chemotherapy, and for patients with advanced, *KRAS G12C*-mutated colorectal cancer who have received prior fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy. The primary objectives of dose escalation are to assess the safety and tolerability of JDQ443 alone and in combinations, and to identify the MTD and/or the RD, and regimens for future studies. The primary objective of dose expansion is to evaluate the antitumor activity via overall response rates for JDQ443, both alone and in combinations, in selected populations. Secondary objectives for both escalation and expansion are to evaluate the antitumor activity and characterize the pharmacokinetics of JDQ443 alone and in combinations, and to assess the immunogenicity of anti–PD-1 in combination with JDQ443 or TNO155. Safety and tolerability will also be further assessed during dose expansion. The study is currently enrolling to the dose-escalation portions of Arm A (JDQ443 monotherapy) and Arm B (JDQ443 + TNO155). NCT04699188

LBA039 Canakinumab as Adjuvant Therapy in Patients With Completely Resected Non-Small Cell Lung Cancer: CANOPY-A Trial Edward B. Garon[1], Andrea Ardizzoni[2], Fabrice Barlesi[3], Byoung Chul Cho[4], Pedro De Marchi[5], Yasushi Goto[6], Dariusz Kowalski[7], Shun Lu[8], David R. Spigel[9], Michael Thomas[10], James Chih-Hsin Yang[11], Sabine Turri[12], Wen

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Background: In the CANTOS study, canakinumab (selective interleukin 1β inhibitor) treatment was associated with reduced incidence and mortality from non-small cell lung cancer (NSCLC) in stable post–myocardial infarction patients with elevated high-sensitivity C-reactive protein (hs-CRP) levels. The CANOPY-A study was designed to investigate the therapeutic role of canakinumab in NSCLC.**Methods:**The CANOPY-A study (NCT03447769) is evaluating the efficacy and safety of canakinumab as adjuvant therapy in adult patients with completely resected NSCLC. Patients with American Joint Committee on Cancer/Union for International Cancer Control version 8 stage IIA-IIIA and IIIB (T >5 cm, N2), any histology, completely resected (R0) NSCLC and completion of adjuvant cisplatin-based chemotherapy (≥2 cycles) and radiotherapy (if applicable) are eligible. Patients must not have had prior neoadjuvant chemotherapy or radiotherapy. Patients (~1500) are randomized 1:1 to receive canakinumab (200 mg) or placebo subcutaneously every 3 weeks for 18 cycles or until disease recurrence as determined by investigator, unacceptable toxicity, or treatment discontinuation at the discretion of the investigator. The primary objective is to compare disease-free survival (DFS) in the canakinumab versus placebo arm per local investigator assessment. Secondary objectives are overall survival (OS), lung cancer–specific survival, safety, pharmacokinetics, immunogenicity, and patient-reported outcomes. Adult patients with stage IIA-IIIA and IIIB (T>5 cm, N2 disease only) NSCLC who are candidates for complete resection surgery (and therefore prospective candidates for the main study) will be asked to participate in a biomarker substudy to understand how resection may impact biomarkers involved in the interleukin 1β inflammatory pathway and mutations present in blood. In the substudy, the levels of hs-CRP, other cytokines, and additional biomarkers in blood will be assessed before and after surgery (endpoint: summary statistics of hs-CRP and other pharmacodynamic biomarkers). For patients who will enroll in the main study, possible associations between pre- and post-surgery biomarker levels with canakinumab efficacy will be assessed (endpoint: DFS and OS by hs-CRP and other pharmacodynamic biomarkers). The CANOPY-A study is currently enrolling. As of August 6, 2021, there are 399 study locations.

DNA repair and modulation

LBA041 Identification and characterization of potent RAD51 inhibitors targeting RAD51-BRCA2 interaction Sanjita Sasmal[1], Sukanya Patra[1], Venkatesham Boorgu[1], Mahesh Yanamandra[1], Shankar Chithaluri[1], Hema Sankar Pathange[1], Deepika Hiremath[1], Sandeep M

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The DNA damage response (DDR) is a complex cellular network that acts as a cell defense mechanism and exerts signal for repair of DNA lesions. It repairs different types of DNA damage such as single-strand (ssDNA) breaks, DNA inter-strand crosslinks and double strand breaks (DSB) caused by endogenous and exogenous factors. These damages are mainly repaired by homologous recombination (HR) repair, and non-homologous end joining (NHEJ). DNA double-strand breaks (DSBs) are the most severe DNA damage and predominantly repaired by HR. As the DDR pathway is critical for tumorigenesis, a high degree of interest has been generated in the recent past to target this network. RAD51 is one of the pivotal enzymes for DNA double-strand break repair by the HR pathway. While PARP inhibitors are extensively used for BRCA2 mutated cancers, their responses in BRCA2 WT is limited. Targeting RAD51 will open a new avenue to treat BRCA2 WT cancer patients. BRCA2 interaction with RAD51 is a prerequisite for RAD51 nuclear translocation to exert its function by binding to DNA. The disruption of this interaction leads to inhibition of DNA repair and in turn inhibits proliferation and tumor growth. As this leads to BRCAness in an otherwise BRCA2 WT background, it is expected to have synergy with PARP1 inhibitors. We report the identification of novel and potent RAD51 inhibitors which disrupt the RAD51:BRCA2 interaction. Our hit compounds show anticancer activity in relevant cancer cell lines across various indications. Further, our compounds inhibit RAD51 nuclear translocation upon induction of exogenous DNA damage. Multiple series have been identified, with a good SAR trend and correlating well with cell based and PD activity. Further profiling of these compounds is ongoing.

Other

LBA042 Male Sex is an Independent Predictor of Recurrence-Free Survival in Middle Eastern Papillary Thyroid Carcinoma KHAWLA S. AL-KURAYA[1], Sandeep Kumar Parvathareddy[1], Abdul K Siraj[1], Felisa De Vera[1], Padmanaban Annaiyappanaidu[1], Saeeda O. Ahmed[1], Saif S. Al-Sobhi[1], Fouad Al-Dayel[1]. [1]King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Background: Disparity between sexes with regards to incidence, disease aggressiveness, and prognosis has been documented in several cancers. Although various reports have documented the association between male sex and aggressive papillary thyroid carcinoma (PTC), the prognostic impact of sex on PTC has been inconsistent. The role of sex in PTC aggressiveness and outcome in Middle Eastern PTC remains unknown. Therefore, our study retrospectively analyzed the data of a large cohort of Middle Eastern PTC patients to address this issue.**Methods:** We compared men and women with respect to clinico-pathological characteristics, disease persistence, structural recurrence, risk stratification and prognosis. We included 1430 patient - 1085 (75.9%) women and 345 (24.1%) men.**Results**: The median follow up was 9.5 years. At diagnosis, 27% (93/345) of men were ≥ 55 years, compared with 17.8% (193/1085) of women ($p = 0.0003$). Men had significantly more advanced disease at presentation: higher stage ($p = 0.0074$), larger tumor size ($p = 0.0069$), higher rates of lymphovascular invasion ($p = 0.0129$), extrathyroidal extension ($p = 0.0086$), regional lymph node metastasis ($p = 0.0279$), and distant metastasis ($p = 0.0101$). There was a higher rate of recurrence ($p < 0.0001$) and *TERT* mutations ($p = 0.0003$) in male PTC patients than in female

patients. Additionally, radioiodine refractoriness was higher in male PTC patients (p = 0.0014). In multivariate analysis, male sex was an independent prognostic factor for poor recurrence-free survival (RFS) (Hazard ratio = 1.59; 95% Confidence interval = 1.21 – 2.07; p = 0.0010).**Conclusions:** Men with PTC are more likely to present with more advanced and aggressive diseases. Importantly, male sex was an independent prognostic factor for RFS. Thus, men may benefit from more aggressive management and therapeutic interventions.

mTOR/PI3-kinase

LBA043 ***mRNA expression of mTOR signaling components as potential predictive biomarker in pancreatic neuroendocrine neoplasms*** Sebastian Wolfshöfer[1], Adrian C Lock[2], Daniel Kaemmerer[3], Ralph M Wirtz[4], Franziska Briest[2], Sven P Haugvik[5], Malte Buchholz[6], Anja Rinke[6], Ruza Arsenic[7], Marianne Pavel[8], Dieter Hörsch[3], Arend Koch[2], Patricia Grabowski[1]. [1]Charité - University Medicine Berlin, Germany, Berlin, Germany, [2]Charité - University Medicine Berlin, Berlin, Germany, [3]Zentralklinik Bad Berka GmbH, Bad Berka, Germany, [4]Stratifyer Molecular Pathology GmbH, Cologne, Germany, [5]Oslo University Hospital, Oslo, Norway, [6]University Hospital of Giessen and Marburg, Marburg, Germany, [7]Charité- Universitätsmedizin Berlin, Berlin, Germany, [8]Universitätsklinikum Erlangen, Erlangen, Germany.

Medical therapeutic options for the treatment of well-differentiated pancreatic neuroendocrine neoplasms (pNENs) include the mTOR pathway inhibitor everolimus (in combination with 5-Fluorouracil). Criteria to assess prognosis in pNEN patients are still limited, as biomarkers to predict the therapeutic response are still unknown. Several studies could show aberrant mTOR signaling pathway activity at different levels of the signaling cascade in pNENs. In this study, we analyzed the mRNA expression of different mTOR pathway components in well-differentiated pNENs, in order to identify potential prognostic and predictive biomarkers. In this retrospective multicenter study, real-time quantitative TaqMan reverse transcriptase-polymerase chain reaction was used to analyze mRNA expression of eleven different mTOR pathway genes (*IGF-1*, *IGFBP-3*, *PIK3CA*, *AKT-1*, *MTOR*, *MLST8*, *DEPTOR*, *RAPTOR*, *RICTOR*, *4EBP1* und *VEGF-A*), in tumor tissue of 75 patients with well-differentiated pNENs in comparison to normal pancreatic tissue. Relative gene expression was determined according to the 40-ΔCT method using *CALM2* as housekeeping gene. The analysis of mRNA expression levels of the aforementioned eleven genes indicated an increased mTOR signaling activity in pNENs. A distinct linear correlation only occurred between *MTOR* and *AKT-1*. Clear association between mRNA expression and a clinical feature was only found for *RAPTOR*: High *RAPTOR* mRNA expression was significantly correlated with better overall survival in unselected patients. No prediction to everolimus treatment response could be deduced by mRNA expression analysis in a small subgroup of 21 patients. Even though mRNA expression of different mTOR pathway components indicated an enhanced activity of this signaling cascade in tumor tissue of pNEN patients, we could not identify a statistical significant parameter useful for prediction of everolimus treatment response. The predictive value of this study is limited due to the low number of patients who received treatment with everolimus. Further prospective studies analyzing the predictive value of the expression of mTOR pathway genes are desirable.

Clinical trials

LBA046 Neoadjuvant and Adjuvant Capmatinib in Resectable Non-Small Cell Lung Cancer With *MET* Exon 14 Skipping Mutation or High *MET* Amplification: GEOMETRY-N Trial Karen Kelly[1], Mark M. Awad[2], Teddy Saliba[3], Nydia Caro[3], Beth Inserra[3], Hiya Banerjee[3], Jay M. Lee[4]. [1]UC Davis Comprehensive Cancer Center, Sacramento, CA, [2]Dana-Farber/Harvard Cancer Center, Boston, MA, [3]Novartis Pharmaceuticals Corporation, East Hanover, NJ, [4]University of California, Los Angeles, Los Angeles, CA.

Background: Neoadjuvant therapy is the earliest opportunity to eliminate micrometastatic disease. Emerging data suggest that neoadjuvant therapy in non-small cell lung cancer (NSCLC) can elicit major pathological responses (MPRs) that translate into prolonged survival outcomes, serving as an early surrogate for efficacy. Adjuvant therapy is well known to improve overall and disease-free survival (DFS) in patients with completely resected NSCLC. DFS observed with osimertinib in patients with early-stage *EGFR*-mutated tumors supports evaluation of other tyrosine kinase inhibitors (TKIs) in the neoadjuvant and adjuvant settings. In early-stage NSCLC, *MET* exon 14 skipping mutation (*MET*ex14) and de novo *MET* amplification (*MET*amp) are estimated to occur in up to 2.8% and 1.7% of patients, respectively. Capmatinib, a selective MET TKI, is FDA approved for patients with metastatic *MET*ex14 NSCLC. It was studied in GEOMETRY mono-1 in patients with advanced/metastatic NSCLC with either *MET*ex14 or *MET*amp. In two treatment-naive *MET*ex14 cohorts, overall response rate (ORR) was 68% and 66%. In a treatment-naive high-level *MET*amp cohort, ORR was 40%. Capmatinib had a tolerable safety profile; most adverse events were reversible with dose adjustments. Based on the response rates and the safety profile observed in GEOMETRY mono-1 in treatment-naive patients with advanced/metastatic *MET*-dysregulated NSCLC, GEOMETRY-N (NCT04926831) a Phase II, two-cohort, two-stage study is evaluating the efficacy and safety of neoadjuvant and adjuvant capmatinib therapy in improving the MPR rate and outcomes beyond those achieved with surgery, chemotherapy, and radiation in patients with *MET*ex14 or high-level *MET*amp NSCLC.**Methods:**Adult patients with resectable, histologically confirmed NSCLC stage IB-IIIA, N2 and select IIIB (T3N2 or T4N2) with either *MET*ex14 irrespective of *MET* gene copy number (GCN) (cohort A) or high-level *MET*amp with GCN ≥10 (cohort B) are eligible. *MET*ex14 must be determined by a Clinical Laboratory Improvement Amendments (CLIA)-certified lab. *MET*amp must be determined by fluorescent in situ hybridization at a CLIA-certified lab or by FoundationOne CDx next-generation sequencing. Prior systemic anticancer therapy is prohibited. Patients will receive capmatinib 400 mg twice daily for 8 weeks prior to surgical resection, followed by 3 years of adjuvant capmatinib. In the two-stage design, stage 1 will enroll 9 patients in each cohort, with MPR evaluated in each cohort after 9 patients have completed neoadjuvant therapy; stage 2, enrolling 10 more patients in a cohort, will proceed only if ≥1 of 9 participants has an MPR. About 42 patients will be enrolled, with 19 evaluable patients per cohort. The primary endpoint is MPR rate based on local investigator assessment. Secondary endpoints are complete pathological response rate (central and local investigator review), ORR (local investigator assessment), DFS, and safety. Following treatment, there will be a 2-year survival follow-up. The expected first patient first visit is September 30, 2021.

Drug delivery

LBA048 Lymph node metastasis targeted intranodal delivery of docetaxel improves treatment outcome Ariunbuyan Sukhbaatar[1], Shouta Sora[1], Mori Shiro[2], Tetsuya Kodama[1]. [1]Tohoku University, Sendai, Japan, [2]Tohoku University Hospital, Sendai, Japan.

Despite recent advances in diagnostic modalities and cancer treatment guidelines, systemic chemotherapy for the lymph node (LN) metastasis has poor treatment efficacy using conventional drugs. Therefore, a novel method, lymphatic drug delivery system (LDDS), has been proposed in a pre-clinical experiment that directly injects the anticancer drug into sentinel LNs to deliver into their downstream LNs, aiming to inhibit tumor cell growth in those LNs. The injection duration of chemotherapy drugs using the LDDS will be less than the intratumoral or intralesion methods used in the clinic, and systemic toxicity will be less than tumor-targeted therapy or systemic chemotherapy. Our previous results indicated that the treatment efficacy increased in LDDS treated groups than systemic delivery. Here, we show the improvement of treatment efficacy by increasing osmotic pressure and viscosity of the drug solvent delivered by LDDS using the metastatic LN mouse model. First, a metastatic LN mouse model was created by inoculating luciferase-expressing tumor cells into subiliac LN (SiLN) of MXH10/Mo/lpr mouse to induce metastasis into the proper axillary LN (PALN). Next, 10 mg/kg docetaxel (DTX) with increased osmotic pressure and viscosity were injected into tumor-inoculated SiLN with an injection rate of 2400 μL/min on day 7 post-inoculation to inhibit tumor cell growth in the inoculation and metastatic site. Luciferase activity decreases in PALNs and SiLNs were observed in groups between 1140 kPa, 1960 kPa, and 2780 kPa (4, 11, and 38 mPa·s) groups without inducing any side effects. By histology, no tumor cells were confirmed in those LNs of the 1960 kPa group. Then, DTX at 1960 kPa, 11 mPa·s was injected into tumor-inoculated SiLN on day 21 post-inoculation with different injection speeds and volumes. We found that DTX at 1960 kPa, 11 mPa·s injected at the injection rate of 200 μL/min with 400 μL volume, can inhibit tumor cell growth in the PALN and delay tumor growth SiLN. Furthermore, the antitumor effects depended on the tumor progression stage in the LNs, and the injection rates and volumes of administered drugs. We anticipate these optimal ranges as a starting point for developing more effective drug regimens to treat metastatic LNs with the LDDS. Moreover, administrable drugs in this novel method of ultrasound-guided LDDS or LDDS at intra-operative or image-guided surgery are not limited only to anticancer agents.

Radiotherapeutics

LBA049 Possibility of total body irradiations for lymph node metastasis and lung metastasis Shouta Sora[1], Radhika Mishra[1], Ariunbuyan Sukhbaatar[1], Maya Sakamoto[2], Shiro Mori[2], Tetsuya Kodama[1]. [1]Graduate School of Biomedical Engineering, Tohoku University, Sendai, Japan, [2]Tohoku University Hospital, Sendai, Japan.

Metastatic lymph nodes (MLNs) are one of the most common causes of distant metastasis, and their presence signals a serious deterioration in the life prognosis of an affected patient. The treatment of MLNs at an early stage is necessary to inhibit metastases to distant LNs and organs. Radiation therapy is an effective therapeutic modality for local tumor control in many cancers, and its effect is enhanced by fractionated radiation therapy or in combination with other

therapeutic regimens. In addition, recent studies have reported that total body irradiation (TBI) increases the activation of immune cells such as NK cells and infiltration of CD4+ T cells to tumor tissue. However, the effects of TBI on LN metastasis remains unclear. This study assessed the effect of TBI by changing the radiation dose and number of irradiations in an MLN mouse model. To induce metastasis into the proper axillary lymph node (PALN) and lung, mouse breast cancer FM3A-Luc cells were inoculated into the subiliac lymph node (SiLN) of MXH10/Mo/lpr mice. Mice were assigned to 4 groups thus: received no TBI; received a single dose of 0.2 Gy as low-dose TBI (single L-TBI); received a single dose of 1.0 Gy as middle-dose TBI (single M-TBI); or received two fractions of 2.0 Gy (fractionated M-TBI). The irradiation was carried out on day 4 post-inoculation in the single L-TBI and M-TBI groups. In the fractionated M-TBI group the irradiation was carried out on days 4 and 7 post-inoculation. Tumor growth was evaluated using an *in vivo* bioluminescence imaging system. In the non-irradiated group, tumor activity in the SiLN and PALN increased over time and lung metastasis was confirmed on day 28 post-inoculation. In addition, the single L-TBI dose did not delay tumor growth in the SiLN and PALN or inhibit lung metastasis. In other words, the tumor activity was not significantly changed compared to the non-irradiated group. Single and fractionated M-TBI doses significantly delayed tumor growth in the SiLN and PALN and inhibited lung metastasis. Furthermore, the fractionated M-TBI group exhibited the greatest delay in tumor growth in the PALN compared to the other three groups. These results suggest that the TBI at a dose > 1.0 Gy can delay tumor growth of MLNs in the early stage and distant metastasis. Moreover, the radiation dose and number of irradiations may be essential factors for enhancing the anti-tumor effects of TBI. We anticipate that TBI will contribute to the prevention and treatment of cancer metastasis via LNs.

Therapeutic agents: other

LBA050 Bioenergetic evaluation of Mito-compound Mito-Met as potent cytotoxic agents in gastric cancer Giovanni A Lineros Franco[1], Yenny Bueno[1], Micael Hardy[2], Olivier Ouari[2], Julio C. Montoya-Villegas[3], Marcos López-Casilla[4], Sandra M. Sanabria-Barrera[1]. [1]Fundación Cardiovascular de Colombia, Floridablanca, Colombia, [2]Marseille University, Paris, France, [3]Universidad del Valle, Cali, Colombia, [4]University of Puerto Rico, San Juan, Puerto Rico.

Gastric cancer (GC) kills around 800,000 people each year around the world, placing it among the deadliest types of cancer. The evolution of intestinal metaplasia is unknown, but there are different factors that are involved in its development, the diet rich in salt, the Epstein-Barr virus, Helicobacter pylori and a wide variety of genes that affect cell activity. Currently, surgical resection with chemotherapy is the main treatment with a 5-year survival of 30% that varies according to the metastatic extension. The mitochondria is now one of the therapeutic targets in the development of new compounds with anticancer capacity and it has been observed that metformin behaves as an insulin sensitizer at high concentrations, inhibiting the mitochondrial complex I. For this reason, Mito-metformin (Mito-Met) was designed, this is a mitochondrial analogue of metformin that is linked to the lipophilic alkyl triphenylphosphonium cation (TPP) with a mitochondrial effect 1000 times greater at low concentrations. In this study, the effects of Mito-Met were evaluated in two GC cell lines (AGS and KATO III) and in the healthy gastric epithelium cell line (HPSEC). Cell viability, cytotoxicity, colony formation and cell migration

assays were performed. Also, it was evaluated whether Mito-Met had the potential to affect mitochondrial bioenergetics by evaluating the OCR oxygen consumption rate, ECAR extracellular acidity rate, glycolytic capacity and mitochondrial fuel oxidation in live cells in real time using the analyzer XFe24 extracellular flux from Seahorse Biosciences. A dose-time-dependent decrease in cell viability was observed with IC_{50} values of 2.9 µM, 19,3 µM and 22,1 µM in AGS, KATO and HPSEC cells, respectively. The results in clonogenic assay showed that Mito-Met strongly affected AGS with an IC_{50} of 11.7 uM and a greater impact was observed in HPSEC than in KATO III, with IC_{50} values of 31.8 uM and 3.6×10^3 uM, respectively. The results of the wound healing assay showed that Mito-Met has a significant time-dependent dose effect on metastasis. Mito-Met affects the mitocondrial bioenergetics, our results showed affectation of basal respiration, maximum respiration, ATP production and reserve capacity in both cell lines. Mito-Met decreased baseline OCR and stressed OCR in a dose-dependent manner in both GC cell lines. Mito-Met increased the metabolic potential in a dose-dependent manner of OCR in AGS, on the contrary, a dose-dependent decrease of ECAR was observed in KATO III. There was no change in HPSEC. These results show that Mito-Met is promising compound for treatment of GC.

Drug delivery

LBA051 Importance of drug osmotic pressure and viscosity for efficient drug delivery using lymphatic drug delivery system Radhika Mishra[1], Ariunbuyan Sukhbaatar[1], Sora Shouta[1], Maya Sakamoto[1], Shiro Mori[1], Tetsuya Kodama[1]. [1]Tohoku University, Sendai, Japan.

Metastasis is known to be the prime cause of cancer associated fatality. It is therefore imperative to treat and prevent metastasis in order to curb cancer-associated morbidity and mortality. Metastatic lymph nodes (MLNs) are known to be crucial diagnostic and prognostic indicators. Furthermore, as per the lymph node mediated hematogenous theory, due to extant communication between the lymphatic network and the venous system, the lymphatic network facilitates the passage and subsequent colonization of tumor cells to distant sites, thus, serving as a starting point for distant metastasis even during the initial stages. Treatment of MLNs, therefore, is imperative. Proposed in 2015, the Lymphatic Drug Delivery System (LDDS) is a novel and promising drug delivery system for the treatment of MLNs that overcomes the limitations of conventional chemotherapy. Unlike systemic chemotherapy, LDDS entails a direct intranodal drug administration to sentinel lymph node, post its identification using radioisotopes and fluorescent molecules, in order to treat it and its downstream lymph nodes. Previous studies have documented the superior efficacy of LDDS as a MLN management strategy as compared to conventional therapy. Higher drug delivery to target sites and prolonged retention with lower systemic toxicity, and thereby stronger anti-tumor effect have been reported to have been achieved upon drug administration via LDDS. In the present study, MXH10/Mo/lpr mice and FM3A-Luc, mouse mammary carcinoma cells expressing the firefly luciferase gene, have been utilized for the establishment of a mouse model of Lymph node metastasis (LNM) to investigate the effect of drug osmotic pressure and viscosity on therapeutic efficacy of the LDDS. Two lymph nodes, exhibiting systemic lymphadenopathy, in the subiliac and axillary region, the subiliac lymph node (SiLN) and proper axillary lymph node (PALN) have been used to model a network of interconnected human lymph nodes. Drug solution of varying osmotic pressure and viscosity were administered via LDDS to the murine model of LNM and subsequently

therapeutic efficacy was monitored using the high frequency ultrasound system, VEVO and bioluminescent imaging system, IVIS, and confirmed through histology. The study revealed an optimized window of drug osmotic pressure and viscosity (π = 1000 - 2000 kPa; μ = 0.9 - 11.5 mPa·s.) where a significantly stronger anti-tumor response was observed. Venturing below or beyond the optimized range results in decreased therapeutic efficacy. The study therefore concluded that drug osmotic pressure and viscosity are important fluid parameters that require careful consideration prior to preparation of drug cocktails as they can severely impact the prognosis. The findings of this study have the potential for translation to clinics and we anticipate that these results can be replicated in clinical trials, positively altering the fate of many cancer patients.