Regular and young investigator award abstracts

Biomarkers, immune monitoring, and novel technologies

1 TUMOUR ASSOCIATED MACROPHAGES IN HPV-RELATED CARCINOMA WITH ADENOID CYSTIC LIKE FEATURES OF THE SINONASAL TRACT: A REVIEW OF THREE CASES

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Background HPV- related carcinoma with adenoid cystic like features of the sinonasal tract is a newly describe entity with histological and immunophenotypic features of surface derived and salivary gland carcinoma. It affects females more than males with age range of 40–70 years and is linked to high risk HPV infection. Most cases present with nasal obstruction and epistaxis. They consist of basaoid cells growing in various sizes separated by fibrocollagenous stroma. It is believed to have a good prognosis. Tumor-associated macrophages (TAMs) are activated macrophages associated with tumor progression in various cancers. TAMs can polarize M1 or M2 type. M1 has a pro-inflammatory function and kills pathogens. Conversely, M2 shows immunosuppressive action and promotes tumor growth. CD68 is known as a pan-macrophage marker. We evaluate the CD68 expression in three cases of HPV-related carcinoma with adenoid cystic like features of the sinonasal tract.

Methods Three cases of HPV-related carcinoma with adenoid cystic like features were retrieved from our archives and stained with p16 and CD68 antibodies. Data was analyzed using spss version 21.

Results Patient ages were 46, 48 and 56 years old respectively, with a female to male ratio of 2:1. Histology showed epithelial surface dysplasia overlying basaoid cell growing in tubular and cribriform patterns. All were strongly positive for p16 stains (figure 1). CD 68 showed intratumoral and peritumoral expression in two cases while, one case showed only peritumoral expression. Infiltration of tumour associated macrophages (M2) CD68 cell in this study is associated with increase recurrence of HPV-related adenoid cystic carcinoma of the sinonasal tract (figure 2).

Conclusions The targeting of TAMs in HPV-related adenoid cystic carcinoma of the sinonasal tract and other cancers should be explored in the future using macrophage targeted approach.

Ethics Approval Health research ethics committee ABUTH/HREC/Y/2017

REFERENCES


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0001

2 THE MULTI-PHYSICS AUTOMATED RECONFIGURABLE SEPARATION (MARS®) SYSTEM PROVIDES HIGH PURITY, HIGH RECOVERY AND HIGH THROUGHPUT ENRICHMENT OF IMMUNE CELLS FOR IMMUNOTHERAPY

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Background Immunotherapies have proven to be a potent disruptor of cancer. Large quantities of purified lymphocytes are needed for expansion and downstream manipulation. This purification step has proven to be a major bottleneck for a streamlined cell production process and will only worsen as CAR-T cell therapies move into the clinic. In particular, autologous CAR-T cell therapies directly from cancer patients frequently undergoing existing therapies will require a cell purification technique that provides high recovery, high purity, and high throughput, while being gentle on the cells to ensure downstream efficacy. We present here an integrated system based on multiple physics principles with built-in technologies to achieve cell purification, concentration, and target cell isolation with high recovery and purity at an unprecedented sample flow rate. This platform – the Multi-physics Automated
Reconfigurable Separation (MARS®) system – combines novel acoustic cell processing and in-flow immuno-magnetic separation technologies with automation of the entire purification workflow for downstream cellular growth, modification, and analysis prior to being administered to patients.

**Methods** As a fully automated system, the MARS® system can isolate T-cells with high purity from lysed whole blood in as little as 11 minutes with up to 98% recovery and 98% viability without the need for Ficoll or centrifugation. The process is scalable to 10ML of blood, with complete purification requiring 1 hour. The system is designed to fit into a culture hood for sterile cell handling and has been used to isolate T-cells for expansion for downstream T-cell uses.

**Results** The tunable microfluidic cell processor is a functional module capable of washing and concentrating various sample types including all white blood cell types from whole blood, bone marrow, and apheresis. Additional uses for thawed frozen PBMC, cultured cells and solid tumor dissociation have also been demonstrated. Comparing with conventional centrifugation process, cell preparation by MARS has demonstrated high level of debris removal (>97%), minimal cell loss (>90% recovery) and high cell viability with full automation. MARS is the first-to-market fully automated system to integrate sample preparation and cell isolation into a single platform and is designed to be a versatile tool for downstream cell analysis workflows.

**Conclusions** MARS is the first to market automatic sample preparation system and is designed to be a versatile tool for downstream cell analysis platforms. The MARS® system is an ideal instrument to prepare CAR-T cells due to its ability to isolate and purify these cells from whole blood with high viability in a completely automated process.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0002

3 **BUTYROPHILIN-3A IS EXPRESSED IN MULTIPLE SOLID TUMORS: TRANSLATIONAL RESEARCH SUPPORTING THE EVICTION STUDY WITH ICT01, AN ANTI-BTN3A MAB ACTIVATING VG9VD2 T-CELLS**

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**Background** Butyrophilin-3A (BTN3A) three isoforms (3A1/3A2/3A3) are widely expressed on a variety of tumors. 3BTN3A1 plays a key role in phosphorylation activation of Vg9Vd2 T-cells, key mediators of innate and adaptive anti-tumor immunity. 4Vg9Vd2 T-cell infiltration into tumor tissues is associated with a positive prognosis across multiple cancers, 5which makes BTN3A an interesting target for enhancing anti-tumor immunity. ImCheck Therapeutics is developing ICT01, an anti-BTN3A mAb that specifically activates Vg9Vd2 T-cells. ICT01 is currently in an international, multi-center Phase 1/2a clinical trial (NCT04243499, EVICTION Study). The level of BTN3A expression required for a clinical response to ICT01 is not known. Therefore, we developed novel immunohistochemistry (IHC) methods to enable a precision-medicine based approach to target population selection for dose escalation and potentially guiding patient selection in the expansion cohorts of the ongoing EVICTION study.

**Methods** A panBTN3A IHC staining that recognizes the three isoforms was developed on Fresh frozen (FF) tissues, while BTN3A2- and BTN3A3-specific IHC methods were developed on formalin-fixed paraffin embedded (FFPE) tissues. BTN3A1-specific staining is still under development. Transfected knock-out/knock-in cell lines and positive tissues were used to assess antibody specificity. BTN3A expression was then analyzed on both normal and associated tumor tissue using tissue microarrays (TMA) and selected frozen blocks from tumor biopsies. 

FACS analyses were also performed on dissociated lung and pancreatic cancer biopsies to determine BTN3A (3 isoforms) membrane expression on tumor-infiltrating immune cells and cancer/stromal cells.

**Results** In normal tissues, BTN3A2 and BTN3A3 specific IHC signals were granular cytoplasmic in epithelial cells, with positive mononuclear and endothelial cells. Higher expression in lung, colon, and small intestine tissues was observed. Regarding panBTN3A expression, inter-indication and inter-patient heterogeneity was observed among head and neck, lung, melanoma, bladder, colon, pancreas, breast, and prostate cancer tissues, with both cytoplasmic and membranous localizations. The major finding was higher expression of BTN3A2 on malignant cells in melanoma, lung, colon, and prostate cancers, as compared to normal tissue. Finally, FACS analyses of lung and pancreatic cancer tissues revealed stronger expression of all BTN3A isoforms at the cell surface of infiltrated immune cells compared to its expression on stromal cells.

**Conclusions** These validated IHC methods supported the selection of cancer indications for the EVICTION trial and will potentially help identify specific tumor subtypes and patients that will most likely benefit from ICT01 treatment.

**REFERENCES**


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4 **MOLECULARLY GUIDED MULTIPLEXED DIGITAL SPATIAL ANALYSIS REVEALS DIFFERENTIAL GENE EXPRESSION PROFILES IN THE WNT-b-CATENIN PATHWAY BETWEEN MELANOMA AND PROSTATE TUMORS**

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**Background** The canonical WNT-b-catenin signaling pathway is vital for development and tissue homeostasis but becomes strongly tumorigenic when dysregulated. and alter the transcriptional signature of a cell to promote malignant transformation. However, thorough characterization of these transcriptional signatures has been challenging because traditional methods lack either spatial information, multiplexing, or sensitivity/specificity. To overcome these challenges, we
MULTIPLE MYELOMA FLOW CYTOMETRY PANEL
VALIDATED FOR CLINICAL MONITORING OF PATIENTS

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Background Multiple myeloma (MM) is an incurable plasma cell malignancy with significant heterogeneity in clinical presentation. Plasma cells are antibody-producing cells of lymphoid origin that are resident in secondary lymphoid organs and in the bone marrow (BM). The detection of circulating malignant plasma cells using flow cytometry has also been described in patients with MM. Enumerating and phenotyping malignant plasma cells in the BM and peripheral blood (PB) may be of value when evaluating the presence of MM antigens targeted by therapies before and during treatment and at relapse. To this end, a flow cytometric panel was developed to enumerate and characterize malignant plasma cells and additional immune subsets.

Methods PB and BM aspirates (BMA) were obtained from healthy donors and MM donors who consented to research testing. MM cell lines were also used to spike into donor samples to detect specific antigens (collected in Cyto-Chex™ blood collection tubes). Samples were then transferred to True-Count tubes to enumerate immune populations. Fluorescently labeled antibodies directed against CD38, CD138, CD56, CD45, BCMA were evaluated to assess parameters such as time and temperature stability of the reportable immune populations by monitoring the frequencies of the populations. In addition, the limit of quantitation, intra- and inter-assay precision were determined.

Results The MM Counting Panel was optimized to leverage antigen expression and fluorophore combinations. A gating strategy enabled enumeration of MM cells based on antigens that can be further subdivided based on BCMA expression. Further testing showed that the precision in frequencies and absolute counts of key reportable populations was deemed acceptable (%CV of <30%). The precision was within the acceptance criteria of %CV <30% for populations with >100 cells. Stability testing revealed that samples were more stable at ambient temperature relative to 4°C, with stability being maintained for 48 h post-collection, where at least 85% of reportable immune readouts were stable (%change <30% relative to baseline), for BMA and PB from various donors (healthy and MM). The panel was ultimately deployed for use with clinical samples from MM clinical trials. Clinical data generated from the MM Counting Panel allowed the identification of malignant plasma cell populations in BMA of patients from trial assessing a BCMAxCD3 bi-specific antibody (NCT03761108).

Conclusions A flow cytometric assay to enumerate and identify normal and malignant plasma cells in MM patients was successfully developed. The approach used can be applied to develop assays for other indications in which patients are treated with therapies.

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PRE-CLINICAL PHARMACODYNAMIC BIOMARKER ASSAYS OF IMMUNE MODULATION CAN TRANSLATE TO INFORM EXPLORATORY ENDPOINTS OF TARGET ENGAGEMENT IN FIRST-IN-HUMAN CLINICAL TRIAL STAGES OF DRUG DISCOVERY

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Background Lack of efficacy is a common cause of failure in Phase I and Phase II clinical trials. Pharmacodynamic (PD) biomarker assays can demonstrate target engagement and proof of mechanism; both key components to improve trial success. Biomarkers established at the pre-clinical phase can serve as exploratory endpoints in early phase clinical trials, to confirm the mode of action of the therapeutic. We show examples of human in vitro assays and murine T cell adoptive transfer models, which can be used to establish potential PD biomarkers for inclusion in the clinical phases.

Methods Human peripheral blood mononuclear cell (PBMC) were incubated with SEB in the presence of Pmbrolizumab or Ipilimumab. IL-2 and IFNgamma levels were quantified by
Luminex. To identify biomarkers of checkpoint inhibition, mice were transfused with a defined population of ovalbumin (OVA)-specific T cells were challenged with OVA antigen or EG7 tumour. Activation and proliferation of antigen-specific T markers was determined and Nanostring gene expression analysis performed. Flow cytometry staining panels for human immune markers including CD4, CD14, CD25 and FOXP3 were established pre-clinically. As part of the assay validation process for a clinical trial, whole blood SEB activation was performed in normal donors, with Luminex analysis of IL-2, IL-17, IFN-gamma and TNAphla.

Results Immune checkpoint inhibitors resulted in increased IL-2 and IFN-gamma secretion in human PBMC stimulated with SEB. In the murine PD model, anti-PD-L1 caused upregulation of CD25, IFN-gamma and granzyme B by antigen-specific CD8 T cells. Gene expression analysis of murine tumours elucidated changes in response to a vaccine. Flow cytometry panel staining determined the frequencies of human Treg and monocytes, which are common targets of immune-modulating therapies. Fit-for-purpose validation was performed for a human SEB activation assay resulting in robust changes in cytokine production.

Conclusions The experiments here show the flow of experiments that can be performed to identify a PD biomarker for use in first in man trials; the pre-clinical human PBMC SEB screening assay provides a simple assay demonstrating that a therapy can enhance T cell function and would be translatable to the clinic. The murine PD model provides a platform to screen for biomarkers of T cell function and monitor gene expression modulation. Biomarkers identified in the murine setting provide a good starting point for exploratory assessment in early phase clinical trials, where inclusion of exploratory PD biomarker endpoints can in confirm proof of mechanism and improve study success rates.

Ethics Approval Human tissues used in this study were collected with ethical approval from UK Research Ethics Committee South West, Bristol (UK), approval number 15/SW/0029.

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class="abstract"

PROGNOSTIC FACTORS FOR OVERALL SURVIVAL IN PATIENTS WITH ADVANCED MELANOMA TREATED WITH ANTI-PD-1 THERAPY – THE MELImmune SCORE

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Background Immune checkpoint inhibitors (ICI) have changed the paradigm of advanced malignant melanoma (MM). Several prognostic factors, mostly linked to inflammation, have been under scope to better select patients for such therapies. We aimed to build and apply a prognostic score in this setting.

Methods Baseline characteristics and outcomes on 147 patients with advanced MM treated with an anti-PD1 (nivolumab or pembrolizumab) in monotherapy, between Jan-2016 and Oct-2019, in the 1st, 2nd or 3rd line setting were collected from two centres in Portugal. Data cut-off for follow-up was May-2020. Cox proportional hazards regression was used to identify independent prognostic factors for OS.

Results With a median FU of 28.93 months (95% CI [22.52–33.54]), mOS for the whole cohort was 14.75 months (95% CI, [10.80–18.71]). Overall, 43 and 104 patients were treated with nivolumab and pembrolizumab, respectively. We identified four adverse prognostic factors that were independent predictors of bad prognosis: number of metastatic sites >2 (p<0.001), baseline PS-ECOG =1 (p<0.001), presence of baseline lymphopenia (over lower limit of normal) (p=0.002) or very high baseline LDH (>2x upper limit of normal) (p<0.001). Patients were separated into three risk categories according to the number of risk factors present: favourable prognosis (no risk factors; n=34), intermediate prognosis (one risk factor; n=65) and poor prognosis (two or more risk factors; n=48). mOS was 43.41 (95% CI [32.13–54.69]), 14.39 (95% CI [6.78–22.01]) and 6.53 months (95% CI [3.61–9.44]), for favourable, intermediate, and poor prognosis group, respectively (p<0.001; figure 1). AUC of ROC curve for OS was 0.737 (95% CI [0.654–0.819], p<0.001).

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class="abstract"

IMMUNE CORRELATES ASSOCIATED WITH CLINICAL OUTCOMES IN PATIENTS WITH ADVANCED MALIGNANCIES TREATED WITHavelumab ANDOX40 AGONIST

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Background We evaluated immune correlates of avelumab in combination with PF-04518600 (OX40 agonist) in a phase I/II study (NCT03217747) in patients with advanced malignancies.

Conclusions Using easily accessible parameters from our daily practice, we propose the MELImmune prognostic score for advanced MM patients treated with anti-PD1 in monotherapy that could be incorporated to the daily clinical practice and clinical trials. We further aim to validate this score in an independent larger sample.

Ethics Approval The study was approved by both institutions’ Ethics Committee.
Methods
Eligible patients received intravenous avelumab 10 mg/kg and PF04518600 100 mg every 2 weeks in a 4-week cycle. Initially, patients received avelumab from cycle 3 day 1 (C3D1), later from cycle 1 day 15 (C1D15). Response was assessed per RECIST 1.1 and irRECIST. Peripheral blood and tumor tissue were obtained from patients at pre-treatment, post-OX40, and post-combo for correlative studies. Translational assays include immunohistochemistry (IHC: PD-L1, OX40 and CD139), multiplex immunofluorescence (mIF: PD-L1/P-1 axis and T-cell activation/regulatory panels), and Nano-string (panCancer Immune Panel) for tumor tissues, and flow cytometry performed on peripheral blood. Fisher’s exact test was used to compare response between the two treatment schedules. Log-rank test was performed to test the difference in overall survival (OS) and progression-free survival (PFS) between groups. Linear mixed-effect model was used to evaluate the effect of schedule and treatment (time) effects on flow and IHC mIF biomarkers.

Results
Twenty-eight patients were treated, 12 received avelumab from C3D1 and 16 from C1D15. Patient characteristics are summarized in table 1 and response data in table 2. The median follow-up time was 17.8 months. The median OS was 7.9 months and PFS was 3.2 months. Patients on C3D1 schedule had superior PFS than patients on C1D15 schedule (4.6 months vs 2.5 months; P=0.032). The biomarkers associated with survival were investigated in the C3D1 group. Patients with following baseline biomarker characteristics had superior PFS (table 3): lower density of total cells expressing CD137 (6.0 vs 3.2 months, P=0.047) and lower percentage of malignant cells expressing OX40+ (5.8 vs 3.2 months, P=0.024). Patients with superior OS had higher frequencies of CD86+HLA-DR+CD141+ dendritic cells and CD3+ cells in circulation at baseline (17.2 vs 7.9 months, P=0.012) (table 4). This combination was not found to expand circulating T regulatory cells. Early data suggests that while higher neutrophil score correlates with PD, higher exhausted CD8+ T cell score correlates with SD. More translational data will be presented at the conference.

Conclusions
With limited data, there is evidence that patients receiving avelumab from C3D1 in combination with PF-04518600 have better response. Antigen presentation machinery showed changes but remained overall intact within the tumor with baseline circulating CD141+CD86+HLA-DR+ DCs positively correlating with OS. Additional studies to evaluate the effect of T-cell agonist on their receptors on malignant cells are needed.

Ethics Approval
The study was approved by The University of Texas MD Anderson Cancer Center Institutional Review Board (FWA #: 00000363).

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9  MESENCHYMAL FEATURES OF A NOVEL 27-GENE ALGORITHM ASSOCIATE WITH CANONICAL TUMOR PROMOTING SIGNALING PATHWAYS WHICH MAY IDENTIFY THERAPEUTIC OPTIONS FOR IMMUNOTHERAPY RESISTANT PATIENTS

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Background
Immune checkpoint inhibitors have emerged as a front-line treatment for cancer in multiple indications. Unfortunately, a majority of patients do not realize durable response as a result of primary resistance to the
A MULTIPARAMETER FLOW CYTOMETRY ASSAY TO IDENTIFY OTHER CELLULAR SUBSETS KNOWN TO BE AMENABLE TO CYTOKINE MODULATION (E.G., CD3 AND CD14) WERE INCLUDED FOR CONCURRENT MONITORING OF T CELL PROLIFERATION AND MONOCYTE ACTIVATION. METHOD VALIDATION FOCUSED ON ANALYTICAL SENSITIVITY, SPECIFICITY AND PRECISION AS KEY CRITERIA OF ASSAY PERFORMANCE USING PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) STIMULATED WITH NK CELL-ACTIVATING CYTOKINES AND RESTING PBMCs FROM HEALTHY DONORS.

RESULTS The assay design allowed for robust quantitation of NK cell, T cell and monocyte functionalities. Lower limit of quantification (LLOQ) of target biomarker population was determined to be 1.0% of the parent population, based upon an analysis of 110 key target populations that displayed a coefficient of variation (CV) of ≤25% and their frequencies ranged from 0.1% to 97.8% of the parent population. Additionally, ≤25% CV was observed in precision assessments, confirming the repeatability and reproducibility of the assay. Clinical trial utility of the assay was verified on cryopreserved PBMCs from patients with a variety of solid tumor malignancies. In these patients, the assay could clearly identify proliferating and activated NK cells, as well as proliferating T cells and activated monocytes, thus demonstrating its suitability for clinical trial applications.

CONCLUSIONS We developed and validated a novel multiparameter flow cytometry assay that allows for simultaneous measurement of proliferation, activation and inhibitory status of key immune cell subsets. Thus, this assay can help shed light on the mode of efficacy of novel therapeutic agents that modulate the immune system, aimed at treatment of cancer and autoimmune diseases.

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10 A MULTIPARAMETER FLOW CYTOMETRY ASSAY TO MONITOR NATURAL KILLER CELL PROLIFERATION AND ACTIVATION IN IMMUNO-ONCOLOGY CLINICAL TRIALS

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BACKGROUND Natural Killer (NK) cells have garnered increasing interest as potential cellular therapies or as targets of biotherapeutic agents due to their ability to kill tumor cells in a non-antigen dependent manner. Hence, measurement of NK cell proliferation and/or activation following treatment can serve as a useful biomarker for assessing the efficacy of immunomodulatory therapies.

METHODS We developed a novel 13-parameter flow cytometry panel incorporating cell differentiation (CD) markers important for identification of NK cell subsets (CD56, CD16), their proliferation (Ki-67), activation (CD25, CD335, NKG2D) and inhibition (CD159a) status. Additionally, CD markers that identify other cellular subsets known to be amenable to cytokine modulation (e.g., CD3 and CD14) were included for concurrent monitoring of T cell proliferation and monocyte activation. Method validation focused on analytical sensitivity, specificity and precision as key criteria of assay performance using peripheral blood mononuclear cells (PBMCs) stimulated with NK cell-activating cytokines and resting PBMCs from healthy donors.

RESULTS The assay design allowed for robust quantitation of NK cell, T cell and monocyte functionalities. Lower limit of quantification (LLOQ) of target biomarker population was determined to be 1.0% of the parent population, based upon an analysis of 110 key target populations that displayed a coefficient of variation (CV) of ≤25% and their frequencies ranged from 0.1% to 97.8% of the parent population. Additionally, ≤25% CV was observed in precision assessments, confirming the repeatability and reproducibility of the assay. Clinical trial utility of the assay was verified on cryopreserved PBMCs from patients with a variety of solid tumor malignancies. In these patients, the assay could clearly identify proliferating and activated NK cells, as well as proliferating T cells and activated monocytes, thus demonstrating its suitability for clinical trial applications.

CONCLUSIONS We developed and validated a novel multiparameter flow cytometry assay that allows for simultaneous measurement of proliferation, activation and inhibitory status of key immune cell subsets. Thus, this assay can help shed light on the mode of efficacy of novel therapeutic agents that modulate the immune system, aimed at treatment of cancer and autoimmune diseases.

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11 A MULTI-PHYSICS APPROACH ENABLING RARE CELL ISOLATION WITH HIGH RECOVERY AND HIGH PURITY

Liping Yu, Silin Sa, Alice Wang*. Applied Cells

BACKGROUND Advancements in fields of multi-omics analysis and cell-based therapies depend upon efficient cell processing tools to isolate rare cancer and immune cells from complex biologic samples as an initial step in sample preparation. Conventional technologies are limited in automation, recovery and purity. We present an integrated system based on multiple physics principles with built-in novel technologies to achieve cell purification, concentration and target cell isolation, with high recovery at an unprecedented flow rate. This platform, the Multi-physics Automated Reconfigurable Separation (MARS), combines tunable, acoustic cell processing and in-flow immuno-magnetic separation technologies, enabling automation of the entire cell sample preparation workflow for proteomics and genomics analysis.

METHODS Circulating tumor cells (CTC) are present in extreme low frequency in blood stream (1-100 in billions of blood cells) thus it has been a challenge to isolate CTCs with high recovery. We have developed protocols on MARS to isolate CTCs from whole blood for multi-color flow cytometry analysis. To demonstrate the extent of enrichment of tumor cells in whole blood, PC3 cells were used for spike recovery. RBC lysed blood sample was then loaded on MARS and automatically processed through cell washing, concentration, and magnetic depletion. Enriched tumor cells were collected and analyzed by flow cytometry.
Results Results show > 4 log enrichment of tumor cells and average recovery of spiked CTC > 85% in the clinical relative range <100 cells per ml of whole blood (R2=0.929) with a throughput of 60 ml/hr. Isolated cells were confirmed to be cancer cells with imaging analysis and single cell genomic sequencing. The protocol was also validated with other cell line cells such as A549. The purity of the cells prepared by MARS are ideal for single cell genomics platforms.

Conclusions The fluids of MARS is also replaceable and can be sterilized to minimize sample to sample contamination. The high molecular debris removal achieved by MARS is ideal for single cell genomics platforms, as is the first-to-market automated and integrated sample preparation and cell separation system designed to be a versatile tool for downstream cell analysis.

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DEVELOPMENT OF AN IN VITRO ASSAY TO ASSESS BISPECIFIC T CELL ENGAGER USING T CELLS FROM CD3E HUMANIZED MICE


Background Bispecific T cell engagers (BiTE) is a fast-growing class of immunotherapies. They are bispecific antibody that bind to T cell-surface protein (for example, CD3e) and a specific tumor associate antigen (TAA) on tumor cells, by which to redirect T cells against tumor cells in a MHC-independent manner. A successful example in the clinical is Blinatumomab, a BiTE antibody against CD3/CD19 approved in 2014 to treat acute lymphoblastic leukemia. Currently, many CD3-based BiTE are in clinical trials, including BCMAXCD3, Her2xCD3, CEaxCD3, and PSMAXCD3. To evaluate the efficacy of BiTE in vitro, human peripheral blood monocyte cells (hPBMC) are commonly being used as a source of T cells to co-culture with tumor cells. The disadvantage of using hPBMC is donor-to-donor variability and the availability of the original donor if a study needs to be repeated.

Methods To overcome this, we proposed to replace hPBMC with T cells from human CD3ε (hCD3) genetically engineered mouse models mice (GEMM) for in vitro coculture assay. T cells were isolated from hCD3 GEMM mice using negative selection mouse T cell isolation kit. Conventional tumor cell lines or luciferase-engineered patient-derived-xenograft (PDX)-derived organoids (PDXO) expressing specific antigens are co-cultured with hCD3 T cells in 96-well plates in the presence of BiTE antibody.

Results We measured the killing of tumor cells using either flow cytometry or luciferase activity as readouts. To analyze tumor-reactivity of T cells to cancer cell line or organoids, IFN-gamma in the culture medium was measured and activation markers on T cells was assessed.

Conclusions Our data showed the feasibility of using humanized mice T cells as a replacement for hPBMCs to assess BiTE antibody in vitro. We are further validating the application of murine hCD3 T cells for in vivo models to test bispecific T cell engagers.

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USE OF ANTI-VIRAL T CELLS TO MODEL HLA-RESTRICTED ANTI-TUMOR CYTOTOXIC LYMPHOCYTE RESPONSES

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Background With the success of T cell checkpoint antagonists in treating cancer, we must better understand treatment response heterogeneity and develop more physiological preclinical models for evaluating the next wave of candidate therapeutics. Several hurdles limit the successful recapitulation of the cellular and molecular interactions between human T cells and tumor cells, not the least of which involves the challenge of access to – and ex vivo manipulation of – bona fide tumor antigen-specific T cells.

Methods In order to improve on our understanding of checkpoint therapy using human model antigens, we developed an antigen-specific T cell-mediated cytotoxicity model using anti-viral human T cells co-cultured with a human tumor cell line expressing viral peptide epitopes.

Results We found that anti-viral T cells could be used to model cytotoxic HLA-restricted anti-tumor responses and these responses varied by donor according to peptide antigen density, antigen quality, T cell numbers, and time. By identifying sub-optimal conditions in a donor-specific fashion, we demonstrated enhanced cytolytic function of T cells in vitro when combined with multiple disparate anti-tumor modalities, including immune checkpoint blockade, growth factor blockade, and chemotherapy. This in vitro model was then successfully adapted to an in vivo tumor model system that demonstrated control of tumor growth in an antigen-dependant manner that was responsive to checkpoint blockade.

Conclusions These in vitro and in vivo systems represent a simple, yet elegant and robust platform for testing human T cell-directed immuno-oncology (IO) therapeutics and IO combinations.

Ethics Approval All animal experiments were conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in accordance with institutional animal care and use committee guidelines and after appropriate approvals.

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NOVEL CD3 EPSILON HUMANIZED N-TERMINAL EPITOPE MODEL FOR ASSESSMENT OF EFFICACY OF T-CELL ENGAGERS

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Background T-cell engagers have proved to be a promising therapeutic strategy in immunotherapy, for redirecting T cells activity against tumor cells. To facilitate the preclinical assessment of novel T-cell engagers and their translatability, we have developed an immunocompetent CD3 epsilon N-terminal epitope humanized mouse model.

Methods This model was developed to express the human epitope of the CD3 epsilon chain, which is recognized by approximately 70% of the T-cell engagers (clone SP34). The rest of the extracellular domain was kept from mouse origin to preserve the amino acids involved in the interaction with CD3 gamma and delta. Similarly, the transmembrane domains
and the intracellular domains where kept murine to enable salt bridges interaction, interaction with the CD3 zeta and the signaling into mouse cells.

**Results** T cells from CD3 epsilon epitope humanized mice are found in comparable frequency in spleen, blood and bone marrow from WT mice. B cells, monocytes, dendritic cells and NK frequencies are also similar to the frequencies of these cell types in WT mice, suggesting that the humanization of the epitope of CD3 epsilon did not alter the immune cells distribution in these mice. Activation of T cells with antibodies targeting human CD3 (clone SP34) induced CD4 and CD8 T cell proliferation, as well as production of IL-2 and IFN-gamma. The CD3 functionality was demonstrated in vitro by the ability of B cells to produce IgM upon activation of T cells, suggesting a proper cooperation between T and B cells. Additionally, a first class of T-cell engagers targeting both human CD3 and a tumoral antigen, induced tumor cell lysis of MC38-Ag in a concentration-dependent manner. A second class of T cell engagers, also targeting CD3 and a tumoral antigen, showed an anti-tumor effect in vivo, and this effect was also shown to be dose-dependent.

**Conclusions** These data suggest that the CD3 epsilon N-terminal epitope humanized mouse model enables the assessment of efficacy and mechanism of action of T-cell engagers. This model is currently being intercrossed with immunostimulatory humanized tumor models to provide new opportunities for assessment of bi-specific antibodies targeting the CD3 and immunostimulatory molecules. This model is the first generation of a broader program aiming at developing a Pan CD3 humanized model, where the gamma, delta and epsilon chains of the CD3 complex will be humanized. The Pan CD3 humanized mice are currently being investigated for immune responses and would provide a broader tool for assessment of T-cell engagers.

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**A NOVEL CD28 HUMANIZED MOUSE MODEL FOR EFFICACY ASSESSMENT OF CD28-TARGETING THERAPIES**

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**Background** Immuno-intervention through targeting of activating and inhibitory immune checkpoints (ICP), has shown promising results in the clinic over the last years. To facilitate these researches, mouse models expressing humanized ICP instead of their mouse counterparts were developed. Herein, we describe a novel CD28 humanized mouse model (hCD28 model), designed to test compounds targeting human CD28 (hCD28).

**Methods** Human and mouse CD28 (mCD28) have different signalling responses, with hCD28 being known for inducing higher levels of pro-inflammatory cytokines upon stimulation with ligands/superagonists. This can be explained by the expression of CD28i, a hCD28 amplifier isoform which is not found in mouse. Additionally, evidences suggested that the different signalling between human and mCD28 relies on one amino acid change in the intracellular domain (ICD).1 Because the hCD28 model was developed to assess hCD28-targeting therapeutics, we decided to keep the expression of both canonical and CD28i isoforms to avoid undermining the biological effects of the testing antibodies. Although keeping the human ICD could favour the evaluation of cytokine production and therefore the safety of the test therapeutics, we decided to keep the mouse ICD to enable a proper interaction of CD28 with its signalling partners, allowing a physiological stimulation of CD28 in efficacy studies.

Results hCD28 mice express hCD28 on T cells and the frequency of CD3 T cells is comparable in both WT and hCD28 mice. Stimulation of hCD28 mice-isolated T cells with hCD28 ligands and agonist antibodies resulted in T cell proliferation and cytokine production, suggesting that hCD28 is functional in mouse cells, MC38 uptake rate and kinetic of growth were comparable in WT and hCD28 mice, suggesting no major defect in the immune response in the hCD28 mice. Importantly, splenocytes and tumor draining lymph nodes cells isolated from tumor-bearing hCD28 mice showed higher production of IL-2 and IFN-gamma upon in vitro re-challenged with MC38 when compared to WT cells. Since the frequency of CD3 cells (Treg, CD4+ and CD8+) is comparable to WT mice, this could be explained by the expression of the amplifier CD28i isoform, which is absent in WT mice.

**Conclusions** The hCD28 model described here supports the efficacy assessment of hCD28-targeting biologics, enabling PK/PD studies as hCD28 expression levels and pattern are physiologically. However, after careful consideration of the CD28 biology, we decided to keep the mouse ICD, although it triggers lower pro-inflammatory cytokine production than CD28 human ICD. As such, this model is not suitable for toxicology/safety studies.

**REFERENCE**


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**ANTIBODY PROFILING OF PROSTATE CANCER PATIENTS REVEALS DIFFERENCES IN ANTIBODY SIGNATURES AMONG DISEASE STAGES AND FOLLOWING TREATMENT**

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**Background** Previous studies of prostate cancer autoantibodies have largely focused on diagnostic applications. So far, there have been no reports attempting to more comprehensively profile the landscape of prostate cancer-associated antibodies. Specifically, it is unknown whether the quantity of antibodies or the types of proteins recognized change with disease progression or treatment.

**Methods** A peptide microarray spanning the amino acid sequences of the gene products of 1611 prostate cancer-associated genes was synthesized. Serum samples from healthy male volunteers (n=15) and prostate cancer patients (n=85) were used to probe the array. These samples included patients with various clinical stages of disease: newly diagnosed localized prostate cancer, castration-sensitive non-metastatic prostate cancer (nmCSPC), castration-resistant non-metastatic prostate cancer (nmCRPC), and castration-resistant metastatic disease (mCRPC). Serial sera samples from individuals who received treatment with either standard androgen deprivation therapy (ADT) or an anti-tumor vaccine were also used to probe the
array, to determine whether we could detect treatment-related changes.

**Results** We demonstrated that this peptide array yielded highly reproducible measurements of serum IgG levels. We found that the overall number of antibody responses did not increase with disease burden. However, the composition of recognized proteins shifted with clinical stage of disease. Our analysis revealed that the largest difference was between patients with castration-sensitive and castration-resistant disease. Patients with castration-resistant disease recognized more proteins associated with nucleic acid binding and gene regulation compared to men in other groups. Our longitudinal data showed that vaccine-treated patients developed increased responses to more proteins over the course of treatment than did ADT-treated patients consistent with the development of antigen spread.

**Conclusions** This study represents the largest survey of prostate-cancer associated antibodies to date. We have been able to characterize the classes of proteins recognized by patients and determine how they change with disease burden. Our findings demonstrate the potential of this platform for measuring antigen spread and studying responses to immunomodulatory therapies.

**Acknowledgements** UW-Madison Medical Scientist Training Program: GM008692UW-Madison Institute for Clinical and Translational Research Predoctoral TL1 Program: TR002375

**Ethics Approval** Study protocols that permitted collection and use of human blood samples were reviewed and approved the University of Wisconsin Human Subjects’ Review Board (IRB). All patients gave written informed consent for use of blood products for research.

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### ACTIVITY SENSORS FOR NONINVASIVE MONITORING OF IMMUNE RESPONSE AND TUMOR RESISTANCE DURING IMMUNE CHECKPOINT BLOCKADE THERAPY

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**Background** Despite the curative potential of immune checkpoint blockade (ICB) therapy, only small subsets of patients achieve tumor regression while many responders relapse and acquire resistance. Monitoring treatment response and detecting the onset of resistance are critical for improving patient prognoses. Here we engineered ICB antibody-sensor conjugates known as ICB-Dx by coupling peptides sensing the activity of granzyme B (GzmB), a T cell cytotoxic protease, directly on αPD1 antibody to monitor therapeutic responses by producing a fluorescent reporter into urine. To develop biomarkers that indicate mechanisms of resistance to ICB, we generated B2m-/- and Jak1-/- tumor models and performed transcriptional analyses to identify unique protease signatures of these resistance mechanisms. We then built a multiplexed library of αPD1-Dx capable of detecting early therapeutic response and illuminating resistance mechanisms during ICB therapy.

**Methods** FITC-labeled GzmB substrates were synthesized (CEM) and conjugated to αPD1 antibody. B2m-/- and Jak1-/- tumors were generated from WT MC38 cells using CRISPR/Cas9. For tumor studies, 10^6 cells were inoculated s.c. in B6 mice. Tumor mice were treated with αPD1 or IgG1 isotype conjugates (0.1 mg), and urine was collected at 3 hours. Tumor RNA was isolated with RNEasy kit (Qiagen) and prepared for sequencing with TruSeq mRNA kit (Illumina).

**Results** To synthesize αPD1-Dx, we coupled FITC-labeled GzmB substrates to αPD1 antibody (figure 1a). In MC38 tumors, systemic administration of αPD1-Dx lowered tumor burden relative to control treatment while producing significantly elevated urine signals that preceded tumor regression (figure 1b, c). To investigate the ability to monitor tumor resistance to ICB, we developed knockout tumors to model B2m and Jak1 mutations, which are observed in human patients. In vivo, B2m-/- and Jak1-/- MC38 tumors were resistant to αPD1 monotherapy (figure 1d). Tumor RNA sequencing revealed that gene expression was altered during αPD1 treatment only in WT tumors. Importantly, B2m-/- tumors showed very different expression profiles than Jak1-/- tumors during αPD1 treatment, indicative of unique regulation of resistance (figure 1e). We used differential expression analyses to discover unique protease signatures associated with these two resistance mechanisms. Finally, a multiplexed library of αPD1-Dx engineered to monitor both tumor and immune proteases detected early on-treatment responses and stratified B2m-/- from Jak1-/- resistance with high diagnostic validity (figure 1f).

**Conclusions** We have engineered activity sensors that accurately detect therapeutic responses and stratify resistance mechanisms noninvasively from urine, thereby potentially expanding the precision of ICB therapy to benefit cancer patients.

**Ethics Approval** All animal studies were approved by Georgia Tech IACUC (A100193).

Abstract 17 Figure 1 Monitoring response and resistance with ICB-Dx (a) αPD1-Dx can reinvigorate T cell response and monitor protease activities in the tumor microenvironment. (b) Growth curves of WT MC38 tumors treated with αPD1- or IgG1-Dx (ANOVA). (c) Urine signals detect treatment response to αPD1 monotherapy (ANOVA). (d) Growth curves of B2m-/- and Jak1-/- tumors treated with αPD1- or IgG1-Dx (ANOVA). (e) TSNE plot showing RNA profiles of WT, B2m-/-, Jak1-/- tumors treated with αPD1 or isotype control. (f) ROC curves of random forest classifiers built from urine signals that differentiate on-treatment response from on-treatment resistance and B2m-/- from Jak1-/- resistance.

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NEW METHOD OF ASSESSING TUMOR HETEROGENEITY UTILIZING BOTH CIRCULATING TUMOR DNA AND TISSUE DNA TO PREDICT THE RESPONSE TO IMMUNOTHERAPY

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Background Tumor heterogeneity assessment may help predict response to immunotherapy. In melanoma mouse models, tumor heterogeneity impaired immune response. In addition, among lung cancer patients receiving immunotherapy, the high clonal neoantigen group had favorable survival and outcomes. Ideal methods of quantifying tumor heterogeneity are multiple biopsies or autopsy. However, these are not feasible in routine clinical practice. Circulating tumor DNA (ctDNA) is emerging as an alternative. Here, we reviewed the current state of tumor heterogeneity quantification from ctDNA. Furthermore, we propose a new tumor heterogeneity index (THI) based on our own scoring system, utilizing both ctDNA and tissue DNA.

Methods Systematic literature search on Pubmed was conducted up to August 18, 2020. A scoring system and THI were theoretically derived.

Results Two studies suggested their own methods of assessing tumor heterogeneity. One suggested clustering mutations with Pyclone, and the other suggested using the ratio of allele frequency (AF) to the maximum somatic allele frequency (MSAF). According to the former, the mutations in the highest cellular prevalence cluster can be defined as clonal mutations. According to the latter, the mutations with AF/MSAF<10% can be defined as subclonal mutations. To date, there have been no studies on utilizing both ctDNA and tissue DNA simultaneously to quantify tumor heterogeneity. We hypothesize that a mutation found in only one of either ctDNA or tissue DNA has a higher chance of being subclonal. We suggest a scoring system based on the previously mentioned methods to estimate the probability for a mutant allele to be subclonal. Adding up the points that correspond to the conditions results in a subclonality score (table 1). In a given ctDNA, the number of alleles with a subclonality score greater than or equal to 2 divided by the total number of alleles is defined as blood THI (bTHI) (figure 1). We can repeat the same calculation in a given tissue DNA for tissue THI (tTHI) (figure 2). Finally, we define composite THI (cTHI) as the mean of bTHI and tTHI.

Conclusions Tumor heterogeneity is becoming an important biomarker for predicting response to immunotherapy. Because autopsy and multiple biopsies are not feasible, utilizing both ctDNA and tissue DNA is the most comprehensive and practical approach. Therefore, we propose cTHI, for the first time, as a quantification measure of tumor heterogeneity.

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WHOLE-EXOME SEQUENCING BASED IMMUNOGENOMIC PROFILING WITH POTENTIAL CLINICAL APPLICABILITY IN CIRCULATING CELL-FREE DNA AND TISSUE FROM ADVANCED STAGE COLORECTAL CANCER PATIENTS

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Background Assessing cfDNA at a whole-exome scale (WES) enables comprehensive immunogenomic profiling and interrogation of tumor heterogeneity. We comprehensively investigate genomic alterations and neoantigens in cfDNA at WES-scale using Personalis’ NeXT Liquid Biopsy™. Genomic alterations, neoantigens, and molecular tumor micro-environment (mTME) from matched solid tumor are evaluated using Personalis’ ImmunoID NeXT Platform®.

Methods Matched plasma, tumor, and adjacent normal tissues were collected from 13 late-stage, treatment-naive CRC patients. cfDNA was extracted and assessed exome-wide, then the mutational landscape and immunogenomic profile were analyzed.2 gDNA extracted from tumor was analyzed by the ImmunoID NeXT Platform, where somatic variants and
neoeantigens were evaluated. RNA analysis of the solid tumor enabled the investigation of the mTME. 

Results The average number of somatic SNVs in plasma samples was 100.5 (Range 50–250). KRAS, APC, PIK3CA, SMAD4, FBXW7, ARID1A were identified. Specifically, two components of SWI/SNF complex, ARID1A and BRD9, were both mutated in plasma samples, suggesting the potential dysregulation of epithelial pathways. RTK-RAS and Notch pathways were also frequently mutated. Further, 1,195 somatic events were found in genes not covered by commercially available targeted panels. 27 of these SNVs are in immuno-oncology related genes, which highlight the importance of somatic evidence observable through an exome-scale cfDNA approach. In solid tumor, the average number of detected somatic SNVs was 133.4 (Range 69–230), with similar mutation landscape. Concordance is observed between tumor and plasma samples (mean: 40.6%; range: 15.13%–94.2%). However, a number of variants are plasma-specific, suggesting that cfDNA WES detects tumor mutations that might be missed by a single site biopsy. We evaluated neoantigens and determined that the fraction of variants predicted as neoantigens are similar between plasma and tumor. Importantly, several of the top neoepitopes are uniquely predicted in plasma, suggesting the potential clinical value of using WES cfDNA. RNA-sequencing of solid tumor samples enabled mTME profiling. CD8 T cell immune infiltration, TCR beta clonality and clone counts were low, suggesting these patients have cold tumors. Myeloid dendritic cells and macrophages demonstrated uniform abundance across samples, while B and T regulatory cells showed variable tumor infiltration.

Conclusions Results demonstrate potential clinical utility and highlight the advantages of whole-exome scale profiling of plasma and matched tumor samples, which enables a systematic interrogation of tumor biology, including mTME. Notably, a whole-exome based liquid biopsy assay offers indispensable insights that might be otherwise missed by a single site tumor biopsy or targeted liquid biopsy panels.

Ethics Approval The study protocol was in accordance with the tenets of the Declaration of Helsinki. Commercial samples used in this study were procured from Bioreclamation IVT and BioChain following protocols approved by the local Institutional Review Board (IRB) committee. Informed consent forms were obtained from all the human subjects in this study.

Consent N/A

REFERENCES


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response in non-small cell lung cancer. Here, we present initial results of a host response-based machine learning classifier that predicts clinical outcome in melanoma patients treated with immune checkpoint inhibitors (ICIs).

**Methods** Plasma samples from melanoma patients (training set; n=32) treated with anti-PD-1 or anti-PD-1 and anti-CTLA-4 combination were obtained at baseline and early on treatment. Response was based on RECIST criteria. Proteomic profiling of the plasma samples was performed using ELISA-based antibody arrays. Machine learning algorithms were used to identify a predictive signature that stratifies between responders and non-responders. The signature was validated on an independent cohort of melanoma patients (validation set; n=14).

In addition, advanced bioinformatic analysis was performed in order to identify biological pathways unique to responders and non-responders.

**Results** A 3-protein signature was identified as a predictor of clinical outcome following immunotherapy with an area under the curve (AUC) of the receiver operating characteristics (ROC) plot of 0.88 (p-value 5.84E-05; confidence interval 0.76 – 1.0), and sensitivity and specificity of 0.65 and 0.95, respectively. This signature was successfully validated with AUC of 0.85 (p-value 0.03; confidence interval 0.63 – 1.0), and sensitivity and specificity of 0.75 and 0.9, respectively. To further explore the biological basis of resistance to immunotherapy, we performed a pathway enrichment analysis. Multiple mechanisms for resistance were identified in the non-responders group, including immunosuppression and inflammation associated pathways. Comparison between the two treatment modalities revealed pathways unique to each treatment that involve extracellular modulation, immunosuppression and processes associated with tumor progression, which may imply important differences between the two regimens.

**Conclusions** Our results demonstrate that analyzing the host response to IC inhibitor therapy using plasma-based proteomic profiling combined with machine learning algorithms serves as a successful approach for predictive biomarker discovery in melanoma. This bioinformatics-based functional analysis provides insights into mechanisms of resistance and may be used to identify potential strategies for improving clinical outcomes.

**Ethics Approval** The study was approved by the Yale University Institutional Review Ethics Board, approval number 060901869.

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23 CIRCULATING TUMOR DNA (CTDNA) SERIAL ANALYSIS DURING PROGRESSION ON PD-1 BLOCKADE AND LATER CTLA4 RESCUE IN PATIENTS WITH MISMATCH REPAIR DEFICIENT METASTATIC COLORECTAL CANCER

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**Background** Patients with mismatch repair defect/microsatellite instability high (dMMR/MSI-High) tumors respond well to immune checkpoint blockade. Pembrolizumab was the first drug to be approved by the FDA in an agnostic fashion for any tumor type with dMMR/MSI-High for the very same reason. The responses in dMMR/MSI-High tumors tend to be brisk, dramatic and durable to the point that the word ‘cure’ is being used for patients who do respond to PD-1 blockade. This year, pembrolizumab now got approval as 1st line therapy for dMMR/MSI-High metastatic colorectal cancers as well.

**Methods** Metastatic colorectal cancer patients enrolled in the expanded access program for tumor informed circulating tumor DNA monitoring (Signatera 16-plex bespoke mPCR NGS assay) who were noted to be dMMR/MSI-High colorectal cancers were identified. Serial monitoring results while they were receiving immune checkpoint blockade therapy is presented. This only includes patients who had progression on PD-1 blockade whereby CTLA-4 rescue was done as part of their treatment strategy.

**Results** Serial monitoring and trends of progression followed by responses are depicted in the patients who had CTLA-4 rescue post PD-1 progression (figure 1). This correlated with radiographic responses in all the patients. The ctDNA decreases in patients showing responses as well as ctDNA increases earlier during progression on PD-1 blockade happened within administration of a single dose. However, a third of patients do not respond. Predictive markers and data for subsequent therapy options are lacking. Here we present for the first time a series of dMMR/MSI-High patients who not only had serial circulating tumor DNA (ctDNA) monitoring during PD-1 blockade/progression, but also were able to get anti-CTLA4 in conjunction with an anti-PD1 (CTLA4-rescue), with ctDNA trends predicting responses weeks ahead of standard imaging.

**Conclusions** To date there is only 1 case report published earlier this year showing the value of ‘immunotherapy after immunotherapy’ in patients with dMMR/MSI-High tumors. Here we not only present a series of patients but also in parallel provide a snapshot on serial ctDNA trends whereby this could serve as a dynamic predictive marker of early response or progression to therapy. Finally, ‘CTLA4-rescue’ needs to be formally included in NCCN and other respective guidelines. Even though nivolumab/ipilimumab is listed as an option for dMMR/MSI-High tumors in addition to single agent pembrolizumab or nivolumab, it is not listed as an option post-PD-1 progression. For all the patients, we have had to fight to get peer to peer approval.

**Ethics Approval** The study is approved at University of Iowa and part of IRB#201202743.

**Consent** Written informed consent was obtained from the patients for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

**REFERENCES**


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UTILITY OF TUMOR-INFORMED MOLECULAR RESIDUAL DISEASE ASSAYS IN PATIENTS WITH COMPLETE RESPONSE TO IMMUNE CHECKPOINT BLOCKADE

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Background Recent data suggests that responses in patients with mismatch repair deficient (dMMR) tumors tend to be durable and potentially curative. Typically immunotherapy is employed and approved only in metastatic settings. However, it is not uncommon now to consider usage in patients with dMMR tumors secondary to the hypermutated nature of these malignancies alongside the concerns that these do not respond to therapy either through a clinical trial or off-label compassionate access programs. As noted, in patients who have these dramatic and durable responses, the question of foregoing surgery and/or radiation comes up. There is no great test to help predict or guide who are these complete responders. Assessment of molecular residual disease or minimal residual disease through tumor-informed assays is one potential test that can be employed in this setting. We here show the feasibility of such an approach in patients with dMMR tumors who got immunotherapy in the advanced but not metastatic setting.

Methods We identified patients who were enrolled in the serial tumor informed molecular residual disease BESPOKE expanded access ctDNA testing program (Signatera 16-plex bespoke mPCR NGS assay) who got immunotherapy in the neoadjuvant setting and were also enrolled in our biobanking program.

Results We were able to serially do ctDNA analysis in 2 patients (1 with advanced but not metastatic esophageal adenocarcinoma and another patient with advanced but not metastatic nearly obstructing rectal adenocarcinoma with extensive nodal metastases in both situations) who got immunotherapy off-label per physician discretion and tumor board discussion. Of note both these patients also had germline Lynch syndrome. Both of them had robust, dramatic and ongoing responses to immune checkpoint blockade. Of note, while they both had some radiographic question of residual disease, repeated endoscopic ultrasounds and random biopsies have yielded no tumor and all just scarred tissue. Circulating tumor DNA in these instances quickly declined to become undetectable and has remained not detectable to date (figure 1). Surgery and radiation is deferred and close follow up with subsequent endoscopic/radiographic assessment in addition to novel usage of ctDNA MRD assays is being employed.

Conclusions Data regarding neoadjuvant usage of immunotherapy is scarce pertaining to mismatch repair deficient tumors. There is a case series of 3 patients with rectal cancer that was just reported this year. Here we not only report a case of an advanced rectal cancer, but also a case of an advanced esophageal cancer who have achieved dramatic responses with no evidence of disease on repeated sampling. It is rare for surgery or radiation to be deferred in these situations. However, with added utility of ctDNA MRD assays, it gives us one more tool in our toolbox in terms of applying it to patients suitable for these ‘watch-&-wait’ approaches.

Ethics Approval The study has been approved by University of Iowa’s Institutional Review Board IRB#201202743.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

REFERENCE


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LINE OF THERAPY ADJUSTMENT IN A PATIENT WITH ADVANCED TRIPLE-NEGATIVE BREAST CANCER (TNBC) BY USING PERSONALIZED CTDNA TEST FOR TREATMENT RESPONSE MONITORING

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Background Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that is most difficult to treat due to the absence of hormone/growth factor receptors. Meta- static TNBC (mTNBC) is particularly challenging, given the limited efficacy and duration of response to chemotherapy. The repertoire of therapeutic options for mTNBC patients continues to increase with chemotherapeutic and immunoncology based treatments and now includes sacituzumab govitecan, a novel antibody-chemotherapy conjugate.

Methods Here we present a case study of a 40-year-old female who on biopsy of her left breast mass was diagnosed with TNBC. The patient underwent neoadjuvant chemotherapy with weekly administration of paclitaxel and carboplatin followed by dose-dense doxorubicin with cyclophosphamide. Following one-month, the patient underwent bilateral mastectomy, showing pathological staging ypT2 pN0. The patient underwent periodic radiological imaging along with the assessment of circulating tumor DNA in blood using a personalized and tumor-informed multiplex PCR, next-generation sequencing assay (Signatera bespoke, mPCR NGS assay) to identify the minimal residual disease (MRD) and treatment response.

Results After surgery, MRD assessment revealed ctDNA positive status (0.41 MTM/mL) prompting PET/CT scan that revealed liver metastasis. Continued ctDNA monitoring...
showed continuous increase in ctDNA concentration (287.09 MTM/mL). Separate analyses indicated MSI-high and PD-L1 positive tumor status, leading to the initiation of the first line of therapy (nab-paclitaxel and Atezolizumab), which resulted in ctDNA decline (39.62 MTM/mL). Weekly ctDNA monitoring noted a rapid increase a month later (178 MTM/mL to 833.69 MTM/mL) within a 2-week interval, which corresponded to disease progression on imaging. Given non-responsiveness with the first-line therapy, the patient was initiated with sacituzumab govitecan. Following this, a rapid decline in the ctDNA level was observed within a week (364.07 MTM/mL) with a downward trend to 73.03 MTM/mL by two weeks. An interval PET/CT scan showed a mixed response. Continued monitoring of ctDNA demonstrated ctDNA levels <5MTM/mL for a period of two months before serially rising again (to 89.27 MTM/mL). PET-CT ordered in response to increasing ctDNA levels confirmed progression involving hepatic and lung lesions. A new line of therapy with nivolumab and ipilimumab was subsequently initiated.

Conclusions Serial monitoring of ctDNA enables early detection of therapy resistance and provides a rationale for treatment change/optimization/discontinuation as compared to periodic imaging that is currently the standard of care. The ease and convenience of using ctDNA-based testing as frequently as every week clearly identified earlier non-responsiveness to IO and also identified earlier acquired resistance to antibody-drug conjugate, enabling a prompt switch to alternative therapy.

METHODS

We report on a 55-year-old female with a PALB-2 germline mutation who presented with a right-sided colonic adenocarcinoma with the involvement of the omentum and liver. The patient received 6 cycles of neoadjuvant FOLFOX, followed by an extended right hemicolectomy, omentectomy, and partial liver resection. The surgical specimen revealed a moderately differentiated adenocarcinoma in the liver with clear margins, and focal omental involvement with adenocarcinoma. The patient subsequently underwent 6 cycles of ‘adjuvant’ FOLFOX, with Oxaliplatin omitted after 3 cycles secondary to peripheral neuropathy. Soon after the patient experienced a recurrence that involved the anterior abdominal wall, between the peritoneum, and stomach, which was subsequently resected with negative margins. Molecular profiling of this metastatic focus revealed a TMB of 15.4 mutations per megabase, proficient Mismatch Repair (pMMR), a PDL1 CPS score of 26, and microsatellite stable (MSS) status. First, ctDNA analysis was performed at the time of recurrence and was found to be positive. Based on the TMB score of 15.4 and an elevated PDL1 score, the patient was initiated on Nivolumab and Ipilimumab. ctDNA measurements were obtained at the patient’s request.

RESULTS

DNA assessment performed after surgery and prior to initiation of immunotherapy revealed an approximate doubling of ctDNA levels, measured in mean tumor molecules (MTM) per mL of plasma, every month. During this period of time and correlating with the rise in ctDNA levels, the patient developed a new and enlarging FDG avid cardiophrenic lymph node. Following 2 cycles of Nivolumab and Ipilimumab, the FDG avid lymph node completely resolved and ctDNA clearance was observed (figure 1).

Abstract 26 Figure 1 ctDNA time-course demonstrating ctDNA kinetics

Time-point A represents the initial ctDNA assay, performed at the time of resection of peritoneal metastasis. An additional time-point (B) drawn a month later reveals a further increase in ctDNA. Time-point C represents a peak in ctDNA levels, concomitant with the new emergence of a PET avid cardiophrenic lymph node. Combination Immunotherapy (IO) was begun shortly after time-point C. Time-point D represents ctDNA clearance and radiographic resolution of lymph node metastasis after two cycles of IO. MTM/mL - mean tumor molecules/ milliliter of plasma
Conclusions Here we present a case of ctDNA clearance correlating with a radiographic resolution of metastatic disease in a patient with MSS mCRC. The data is provocative and suggests a possible contributory role of ctDNA-based testing as an additional monitoring parameter to measure disease-responsiveness to immunotherapy. Further investigation is warranted.

Ethics Approval N/A

Consent N/A

REFERENCES


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27 THE ROLE OF LIGANDS OF ACTIVATORY RECEPTOR NKG2D IN THE IMMUNE-DEPENDENT PATHOGENESIS AND EVOLUTION OF INFLAMMATORY BOWEL DISEASE (IBD)

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Background Long-term inflammation in IBD is mediated by several immune cells, including T lymphocytes and natural killer (NK) cells, through the engagement of NK group 2D (NKG2D) receptors. Allergic variations of NKG2D ligands (NKG2DLs, MICA/B, ULBP1-3) influence differential levels and localization of protein expression or the release of soluble isoforms. The affinity of interaction with NKG2D can be also affected, modulating the cytotoxic activity of the target cell. Evaluation of these molecular pathways and soluble ligands presents the potential use a clinical biomarker for patient outcomes.

Methods Gut tissue biopsies (left and right sides) and peripheral blood were collected from patients. 10 pediatric and 11 adult patients with IBD, 10 patients with gut malignancies and history of IBD were included in the study. Plasma form IBD patients and 10 healthy donors as controls, was used to quantify soluble NKG2DLs (sNKG2DLs) by ELISA (R&D Systems Duo Set). Nucleic acids were extracted from gut biopsies using the BioMasher II (Kimble) and All Prep DNA/RNA universal kit (Qiagen). Single nucleotide polymorphisms (SNPs, N=26) and relative gene expression of NKG2DL genes were conducted by qPCR using Taqman assays.

Results 9/11 adult patients had diagnosis of ulcerative colitis, compared to 3/10 pediatric. 5/10 pediatric had Crohn’s disease and 2/10 uncategorized IBD. A trend of prevalence of some allelic variants was detected for most of NKG2DLs. In addition, mRNA encoding for NKG2DLs was detected commonly, although with heterogeneous quantifications, in all the tissues, including the retrospectively collected malignancies with history of IBD. Interestingly, the levels of sNKG2DLs were higher in pediatric (p<0.001) as compared to adult patients. No or low levels of sNKG2DLs were detectable in healthy donors. Moreover 3/5 patients with the highest level (700–1500 pg/ml) of sMICA had homozygosity at least in one of the rs1051792 or rs1051794 polymorphic site (GG allele variant MICA-129Val or MICA-250Val) that have been reported to be associated with soluble form of MICA.

Conclusions These results, although preliminary and further investigations are ongoing, suggest the relevance of NKG2D/NKG2DL pathway in the development and evolution of IBD. sNKG2DLs could be detected in most of patients, with different levels and highest concentrations in pediatric patients. In some cases, the presence of sNKG2DLs in the plasma could be associated with defined polymorphisms in genes encoding for these proteins.

Ethics Approval This study was approved by Sidra Medicine and Hamad Medical Corporation Ethics Boards; approval number 180402817 and MRC-02-18-096, respectively.

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28 RETROSPECTIVE POOLED ANALYSIS OF EPACADOSTAT CLINICAL STUDIES IDENTIFIES DOES REQUIRED FOR MAXIMAL PHARMACODYNAMIC EFFECT IN ANTI-PD-1 COMBINATION STUDIES

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Background IDO1 is the initial rate-limiting enzyme in one breakdown pathway of tryptophan. It reduces tryptophan levels and generates metabolites (e.g., kynurenine [KYN]) that contribute to tumor-associated immune suppression. Epacadostat (EPA) is a novel, potent, selective, reversible inhibitor of IDO1 studied in clinical trials in combination with anti-PD-1 antibodies. Epacadostat-induced decreases in plasma KYN have been used as a pharmacodynamic measure of drug activity and have aided in dose selection for clinical studies. Despite encouraging signs of efficacy in combination with pembrolizumab (PMB) in the ECHO-202 study, a large phase 3 study in melanoma (ECHO-301) failed to reproduce this outcome.1

Methods Longitudinal plasma samples were obtained from participants in EPA clinical studies. Plasma KYN and EPA concentrations were measured by validated liquid chromatography tandem mass spectrometry. Quantitative mass spectrometry imaging (qMSI) of intratumoral tryptophan metabolites was also performed.

Results Analysis of plasma KYN levels demonstrated that PMB monotherapy significantly elevated KYN. While blocking the PMB-induced increase, EPA (100 mg BID) in combination with PMB failed to normalize KYN to healthy control levels as was reported for EPA monotherapy;2 Because anti-PD-1 treatment can induce interferon gamma (IFN-γ) production and IDO1 expression is IFNγ inducible,3 we hypothesize that PMB-induced IFN-γ may be responsible for the observed increase of plasma KYN levels. Combined analysis of plasma KYN from additional EPA/anti-PD-1 combination (ECHO-202; EPA/PMB, ECHO-204; EPA/nivolumab) and monotherapy (ECHO-210) studies, with EPA doses ranging from 50 to 600 mg BID, suggested that higher EPA doses (≥600 mg BID) may be necessary to overcome the anti-PD-1-associated KYN elevation. Doses ≥600 mg BID are projected to cover the EPA IC90 value for 24h. The POD1UM-102 study is currently evaluating the combination of a novel anti-PD-1 monoclonal antibody (retifanlimab) plus EPA at doses up to 900 mg BID. Preliminary results from this study indicate that 600 mg BID is the maximally tolerated dose and is capable of maintaining suppression of KYN to healthy control levels through treatment cycle 4. Additionally, qMSI of paired
pre-treatment and on-treatment biopsies demonstrated intratumoral suppression of KYN production with EPA 600 mg BID.

**Conclusions** Using suppression of plasma KYN as a pharmacodynamic marker of EPA activity, we demonstrated that maximal blockade of IDO1 activity in the context of anti-PD-1 treatment requires doses of EPA substantially higher than those tested in prior clinical studies. These findings are now informing additional proof of concept clinical studies.

**Acknowledgements** These studies were sponsored by Incyte Corporation (ECHO-210, POD1UM-102) and in collaboration with MSD (ECHO-301, ECHO-202) and Bristol Myers Squibb (ECHO-204).

**Trial Registration** ECHO-202 [NCT NCT02178722]; ECHO-204 [NCT02327078]; ECHO-210 [NCT01685255]; ECHO-301 [NCT02752074]; POD1UM-102 [NCT03589651]

**Ethics Approval** These studies were each approved by the institutional review board or independent ethics committee of participating institutions.

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**30**

**PROFILING TUMOR CIRCULATING CELL-FREE DNA WITH AN ENHANCED WHOLE-EXOME TO ENABLE SENSITIVE ASSESSMENT OF SOMATIC MUTATIONS**

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**Background** An increasing number of studies have demonstrated the potential use of circulating cell-free DNA (cfDNA) for diagnosis, prognosis, disease progression, and treatment monitoring. However, many of these studies use assays covering a limited set of genes and therefore miss biologically and clinically relevant genetic alterations involving immuno-modulatory pathways which confer treatment resistance, and leading to changes in neoantigen status. To address this, we developed a whole-exome scale cfDNA platform, NeXT Liquid Biopsy™, that enables sensitive detection and tracking of mutations in approximately 20000 genes.

**Methods** To enable sensitive detection across the exome, our enhanced exome assay and chemistry augments hard-to-sequence genomic regions, such as regions of high GC content, to enable more uniform coverage across the exome. We achieved a high mean sequencing depth of approximately 2000X exome-wide, with additionally boosted depth for 248 clinically relevant oncogenic and tumor suppressor genes to further enhance sensitivity. We developed a computational pipeline for our NeXT Liquid Biopsy assay optimized to lower the noise floor for variant detection, providing sensitive monitoring and de novo detection of variants over multiple time points.

**Results** We evaluated the sensitivity of our NeXT Liquid Biopsy platform in three ways. First, we evaluated the sensitivity within the coverage boosted regions using the SeraCare reference materials at multiple allele frequency (AF) dilutions. Our platform identified all 8 and 25 Horizon and SeraCare SNV events at 1% AF and above, respectively, and detected 24 out of 25 events at 0.5% for the SeraCare samples. Additionally, to enable sensitivity analysis at the whole-exome scale, we then developed a cell culture media system that models the shedding of tumor DNA fragments seen in human plasma samples and created tumor/normal dilution series in vitro. We achieved >95% sensitivity for variants with AF≥2%, and between 85% to 92% for mutations with AF of 1%-2%. Second, we evaluated false-positive rates on 12 cancer patients using digital droplet PCR. Third, we demonstrated our ability to longitudinally monitor treatment response using a clinical cancer cohort on checkpoint therapy, profiling putative tumor evolution while on therapy.

**Conclusions** In conclusion, we have developed a whole-exome scale liquid biopsy platform, NeXT Liquid Biopsy, that enables sensitive monitoring and detection of somatic SNVs from cfDNA across ~20000 genes. The platform enables broader monitoring of changes in response to cancer therapy, acquired mechanisms of resistance, and intra- and inter-tumor heterogeneity.

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**Background** Elevated baseline neutrophil lymphocyte ratios (NLR) are now well established as a poor predictor of survival in renal cell carcinoma (RCC) and other cancers. Platelet Lymphocyte Ratios (PLR) have also recently shown similar effects. Despite these findings, the practical use of these ratios is still somewhat limited. We have previously shown that higher NLRs may be associated with increased concentrations of myeloid derived suppressor cells (MDSC). We hypothesized that increases in NLR or PLR (NLR/PLR failure) at 2 months while on immunotherapy could be a predictor of eventual treatment failure and overall survival.

**Methods** We analyzed patients who received nivolumab therapy for RCC at our institution from 3/2016 to 6/2019. Patients with complete data on NLR and PLR at time = 0 and +2 months and those who had accurate response and overall survival information available were selected (n = 37). Information on comorbidities, previous therapy, demographics were collected for adjusted analysis. NLR failure was defined as an increase of 3 or more compared to baseline NLR. Cox proportional hazard models were used to analyze the risk of progression and death with NLR/PLR failure at 2 months (±2 weeks). Kaplan Meier graphs were constructed to trace survival functions for PFS and OS by NLR.

**Results** NLR failure was associated with a statistically significant increase in the risk of progression on nivolumab therapy (HR 4.26, 95% CI [1.47–12.3], p = 0.007), in an adjusted cox regression model that included baseline NLR. In this adjusted model, the value of baseline NLR to predict
Background Immune-checkpoint inhibitors (ICIs) have revolutionized the treatment of advanced/metastatic non-small cell lung cancer patients (NSCLC), however, only a small subset of patients derives clinical benefit.1-3 To date, PD-L1 immunohistochemical evaluation is the gold-standard assay and the only approved biomarker, but associated with several limitations due to technical and biological factors such as spatial and temporal sampling variability.4-5 In this context, liquid biopsies emerge as novel powerful tools that could allow the non-invasive real-time characterization of the tumor PD-L1 status. In particular, extracellular vesicles (EVs), defined as cell-derived double-membrane structures involved in cell communication, hold strong potential as tissue surrogates. Recent studies have suggested that EV PD-L1 could stratify melanoma patients receiving ICIs, but none has showed the predictive value of this biomarker in NSCLC patients.6-7 We hypothesize that EV PD-L1 cargo can serve to stratify the response to ICIs in NSCLC patients.

Methods This study enrolled advanced/metastatic NSCLC patients receiving ICI treatment. Plasma samples were obtained at baseline (T1) and at 8 weeks (T2) during the first response evaluation. Patients were classified as responders when showing partial, stable or complete response or as non-responders when manifesting progressive disease following RECIST v1.1.8 Plasma EVs were isolated by standard serial ultracentrifugation methods and characterized according to ISERV recommendations.9-10 Tissue PD-L1 expression was measured by immunohistochemistry while EV PD-L1 expression was measured by immunoblot. A predictive model was created by logistic-regression and a bootstrap corrected ROC curve to validate the results.

Results Paired plasma samples from 21 patients were analyzed. PD-L1 tissue expression was not correlated with treatment response (p=0.394) nor matched the baseline EV PD-L1 levels (p=0.337) (figure 1.A). However, the dynamics of EV PD-L1 (T1-T2) correlated with the treatment response, observing an increase of PD-L1 expression in non-responders and a decrease or stable levels in responders (p=0.043) (figure 1.B). The predictive model reported an AUC=0.85, 90% CI=0.72–0.97, with 74.2% sensitivity and 73.5% specificity (figure 1.C). Moreover, the increase of EV PD-L1 was associated with shorter overall survival (HR=4.34, p=0.037) and shorter progression-free survival (HR=5.06, p=0.025) (figure 1D & E).

Conclusions Our preliminary-study showed, for the first time, the predictive and prognostic value of EV PD-L1 dynamic changes in immunotherapy-treated NSCLC patients. Although larger studies are needed to validate these results, this promising biomarker could have important clinical implications, guiding treatment decisions in near real-time and improving the outcome of patients that could benefit from ICIs.

Acknowledgements We would like to extend our gratitude to all the patients that participated in the study.

Ethics Approval All patients consented to an Institutional Review Board–approved protocol (A.O. Papardo, Messina, Italy). Biological material was transfer to the University of Maryland, USA under signed MTA between both institutions (MTA/2020-13111).

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32 C-REACTIVE PROTEIN (CRP) AS A PROGNOSTIC BIOMARKER IN ADVANCED NON-SMALL CELL LUNG CANCER TREATED WITH IMMUNE CHECKPOINT INHIBITORS. RESULTS FROM A MULTI-CENTER INTERNATIONAL OBSERVATIONAL STUDY

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Background CRP is an acute-phase protein produced primarily in response to interleukin-6 via transcriptional activation of the STAT3. Recent data have provided mechanistic insights into the immune suppressive role of elevated CRP by elucidating its influence on effector T-cell function and antigen presentation. Furthermore, melanoma patients in CheckMate-064 and 067 with high baseline and on-treatment CRP were seen to have a lower response rate and shorter survival to immune checkpoint inhibitors (ICIs). Given these observations, we sought to evaluate the role of CRP as a prognostic biomarker in advanced NSCLC treated with ICIs from a multi-center international cohort.

Methods Between 2015–2019, 420 adult patients with advanced NSCLC treated with ICIs alone or with concurrent chemotherapy (Chemo-ICI) were identified at four (1 US and 3 European) academic centers. CRP level in peripheral blood samples collected up to 2 weeks before starting ICI based treatments was considered as baseline. Based on previously validated data, a CRP cutoff of 10 mg/l was used to define CRP-normal (CRP-N) and CRP-high (CRP-H). Association of baseline CRP with median progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method and multivariate proportional hazards regression adjusted for multiple variables.

Results Baseline CRP value was available in 75.5% of patients, with 66% having CRP-H. The median CRP was 21.0 mg/l. Single-agent nivolumab (44%) and Chemo-ICI (33.3%) were the two most common therapies. CRP-H showed a trend for stronger association with squamous histology (73.7% vs 63.3%; p = 0.063) and female sex (70.8 vs 60.0%; p=0.062) but did not show an association with PD-L1 status (0%, 1–49%, or ≥50%). Patients with CRP-H had a lower objective response rate compared with patients with CRP-N (26.9% vs. 47.6% PR; p=0.029). Compared to those with CRP-N (figure 1), patients with CRP-H had a significantly shorter median PFS [3.9 vs. 6.6 months, HR 1.41 95% CI: (1.07–1.86); p=0.0138] and OS (8.6 vs. 14.8 months, HR 1.55 95% CI [1.13–2.14]; p=0.0060). In Cox regression analysis, CRP-H was again found to be independently associated with shorter median PFS and OS.

Conclusions This is the largest international real-world dataset demonstrating significantly inferior outcomes associated with CRP > 10 mg/l in NSCLC patients treated with ICI based therapies. The potential influence of the immune suppressive effects of elevated CRP and IL-6 on the anti-tumor efficacy of ICIs needs prospective evaluation and could potentially be exploited as a therapeutic avenue in NSCLC.

Acknowledgements

BACKGROUND

C-REACTIVE PROTEIN (CRP) AS A PROGNOSTIC BIOMARKER IN ADVANCED NON-SMALL CELL LUNG CANCER TREATED WITH IMMUNE CHECKPOINT INHIBITORS. RESULTS FROM A MULTI-CENTER INTERNATIONAL OBSERVATIONAL STUDY

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33 DYNAMIC MONITORING OF RESPONSE TO IMMUNE CHECKPOINT BLOCKADE THROUGH DEEP-LEARNING EMPOWERED ULTRA-SENSITIVE LIQUID BIOPSY IN MELANOMA

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Background Clearance of circulating tumor DNA (ctDNA) following checkpoint blockade (CB) can precede radiographic response,1 2 though current state of the art ctDNA detection via targeted panels faces limited sensitivity in low burden disease (figure 1). We previously showed that whole genome sequencing (WGS) of plasma can overcome low input of ctDNA to dynamically track low volume malignancy using matched tumor tissue.3 We therefore sought to evaluate

Abstract 32 Figure 1 Kaplan-Meier Curves with 95% CI for PFS and OS

Significantly inferior median PFS and OS were seen for patients with CRP-H vs. CRP-N.

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ctDNA for tracking early response to checkpoint blockade (CB) in melanoma, and developed a novel classifier that allows us to track disease without matched tumor tissue for expanded applicability in immunotherapy.

Methods To identify ctDNA sparsely diluted in noncancerous plasma cell free DNA (cfDNA), we developed Phoenix, a deep-learning classifier that uses genomic and epigenomic features to distinguish single nucleotide variants (SNVs) in melanoma from sequencing noise. We evaluated Phoenix on a retrospective cohort of serially sampled plasma from patients with advanced cutaneous melanoma on CB (nivolumab alone or with ipilimumab). Plasma was collected at 0, 3, 6 and 12 weeks after first dose of immunotherapy. ctDNA dynamics were compared to radiographic imaging results at 12 weeks.

Results We trained Phoenix on tumor-confirmed SNVs in plasma from a single patient with high tumor mutational burden (TMB) melanoma and cfDNA from age-matched patients without known cancer. Overall ctDNA signal-to-noise enrichment ranged from 100 - 260x in validation patients (n=2) with bulky disease. Phoenix learned key features of melanoma ctDNA including the UV mutational signature and short fragment size (figure 2), and sensitively tracked persistent low burden disease seen on imaging (figure 3). To validate these findings, we expanded our cohort (n= 15) of serially tracked tumors. In our preliminary analysis of 12 patients, Phoenix detected pretreatment ctDNA in 92% of patients at a specificity of 97% (figure 4), compared with only 17% with the benchmark in the field (iChorCNA, a plasma-based WGS liquid biopsy tool; table 1). Phoenix detected a decrease in ctDNA 3 weeks after initiation of CB in 80% of patients (figure 5) with an objective response on imaging. No change in ctDNA was seen in patients who did not respond to treatment.
Conclusions

Phoenix successfully identified pretreatment melanoma ctDNA without matched tumor tissue and identified response to CB as early as 3 weeks after treatment. Our ongoing studies aim to optimize this technology for early identification of CB response in clinical practice.

Acknowledgements

Thanks to support from the Conquer Cancer Foundation.

Ethics Approval

Use of human data in this study was approved by Memorial Sloan Kettering’s IRB, Assurance Number FWA0000499.

REFERENCES


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Abstract 33 Table 1

Characteristics of patients at baseline and ctDNA dynamics

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<th>Patient ID</th>
<th>Age/sex</th>
<th>Sites of metastasis</th>
<th>Pre-treatment detected by Phoenix</th>
<th>Total ctDNA detected by Phoenix</th>
<th>Therapy</th>
<th>Objective response to therapy</th>
<th>ctDNA response detected at 3 weeks</th>
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Baseline characteristics for preliminary validation cohort (n=12)

Conclusions

Phoenix successfully identified pretreatment melanoma ctDNA without matched tumor tissue and identified response to CB as early as 3 weeks after treatment. Our ongoing studies aim to optimize this technology for early identification of CB response in clinical practice.

Acknowledgements

Thanks to support from the Conquer Cancer Foundation.

Ethics Approval

Use of human data in this study was approved by Memorial Sloan Kettering’s IRB, Assurance Number FWA0000499.

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Abstract 33 Figure 4

Phoenix detects pre- and intratreatment melanoma ctDNA

We evaluated Phoenix post-filter sample-level detection rate. Phoenix detects ctDNA in 92% of pretreatment melanoma plasma samples (green, n=12) at a specificity of 97% relative to held-out noncancerous controls (blue, n=38). Phoenix detected ctDNA in 84% of posttreatment plasma samples (n=38, yellow), indicating full ctDNA clearance in 7/38 samples.

Abstract 33 Figure 5

cDNA response to checkpoint blockade after 3 weeks

Serial plasma samples were taken from patients on checkpoint blockade (nivolumab alone or with ipilimumab). ctDNA burden was measured as detection rate among post-filter candidate SNVs and compared to a 97% specificity boundary among a panel of healthy controls. Phoenix detects a response to checkpoint blockade, measured as a decrease in ctDNA detection rate, as early as 3 weeks as shown in 3 patients (MSK-38, MSK-40, MSK 42).
screened on tissue microarrays (TMAs) composed of healthy and diseased tissues to ensure that they will perform as expected in real samples and yield sufficient signal over background. Finally, after antibodies pass functional validation, we assess the performance of antibodies within panels of antibodies that will be commercialized.

**Results**

In total, approximately 60% of off-the-shelf antibodies tested for use in GeoMx assays pass the entire validation process and are put into commercial assays. Passing requirements include exhibiting a maximum positive signal divided by the limit of detection, plus two standard deviations (SD) that is greater than or equal to 5 in both CPAs and TMAs for individual antibodies; such a threshold gives a false positive rate of less than 10%.

**Conclusions**

Unvalidated or poorly validated antibodies can result in false positives and non-reproducible results. Following the robust validation process outlined here, approximately 40% of off-the-shelf antibodies are removed from panels, underscoring the importance of antibody validation prior to incorporating new antibodies into experiments.

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**TARGETED NON-VIRAL INTEGRATION OF LARGE CARGO IN PRIMARY HUMAN T CELLS BY CRISPR/CAS9 GUIDED HOMOLOGY MEDIATED END JOINING**

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**Background**

Engineered immune cells hold tremendous promise for the treatment of advanced cancers. As the scale and complexity of engineered cell therapies increase, reliance on viral vectors for clinical production limits translation of promising new therapies. Here, we present an optimized platform for CRISPR/Cas9-targeted, non-viral engineering of primary human T cells that overcomes key limitations of previous approaches, namely DNA-induced toxicity and low efficiency of integration of large genetic cargos.

**Methods**

A systematic optimization of nucleic acid delivery, editing reagent composition, and culture protocol was performed to overcome DNA toxicity. Targeted knockin (KI) at AAVS1 and TRAC was compared across multiple vector configurations with genetic cargos ranging from 1 to 3 kilobases (kb) in size. Integration efficiency was measured by flow cytometry and sequencing. Off-target editing and integration were evaluated using GUIDE-seq and targeted locus amplification (TLA), respectively. Phenotype and function of non-virally and lentivirus engineered CAR-T cells was compared using flow cytometry, cytokine profiling and cytotoxicity assays.

**Results**

We identified a temporal window following T cell activation where transfection efficiency, cell-cycle-status, and cytotoxic DNA sensor expression were optimal for targeted DNA integration and reduced toxicity. Within this window, we targeted a 1kb GFP reporter to the AAVS1 locus with an efficiency of ~45% using homologous recombination (HR). Efficiency was reduced to ~11% with a larger ~3kb TCR cassette targeted to the TRAC locus, consistent with previous reports.1-3 To improve large cargo integration we employed homology mediated end-joining (HMEJ) and short homology design (48bp vs. ~1kb for traditional HR).4 Using HMEJ, knockin of the 1kb GFP cassette at AAVS1 reached ~70%. Strikingly, integration of the 3kb TCR at TRAC reached ~50% using HMEJ. Additional optimization of the culture protocol doubled post-engineering survival and proliferation (up to ~35-fold expansion in 7 days). Non-virally engineered TRAC KI CAR-T cells were phenotypically and functionally equivalent to lentivirally engineered T cells in vivo. In vivo assays in xenograft models are underway and results will be presented.

**Conclusions**

Comprehensive, orthogonal optimization of parameters impacting nucleic acid delivery and DNA-toxicity in combination with novel modalities for integration achieved knockin of TCR and CAR cargo at efficiencies equivalent to that of current viral vector platforms without compromising expansion or function. Our protocol is suitable for clinical scale production under GMP conditions and offers an improved methodology over previous methods for non-viral engineering of human T cells.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0035

**MOLECULAR EVENTS REGULATING SOLID TUMOR CELL RESPONSES TO NATURAL KILLER CELLS**

Michal Sheffer*, Constantine Mitsiades, Dana-Farber Cancer Institute, Boston, MA, USA

**Background**

Natural killer (NK) cells exhibit potent activity in pre-clinical models of diverse hematologic malignancies and solid tumors and infusion of high numbers of NK cells, either autologous or allogeneic, after their ex vivo expansion and activation, has been feasible and safe in clinical studies. **Methods**

To systematically define molecular features in human tumor cells which determine their degree of sensitivity to human allogeneic NK cells, we quantified the NK cell responsiveness of hundreds of molecularly-annotated ‘DNA-barcoded’ solid tumor cell lines in multiplexed format (PRISM; Profiling Relative Inhibition Simultaneously in Mixtures approach),1 correlating cytotoxicity scores for each cell line with the CCLE transcriptional data2 (RNA-seq), to reveal genes that are

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0035
associated with resistance or sensitivity to NK cells. In addition, we applied genome-scale CRISPR-based gene editing screens in several solid tumor cell lines to interrogate, at a functional level, which genes regulate tumor cell response to NK cells. Figure 1 schematically depicts the two screens. c, Histogram of gene fold changes (z-scores). Listed are selected genes with most prominent p-values across more than one screen.

**Results** Based on these orthogonal studies, NK sensitive tumor cells tend to exhibit high levels of the NK cell-activating ligand B7-H6 (NCR3LG1); low levels of the inhibitory ligand HLA-E; microsatellite instability (MSI) status; high transcriptional signature for chromatin remodeling complexes and low antigen presentation machinery genes. Treatment with HDAC inhibitor reduced the sensitivity of SW620 colon cancer cells, increased antigen presentation machinery, including HLA-E, and reduced B7-H6. Importantly, transcriptional signatures of NK cell-sensitive tumor cells correlate with immune checkpoint inhibitor resistance in clinical samples. Widespread analysis of CCLE transcriptional signatures revealed that cell lines with mesenchymal-like program tend to be more sensitive to NK cells, compared with epithelial-like cell lines. Indeed, mesenchymal tumors tend to have lower expression of antigen presentation machinery in both CCLE and TCGA.

**Conclusions** This study provides a comprehensive map of mechanisms regulating tumor cell responses to NK cells, with implications for future biomarker-driven applications of NK cell immunotherapies. The integration of PRISM and CRISPR identified potential regulators of tumor cell response to NK cells, which upon further validation, may serve as biomarkers in future NK cell-based studies. Moreover, NK cells may complement T-cells, killing tumor cells that do not respond to immune checkpoint inhibitors.

**Acknowledgements** This work was supported by Stand Up To Cancer (SU2C) Convergence 2.0 Grant; SU2C Phillip A. Sharp Award for Innovation in Collaboration; Claudia Adams Barr Program in Innovative Basic Cancer Research; Human Frontier Science Program Fellowship; and Leukemia and Lymphoma Society Scholar Award.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0036
**Background** Determination of programmed death-ligand 1 (PD-L1) level in tumor by immunohistochemistry (IHC) is widely used to predict response to check point inhibitor therapy. In particular, the Dako PD-L1 (22C3) antibody is a common companion diagnostic to the monoclonal antibody drug Keytruda® (pembrolizumab) in non-small cell lung cancer (NSCLC). However, for the practicing pathologist, interpretation of the PD-L1 (22C3) assay is cumbersome and time consuming. Manual pathologist scoring also suffers from poor intra- and inter-pathologist precision, particularly around the cut-off point. In this clinical validation study, we developed an image analysis (IA) based solution to accurately and precisely score digital images obtained from PD-L1 stained NSCLC tissues for making clinical enrollment decisions.

**Methods** 10 NSCLC tissue samples were purchased from a qualified vendor and IHC stained for PD-L1; 4 of these samples had serial sections stained on two separate days. Stained slides were scanned at 20X magnification and analyzed using Flagship Biosciences’ IA solutions that quantify PD-L1 expression and separate tumor and stromal compartments. Resulting image markups of cell detection and PD-L1 expression were reviewed by an MD pathologist for acceptance. PD-L1 staining was evaluated by digital IA in the sample’s tumor compartment for Total Proportion Score (TPS,%). Assay specificity was defined by ≥90% of the tissue compartment exhibiting appropriate cell recognition (≥90% cells correctly recognized as determined by the pathologist), with ≤10% false positive rate for staining classification. Sensitivity was defined by ≥90% of the cohort exhibiting appropriate cell identification (≥90% cells correctly identified), with ≤10% false negative rate for staining classification. Accuracy was defined by the combination of sensitivity and specificity and precision was defined by concordance of the binned TPS (<1%, ≥1%, ≥50%) in ≥80% of the samples stained on multiple days.

**Results** The preliminary results show that IA can yield high analytical sensitivity, specificity, accuracy, and precision in the determination of the PD-L1 score. 100% of the tissue cohort met criteria for analytical specificity, sensitivity, and accuracy and 100% of the samples stained on multiple days met the precision criteria.

**Conclusions** This data demonstrates the feasibility of an IA approach as applied to PD-L1 (22C3) scoring. Ongoing experiments include application of the developed 22C3 algorithm on a separate cohort of 20 NSCLC samples to determine the correlation of digital scoring and scoring obtained by three pathologists. Additionally, we will evaluate the precision obtained by digital scoring in relation to the intra- and inter-pathologist concordance.

**REFERENCES**

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0038

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**Background** While the quantities and types of immune, tumor, and structure-related cells present in the tumor-immune micro-environment (TiME) are important for understanding aspects of cancer progression and potential responses to therapy, spatial locations and relationships of these cells also play a critical role. Emerging single-cell imaging modalities, such as multiplex immunohistochemistry (mIHC), provide phenotypic and functional state information for each cell present in the TiME while maintaining the spatial context of tissue architecture. We performed a quantitative analysis of mIHC images to characterize the cellular composition and spatial organization of human head and neck squamous cell carcinomas (HNSCC) and identified features correlated with patient survival.

**Methods** mIHC is an immunoassay-based imaging platform that evaluates sequentially stained immune lineage epitope-specific antibodies for immunodetection on FFPE tissue sections to phenotype single cells as tumor, stromal (mesenchymal), or one of more than 20 different immune cell lineages, all while maintaining the Cartesian coordinates of each cell. Matched primary and recurrent HNSCC tumors from nine patients were assayed via mIHC. Using unsupervised hierarchical clustering and principal component analysis, we interrogated the heterogeneity in cellular composition of each tumor section. We further quantified the spatial organization of tumors and identified prognostic tumor and immune cell architectures, as well as cellular neighborhoods that clustered together based on similar compositions and physically grouped together to reveal common spatial features across tumors.

**Results** Regions from the same tumor and tumors from the same patient clustered together more in their cellular composition than tumors from different patients. We also observed a decrease in the fraction of B cells present in recurrent tumors following therapy for all patients (p=0.024). While common biomarkers for HNSCC, such as CD8+ T cell density and tumor cell abundance were not associated with outcome, the tumor-immune spatial relationship was prognostic. Tissue regions of compartmentalization between immune and tumor cells were associated with higher fractions of αSMA+ stromal cells and had a greater proportion of Ki-67+ lymphocytes present, as compared to mixed regions. Patients with more compartmentalization in their primary tumors demonstrated longer progression free survival than those with more mixing between these cell types (p=0.027).

**Conclusions** Our results provide insight into the spatial organization of HNSCCs, highlighted by the result that compartmentalization between immune and tumor cells is associated with improved outcomes. This study provides spatial analysis methods and hypotheses that can be used as a framework for analysis of larger cohorts.

**Ethics Approval** This study was approved by Oregon Health and Science University’s IRB (protocol #809 and #3609), and written informed consent was obtained.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0039

40 THE ORION™ PLATFORM FROM RARECYTE® ENABLES SAME-DAY 21-PLEX FLUORESCENCE TISSUE ANALYSIS

Daniel Campton, Jeremy Cooper, Steven Reese, Kyla Teplitz, Jeffrey Webrin, Joshua Nordberg*, Eric Kaldjian, Tad George. RarCyte, Seattle, WA, USA

Background Tissue consists of heterogenous cell types, each with diverse functions and functional states, arranged spatially in a way that impacts patient health status. Resolving this complexity at the subcellular level has historically been challenged by fluorescence overlap, autofluorescence, a limited number of targets that can be simultaneously assessed, and low throughput. Orion technology breaks these barriers, providing rapid, straightforward, highly multiplexed whole slide tissue analysis. The recently announced Orion platform rapidly generates high resolution 21-channel images to enable comprehensive phenotypic profiling and characterization of tissue architecture including micro-anatomy, analysis of tumor heterogeneity and the complex tissue microenvironment.

Methods FFPE tissue samples are stained with TissuePlex™ reagents and whole slides are imaged on the Orion Instrument. Resulting 21-channel datasets are reviewed by a pathologist to verify staining specificity. The TissuePlex core panel includes antibodies against CD45, CD4, FoxP3, CD8A, CD11b, LAG-3, PD-L1, CD11c, CD163, CD68, PD-1, Ki-67, CD3d, and CD20. Single marker IHC is used as the gold standard to evaluate TissuePlex reagents and instrument spectral isolation performance is evaluated using fluorescent microspheres.

Results We will present the novel Orion spatial biology platform, provide a technology and workflow overview, demonstrate instrument validation results, and present sample datasets.

Conclusions The Orion platform enables rapid and deep phenotypic analysis of tissue samples for high resolution, whole slide sample analysis.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0040

41 OPTIMIZATION OF AN ULTRASENSITIVE, QUANTITATIVE IMMUNOASSAY FOR DETECTION OF CD20 IN NON-HODGKIN’S LYMPHOMA (NHL) FFPE SAMPLES

1Apolina Goel*, 1Michael Ross, 1Jeanette Rheinhardt, 1Peter Duval, 1Michael Maker, 1Hirojiuki Yokota, 1Kenneth Bloom, 2Ann Ranger, 1Joseph Krueger. 1Apollina Goel*, 2Michael Ross, 1Jeanette Rheinhardt, 1Peter Duval, 1Michael Maker, 1Hirojiuki Yokota, 1Kenneth Bloom, 2Ann Ranger, 1Joseph Krueger. 1Invicro, a Konica Minolta Company, Boston, MA, USA; 2RarCyte, Seattle, WA, USA

Background CD20, a membrane B cell marker, is expressed on the majority of mature B cell neoplasms, including diffuse large B cell lymphoma and follicular lymphoma. Importantly, CD20 is the target of rituximab as well as autologous T cell and BiTE® therapies in clinical development. Studies show that one mechanism of resistance to rituximab-containing therapies is downregulation of CD20. 1, 2 Development of an assay that provides highly sensitive and accurate detection of CD20 levels in the tissue context may help to assess whether there is a minimum CD20 threshold associated with response to rituximab or other CD20-targeted therapies. Here, we describe the development of a novel Quanticell™ assay for sensitive and quantitative detection of CD20 expression in formalin-fixed paraffin-embedded (FFPE) biopsy samples from NHL patients.

Methods A CD20 (Abcam, clone SP32) Quanticell-based assay, which utilizes Konica Minolta’s novel fluorescent phosphor-integrated dots (PIDs)3 was optimized on a panel of B lymphoma cell lines. Flow cytometry was performed to benchmark assay performance. Next, a human B lymphoma tissue microarray (TMA, n=39 cores) was stained using DAB-IHC to evaluate CD20 expression. Tumor cores (n=10) showing CD20highCD19high expression by DAB-IHC and immunofluorescence (IF)-IHC were selected for further evaluation. Human tonsil tissue was used to assess CD20 assay performance as a Quanticell singleplex or duplexed with CD19 IF-IHC. The TMA was stained with CD20 Quanticell plus CD19-AF488 to measure CD20 expression on a per cell basis. To assess sensitivity of CD20 Quanticell detection, a CD19 negative non-B cell core was analyzed. CD20 expression determined by Quanticell was compared to results generated with a commercially available method enabling digital profiling of CD20 protein in FFPE sections.

Results Analytical comparison between the Quanticell assay and flow cytometry on cell lines showed strong concordance between the two methods (CD20 Quanticell score versus CD20 receptor number). The Quanticell method demonstrated a broader dynamic range in CD20 expression in the TMA samples compared to DAB-IHC. Both the Quanticell and digital protein detection assays appropriately clustered cores into CD20low and CD20high categories. Notably, the CD20 Quanticell assay demonstrated the ability to measure CD20 expression accurately and precisely over a broader dynamic range when compared to the digital method.

Conclusions Relative to DAB IHC, the novel CD20 Quanticell assay provides significantly enhanced detection and quantification of CD20 in FFPE tissue samples. This technology may be useful to assess whether there are critical antigen densities associated with response to CD20-targeting therapies.

Acknowledgements The authors gratefully acknowledge technical assistance from Ankit Gandhi and Marie Zamanis. The authors also thank Sean Gerrin for technical writing review.

Trial Registration N/A

Ethics Approval N/A

Consent N/A

REFERENCES


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0041
INTRAVITAL MULTIPHOTON MICROSCOPY OF INFILTRATING T CELL AND TUMOR CELL METABOLISM IN A MURINE MELANOMA MODEL

1Alexa Heaton*, 2Tiffany Heaster, 3Anna Hoefges, 4Alexander Rakhmilevich, 5Amy Erbe, 2Paul Sondel, 1Melissa Skala. 1University of Wisconsin and Morgridge Institute for Research, Madison, WI, USA; 2University of Wisconsin, Madison, WI, USA

Background Intravital multiphoton microscopy (IMM) provides single-cell imaging within intact living systems. IMM of the autofluorescent metabolic co-enzymes NAD(P)H and FAD, or optical metabolic imaging (OMI), provides in vivo label-free imaging of metabolic changes. The metabolism of tumor cells and immune cells is closely associated with cancer progression, so we aim to study metabolic trends before and after administration of an established, effective, triple-combination immunotherapy within murine melanoma tumors. This therapy includes 12 Gy external beam radiation, intratumoral administration of a hu14.18-IL2 immunocytokine (anti-GD2 mAb fused to IL2), and intraperitoneal administration of anti-CTLA-4 leading to in situ vaccination and cure of GD2+ murine tumors. Previous work has shown that a T cell response is critical to the efficacy of this therapy, so we created mCherry-labeled T cell mouse models to study T cell response. Here, IMM was used to image concurrent tumor cell and T cell metabolic trends, T cell infiltration, and tumor microenvironment composition.

Methods We created mCherry-labeled T cell mouse models through CRISPR/Cas9 knock-in and Cre-lox genetic modifications. We then implanted syngeneic B78 (GD2+) melanoma cells intradermally into the flanks of C57BL/6 mice to induce measurable tumors. Mice were anesthetized, skin flap surgery performed, and tumors imaged at varying time points. IMM was performed using 750–1040 nm to excite NAD(P)H, FAD, and mCherry through a 40X (1.15 NA) objective. Fluorescence lifetime data was collected using time correlated single photon counting electronics. Murine tissues were later harvested and analyzed via flow cytometry and immunohistochemistry to confirm mCherry expression in mouse models and IMM findings.

Results Here we demonstrate the feasibility of our IMM platform to perform single-cell resolution imaging in vivo. We establish that our genetically engineered mouse models enable clear identification and tracking of mCherry T cell populations. In addition, we show that label-free OMI provides metabolic trends and structural information in vivo (figure 1). Overall, we demonstrate concurrent imaging of intravital tumor cell and T cell populations within the tumor microenvironment.

Conclusions Our preliminary results suggest that the combination of IMM and our mCherry mouse models with OMI allows for concurrent imaging of T cell infiltration and metabolic trends. With continued work, this imaging platform has the potential to provide dynamic, metabolic information on tumor cell and immune cell populations to inform further immunotherapy development.

Acknowledgements This work is supported by the Morgridge Institute for Research (Interdisciplinary Fellowship awarded to A.R.H.) and the NIH (R01 CA205101 and R35 CA197078). The authors thank the University of Wisconsin Carbone Cancer Center (UWCCC) Support Grant P30 CA014520, the UWCCC Translational Research Initiatives in Pathology Laboratory - supported by the UW Department of Pathology and Laboratory Medicine and the Office of The Director NIH (S10OD023526), the UWCCC Flow Cytometry Laboratory, and the Genome Editing and Animal Models Laboratory for core services.

Ethics Approval All animal work was approved by the University of Wisconsin Institutional Animal Care and Use Committees.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0042

Abstract 42 Figure 1 Representative intravital multiphoton microscopy images of a B78 syngeneic melanoma growing in a mouse with mCherry-labeled T cells. (A) Fluorescence intensity image of all fluorophores shows mCherry-labeled T cells (red) infiltrating untreated tumor tissue and vasculature as well as metabolic coenzymes NAD(P)H (blue) and FAD (green) expressed by the tumor. (B) Fluorescence intensity image of NAD(P)H alone shows NAD(P)H landscape as well as tumor boundaries and winding vasculature filled with red blood cells. (C) Fluorescence lifetime image shows mCherry-labeled T cell populations and their corresponding mean lifetime (tau m) values. Fluorescence lifetime values help distinguish mCherry-labeled T cells (typical tau m = 1,400 ps) from nonspecific red autofluorescence in vivo. (D) Fluorescence lifetime image shows NAD(P)H expression and corresponding mean lifetime values which give insight into tumor metabolism and microenvironment.
Abstracts

43 HIGHLY MULTIPLEXED DIGITAL SPATIAL PROFILING OF THE TUMOR MICROENVIRONMENT OF NON-SMALL-CELL LUNG CANCER (NSCLC)

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Background Profiling the tumour microenvironment (TME) has been informative in understanding the underlying tumour-immune interactions. Multiplex immunohistochemistry(mIHC) coupled with molecular barcoding technologies have revealed greater insights into the TME.

Methods In this study, we utilised the Nanostring GeoMX Digital Spatial Profiler (DSP) platform to profile a NSCLC tissue microarray for protein markers across immune cell profiling, immuno-oncology(IO) drug target, immune activation status, immune cell typing, and pan-tumour protein modules. Regions of interest (ROIs) were selected that described tumour, TME and normal adjacent tissue (NAT) compartments.

Results Our data revealed that paired analysis (n=18) of patient matched compartments indicated that the TME was significantly enriched in CD27, CD3, CD4, CD44, CD45, CD45RO, CD68, CD163, and VISTA relative to tumour. Unmatched analysis indicated that the NAT(n=19) was significantly enriched in CD34, fibronectin, IDO1, LAG3, ARG1 and PTEN when compared to the TM E(n=32). Univariate Cox proportional hazards indicated that the presence of cells expressing CD3 (HR:0.5, p=0.018), CD34(HR:0.53, p=0.004) and ICOS (HR:0.6, p=0.047) in tumour compartments were significantly associated with improved overall survival (OS).

Conclusions We implemented both high-plex and high-throughput methodologies to the discovery of protein biomarkers and molecular phenotypes within biopsy samples and demonstrate the power of such tools for a new generation of pathology research.

Acknowledgements This study was funded by the Princess Alexandra Hospital Foundation grant for KOB. AK is supported by an NHMRC ECF Fellowship (APP1157741) and Cure Cancer (APP1182179).

Ethics Approval The study was approved by the QUT Human Research Ethics Board

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0043

44 BODY COMPOSITION MAY BE PROGNOSTIC AND PREDICTIVE OF CLINICAL OUTCOMES IN METASTATIC RENAL CELL CARCINOMA (mRCC) PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITORS (ICI)

1Dylan Martin*, 1Anders Olsen, 2Subir Goyal, 3Yuan Liu, 4Sean Evans, 5Benjamin Magod, 6Jacqueline Brown, 7Lauren Fantoni, 8Greta Rusler, 9Sarah Caulfield, 10Jamie Leary, 2Rahul Ladwa, 3Majid Warkiani, 1Kenneth O’byrne, 1Emory University, Atlanta, GA, USA; 2Emory University School of Medicine, Atlanta, GA, USA; 3Winship Cancer Institute, Atlanta, GA, USA

Background Immune checkpoint inhibitors (ICI) have revolutionized the treatment of metastatic renal cell carcinoma (mRCC). Biomarkers for mRCC patients treated with ICI are limited, and body composition is underutilized in mRCC. We investigated the association between body composition and clinical outcomes in ICI-treated mRCC patients.

Methods We performed a retrospective analysis of 79 ICI-treated mRCC patients at Winship Cancer Institute from 2015–2020. Patients with CT scans within 2 months of ICI-initiation were included. Baseline CT images were collected at mid-L3 and segmented using SliceOMatic v5.0 (TomoVision). Density of skeletal muscle (SM), subcutaneous fat, inter-muscular fat, and visceral fat were measured and converted to indices by dividing by height(m^2) (SMI, SFI, IFI, and VFI, respectively). Total fat index (TFI) was defined as the sum of SFI, IFI, and VFI. Patients were characterized as high versus low for each variable at gender-specific optimal cuts using overall survival (OS) as the primary outcome. A prognostic risk score was created based on the beta coefficient from the multivariable Cox model (MVA) after best subset variable selection. Body composition risk score was calculated as IFI + 2*SM mean + SFI, and patients were classified as high (0–1), intermediate (2), or low-risk (3–4). Kaplan-Meier method and Log-rank test were used to estimate OS and PFS and compare the risk groups. Concordance statistics (C-statistics) were used to measure the discriminatory magnitude of the model.

Results Most were male (73%), and median age was 61 years. Patients were primarily intermediate (54%) or poor-risk (30%) per IMDC criteria and most received ICI as first (35%) or second-line (51%) therapy. The body composition high-risk patients had significantly shorter OS (HR: 6.37, p<0.001), PFS (HR: 4.19, p<0.001), and lower chance of CB (OR: 0.23, p=0.044) compared to low-risk patients in MVA (table 1). Patients with low TFI had significantly shorter OS (HR: 2.72, p=0.002), PFS (HR: 1.91, p=0.025), and lower chance of CB (OR: 0.25, p=0.008) compared to high TFI patients in MVA. The C-statistics were higher for body composition risk groups and TFI compared to IMDC and BMI (table 2). The median OS and PFS were shorter for high-risk versus intermediate and low-risk patients (figures 1–2).

Abstract 44 Table 1 MVA* of association between body composition risk groups and TFI with clinical outcomes

| Body Composition Risk Group Analysis | OS | | PFS | | CB |
|------------------------------------|----|---|---|---|
| High Risk: Risk Score=5-6 n=20     |    |   |    |    |
| HR (CI)                            | 6.37 (2.40-16.92) | <0.001** |    | | |
| Median Survival:                   |    |   |    |    |
| 6.3 months                         |    |   |    |    |
| Intermediate Risk: Risk Score=2 n=42|    |   |    |    |
| HR (CI)                            | 1.56 (0.61-3.95)  | 0.350 | 2.05 (0.98-4.29) | 0.057 | 0.49 (0.15-1.59) | 0.238 |
| Median Survival:                   |    |   |    |    |
| 24-Month Survival:                 |    |   |    |    |
| 29.2%                              |    |   |    |    |
| 12-Month Survival:                 |    |   |    |    |
| 15.0%                              |    |   |    |    |
| Low Risk: Risk Score=3-4 n=18     |    |   |    |    |
| HR (CI)                            | 1   | 1 | 1 |    |
| Median Survival:                   |    |   |    |    |
| 44.5 months                        |    |   |    |    |
| 12-Month Survival:                 |    |   |    |    |
| 53.1%                              |    |   |    |    |

Categorical Total Fat Index (TFI) Analysis**

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=34</td>
<td>n=4</td>
</tr>
<tr>
<td>2.72 (1.43-5.17)</td>
<td>2.05 (0.98-4.29)</td>
</tr>
<tr>
<td>0.002**</td>
<td>0.025**</td>
</tr>
<tr>
<td>1.91 (1.09-3.35)</td>
<td>0.25 (0.09-0.70)</td>
</tr>
<tr>
<td>0.088**</td>
<td>0.006**</td>
</tr>
</tbody>
</table>

* MVA controlled for race, gender, clear cell RCC, Baseline BMI, Age, anti-PD1 monotherapy, IMDC risk groups and number of prior lines of therapy.
** Statistically significant at the level of p<0.05
***High vs low TFI determined by optimal cut analysis
Conclusions Risk stratification using the body composition variables IFI, SM mean, SFI, and TFI may be prognostic and predictive of clinical outcomes in mRCC patients treated with ICI. Larger, prospective studies are warranted to validate this hypothesis-generating data.

Acknowledgements Research reported in this publication was supported in part by the Breen Foundation and the Biostatistics Shared Resource of Winship Cancer Institute of Emory University and NIH/NCI under award number P30CA138292. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Trial Registration Not applicable

Ethics Approval This retrospective study was approved by the Emory University Institutional Review Board.

Consent Not applicable

REFERENCES

Not applicable

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0044

Abstract 44 Figure 1 Comparison of Kaplan-Meier curves between IMDC risk groups (Top panel) and body composition risk groups (Bottom panel) for overall survival (OS)

Abstract 44 Figure 2 Comparison of Kaplan-Meier curves between IMDC risk groups (Top Panel) and body composition risk groups (Bottom Panel) for progression-free survival (PFS)

Abstract 44 Table 2 Comparison of C-statistics between body composition risk groups, TFI, IMDC, and BMI

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>IFI C-statistic</th>
<th>IMDC C-statistic</th>
<th>BMI C-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMDC</td>
<td>NA</td>
<td>0.760</td>
<td>0.606</td>
</tr>
<tr>
<td>BMI</td>
<td>0.710</td>
<td>NA</td>
<td>0.743</td>
</tr>
<tr>
<td>TFI</td>
<td>0.694</td>
<td>0.678</td>
<td>0.696</td>
</tr>
</tbody>
</table>

*Statistically significant at the level of p<0.05
Baseline CT images were collected at mid-L3 and muscle and fat compartments were segmented using SliceOMatic v5.0 (TomoVision). The density of subcutaneous fat, inter-muscular fat, visceral fat, and skeletal muscle (SM) were measured and converted to indices by dividing by height(m)² (SFI, IFI, VFI and SMI, respectively). Attenuated SM mean (Hounsfield Units) was also collected. Myosteatosis was calculated by IFI/SMI*100%. Gender-specific optimal cuts were used to dichotomize patients as high or low for each variable using OS as the primary outcome. A prognostic body composition risk score was created based on the beta coefficient from the multivariable Cox model (MVA) following best-subset variable selection. Our body composite risk score was SMI + 2*SM mean + VFI and patients were categorized as high (0–1), intermediate (2–3), or low-risk (4). Comparison of OS and PFS between the risk groups was performed via Kaplan-Meier method and Log-rank test. Concordance statistics (C-statistic) were used to quantify the discriminatory magnitude of the predictive model.

Results Most patients (70%) were male and more than one-quarter (26%) had an ECOG PS ≥ 2. The majority received ICI in the second (46%) or third-line (21%) setting. Body composite poor-risk patients had significantly shorter OS (HR: 6.18, p<0.001), PFS (HR: 5.91, p<0.001), and lower chance at CB (OR: 0.02, p=0.004) compared to low-risk group in MVA (table 1). Patients with low myosteatosis had significantly longer OS (HR: 0.35, p=0.002), PFS (HR: 0.32, p<0.001), and higher chance at CB (OR: 20.47, p=0.034) compared to high myosteatosis patients in MVA. The C-statistics for our body composition risk group and myosteatosis analyses were higher than BMI for all clinical outcomes (table 2). High and intermediate-risk patients had significantly shorter OS and PFS compared to low-risk patients per Kaplan-Meier estimation (figure 1).

Conclusions Body composition variables such as SMI, SM mean, VFI and myosteatosis may be predictive of clinical outcomes in ICI-treated advanced UC patients. Larger, prospective studies are warranted to validate this hypothesis-generating data.

Acknowledgements Research reported in this publication was supported in part by the Biostatistics and Bioinformatics Shared Resource of Winship Cancer Institute of Emory University and NIH/NCI under award number P30CA138292. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Trial Registration Not applicable

Ethics Approval This retrospective study was approved by the Emory University Institutional Review Board.

Consent Not applicable

Abstract 45 Table 2 Comparison of C-statistics between body composition risk groups, myosteatosis and BMI

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>OS Statistic</th>
<th>p-value (comparison to BMI)</th>
<th>PFS Statistic</th>
<th>p-value (comparison to BMI)</th>
<th>CB Statistic</th>
<th>p-value (comparison to BMI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>0.004</td>
<td>0.001*</td>
<td>0.005</td>
<td>0.001*</td>
<td>0.001</td>
<td>0.001*</td>
</tr>
<tr>
<td>Low</td>
<td>0.005</td>
<td>0.001*</td>
<td>0.005</td>
<td>0.001*</td>
<td>0.001</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Abstract 45 Table 1 MVA* of association between risk groups and myosteatosis with clinical outcomes

<table>
<thead>
<tr>
<th>Body Composition Risk Group Analysis</th>
<th>HR (CI)</th>
<th>p-value</th>
<th>HR (CI)</th>
<th>p-value</th>
<th>OR (CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Risk: Risk Score=0-1</td>
<td>6.81 (2.49-15.96)</td>
<td>&lt;0.001**</td>
<td>5.01 (2.49-14.90)</td>
<td>&lt;0.001**</td>
<td>0.02 (0.00-0.30)</td>
<td>0.094**</td>
</tr>
<tr>
<td>Median Survival: 2.7 months</td>
<td>12 Month Survival: 19.7%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate Risk: Risk Score=2-3</td>
<td>5.56 (0.87-3.58)</td>
<td>0.098</td>
<td>2.90 (1.32-6.40)</td>
<td>0.008**</td>
<td>0.05 (0.01-0.38)</td>
<td>0.083**</td>
</tr>
<tr>
<td>Median Survival: 8.9 months</td>
<td>12 Month Survival: 7.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Risk: Risk Score=4-6</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>Median Survival: Not Reached</td>
<td>12 Month Survival: 45.1%</td>
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</table>

Abstract 45 Figure 1 Kaplan-Meier curves of association between body composition risk groups and overall survival (OS, top panel) and progression-free survival (PFS, bottom panel)
Background Hematoxylin and eosin (H&E) staining is a traditional and widely used histological stain for elucidating tissue morphology for pathological review. However, H&E staining is not fully removable and prevents or severely limits any further use of the same tissue section. We have developed a method for accurately simulating the H&E staining pattern via removable fluorescent dyes that allows for subsequent re-use of the same tissue section for multiplexed immunofluorescent (mIF) staining methods with no decrease in performance. This workflow allows for the pathological pre-screening, annotation, and triaging of samples to undergo multiplexed IHC.

Methods Serial FFPE lung cancer sections were used in this study. After deparaffinization and rehydration, these slides were divided into 3 groups. The first group was stained with a traditional H&E protocol. The second group was stained using a MOTif™ PD-1/PD-L1 Panel kit (Akoya Biosciences, Inc.). The third group was stained with H&E simulation staining reagents, imaged and re-stained using a MOTif™ PD-1/PD-L1 Panel kit (Akoya Biosciences, Inc.) after removal of the H&E simulation reagents. Multispectral fluorescence imagery was acquired on a Vectra Polaris® automated imaging system using Phenochart and inForm® software (Akoya Biosciences, Inc.) to computer scans, as well as exclude ROI containing necrosis, hemorrhage, blood vessels, and autofluorescence. Those pathologist-selected images are then quantified by digital pathology software such as Automated QUantitative Analysis (AQUA®) technology. Finally, pathologists also provide interpretation and summarize findings relevant to the clinical study during the post-analytical phase.

Results Case studies representing distinct malignancies, such as melanoma, non-small cell lung cancer, squamous cell carcinoma of head and neck and diffuse large B-cell lymphoma, illustrating the role of pathologists and especially in rescuing challenging cases and interpreting biomarkers scores from mFIHC assays will be presented.

Conclusions With the advancement in technologies to detect increasing number of biomarkers in a single tissue section and accompanied growth of mFIHC assays in immuno-oncology studies, there is a clear transition from conventional pathology (HI) to computer-aided pathology (AI+HI) that will ultimately ensure greater accuracy, reproducibility and standardization of clinical trial testing, and enable approval of more effective therapies and better patient care.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0047
Background Multiplexed ion beam imaging (MIBI) combines time-of-flight secondary ion mass spectrometry (ToF-SIMS) with metal labeled antibodies to image 40+ proteins in a single scan at subcellular spatial resolution. Here, we show that the recently released MIBIscope provides improved sensitivity for detecting immune checkpoint markers and offers greater throughput at higher resolution than the alpha instrument.

Methods Serial sections from three FFPE NSCLC samples, in addition to a control slide consisting of various unremarkable tissues, were stained with a panel of 25 metal labeled antibodies. The tissue was imaged at subcellular resolution using the MIBIscope and the alpha instrument. Masses of detected species were assigned to target biomolecules given the unique label of each antibody and multi-step processing was used to create images. Cell classification was performed using two complementary methods that differed in the need for cell segmentation to phenotypically characterize the tissue environment and quantify marker expression.

Results Replicate regions of interest (ROIs) were collected on both instruments with similarly sized ROIs acquired in 17 minutes with the MIBIscope compared to 280 minutes with the alpha instrument. Fourier Ring Correlation (FRC) showed the resolution to be greater on the MIBIscope as compared to the alpha instrument with FRC also demonstrating uniform resolution across an ROI 2.5X greater in size. Even with the 16X greater speed of the MIBIscope, the signal of the 25 markers across replicate ROIs was increased (y=x^1.07) and showed similar expression patterns to those observed on the alpha instrument (figure 1). This resulted in greater sensitivity to markers with low expression, such as checkpoint markers. Eleven cell populations were classified across the ROIs utilizing two methods, with both methods showing a similar frequency of tumor cells and B, T, and myeloid cell subsets between instruments. Segmentation enabled the number of cells within a population to be calculated but defining boundaries is laborious and signal from neighboring cells can result in misclassification. Performing classification at the pixel level, without segmentation, enabled the fraction of the tissue that is tumor or any other cell type to be rapidly determined.

Conclusions The MIBIscope enables the phenotypic characterization of tumor and non-tumor microenvironments. Co-expression of markers can be used to classify tumor and immune populations and to quantify the expression of markers associated with immune suppression. The increased sensitivity and throughput of the MIBIscope, in combination with the 40-parameter capability and subcellular resolution, provides a platform uniquely suited to understanding the complex tumor immune landscape.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0048

Abstract 48 Figure 1 Comparison of images acquired between instruments. The signal intensity is greater on the MIBIscope and shows a similar staining pattern as achieved by the alpha instrument. Shown are 3 overlays from a single scan from replicate ROIs of an NSCLC sample displayed with the same contrast settings.

IN SITU PHENOTYPIC ANALYSIS OF T CELLS IN THE TUMOR MICROENVIRONMENT OF A PRE-CLINICAL MODEL OF NON-SMALL CELL LUNG CANCER (NSCLC) BY TISSUE SECTIONING AND WHOLE-MOUNT IMMUNOFLUORESCENCE IMAGING

Elen Torres*, Stefani Spranger. The Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA, USA

Background Understanding the interactions between tumor and immune cells is critical for improving current immunotherapies. Pre-clinical and clinical evidence has shown that failed T cell infiltration into lung cancer lesions might be associated with low responsiveness towards checkpoint blockade.1 For this reason, it is necessary to characterize not only the phenotype of T cells in tumor-bearing lungs but also their spatial location in the tumor microenvironment (TME). Multiplex immunofluorescence staining allows the simultaneous use of several cell markers to study the state and the spatial location of cell populations in the tissue of interest. Although this technique is usually applied to thin tissue sections (5 to 12 μm), the analysis of large tissue volumes may provide a better understanding of the spatial distribution of cells in relation to the TME. Here, we analyzed the number and spatial distribution of cytotoxic T cells and other immune cells in the TME of tumor-bearing lungs, using both 12 μm sections and whole-mount preparations imaged by confocal microscopy.

Methods Lung tumors were induced in C57BL/6 mice by tail vein injection of a cancer cell line derived from KrasG12D/+ and Tp53-/- mice. Lung tissue with a diverse degree of T cell infiltration was collected after 21 days post tumor induction. Tissue was fixed in 4% PFA, followed by snap-frozen for sectioning. Whole-mount preparations were processed according to WeiZhe Li et al. (2019) 2 for tissue clearing and multiplex volume imaging. T cells were labeled with CD8 and FOXP3
antibodies to identify cytotoxic or regulatory T cells, respectively. Tumor cells were labeled with a pan-Keratin antibody. Images were acquired using a Leica SP8 confocal microscope. FIJI\textsuperscript{3} and IMARIS\textsuperscript{3} were used for image processing.

**Results** We identified both cytotoxic and regulatory T cell populations in the TME using thin sections and whole-mount. However, using whole-mount after tissue clearing allowed us to better evaluate the spatial distribution of the T cell populations in relation to the tumor structure. Furthermore, tissue clearance facilitates the imaging of larger volumes using multiplex immunofluorescence.

**Conclusions** Analysis of large lung tissue volumes provides a better understanding of the location of immune cell populations in relation to the TME and allows to study heterogeneous immune infiltration on a per-lesion base. This valuable information will improve the characterization of the TME and the definition of cancer-immune phenotypes in NSCLC.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0049

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**50 SPATIALLY RESOLVED MOLECULAR INVESTIGATION OF TRIPLE NEGATIVE BREAST CANCER AND ITS IMMUNE MICROENVIRONMENT**

Stephen Williams*, Cedric Uytingco, Neil Weisenfeld, Nigel Delaney, Solongo Ziraldo, Yifeng Yin, Jennifer Chew, Sharmila Chatterjee, Daniel Riordan, Zachary Bent. x Genomics, Pleasanton, CA, USA

**Background** Triple negative breast cancer (TNBC) accounts for 10–20% of all diagnosed breast cancer cases in the US and is characterized by loss of HER2, estrogen receptors, and progesterone receptors. TNBC is an aggressive, complex disease with a poor prognosis due to resistance to traditional therapies. Understanding the underlying biology and tumor microenvironment is critical to the development of diagnostic biomarkers and to guide the search for effective therapies. Here, we demonstrated the ability of the 10x Genomics Visium Spatial Gene Expression Solution to elucidate the immunological profile and microenvironment of TNBC samples in conjunction with standard pathological techniques.

**Methods** Spatial transcriptomics technology complement pathological examination by combining the benefits of histological stains with the throughput and deep biological insight of RNA-seq. We investigated serial sections of TNBC by using the 10x Genomics Visium Spatial Gene Expression Solution to spatially resolve the samples’ cellular composition and expressed microenvironment. Visium incorporates ~5000 molecularly barcoded, spatially encoded capture probes in expressed microenvironment. Visium incorporates ~5000 molecularly barcoded, spatially encoded capture probes in

**Results**

Images were acquired using a Leica SP8 confocal microscope. Tumor cells were labeled with a pan-Keratin antibody. Images were acquired using a Leica SP8 confocal microscope. FIJI\textsuperscript{3} and IMARIS\textsuperscript{3} were used for image processing.

**Conclusions** Analysis of large lung tissue volumes provides a better understanding of the location of immune cell populations in relation to the TME and allows to study heterogeneous immune infiltration on a per-lesion base. This valuable information will improve the characterization of the TME and the definition of cancer-immune phenotypes in NSCLC.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0049

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**51 SMALL CELL/LYMPHOHISTIOCYTIC MORPHOLOGY IS ASSOCIATED WITH CD8 POSITIVITY, RETAINED T CELL MARKERS, A TREND OF DECREASED PD-L1 EXPRESSION, BUT NOT OUTCOME IN ADULTS WITH ALK + ALCL**

Mahsa Khanlari*, Shaoying Li, Roberto N Miranda, Swaminathan Iyer, Cameron Yin, Pei Lin, Guilin Tang, Sergej N Konoplev, L Jeffrey Medeiros, Jie Xu. MD Anderson Cancer Center, Houston, TX, USA

**Background** Several morphologic patterns of ALK+ anaplastic large cell lymphoma (ALCL) are recognized: common, small cell, lymphohistiocytic, Hodgkin-like, and composite patterns. Small cell (SC) and lymphohistiocytic (LH) patterns are thought to be closely associated with poorer outcome in children with ALK+ ALCL\textsuperscript{2}. However, the clinicopathologic and prognostic features of SC/LH patterns of ALK+ ALCL are not yet reported in adults. Recently, we found PD-L1 expression in a large subset of ALK+ ALCL cases, however, PD-L1 expression in SC/LH versus non-SC/LH ALCL has not been reported.

**Methods** Among 102 adult patients with ALK+ ALCL seen at our institution from January 1, 2007 through August 30, 2018, 18 (18%) cases had a SC and/or LH pattern. The clinical, pathologic, and outcome data were compared between SC/LH and non-SC/LH ALK+ ALCL cases using Fisher’s exact test. Overall survival (OS) was analyzed using the Kaplan-Meier method and compared using the log-rank test.

**Results** There were no significant differences in clinical features including age, gender, nodal versus extranodal involvement, B symptoms, stage, leukocytosis/lymphocytosis, and serum LDH levels between patients with SC/LH versus non-SC/LH ALK+ ALCL. Compared to non-SC/LH cases, SC/LH ALCL was more often positive for CD2 (92% vs. 36%, \( p = 0.0007 \)), CD3 (81% vs. 15%, \( p = 0.0001 \)), CD7 (80% vs. 37%, \( p = 0.03 \)), and CD8 (54% vs. 7%, \( p = 0.0006 \)). SC/LH ALCL showed a trend of decreased PD-L1 expression than non-SC/LH cases (24% vs. 46%, \( p = 0.11 \)). There were no significant differences in the expression of CD4, CD5, CD25, CD43, CD45, CD56,
TCR A/B, TCR G/D, cytotoxic markers, EMA, Ki-67 proliferation index. The induction chemotherapy and response rate in patients with SC/LH ALK+ ALCL were similar to patients with non-SC/LH ALK+ ALCL. After a median follow-up of 30.5 months (range, 0.3–224 months), there was no significant difference in OS between patients with SC/LH versus non-SC/LH ALK+ ALCL (p = 0.88).

Conclusions In adults with ALK+ALCL, the SC/LH morphological pattern is associated with a CD8+ T cell immunophenotype and retention of expression of T cell markers (CD2, CD3, and CD7). The trend of decreased PD-L1 expression in SC/LH ALCL suggests that these patients may not be ideal candidates for PD-L1 immunotherapy. The SC/LH patterns of ALK+ ALCL have no impact on the prognosis of adult patients which is in contrast to the reported association of the SC/LH patterns with poorer outcome in children with ALK+ ALCL.

Ethics Approval The study was approved by the Institutional Review Board at MD Anderson Cancer Center, Approval number: PA16-0897.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0051

ADVANCED T LYMPHOCYTE ANALYSIS SYSTEM (ATLAS) FOR IN-DEPTH IMMUNOLOGICAL INTERROGATION IN REAL-WORLD CONDITIONS, A METHODOLOGICAL STRATEGY

Background Humans are genetically diverse and possess a rich immunological history. It is logical to consider that these factors may lead to differences in individual immunological responses to therapy when diagnosed with cancer. The successful implementation of immune-based therapies against cancer has brought the need to develop strategies to create meaningful profiles that faithfully depict the patient’s immunological status. We report an in-depth immunological interrogation methodology, termed ATLAS. This system was designed to generate an accurate representation of the patient’s immunological landscape that can be used during various time points during immune-checkpoint inhibitor (ICI) therapy.

Methods We selected data from our prospective registry trial at Loyola University Medical Center to design individual immunological profiles of patients diagnosed with locally advanced or metastatic solid tumors planning to receive ICI. Only metastatic melanoma patients samples pre-ICI therapy are included in this first analysis. Twenty mL of peripheral blood were collected. Giving consideration to scientific rigor and limited sample availability, the assays were designed in miniaturized forms. ATLAS includes classical peripheral blood mononuclear cells (PBMCs) composition and T cell phenotypic and transcriptional analysis. To depict T cell functionality, we examined multiple parameters such as T cell receptor (TCR) signaling threshold, cell proliferation and NF-κB activation, at steady-state and in response to cell activation. To obtain both a broad and T cell-specific view, we quantified circulating chemokines and cytokines in plasma and from activated T cells.

Results For this first methodologic demonstration, patient characteristics are depicted in table 1. Data from different ATLAS assays were used to create individual immunological profiles presented as a dashboard for each patient. Distributional plots and measures of center (mean, median) and spread (range, variance) were used to eliminate low-information parameters from the figures. Data visualizations compared individual patients to the sample median for continuous parameters and

<table>
<thead>
<tr>
<th>Table 1: Patient characteristics</th>
<th>Overall, n=11</th>
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<tr>
<td>Age, mean (SD)</td>
<td>56.5 (13.2)</td>
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<tr>
<td>Female, n (%)</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>Comorbidities and medical history, n (%)</td>
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</tr>
<tr>
<td>Diabetes</td>
<td>1 (9.1)</td>
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<tr>
<td>Hypertension</td>
<td>4 (36.4)</td>
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<tr>
<td>Hyperlipidemia</td>
<td>1 (9.1)</td>
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<tr>
<td>Autoimmune</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>BPH</td>
<td>1 (9.1)</td>
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<tr>
<td>Multinodular Goiter</td>
<td>1 (9.1)</td>
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<tr>
<td>Breast Cancer</td>
<td>1 (9.1)</td>
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<tr>
<td>Arthritis</td>
<td>1 (9.1)</td>
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<tr>
<td>Tobacco use</td>
<td>4 (36.4)</td>
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<tr>
<td>Alcohol use</td>
<td>2 (18.2)</td>
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<tr>
<td>Lab values, mean (SD)</td>
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</tr>
<tr>
<td>Hemoglobin</td>
<td>13.2 (1.6)</td>
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<tr>
<td>Platelets</td>
<td>279 (116)</td>
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<tr>
<td>Neutrophils</td>
<td>5.4 (2.5)</td>
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<tr>
<td>LDH</td>
<td>354 (176)</td>
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<tr>
<td>BUN</td>
<td>15.7 (5.5)</td>
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<tr>
<td>Creatinine</td>
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<td>Calcium</td>
<td>9.4 (0.4)</td>
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<tr>
<td>Bilirubin</td>
<td>0.91 (0.34)</td>
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<tr>
<td>Started ICI, n (%)</td>
<td>9 (81.8)</td>
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<tr>
<td>No chemotherapy before ICI, n (%)</td>
<td>3 (27.3)</td>
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<tr>
<td>Metastases location, n (%)</td>
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<tr>
<td>Brain</td>
<td>3 (27.3)</td>
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<tr>
<td>Lung</td>
<td>7 (63.6)</td>
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<tr>
<td>Cer. Lymph Nodes</td>
<td>1 (9.1)</td>
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<tr>
<td>Tho. Lymph Nodes</td>
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<tr>
<td>Med. Lymph Nodes</td>
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<tr>
<td>Abd. Lymph Nodes</td>
<td>2 (18.2)</td>
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<tr>
<td>Pol. Lymph Nodes</td>
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<tr>
<td>Liver</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Bone</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (27.3)</td>
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<tr>
<td>ECOG Status Pre-ICI, n (%)</td>
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<tr>
<td>0</td>
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<tr>
<td>1</td>
<td>4 (36.4)</td>
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<tr>
<td>2</td>
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</table>
compared patients’ percentages to sample average relative abundance. Two patients, P011 shown in figure 1 and P021 shown in figure 2, are depicted using this approach.

Conclusions ATLAS can be used in real-world conditions to generate comprehensive immunological profiles of cancer patients. Individual profiles indicate that immunological constitution is heterogeneous among patients, even with the same tumor type. We propose that the addition of ATLAS to our clinical and immunological toolbox may help stratify patients to articulate truly personalized oncologic therapies.

Ethics Approval The study was approved by Loyola University Medical Center and Loyola University Chicago Ethics Board and Institutional Review Board, approval number 209364.

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53 PREDICTORS OF RESPONSE TO IMMUNE CHECKPOINT INHIBITOR THERAPY IN METASTATIC SOLID TUMORS: REAL WORLD EVIDENCE

Aasems Jacob*, Jianrong Wu, Jill Kolesar, Eric Durbin, Aju Mathew, Susanne Arnold, Aman Chauhan. University of Kentucky, Lexington, KY, USA

Background Immune checkpoint inhibitor (ICI) therapy is increasingly being used in oncology and novel predictive biomarker for efficacy and side effects are an unmet need.1, 2 The study aims to do a comprehensive analysis of factors affecting outcome from ICI therapy with real-world data and identify potential predictive biomarkers in diverse populations.

Methods We performed a retrospective analysis of patients with metastatic solid tumors who received ICI and underwent molecular profiling with FoundationOne® CDx panel between 2016 and 2020 at Markey Cancer Center, Lexington KY. Progression-free survival (PFS), radiological response, and autoimmune side effects were analyzed and compared with various molecular biomarkers (figure 1). Logistic regression, Fisher’s exact test, Kaplan-Meier method, log-rank test, and Cox regression were used to analyze clinical features and efficacy outcomes.

Results 69 patients were included in the study (tables 1 and 2). A statistically significant improvement in PFS was observed in the PIK3 mutated cohort (median 123 vs. 23 weeks. HR=2.51, 95%CI 1.23, 5.14; table 3 and figure 2). This was independent of tumor mutational burden (TMB) status or PD-L1 expression status (HR 3.24, p=0.016). PIK3 mutants had a higher overall response rate (ORR) than the wild type (69.6% vs. 43.5%, OR 0.34; p=0.045; tables 3 and 4). PIK3 mutants had a higher risk of developing immune-related adverse events (IRAEs) (73.9% vs. 37%, p=0.004). PIK3 mutation did not associate with TMB, PD-L1 expression or microsatellite stability status. Median PFS was higher in the high TMB cohort compared to the low-intermediate group and reached statistical significance (median not reached vs. 26
weeks; HR = 0.37, 95% CI 0.13, 0.95). PDL1 expression had no significant effect on the radiologic response, but PFS improvement in patients with tumors expressing PDL1 trended towards statistical significance (median 18 vs. 40 weeks, HR = 1.43, 95% CI 0.93, 4.59). BRAF mutation conferred shorter PFS (median 17 vs. 39 weeks, HR = 0.35, 95% CI 0.14, 0.91) (figure 2).

Conclusions High tumor mutational burden and PIK3 mutation conferred better progression-free survival with immunotherapy across cancer types. The improvement in PFS in PIK3 mutated patients was independent of PDL1 status or TMB. The results should prompt further evaluation of these potential biomarkers and more widespread real-world data publications to help determine biomarkers that could benefit specific populations.

Ethics Approval The study was approved by University of Kentucky Institutional Review Board, approval number 49450.
PATIENT STRATIFICATION USING CLINICAL PROTEOMICS – VALIDATED MULTIPLEXED MRM ASSAYS TO QUANTIFY HER2 AND OTHER BIOMARKERS IN CLINICAL FFPE TISSUES

Maxim Isabelle*, Michael Schirm, Gwenaël Pottiez, Rudolf Gulbaud, Lorella Di Donato.
Caprion Biosciences, Montreal, Canada

Background The advent of precision oncology has led a shift towards biomarker-driven clinical trial designs and molecular profiling of individual patients. Identification of patients with the target biomarker profile may be useful in guiding patient selection as an enrichment strategy for clinical trials. Targeted multiple reaction monitoring mass spectrometry (MRM-MS) analysis for multiplexed quantitation of biomarker proteins in FFPE tissue provides direct, more accurate and precise quantification over current gold standard immunohistochemistry (IHC) methods. However, MRM-MS has not yet been broadly applied to clinical trials. In this study, we demonstrate the systematic development, optimization and analytical validation of quantitative, multiplexed MRM-MS assays for robust biomarker quantification in clinical FFPE tissues, including sample analysis under GLP. Results from an MRM panel targeting 8 clinically relevant biomarker proteins will also be shown, including the measured HER2 levels in FFPE breast tumors classified by IHC as 0, 1+, 2+ or 3+.

Methods MRM-MS biomarker panels were developed and optimized for multiplexed quantification of ≤12 proteins, in which unique peptides derived from each target protein were monitored as a surrogate measure of protein levels. Tumor regions from FFPE tissue sections were dissected using laser capture or macrodissection, solubilized, digested with trypsin to generate peptides for analysis, spiked with fixed levels of stable isotope labeled (SIL) peptide standards, and analyzed by MRM-MS. Analytical validation was performed per NCI CPTAC guidelines, including response curves, assay repeatability, selectivity, stability, and reproducibility of endogenous detection. Clinical performance was assessed using commercially sourced FFPE-tumor tissues, including a cohort of breast tumor tissues with a wide range of HER2 expression.

Results Assay performance results were protein/peptide dependent, with sensitivity in the low pg/µg total protein range. For HER2, assay linearity was demonstrated over 2.5 to 3 orders of magnitude, with a precision and accuracy of <15% over 3 independent runs. In sample analysis, the MRM-MS was sufficiently sensitive to detect HER2 in 1 µg total protein from FFPE breast tumor classified by IHC as negative (0).

Conclusions GCLP-compliant quantitative multiplexed large-scale clinical analysis of protein biomarkers by MRM-MS in FFPE tissue is feasible and enables precise and accurate quantitation of proteins when IHC methods are unsuitable or unavailable. Data can be used for patient stratification, optimization of treatment outcomes, drug resistance prediction, and to support clinical development of novel therapeutics.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0054

THOUSANDS OF ANTIGENS ARE RECOGNIZED IN MICE VIA ENDOGENOUS ANTIBODIES AFTER BEING CURED OF A B78 MELANOMA VIA IMMUNOTHERAPY

1Anna Hoeffer*, 1Amy Erbe, 1Sean McVain, 1Trang Le, 1Angie Xu, 1Nicholas Mathers, 1Eric Zhang, 1Andrew Melby, 1Claire Baniel, 1Alexander Rahmilevich, 2Jacquelyn Hank, 2Richard Pinapati, 2Brad Garcia, 2Jigar Patel, 2Zachary Morris, 1Irene Org, 1Paul Sondel. 1University of Wisconsin Madison, Madison, WI, USA; 2Nimble Therapeutics, Madison, WI, USA

Background Antibodies can play an important role in innate and adaptive immune responses against cancer. Using a high-density peptide array, we assessed potential protein-targets for antibodies detected in mice cured of melanoma through a combined immunotherapy regimen. Our goal was to determine the linear peptide sequences recognized by anti-tumor antibodies produced in mice cured of melanoma following immunotherapy.

Methods Mice with GD2-expressing syngeneic B78 melanoma were treated with a combination immunotherapy (local radiation therapy + intratumoral anti-GD2 mAb linked to IL2) capable of inducing an ‘in situ vaccine’ effect (ISV), enabling mice to be cured of their tumors with long-term immune memory. Naive and immune sera were collected from these mice. Using flow cytometry, immune sera showed strong antibody-binding against B16 (parental cell line of B78 without GD2 expression). These sera were then used on a Nimble Therapeutics’ peptide-array (either whole proteome or a curated list of ~650 proteins) to determine specific antibody-binding sites, and data were analyzed using a dynamic programming method that scans adjacent peptides to determine whether a peptide is bound by antibodies. Proteins were selected if peptides were bound using immune sera but not bound with the sera from naïve or non-responding tumor-bearing mice.

Results Multiple proteins were selectively identified by immune sera that were not detected by sera from naïve or non-responding tumor-bearing mice. When focusing on the whole mouse proteome data, thousands of peptides were targeted by 2 or more mice and exhibited strong antibody binding only by immune sera. We also identified a few proteins that elicited an immune response in the naïve mouse sera that showed a significantly stronger signal in the immune sera of the same mice indicating that the cancer and/or the received therapy strengthened the immune response to these proteins.

Conclusions We are able to detect selective antibody binding to immune sera. However, we are continuing to refine our analytical methods and are further investigating the identified proteins. These peptides may potentially serve as targets for antibody-based or cellular therapies. In addition, we are examining whether some of the identified tumor-specific endogenous antibodies might be used as biomarkers to predict response to our ISV regimen and potentially other immunotherapy treatments.

REFERENCE


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0055
Background Precision immuno-oncology is increasingly relevant to cancer therapy given the ascendance of immunotherapy. While next-generation sequencing (NGS) based algorithms may elucidate immunotherapeutic response, many such algorithms require highly accurate Class I HLA typing. One major challenge of HLA type derivation resides in highly polymorphic HLA allelic diversity, which conventional exome sequencing technologies poorly capture. Further, accurate HLA typing requires definitive distinction between thousands of potential HLA alleles. These challenges may cause widely used NGS HLA typing tools, such as Polysolver and Optitype, to perform inaccurate HLA typing. Poor HLA coverage poses the risk of silently mistyping HLA alleles, yielding inaccurate downstream HLA loss of heterozygosity (LOH) detection and neoepitope predictions.

Methods We designed the ImmunoID NeXT Platform to more comprehensively profile the HLA region. To evaluate the accuracy of conventional NGS-based Class I HLA typing, a widely used dbGaP project (phs000452, n=160) of melanoma NGS data was evaluated alongside a set of over 500 solid tumor cancer patient samples sequenced on the ImmunoID NeXT Platform. Read coverage was derived from both GRCh38 and HLA allele database alignments. To test whether Polysolver over represents specific HLA alleles under reduced read conditions, a Monte Carlo bootstrap approach predicted theoretical allele frequency ranges.

Results Below 20x read coverage, nearly 50% of Polysolver HLA calls (phs000452) are homozygous, representing a divergence from typical HLA homozygous rates of between 10–20%, with $p<10^{-15}$ (Fisher’s Exact) compared to reference 1000 Genomes homozygous rates. Polysolver’s homozygous, heterozygous, and no-calls demonstrated a statistically significant difference in coverage ($p<10^{-6}$, Kruskal-Wallis) across all Class I HLA genes per Polysolver and public exome data (phs000452). The Personalis ImmunoID NeXT™ cohort did not demonstrate such a trend despite a similar exome-wide sequencing depth. Further, sixteen rare HLA alleles were identified with sample frequencies greater than expected from the dbGaP data set, with no such alleles identified from the Personalis ImmunoID NeXT data set.

Conclusions HLA typing may silently fail in the context of reduced read coverage without HLA-specific platform augmentation. This silent failure can have large implications for accurate neoantigen prediction and HLA LOH detection, both of which are becoming increasingly important for immuno-oncology treatment modalities such as personalized cancer vaccines, adoptive cell therapies, and blockade therapy response biomarkers. Studies utilizing neoepitope and HLA LOH prediction require careful validation for HLA calls, including assessments of coverage and homozygous rates, and may benefit from increased HLA locus coverage.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0056

Abstracts

56 IMPROVED NEXT GENERATION SEQUENCING BASED CLASS I HLA TYPING THROUGH EXOME ENHANCEMENT


Background Accurately identified neoantigens can be effective therapeutic agents in both adjuvant and neoadjuvant settings. A key challenge for neoantigen discovery has been the availability of accurate prediction models for MHC peptide presentation. We have shown previously that our proprietary model based on (i) large-scale, in-house mono-allelic data, (ii) custom features that model antigen processing, and (iii) advanced machine learning algorithms has strong performance. We have extended upon our work by systematically integrating large quantities of high-quality, publicly available data, implementing new modelling algorithms, and rigorously testing our models. These extensions lead to substantial improvements in performance and generalizability. Our algorithm, named Systematic HLA Epitope Ranking Pan Algorithm (SHERPA™), is integrated into the ImmunoID NeXT Platform®, our immuno-genomics and transcriptomics platform specifically designed to enable the development of immunotherapies.

Methods In-house immunopeptidomic data was generated using stably transfected HLA-null K562 cells lines that express a single HLA allele of interest, followed by immunoprecipitation using W6/32 antibody and LC-MS/MS. Public immunopeptidomics data was downloaded from repositories such as MassIVE and processed uniformly using in-house pipelines to generate peptide lists filtered at 1% false discovery rate. Other metrics (features) were either extracted from source data or generated internally by re-processing samples utilizing the ImmunoID NeXT Platform.

Results We have generated large-scale and high-quality immunopeptidomics data by using approximately 60 mono-allelic cell lines that unambiguously assign peptides to their presenting alleles to create our primary models. Briefly, our primary ‘binding’ algorithm models MHC-peptide binding using peptide and binding pockets while our primary ‘presentation’ model uses additional features to model antigen processing and presentation. Both primary models have significantly higher precision across all recall values in multiple test data sets, including mono-allelic cell lines and multi-allelic tissue samples. To further improve the performance of our model, we expanded the diversity of our training set using high-quality, publicly available mono-allelic immunopeptidomics data. Furthermore, multi-allelic data was integrated by resolving peptide-to-allele mappings using our primary models. We then trained a new model using the expanded training data and a new composite machine learning architecture. The resulting secondary model further improves performance and generalizability across several tissue samples.

Conclusions Improving technologies for neoantigen discovery is critical for many therapeutic applications, including personalized neoantigen vaccines, and neoantigen-based biomarkers for immunotherapies. Our new and improved algorithm (SHERPA) has significantly higher performance compared to a state-of-the-art public algorithm and furthers this objective.

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EXCLUDING TREG EPITOPE AND INTEGRATING CD8 AND CD4 EFFECTOR NEOEPITOPE CONTENT IMPROVES PROGNOSTIC BIOMARKER TOOL IN BLADDER CANCER

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Background Improvement of current prognostic biomarkers will enhance our ability to identify cancer patients at higher risk of recurrence and will further advance the personalization of patient monitoring and treatment. We hypothesized that the presence of a mutation alone is not sufficient to generate an immunogenic neoepitope, but that significant differences must exist between the Human Leukocyte Antigen (HLA)- and/or T Cell Receptor (TCR)-interfaces of the neoepitope and its non-mutated form, or with other self-epitopes, in order to be recognized as non-self by the immune system. As such, cancer patient clinical outcomes may be better understood by neoepitope analyses that integrate these considerations.

Methods We analyzed large-scale, genomic data from The Cancer Genome Atlas (TCGA) using Aner, an automated machine-learning-based pipeline we designed for neoantigen screening and vaccine design. Aner shares components with other commercial-grade screening platforms used routinely in immunogenicity assessments of biologics and infectious disease antigens, such as the EpiMatrix algorithm for HLA-I and HLA-II neoepitope identification, and the JanusMatrix algorithm for tolerated, tolerogenic, and cross-reactive T cell epitope identification. Evaluation of patient survival with Aner was compared to other analyses employing tumor mutational burden (TMB) or neoepitopes identified with the commonly used NetMHCpan-4.0 and NetMHCIIpan-3.1 T cell epitope prediction tools.

Results We stratified bladder patients based on their Aner HLA-I and HLA-II neoepitope burdens and observed significantly prolonged disease free and overall survival in patients whose tumor contained both high numbers of HLA-I and HLA-II neoepitopes compared to other individuals. Stratifications performed with Aner were superior to comparative analyses performed with TMB or with NetMHCpan and NetMHCIIpan. In addition, we showed that Aner’s precise filtering and characterization of mutated epitopes contributed to this increased association with survival, as showcased by gradual improvements in survival analyses performed after each of its filtering step. Multivariate survival analyses indicated that Aner neoepitope content remained a significant factor in patient overall survival even when adjusted for TMB, and other clinical covariates such as age at diagnosis and disease stage, unlike analyses involving NetMHCpan and NetMHCIIpan neoepitopes.

Conclusions Our analysis suggests that enhanced presence of CD8, CD4 T cell epitopes, and limited inclusion of Treg epitopes in the tumor genome plays a key role in cancer survival. Aner scoring provides a predictive method for predicting patient outcomes, by defining the number of true neoepitopes and by identifying Treg epitopes that would interfere with T cell-based immune activation and response to the tumor.

INTEGRATING DEEP PROTEOMICS PROFILING WITH SURVIVAL ANALYSIS TO IDENTIFY NOVEL BIOMARKERS OF RESPONSE TO PD-1 BLOCKADE IN NSCLC PATIENTS

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Background Immune checkpoint inhibitors have improved clinical responses and overall survival for patients with non-small cell lung cancer (NSCLC). However, the response is not equal and known NSCLC biomarkers are not sufficient in predicting therapy outcome. Deep proteomic analysis of NSCLC patient’s plasma treated with anti-PD-1-blockade using a state-of-the-art data independent acquisition mass spectrometry (DIA-MS) is a powerful and unbiased way of identifying protein signatures associated with disease stage or response to treatment. However, to unravel these associations large-scale omics data should be analyzed with respect to available clinical information. To achieve this goal, we have used an approach previously applied by Uhlen et al., 20171 for transcriptomic datasets. In this approach survival data is used to set the most optimal thresholds for candidate biomarkers.

Methods 125 plasma samples were analyzed by capillary flow liquid chromatography coupled to DIA-MS. Data were extracted with latest Spectronaut TM and proteins were quantified. Each recorded protein intensity was used as a threshold for two groups of samples for which Kaplan-Meier estimates were generated using ‘survival’ package in R. Benjamini-Hochberg correction was applied and p-values with corresponding intensity cut-offs were extracted to generate panels of potential biomarkers.

Results 125 plasma samples (in total 75 baseline and 50 after 8-weeks treatment) from advanced NSCLC patients treated with an anti-PD-1 inhibitor following at least 1 prior line of treatment were analyzed. 727 unique proteins were quantified across all samples. Data analysis was performed separately for each line of treatment and treatment status resulting in more than 100’000 p-values. For each group, panels of proteins with best performance in separating progression free survivals were defined at FDR of 0.10, giving 64 unique proteins which were mapped to acute phase response, platelet degranulation and complement activation. Several of these proteins were listed in the Early Detection Research Network database of the National Cancer Institute, and one of them – LYPD3, was a potential therapeutic target in a preclinical study for NSCLC treatment. Selected proteins were then used to cluster patients into cohorts that showed association with the response to therapy.

Conclusions Deep proteomic profiling of plasma samples using DIA-MS in conjunction with clinical outcome enables a holistic and stringent analysis of potential circulating biomarkers. Such analysis generates functional insights into the plasma proteome that enable deeper understanding and comprehensive integration of clinical data with proteomics markers at different disease stages and treatment phases.

REFERENCES

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A37
DEVELOPMENT AND VALIDATION OF BLOOD TUMOR MUTATIONAL BURDEN REFERENCE STANDARDS

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Background Tumor mutational burden (TMB), as measured by exome or panel sequencing of tumor tissue (tTMB) or blood (bTMB), has been identified as a potential predictive biomarker for treatment benefit in patients with various cancer types receiving immunotherapy targeting checkpoint inhibitors (e.g., PD-1, PD-L1, CTLA-4). However, significant variability in TMB measurement has been reported due to differences in pre-analytical and laboratory methods, panel size, number of genes covered and bioinformatics pipelines. Reference standards have been proposed and evaluated for tTMB analysis by the Friends of Cancer Research (FoCR) to enable harmonization and standardization across different tTMB panel providers. Reference standards for bTMB are likely to be even more important given the unique challenges and higher sensitivity required for bTMB assays.

Methods Contrived bTMB reference materials with 0.5% and 2% tumor content were developed using DNA from tumor cell lines and donor-matched lymphoblastoid cell lines fragmented and size-selected to mimic cell-free DNA with TMB scores of 7, 9, 20 and 26 mut/Mb. Mutation coverage, mutant allele frequency (MAF) and bTMB scores were assessed using the PredicineATLAS and GuardantOMNI next-generation sequencing (NGS) platforms.

Results The DNA fragment size for the contrived samples was similar to naturally occurring circulating cell-free tumor DNA and mutation patterns were aligned with those from parental tumor lines. As anticipated, low frequency artefactual MAF variants were observed, requiring removal by bioinformatic filtration. For samples with 2% tumor content, standards for 7, 20 and 26 mut/Mb were found to have as-expected bTMB scores across both evaluation platforms, with good reproducibility, following removal of low frequency MAFs. Results for 0.5% tumour content were also promising, although with greater variability in post-filtration bTMB scores observed.

Conclusions The findings demonstrate it is feasible to produce bTMB reference standards across a range of bTMB levels. The data highlight the importance of data filtration to account for underlying low MAFs in such cell-line derived samples and that this reference material can control for variant sensitivity though not variant specificity. bTMB reference standards reported here could support the calibration and validation of bTMB platforms and help harmonization between panels and laboratories, thus improving the accuracy of testing to aid treatment decisions in oncology.

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TUMOR MUTATIONAL BURDEN ASSESSMENTS BY TWO COMMERCIAL TARGETED SEQUENCING ASSAYS

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Background Tumor mutational burden (TMB) is a key biomarker for immune checkpoint inhibitor across several cancer types. While TMB as calculated from whole exome sequencing of the tumor tissue is still the gold standard, enabling TMB in clinical labs requires targeted sequencing panels for faster turnaround time and low input requirements. Herein, we assess two commercially available, targeted sequencing (research-use-only) TMB assays for the possibility of offering in the Medgenome labs.

Methods Two assays, Oncomine Tumor Mutation Load Assay (or Oncomine) by Thermo Fisher Scientific and QIAseq Tumor Mutational Burden Panel (or QIAseq) by Qiagen, were studied. One negative control (NA12878), five positive control (A549, lung; T47D, breast; SKMEL2, skin; HCT-15, large intestine; HCT116, large intestine) cell lines, and 18 FFPE (13 colon, 1 lung, 1 testicular, and 1 oral cancer; 2 healthy) samples were ran on both assays. Sample QC was performed through measuring DNA fragmentation on TapeStation and concentration on Qubit. Failure rates on FFPE samples were investigated. TMB values by both assays were compared on all samples, as well as with expected TMB on cell line samples. Expected TMB on the negative control was considered zero; expected TMB for positive cell lines was calculated by restricting somatic mutations (from cBioPortal.com) to each panel, normalizing by panel size, and averaging. TMB values of 3 samples with known MSI were evaluated and signature patterns of relatively high TMB samples were studied.

Results On cell line samples, high correlation (r² = 0.9994) was observed between TMB values by both assays. TMB values were consistently zero on negative control by both assays. Both assays estimated lower than expected TMB on positive control samples. 6/18 FFPE samples failed on both assays, with Oncomine’s error mode was high deamination (i.e., number of C:G>T:A mutations at low allelic frequency) and QIAseq’s was low confidence (i.e., < 0.9 Mb sequenced panel). All 6 failed samples showed either low DNA integrity (DIN<2) or low concentration (<6 ng/µl). A combined analysis of all QC pass samples showed high correlation (r² = 0.97) between two assays. TMB values on two MSI cell lines was > 50 by both assays, but 14 by QIAseq and 33 by Oncomine on one MSI FFPE sample. Four out of five FFPE samples with > 25 TMB by both assays displayed MSI signature patterns from COSMIC or incorporated a pathogenic mutation in MLH1 gene.

Conclusions Preliminary analyses showed comparative accuracy and failure rates on FFPE samples. Future analyses will aim at comparison with WES based TMB on reference cell line and FFPE material.
Background Recent advancements in immunotherapy are revo-
lutionizing the landscape of clinical immuno-oncology and have significantly increased patient survival in a range of can-
cers. Notably, immune checkpoint blockade therapies have
induced durable responses and provided tremendous clinical
benefits to previously untreated patients. However, unmask-
ing immune system against cancer also disrupts the immuno-
logic homeostasis and induce inflammatory responses, resulting
immuno-related adverse events. The precise mechanisms under-
lying immune-related adverse events (irAEs) remain unknown.
Furthermore, it is unclear why immune checkpoint blockade
therapies only induce irAEs in some patients but not the others.
In this study, we systematically characterize the func-
tional impacts of immune checkpoint blockade on the patient
immune system at single-cell resolution.

Methods The peripheral blood mononuclear cells (PBMCs)
from seven cancer patients with melanoma, non-small cell
lung cancer, or colon cancer (MSI-H) receiving immune
checkpoint inhibitors (CPIs), i.e. anti-PD-1+anti-CTLA4
combo or anti-PD-1 single agent, were collected at three
serial time points (T1, T2, and T3). During the immunother-
apy, four patients developed irAEs, including colitis (2X),
pneumonitis (1), hyper/hypothyroidism (1), while three
patients showed no signs of irAEs. In total, we generated
and characterized single cell gene expression profiles for
more than 65,000 cells from 21 PBMC libraries. Further-
more, we simultaneously measured TCR and BCR from nine
selected samples, thus generating a comprehensive profile of
Immune repertoire upon CPIs.

Results We systematically characterized T cells, B cells, mono-
cytes, NK cells, and platelets from PBMCs. Both checkpoint
blockade and patient comorbidity affect PBMC populations.
We found that irAEs are often associated with an acute
increase in monocytes and decrease in T cells. After repeated
CPI treatment, PBMC populations remained relatively stable.
We characterized specific subsets within each cell type that are
associated with CPI treatment as well as patient clinical condi-
tions, and identified signature genes for each subset. For ex-
ample, Mucosal-Associated Invariant CD8 T cells were
strongly enriched in the PBMC population of the colon cancer
patient. In the melanoma patient who received anti-PD-1+
+anti-CTLA4 combo but didn’t develop colitis, we found
enriched NK cell subsets expressing chemokine such as XCL1
and CCL4. Furthermore, we found prominent T cell clonal
expansion in this patient compared to the two melanoma
patients who developed colitis. The administration of steroids
after irAEs led to massive anti-inflammatory responses in
PBMCs, often characterized by the prominent expression of
AREG.

Conclusions Our study characterized the functional impact of
CPIs on patient PBMCs. Our data demonstrated that single
cell RNA sequencing provides a powerful tool to dissect and
identify clinically actionable biomarkers for response prediction
and side effects alleviation in patients receiving immunother-
apy in the era of precision medicine.

Ethics Approval This study was approved by the Institutional
Review Board (#1050678) at Intermountain Healthcare (Salt
Lake City, UT USA)

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Background Cancer testis antigens (CTAs) are tumor antigens that have a highly tissue-restricted expression but are often expressed in diverse malignancies. With their highly immunogenetic expression limited to tumor cells, CTAs have become a prime target for cancer vaccinations and T-cell-based therapy with chimeric T-cell receptors. In this study, we investigated the landscape of 17 CTA (NY-ESO-1, LAGE-1A, and 15 other CTAs) in the context of the tumor immune microenvironment of real-world clinical tumors spanning multiple histologies.

Methods RNA-seq was performed on 5450 FFPE tumors, and the expression of each of the 17 CTAs were classified as Positive (nRPM≥20) or Negative (nRPM<20). Pearson correlation analysis was conducted on the nRPM values for each CTA to determine co-expression relationships between any of the 17 CTAs. In order to visualize patterns in the CTA expression landscape, heatmap analysis was performed, using hierarchical clustering with Pearson’s correlation as a distance measure to reveal patterns in CTA status across all samples and CTAs.

Results 5450 tumor samples analyzed in this study spanned 39 histologic types of tumor and were predominantly composed of lung cancer (40.4%) followed by colorectal cancer (10.6%) and breast cancer (8.6%). Positive CTA prevalence ranged from 2.4% (GAGE13) to 31.5% (XAGE1B). A high degree of significant correlation between the expression of all CTAs was observed, with only GAGE10, XAGE1B, MLANA, MAGEA4, GAGE13, and SSX2 having a non-significant correlation with at least one other CTA. Three key groups of co-expressed CTAs were observed: 1) NY-ESO-1, LAGE-1A, MAGEA12, MAGEA3, MAGEA1, MAGEA10, and MAGEA4 (0.36≤R≤0.82); 2) GAGE12J, GAGE2, GAGE13, GAGE13, and SSX2, BAGE, MAGEC2 (0.4≤R≤0.58). The three remaining CTAs (GAGE10, XAGE1B, and MLANA) had little or no (R<0.22) correlation with any other CTA or each other. Clustering CTAs across all samples revealed three CTA expression clusters: 1) samples that express a collection of multiple CTAs; 2) samples that express mostly XAGE1B, over-represented by lung cancer (p=1.51e-296); 3) samples that express mostly GAGE10, over-represented by neuroendocrine tumors (p=1.64e-05).

Conclusions Across multiple cancer subtypes, the expression of a CTA occurs in the context of other CTAs, and specific groups of CTAs are likely to co-express, forming expression patterns characteristic to tumor subgroups. These findings provide a scientific base for selecting appropriate CTAs and designing multiplex vaccination in immunotherapies of a variety of tumors. Further studies are needed to understand the relationship of these CTAs with traditional and emerging immune-oncology biomarkers.

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66  COMPLEX MARKERS OF SURVIVAL FROM PEMBROLIZUMAB: THE POTENTIAL PREDICTIVE ROLE OF TUMOR MUTATIONAL BURDEN (TMB) AND KRAS

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Background Pembrolizumab, with or without chemotherapy, is NCCN guideline-recommended treatment for NSCLC cancer patients depending on tumor PD-L1 status by IHC. PD-L1 IHC provides guidance for treatment selection for response, but does not accurately predict survival benefit from pembrolizumab. Emerging evidence suggests TMB and other genomic markers (KRAS, STK11, TP53 mutations), may have clinical utility for predicting survival benefit.

Methods We identified a cohort of metastatic EGFR/ALK wild type NSCLC patients (n=116) whose tumors underwent comprehensive profiling (June 2017-March 2019) for genomic variants, TMB and PD-L1 IHC prior to selection of pembrolizumab (n=43), pembrolizumab + chemotherapy (n=41), or chemotherapy only (excluding subsequent targeted therapy or immunotherapy) (n=32) at Roswell Park Comprehensive Cancer Center, with at least one year of follow up. TMB was assessed using a 1.75 Mb capture of 409 oncogenes with full exon coverage (DNA-Seq), with 'high' TMB interpreted as ≥10 mutations/Mb. Electronic pharmacy records were curated to create pre and post-test treatment histories for each patient. Cox regression analysis evaluated OS with pembrolizumab monotherapy or pembrolizumab + chemotherapy vs chemotherapy only, adjusting for covariates including oncogenic driver mutations, TMB, PD-L1 IHC demographics, clinicopathologic characteristics, prior treatment, and performance status. Using the same model, we then assessed overall survival for each treatment group by TMB, KRAS, STK11, and TP53 mutant status.

Results Overall, 47% of tumors were PD-L1 high, 47% TMB high, 34% KRAS mutant (codons 12, 13, 60, 61), 52% TP53 mutant and 16% STK11 mutant. As expected, pembrolizumab with or without chemotherapy significantly improved overall survival (OS) compared to chemotherapy alone; with TMB, smoking, and ECOG status identified as significant covariates. PD-L1 IHC status was not associated with OS for any treatment. TMB high status was significant for OS benefit with pembrolizumab either as monotherapy [HR=0.02; CI=0.01–0.40; p=0.01] or in combination with chemotherapy [HR=0.20; CI=0.04–0.95; p=0.04]. KRAS mutant status was independently significant for OS benefit from pembrolizumab + chemotherapy [HR=0.01; CI=0.01–0.79; p=0.04] but not for pembrolizumab monotherapy or chemotherapy alone. Among patients who received pembrolizumab monotherapy, there was a trend toward increased risk of death in those with STK11 mutations [HR=1.75; CI=0.35–1.00; p=0.15], whereas TP53 mutant status trended toward survival benefit [HR=0.18; CI=0.02–1.53; p=0.11].

Conclusions Data comparing pembrolizumab treatments with chemotherapy and independent marker associations suggest TMB has predictive power for determining overall survival benefit from pembrolizumab, while KRAS, STK11, and TP53 mutational status demonstrated potential prognostic relevance for NSCLC.

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67  B-CELL RECEPTOR HEAVY CHAIN REPertoire PROFILING USING AN AUGMENTed TRANSCRIPTOME


Background Comprehensive profiling of the tumor and tumor microenvironment (TME) is a critical tool for furthering our understanding of tumor progression and response to treatment, including immunotherapies. To address this challenge, we developed an augmented, immuno-oncology-optimized exome-transcriptome platform, ImmunoID NeXTTM, which provides a more comprehensive view of the tumor and TME from limited FFPE tumor biopsies. We have recently added the ability to profile the B-cell receptor (BCR) heavy chain. Here, we show that ImmunoID NeXT is now able to accurately and reproducibly profile abundant B-cell clones and provide information on the diversity of B-cells in tumor samples.

Methods We analyzed multiple replicates of PBMCs to examine the reproducibility of BCR sequence identification using ImmunoID NeXT. Utilizing a standalone BCR sequencing approach, we further evaluated the concordance of top clones to those identified by ImmunoID NeXT. In addition, we analyzed the reproducibility of BCR sequences in patient-derived FFPE samples. Finally, we used ImmunoID NeXT to profile the B-cell clonal diversity across over 500 solid tumor samples.

Results Reproducibility in PBMC samples was very high, with abundances of clones shared between replicates being very concordant (R²>0.92, R²>0.86, and R²>0.97 for IgG, IgM, and IgA, respectively). When comparing to a standalone BCR sequencing method that profiles IgM and IgG, we observed highly concordant abundances (R²>0.72 and R²>0.82 in IgM and IgG, respectively), as well as strong overlaps of top clones. When comparing subsequent curls of a tumor FFPE sample, we also achieved a high concordance of clonal abundances (R²>0.92, R²>0.93, and R²>0.76 for IgG, IgM, and IgA, respectively). Finally, we observed differences in clonal diversity of B-cell repertoires across over 500 solid tumor samples.

Conclusions We demonstrate that ImmunoID NeXT can be used to reproducibly, sensitively, and accurately profile high-abundance BCR heavy chain clones, including coverage of all major isotypes. In addition, we show how ImmunoID NeXT can profile the diversity of the BCR repertoire across a variety of tumor samples. Combined with the platform’s TCR profiling capabilities, ImmunoID NeXT can provide insight into the diversity of the immune repertoire, contributing to its ability to provide comprehensive analysis of both the tumor and TME from a single FFPE sample.

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68  THE PROGNOSTIC AND PREDICTIVE IMPLICATIONS OF THE 12-CHEMOKINE SCORE IN MUSCLE INVASIVE BLADDER CANCER

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Background Emerging evidence from studies in sarcoma and melanoma immune checkpoint blockade (ICB) trials...
demonstrated enhanced efficacy in tumors harbouring tertiary lymphoid structures (TLS)\(^1\)-\(^3\) - lymph node like aggregates in the tumor microenvironment postulated to recruit lymphocyte infiltration and coordinate tumor antigen presentation and lymphocyte priming. Previously, our group described a 12-chemokine metagene signature that reflects strong intratumoral chemotactic signalling and accurately predicts the presence of TLS in colorectal carcinoma.\(^4\) Grounded in these works, we attempted to correlate high 12CK signature with TLS formation in the context of muscle invasive bladder cancer (MIBC), and then sought to define its prognostic implications and its ability to predict response to ICB.

**Methods** A total of 130 MIBC samples were arrayed on Affymetrix microarrays and 12CK scores were assessed. Scores were normalized using 12CK\(>1\) to denote 12CK-High\((n=24)\). We then investigated the presence of TLS with the associated immune cellular infiltration evaluated by immunohistochemistry and gene signature deconvolution method (\(x\)Cell).\(^5\) 12CK scores were also correlated with survival in our institutional cohort and validated using data from TCGA. Finally, 12CK scores were extracted from the IMVIGOR210 study to examine its ability to predict response to ICB.\(^6\)

**Results** Type III TLS, consisting of germinal center-like structures and discrete T-cell zones were found in 7/22 12CK-High vs. 0/21 12CK-Low tumors \((p=0.009)\) (figure 1a). Additionally, a more robust immuno-environment was seen in 12CK-High tumors, consisting of increased infiltration of CD4\(^+\) T lymphocytes \((p=0.1)\), CD8\(^+\) T lymphocytes \((p=0.02)\), activated dendritic cells \((p=0.047)\), and B lymphocytes \((p=0.006)\) on immunohistochemistry (figure 1b). Furthermore, on \(x\)Cell deconvolution, M1 macrophage, NK cells, CD8\(^+\) Tem, CD4\(^+\) Tem, and memory B cells were enriched in 12CK-High tumors, suggesting both a heightened innate and adaptive immune response (figure 1c, d). Kaplan-Meier survival analyses of our internal cohort revealed improved PFS (HR 0.25, \(p=0.003\)), CSS (HR 0.25, \(p=0.003\)), and OS (HR 0.55, \(p=0.03\)) amongst 12CK-High patients (figure 2a-c). From the TCGA, similar improvements were found in PFS (HR 0.55, \(p=0.007\)), CSS (HR 0.40, \(p=0.002\)), and OS (HR 0.59, \(p=0.01\)) in 12CK-High patients (figure 2d-f). From the IMVIGOR 210 study, complete responders exhibited significantly higher 12-CK scores than all other groups (figure 2g). Strikingly, the 12CK-High signature conferred a median overall survival benefit of almost 1 year in the atezolizumab-treated patients (figure 2h).

**Conclusions** In muscle invasive bladder cancer, 12CK-High scores corresponded with formation of TLS in the TME; favourable prognosis in surgically treated MIBC patients; and CR in atezolizumab-treated patients.

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### Abstract 68 Figure 1
High 12-chemokine score (12-CK) is associated with the presence of tertiary lymphoid structures (TLS) in the tumor microenvironment (TME) and a more robust peritumoral inflammatory response. (A) H&E and immunohistochemistry (IHC) stains were performed against CD4, CD8, CD20, and LAMP3 to define populations of CD4\(^+\) T lymphocytes, CD8\(^+\) T lymphocytes, CD20\(^+\) B lymphocytes, and activated dendritic cells in the TME, respectively. Type III tertiary lymphoid structures, consisting of prominent B-cell follicles with germinal center-like structures and discrete T-cell zones, were found within tumors as well as at the tumor invasive front. (B) Higher densities of CD4\(^+\) T lymphocytes \((p=0.1)\), CD8\(^+\) T lymphocytes \((p=0.02)\), B lymphocytes \((p=0.008)\), and activated dendritic cells \((p=0.047)\) were found in the 12CK-High TME. (C) Findings using the deconvolution tool \(x\)Cell demonstrated heightened signatures related to immune cells found within the (C) adaptive and (D) innate immune responses. 12-CK=12-Chemokine score; TLS= Tertiary lymphoid structure; GC = germinal center; * \(p<0.05\); ** \(p<0.01\); *** \(p<0.001\); **** \(p<0.0001\).

### Abstract 68 Figure 2
The prognostic and predictive implications of 12-CK score. Kaplan-Meier survival analyses revealed improved overall survival (OS; HR 0.55, \(p=0.03\)) (A), disease-specific survival (DSS; HR 0.25, \(p=0.003\)) (B), and progression-free survival (PFS; HR 0.25, \(p=0.003\)) in 12CK-High muscle invasive bladder cancer patients treated with radical cystectomy at Moffitt Cancer Center. The favorable prognosis in OS (HR 0.59, \(p=0.010\)) (D), DSS (HR 0.40, \(p=0.002\)) (E), and PFS (HR 0.55, \(p=0.007\)) (F) were confirmed in patients from TCGA. (G) From the IMVIGOR-210 study testing the efficacy of atezolizumab on immunohistochemistry (figure 1b). Furthermore, on \(x\)Cell deconvolution, M1 macrophage, NK cells, CD8\(^+\) Tem, CD4\(^+\) Tem, and memory B cells were enriched in 12CK-High tumors, suggesting both a heightened innate and adaptive immune response (figure 1c, d). Kaplan-Meier survival analyses of our internal cohort revealed improved PFS (HR 0.25, \(p=0.003\)), CSS (HR 0.25, \(p=0.003\)), and OS (HR 0.55, \(p=0.03\)) amongst 12CK-High patients (figure 2a-c). From the TCGA, similar improvements were found in PFS (HR 0.55, \(p=0.007\)), CSS (HR 0.40, \(p=0.002\)), and OS (HR 0.59, \(p=0.01\)) in 12CK-High patients (figure 2d-f). From the IMVIGOR 210 study, complete responders exhibited significantly higher 12-CK scores than all other groups (figure 2g). Strikingly, the 12CK-High signature conferred a median overall survival benefit of almost 1 year in the atezolizumab-treated patients (figure 2h).

**Conclusions** In muscle invasive bladder cancer, 12CK-High scores corresponded with formation of TLS in the TME; favourable prognosis in surgically treated MIBC patients; and CR in atezolizumab-treated patients.

**REFERENCES**

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0068
Background Identifying potential predictive biomarkers for immune related adverse events (irAEs) following checkpoint blockade inhibition (CPI) remains an outstanding goal of immune-oncology translational research. Polymorphism with the T cell receptor variable gene (TRBV) has been proposed as a potential risk factor for irAEs owing to a potential link between TRBV polymorphism and chronic autoimmune disease. Efforts to interrogate the potential biomarker utility of TRBV polymorphism have been hampered by the repetitive nature of the TRB locus. Our research has demonstrated a method for inferring TRB locus haplotypes from long-amplicon TCRB chain sequencing data, which we used to identify major haplotype groups in from nucleic acid. Here we present our research for a potential automated method for haplotype group assignment from TCRB chain sequencing data.

Methods Rearranged TCRB chains from 10 blood samples were amplified and sequenced from 25ng peripheral blood total RNA via the Oncomine™ TCRB-LR assay using the Genexus™ Integrated Sequencer. 12 samples were run per chip with 4 samples run in each lane. TCRB clonotyping and repertoire feature analysis was performed using Genexus™ analysis software. Automated haplotype group assignment was performed by generation and comparison of TRBV allele profiles to those presented previously. For context, TCR evenness, convergence, and haplotype group assignment were compared to values obtained following analysis of the same samples via the GeneStudio™ S5 platform and Ion Reporter™ 5.12 software.

Results TCR Evenness and convergence values were highly correlated across replicates run on the Genexus™ Integrated Sequencer (Spearman correlation >0.95 and >0.70, respectively). Evenness at equivalent clone count and convergence at equivalent sequencing depth were not significantly different across platforms (Spearman correlation >0.88). Haplotype group assignments demonstrated 100% agreement across replicates on both platforms.

Conclusions Our research has demonstrated a potential automated and reproducible method for TRB haplotype group assignment via the Oncomine™ TCR-Beta LR Assay, GX run on the Genexus™ Integrated Sequencer. Future studies will be needed to evaluate the potential biomarker utility of TRB haplotype for the prediction of irAEs. For research use only not for diagnostic procedures.

REFERENCE

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WHOLE EXOME SEQUENCING OF INDIVIDUALS PRESENTING EXTREME PHENOTYPES OF HIGH AND LOW-RISK OF DEVELOPING TOBACCO-INDUCED LUNG ADENOCARCINOMA: RELEVANCE OF IMMUNE AND DNA-REPAIR RELATED PATHWAYS

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Background Individual susceptibility to carcinogens may depend on genetic background. We performed for the first-time Whole Exome Sequencing (WES) of germline DNA from individuals presenting phenotypes of extreme sensitivity and resistance to developing tobacco-induced lung adenocarcinoma, in order to characterize the genetic background associated with these relevant phenotypes.

Methods We performed WES of germline DNA from heavy smokers (≥15 pack-years) who either developed lung adenocarcinoma at an early age (≤55 years, extreme cases, n=50) or did not present lung adenocarcinoma or other tumors at an advanced age (≥72 years, extreme controls, n=50). We selected non-synonymous variants (missense and non-sense) located in the coding regions and consensus splice sites of the genes showing significantly different allelic frequencies between both cohorts. We validated our results in germline data from 52 additional extreme cases selected from TCGA using the same criteria (diagnosis of lung adenocarcinoma at ≤55 years, tobacco consumption ≥15 pack-years).

Results The mean age for the extreme cases and controls was respectively 49.7 and 77.5 years. Mean tobacco consumption was 43.5 and 54.4 pack-years. We identified 619 significantly different variants between both cohorts, and we validated 107 of these in 52 extreme cases selected from TCGA (mean age 49.3 years, mean tobacco consumption 37 pack-years). Nine validated variants, located in relevant cancer related genes, such as PARP4 (DNA repair), HLA-A (antigen presentation) or NQO1 (detoxification) among others, achieved statistical significance in the False Discovery Rate test (FDR) (table 1). The most significant validated variant (p=4.48 × 10−5) was located in the tumor-suppressor gene ALPK2. The Reactome Pathway Database analysis showed that the genes harboring the most significant validated variants were significantly related to antigen processing and presentation, interferon and cytokine signaling and immune regulation, also achieving statistical significance in the FDR test (table 2).

Conclusions We describe for the first time genetic variants associated with extreme phenotypes of high and low-risk for the development of tobacco-induced lung adenocarcinoma, assessed with WES. The most significant validated variants were related with antigen presentation, immune regulation and DNA repair. Our results and our strategy warrant further development to characterize these clinically relevant phenotypes.

Ethics Approval The study was approved by the Investigational Review Board of Clinica Universidad de Navarra, approval number 021/2009.

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Routine Use of Comprehensive Genomic Profiling to Assess Tumor Mutational Burden Across a Community Health System

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Background Tumor mutational burden (TMB), defined as the average number of somatic mutations per megabase (mut/Mb) of DNA in tumor cells, has emerged as a predictive biomarker for response to immune checkpoint inhibitor (ICI) therapy. With more widespread adoption of comprehensive genomic profiling (CGP) assays in the clinic, it is now possible to routinely assess TMB across a wide variety of advanced cancers. Here we performed a retrospective study of routine TMB results assessed from CGP testing across a large community health system to reveal novel insights into the proportion of patients that may benefit from ICI treatment.

Methods Patients in the Providence St. Joseph Healthcare system diagnosed with advanced or metastatic solid tumors and tested for TMB using CGP tests (TruSight Oncology 500, research use only) between July 2019 and July 2020 were considered in this study. Deidentified electronic medical record data and CGP results were abstracted for downstream study.

Results A total of 1300 patients had one or more CGP tests with a TMB calculation. The median age of patients was 66 years, 51% were female, and 59% were white. TMB values ranged from 0–536 mutations per mut/Mb. Across tumor types, the proportion of patients with TMB ≥10 mut/Mb was 26% (n=341) and with TMB 5–9 mut/Mb was 27% (n=353). The proportion of patients with TMB ≥10 mut/Mb varied by tumor type: Melanoma (60%), NSCLC (42%), CRC (24%), pancreatic (5%). Of all the TMB-tested patients, 90 (7%) received IO therapy post testing. IO therapy use was highest among patients with TMB ≥10 mut/Mb (12%), followed by 7% with TMB of 5–9 mut/Mb, and 4% with TMB of 0–5 mut/Mb. Twenty-nine percent of TMB ≥10 also had high PD-L1 expression by IHC as compared to 8% of TMB <10. ICI therapy choice in this retrospective cohort appeared to be largely driven by other considerations (PD-L1 immunohistochemistry etc.) independent of TMB.

Conclusions A minority of TMB ≥10 patients assessed in this study received an ICI therapy, a result that is likely reflective of the lack of definitive guidelines for this emerging biomarker. As the adoption of TMB increases as a biomarker of...
immunotherapy response, there is a greater need to expedite the standardization of sample collection, processing, and bioinformatics in TMB assessment.

Ethics Approval This study was approved by the Providence St. Joseph Health Institutional Review Board, approval number STUDY2019000048.

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73 ORTHOGONALLY AND FUNCTIONALLY VALIDATED ALGORITHM FOR DETECTING HLA LOSS OF HETEROZYGOSITY


Background Human leukocyte antigen (HLA) genes facilitate communication between tumor cells and the immune system through the cell surface presentation of a diverse set of peptides. HLA loss of heterozygosity (LOH) has been associated with reduced immune pressure on neoantigens and impaired response to checkpoint blockade immunotherapy. Although HLA LOH is emerging as a key biomarker for response to immunotherapy, few tools exist to detect HLA LOH. Moreover, the accuracy of these tools is not well understood due to lack of orthogonal validation approaches. Here, we briefly describe DASH (Deletion of Allele-Specific HLAAs), an algorithm to detect HLA LOH from exome sequencing data, and present a three-pronged validation approach to assess its performance.

Methods In-silico evaluation of the limit of detection (LOD) of DASH was performed by deeply sequencing a tumor-normal paired cell line with HLA LOH and mixing reads at different proportions to simulate variable tumor purity and clonality. Direct genomic validation was performed using digital PCR (dPCR) with allele-specific primers targeting both predicted kept and lost alleles in ten patient samples and one cell line. Quantitative immunopeptidomics was performed to compare peptides presented by HLA alleles in tumor cells and adjacent normal cells. The relative increase or decrease of peptide presentation per allele was estimated by predicting the binding of each peptide to the patient-specific alleles.

Results DASH is a machine learning model built upon the HLA-enhanced ImmunoID NeXT Platform®. We validated the performance of DASH using three orthogonal approaches to better understand the factors driving sensitivity and specificity of the algorithm. Evaluation using cell line mixtures that simulate LOH at various dilutions helped establish the LOD of DASH. For fully clonal tumors, DASH showed 100% sensitivity at all tumor purity levels above 8% and 100% specificity at tumor purity levels higher than 24%. Patient-specific and allele-specific dPCR assays provided sensitive, direct evidence of HLA LOH. All samples predicted to have HLA LOH by DASH with high confidence were confirmed by dPCR. Finally, a quantitative immunopeptidomics experiment in one patient with HLA LOH revealed a large decrease in the peptides presented by deleted alleles, revealing the functional implications of HLA LOH.

Conclusions HLA LOH detection methods need to be rigorously validated in order to be used as a clinical biomarker. Here, we introduced three methods to assess performance, demonstrated the strong predictive power of DASH, and highlighted the need to consider tumor purity in such assessments.

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74 NOVEL APPROACH FOR PROFILING IMMUNE-TUMOR CELL INTERACTIONS AND MUTATIONS IN THE SAME TUMOR SECTION BY MULTIPLE IMMUNOHISTOCHEMISTRY AND NGS IN IMMUNO-Oncology TRIALS

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Background Both proteins (e.g., PD-L1 IHC) and tumor mutation burden (NGS-based) are known to independently predict clinical response to anti-PD-1/PD-L1 therapies. In a meta-analysis of tumor specimens from 8,135 patients treated with PD-1/PD-L1 blockers, multiplex fluorescence immunohistochemistry (mFIHC) had significantly higher diagnostic accuracy than PD-L1 IHC, tumor mutational burden (TMB), or gene expression profiling alone in predicting clinical response1 or equivalent to a multimodality approach (e.g., PD-L1 IHC + TMB). While the benefits of combining mFIHC (tumor-immune interplay) and NGS approaches in selection of patients for next generation immunotherapies is appealing, tumor tissue is a key limiting factor for multimodality analyses in clinical trials. To address this critical limitation, we developed a novel approach for sequential profiling of tumor and immune cell interactions by 7-parameter mFIHC assays, followed by analyses of nucleic acid extracted from same tissue sections.

Methods Formalin-fixed paraffin-embedded (FFPE) tumor tissue and cell line blocks were sectioned, and then stained using mFIHC followed by isolation of nucleic acids, or direct isolation of total nucleic acids. NanoString, qPCR, and NGS were performed on isolated nucleic acids. Nucleic acid quality, transcript abundance, and TMB scores were compared before and after mFIHC staining.

Results mFIHC revealed a broad range of immune cell phenotypes and spatial interactions, including T cells, B cells, NK cells, monocytes, neutrophils, and their functional status. Isolation of testable quantities of DNA from mFIHC treated slides was achieved when using a DNA-only isolation method, and TMB scores were robust across tested conditions. Cell phenotypes identified by mFIHC were compared to TMB scores across the tested samples. Following mFIHC treatment, RNA yields were reduced relative to the non-mFIHC treated replicates, but still sufficient for optimal input into a 770-target NanoString gene expression panel. However, for mFIHC treated samples, transcript levels were not distinguishable from background for the assessed targets.

Conclusions In summary, integrating mFIHC testing and TMB analysis on the same samples allows for comprehensive biomarker evaluation. The real world benefits of the combined approach will be described in upcoming clinical trials.

REFERENCE

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Background Multiple genomics-based biomarkers of response to immune checkpoint inhibition have been reported or proposed, including tumor mutation/neoantigen frequency, PD-L1 expression, T cell receptor repertoire clonality, interferon gene expression, HLA expression, and others. Although genomics associations of response have been reported, the primary studies have used a variety of data generation and processing techniques. There is a need for data harmonization and assessment of generalizability of potential biomarkers across multiple datasets.

Methods We acquired patient-level RNA sequencing FASTQ data files from 10 data sets reported in seven pan-cancer PD-1 and CTLA-4 immune checkpoint inhibition trials with matched clinical annotations. We applied a common bioinformatics workflow for quality control, mapping to reference (STAR), generating gene expression matrices (SALMON), T cell receptor repertoire inference (MiXCR), extraction of immune gene signatures and immune subtypes, and differential gene expression analysis (DESeq2). We analyzed i) immunogenomics features proposed as biomarkers, and ii) gene expression signatures built from each trial for association with overall survival across the set of trials using univariable Cox proportional hazards regression. In all, we assessed 9 total immunogenomics features/signatures. P-values were adjusted for multiple testing using the Benjamini-Hochberg method.

Results Of the 9 immunogenomics features assessed, cytolytic activity score and expression of the Follicular Dendritic Cell Secreted Protein gene (FDCSP) were associated with survival in two of seven studies, respectively (adjusted p < 0.05) (figure 1). No proposed biomarkers were significantly associated with survival in more than two studies. The sets of genes significantly associated with clinical benefit across the studies were highly disjoint, with only three genes significant in three studies and thirteen genes significant in two studies (figure 2). No genes were significantly associated with clinical benefit in more than three of seven studies.

Conclusions No proposed biomarkers were highly generalizable across studies. We expect that integrated modeling incorporating multiple immunogenomics features will be required to build a robust and generalizable biomarker for ICI response. Further work is needed to analyze determinants of response and clinical benefit.

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REFERENCES
Background Tislelimub, an anti-PD-1 monoclonal antibody, has demonstrated clinical benefit for patients with NSCLC. The underlying response and resistance mechanisms to tislelimub treatment remain unknown.

Methods Baseline tumor samples from 59 nonsquamous (NSQ) and 41 squamous (SQ) NSCLC patients treated with tislelimub monotherapy (NCT02407990 and NCT04068519) were tested for gene mutations using large panel next generation sequencing and RNA expression using gene expression profiling (GEP). Precision Immuno-Oncology Panel, HTG Molecular

Diagnoses). GEP analyses of NSQ and SQ NSCLC were performed separately due to different gene expression patterns. Results The ORR, mPFS, and mOS in this pooled NSCLC cohort were 15.2% (95% CI: 9.0, 23.6), 4.1 months (95% CI: 2.20, 6.11), and 15.1 months (95% CI: 11.20, NE), respectively, with a median study follow-up of 15.3 months (95% CI: 14.06, 15.90). Non-responders (NRs) exhibited distinct tumor and immune gene signature profiles and could be clustered into two subgroups: NR1 and NR2. Compared with responders, NR1 had elevated cell cycle signatures in both NSQ (P=0.2) and SQ (P=0.03) cohorts, and a trend of decreased inflamed gene signature profiles. However, NR2 showed comparable or even higher tumor inflammation (18-gene), and CD8+ T-cell signature scores in both NSQ and SQ cohorts and could be classified as immune hot. To explore the resistance mechanisms of immune hot NRs, differentially expressed gene analyses between immune hot NR2 and responders were performed. M2 macrophage and Treg signature scores were higher in NR2 in both NSQ (M2, P=0.05; Treg, P=0.03) and SQ (M2, P=0.05 [subgroup of NR2]; Treg, P=0.03) cohorts; significantly higher expression of immune regulatory genes included PIK3CD, CCR2, CD244, IRAK3, and MAP4K1 (P<0.05) in NSQ and PIK3CD, CCR2, CD40, CD163, and MMP12 (P<0.05) in SQ. Significantly higher epithelial–mesenchymal transition (EMT) and angiogenesis gene expression, including SNAI1, FAP, VEGFC, and TEK (P<0.05) genes, were also observed in SQ NR2. Moreover, gene mutation analysis identified seven immune hot NR patients harboring either driver mutations (RET fusion, ROS1 fusion, BRAF, and PIK3CA amp) or well-established resistance mutations (loss of function mutation in EGFR, ALK, and PTEN). Conclusions Despite the presence of immune hot features, a subgroup of tislelimub NRs with NSCLC were identified. High levels of immune suppressive factors, such as M2 macrophage and Treg signatures, angiogenesis, and EMT genes, as well as the existence of driver/resistance mutations, may indicate mechanisms of resistance of immune hot NRs, highlighting potential novel treatment targets.

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Background POTENTIAL MECHANISMS OF RESISTANCE IDENTIFIED THROUGH ANALYSIS OF MULTIPLE BIOMARKERS IN IMMUNE HOT NON-RESPONDERS WITH NON-SMALL CELL LUNG CANCER (NSCLC) TREATED WITH TISLELIMUB

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Abstracts
sorafenib treatment. Tislelizumab, an anti-PD-1 monoclonal antibody, has demonstrated single-agent antitumor activity in patients with advanced, previously treated HCC in two early phase studies (NCT02407990, NCT04068519). Association of biomarkers, including PD-L1 expression and gene expression profiles (GEP), with response and resistance to tislelizumab were explored.

Methods PD-L1 expression was evaluated on tumor cells (TC) using the VENTANA PD-L1 (SP263) assay in baseline tumor samples collected before or after sorafenib treatment. GEP were assessed using the 1392-gene HTG GEP EdgeSeq panel. Signature scores were calculated using the Gene Set Variation Analysis package with publicly available gene signatures (GS). Wilcoxon rank-sum test was used to analyze differential gene signatures (DEGs); GS association with PFS and OS was evaluated using Cox proportional hazards models.

Results Single-agent tislelizumab demonstrated antitumor activity in advanced, previously treated HCC (ORR=13%; CB [PR + SD > 6 months]=31%, median PFS=3.3 months; median OS=13.3 months). PD-L1+ (TC≥1%) prevalence and GEP showed different patterns in samples collected before and after sorafenib exposure (figure 1). While non-exposed samples (n=16) were enriched for immune suppressive signatures, sorafenib-exposed samples (n=41) showed higher PD-L1+ prevalence (53.7% vs 23%; P=0.08) and immune-cell activation signatures along with co-inhibition molecules. In sorafenib-exposed samples, PD-L1 expression was positively correlated with CB (P=0.0027) and a trend of longer PFS (HR=0.56, 95% CI:0.17–1.13). ORR was higher in PD-L1+ than PD-L1− sorafenib-exposed samples (23.8% vs 0%; P=0.049). DEG analysis in sorafenib-exposed samples demonstrated that NK-mediated cytotoxicity GS was positively correlated with CB (P=0.03), as well as a trend of longer PFS (HR=0.43, 95% CI:0.17–1.06). Across the different analyses, no correlation with OS was observed. Patients considered non-responders (NRs) were found clustered into three distinct GEP subgroups (NR1, NR2, NR3). Compared with responders, NR1 had enhanced angiogenesis signatures (P=0.01), including TEK, KDR, HGF, and EGR1. Despite high inflamed tumor signatures, NR2 had increased expression of T-cell inhibition GS scores (P=0.01), including CD274, CTLA4, TIGIT, and CD96. The NR3 subgroup showed higher cell-cycle GS levels compared with responders (P=0.05), including E2F7, FOXA1, and FANCDC2.

Conclusions Prior sorafenib exposure appears to be associated with increased PD-L1 expression and tumor microenvironment-related GS, as well as response and PFS from tislelizumab in advanced HCC patients. Elevated angiogenesis, immune exhaustion, and cell-cycle GS levels may indicate resistance to single-agent PD-1 inhibitors and is suggestive of potential treatment strategies. Validation is warranted in future clinical trials (NCT03412773).

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Trial Registration NCT02407990, NCT04068519

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improvement in clinical efficacy (40% objective response rate [ORR], 5.26 month median progression-free survival [PFS], and 15.2 month median OS) than other subgroups (table 1).

In addition to immune-related genes in the microenvironment, DEG analysis also revealed that tumor-related genes were highly expressed in non-responders, such as intrinsic genes related to angiogenesis [VEGFA (P = 0.07), KDR (P = 0.07)], the mTOR pathway [MTOR (P = 0.015)], and DNA damage repair (REV3L (P = 0.007)]. MTOR and REV3L were associated with shorter PFS (P = 0.02; P = 0.003) and OS (P = 0.03; P = 0.008), respectively.

Conclusions By using GEP, T-cell and MHC I GS were identified as potentially predictive biomarkers of response to tislelizumab monotherapy in PD-L1+ UC in this retrospective analysis. By combining these two GS scores, patients with optimal efficacy responses could be identified. Conversely, high expression of tumor intrinsic genes related to angiogenesis and the mTOR pathway may indicate resistance and suggest potential future drug combinations for these patients. Both findings warrant further validation in a phase 3 study (NCT03967977).

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Trial Registration CTR20170071

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TUMOR-IMMUNE SIGNATURES ASSOCIATED WITH RESPONSE OR RESISTANCE TO TISLELIZUMAB (ANTI-PD-1) IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA (ESCC)

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Background Tislelizumab, an anti-PD-1 monoclonal antibody, showed promising clinical outcomes for patients with ESCC. Here, the tumor and immune microenvironment is investigated using gene expression profiles (GEP) and gene signatures associated with clinical efficacy in patients with ESCC receiving tislelizumab either as monotherapy (NCT02407990, NCT04068519) or in combination with chemotherapy (5-fluorouracil plus cisplatin; NCT04068519). Variability in the immune and tumor-related features may be considered for validation in phase 3 studies (NCT03430843, NCT03783442).

Methods Baseline tumor samples were subjected to GEP using an 1392-gene HTG EdgeSeq panel. Signature scores were calculated using the Gene Set Variation Analysis package with publically available gene signatures. Differential gene signature (DEG) analysis was performed between responders and non-responders using the Wilcoxon rank-sum test. Associations between gene signatures and survival were evaluated using the Cox proportional hazards model.

Results In GEP- evaluable patients receiving monotherapy (n=43), DEG analysis showed toll-like receptor (TLR) signature scores, driven by TLR8, TLR6, TIRAP, and TLR4, were positively correlated with response and survival, while Treg scores, driven by FOXP3, EBI3, TNFRSF18, and BATF, showed a negative correlation. After combining TLR-high and Treg-low scores

Tumor-immune signatures associated with clinical efficacy of tislelizumab in patients with ESCC

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>GEP-evaluable</th>
<th>Single TLR signature</th>
<th>Single Log signature</th>
<th>Combined signature</th>
</tr>
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<tbody>
<tr>
<td>Non-responders (NR)</td>
<td>27</td>
<td>17</td>
<td>30</td>
<td>47</td>
</tr>
<tr>
<td>Responders (R)</td>
<td>28</td>
<td>14</td>
<td>27</td>
<td>41</td>
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As defined by median cutoff, the prediction of clinical efficacy was further improved (table 1). In addition to Treg scores, non-responders (NR) to tislelizumab monotherapy could be further clustered into four subgroups (NR1, NR2, NR3, and NR4), each exhibiting distinct resistance signatures. Despite a high level of immune infiltration, NR1 expressed a higher exhaustion signature (CD96, CTLA4, TIGIT, HAVCR2, etc.) versus responders (P=0.01). Both NR2 and NR3 demonstrated a trend of enhanced cell-cycle signatures versus responders (P=0.07 and P=0.08, respectively), accompanied by a lower NK signature (KIR2DS4, KIR,panL, CD56) in NR2 and a lack of immune infiltration in NR3. In the NR4 subgroup, a trend toward higher TH17 (P<0.01) and IL-17F signatures (Log2FC=0.56, P=0.10) versus responders was observed. GEP- evaluable patients (n=12) receiving tislelizumab in combination with chemotherapy had an objective response rate of 58% (n=7), with a different gene signature pattern than those observed in patients receiving monotherapy. Responders to combination therapy showed higher DNA repair expression versus NR (P=0.07), while angiogenesis signatures were significantly higher in NR vs responders (P=0.01). Consistent with this, NR exhibited higher expression of VEGF at a single gene level (Log2FC=2.46, P<0.01).

Conclusions While higher TLR signaling was associated with clinical benefit of tislelizumab monotherapy, elevated Treg, exhaustion, cell cycle, and TH17 signatures may indicate resistance. Signatures predictive for combination therapy may vary. Both immune- and tumor-related features may be considered for validation in phase 3 studies (NCT03430843, NCT03783442).

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Trial Registration NCT2407990, NCT04068519, NCT03469557

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EVALUATION OF THE TRUSIGHT ONCOLOGY 500 ASSAY FOR ROUTINE CLINICAL TESTING OF TUMOR MUTATIONAL BURDEN (TMB) AND CLINICAL UTILITY FOR PREDICTING RESPONSE TO PEBROLIZUMAB

Bo Wei*, John Kang, Hisao Kikubawa, Gladys Areaza, Maureen Maguire, Lei Chen, Ping Qiu, Liang Liang, Deepthi Aurora-Gang, Razvan Cristescu, Diane Levitan, Merck Co, Inc, Kenilworth, NJ, USA

Background Various biomarkers have been investigated for their ability to identify patients more likely to respond to
immunotherapy. Recently, the PD-1 inhibitor pembrolizumab was approved by the FDA for treating patients with unresectable or metastatic solid tumors with high TMB (TMB-H) who have no satisfactory alternative treatment options following progression on prior treatment. The FDA contemporaneously approved the FoundationOne®CDx (F1CDx; Foundation Medicine) as the companion diagnostic for TMB assessment for pembrolizumab. However, multiple comprehensive genomic profiling panels that can measure TMB are currently available or in development. We evaluated the performance of TruSight™ Oncology 500 (TSO500; Illumina) for assessing TMB and its clinical utility using F1CDx and whole exome sequencing (WES) as reference methods.

**Methods** Pretreatment archival tumor samples from patients enrolled in 8 clinical trials of pembrolizumab monotherapy were evaluated for TMB by TSO500, F1CDx QSR pipeline v3.2.0, and WES. Correlation was assessed using Spearman’s rank correlation coefficient (ρ). The F1CDx and WES TMB cutpoints were 10 mut/Mb and 175 mut/exome, respectively. The TSO500 cutpoint was selected using the Youden index criterion. Concordance was assessed by calculating area under the receiver-operating curve (AUROC), positive percentage agreement (PPA), and negative percentage agreement (NPA). Statistical significance of the association of TMB measured by TSO500 with ORR was assessed using logistic regression adjusted for ECOG performance status and cancer type. Clinical utility of the selected TSO500 TMB cutpoint for discriminating responders and nonresponders was assessed by calculating sensitivity, specificity, positive predictive value, negative predictive value, ORR enrichment, and prevalence.

**Results** TMB scores were valid for 294/294 patients assessed by TSO500, 269/270 assessed by F1CDx, and 293/294 assessed by WES. TMB assessed by TSO500 had good correlation with TMB assessed by F1CDx (ρ=0.76) and WES (ρ=0.74). Using Youden index criterion, 10 mut/Mb was the TSO500 cutpoint that corresponded with both the F1CDx and WES cutpoints. TSO500 reliably predicted TMB-H and non–TMB-H status as determined by the F1CDx (AUROC=0.99, PPA=97.4%, NPA=93.0%) and WES (AUROC=0.95, PPA=76.2%, NPA=96.1%) cutpoints. TMB measured by TSO500 was significantly associated with ORR (one-sided P<0.0001). Clinical utility metrics were generally similar for TSO500 and F1CDx (table 1) and TSO500 and WES (table 2).

**Conclusions** TMB assessed by TSO500 is highly correlated and concordant with TMB assessed by F1CDx and WES. Similar to the validated and approved F1CDx TMB cutpoint of 10 mut/Mb, the TSO500 TMB cutpoint of 10 mut/Mb is predictive of response to pembrolizumab monotherapy.

**Acknowledgements** This analysis and all included studies were sponsored by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

**Trial Registration** NA

**Ethics Approval** The protocols and all amendments for the studies included in this analysis were approved by the appropriate ethics committee at each participating institution.

**Consent** NA

**REFERENCE**

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0080

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**Abstract 80 Table 1 Clinical Utility Metrics for the TSO500 TMB Cutpoint Compared with the F1CDx TMB Cutpoint (n=269)**

<table>
<thead>
<tr>
<th>TSO500 at 10 mut/Mb</th>
<th>F1CDx at 10 mut/Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.425</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.868</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.315</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.389</td>
</tr>
<tr>
<td>ORR enrichment</td>
<td>8.85</td>
</tr>
<tr>
<td>Prevalence of TMB-H</td>
<td>20.0%</td>
</tr>
</tbody>
</table>


**Abstract 80 Table 2 Clinical Utility Metrics for the TSO500 TMB Cutpoint Compared with the WES TMB Cutpoint (n=293)**

<table>
<thead>
<tr>
<th>TSO500 at 10 mut/Mb</th>
<th>WES at 175 mut/exome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.419</td>
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<tr>
<td>Specificity</td>
<td>0.844</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.316</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.317</td>
</tr>
<tr>
<td>ORR enrichment</td>
<td>2.981</td>
</tr>
<tr>
<td>Prevalence of TMB-H</td>
<td>15.9%</td>
</tr>
</tbody>
</table>


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**Background** PD-L1 IHC 22C3 pharmDx uses Tumor Proportion Score (TPS) and Combined Positive Score (CPS) scoring algorithms for the immunohistochemical (IHC) evaluation of PD-L1 protein expression in human cancer tissues; both algorithms include PD-L1 staining tumor cells (TC) in scoring and CPS also includes scoring of PD-L1 staining mononuclear inflammatory cells to aid in the identification of patients for treatment with pembrolizumab (KEYTRUDA®) using indication-specific diagnostic cut-offs. This study evaluated contribution of TC in determining specimen diagnostic status based on the CPS scoring algorithm by looking into four tumor indications approved for use with KEYTRUDA®: gastric or gastroesophageal junction (GEJ) adenocarcinoma (GC/GEJ), urothelial carcinoma (UC), head and neck squamous cell carcinoma (HNSCC), and esophageal squamous cell carcinoma (ESCC). Detection of specimens expressing PD-L1 is significantly dependent on the PD-L1 staining TC component.

**Methods** A retrospective analysis was done looking at Dako’s internal tumor bank of the mentioned indications that were all stained with PD-L1 IHC 22C3 pharmDx and scored using the TPS, CPS and Quantitative Immune Cell Density (QID) methods described in figure 1. Statistical analysis encompassed looking at the scores generated that met the following criteria: CPS>0, TPS>0 and CPS≠TPS and then evaluating the percentage of those samples that changed from positive to negative diagnostic status upon removal of the TC component from the scoring.

**Results** A noticeable downward trend was observed in all four indications in the total number of positives with the removal of the TC component. Table 1 aptly captures this by showing the number of specimens for each indication that had changed from positive to negative around each indication’s diagnostic cut-offs(s). The three indications that showed the highest...
percentages of diagnostic status change were HNSCC (CPS ≥20) with a remarkable 83.3% (130) followed by UC (CPS ≥10) at 46.3% (57) and ESCC (CPS ≥10) at 36.6% (45) of the specimens reclassified as negative.

\[ a) \quad TPS = \left( \frac{PD-L1 \text{ Staining TC}}{\text{Total Number of Viable TC}} \right) \times 100 \]

\[ b) \quad CPS = \left( \frac{PD-L1 \text{ Staining Cells (TC, lymphocytes and macrophages)}}{\text{Total Number of Viable TC}} \right) \times 100 \]

\[ c) \quad QID = CPS - TPS \]

Abstract 81 Figure 1 PD-L1 Scoring Algorithms

The TPS algorithm (a) is defined as the number of PD-L1 staining tumor cells divided by the total number of viable TC, multiplied by 100. The CPS algorithm (b) includes TC and IC and is defined as the number of PD-L1 staining cells (TC, lymphocytes and macrophages) divided by the total number of viable TC, multiplied by 100. In addition to TPS and CPS, QID (c) was also calculated to quantify the contribution from PD-L1 expressing IC, QID is defined as the CPS minus the TPS.

Abstract 81 Table 1 Agilent Tumor Bank CPS and QID

| Indication | Diagnostic Cut-off | Number of Specimens | Number CPS Positive Cases | Number QID (CPS - TPS) Positive Cases | Number of Specimens Flipped at Cut-off | Proportion of Samples Flipped (%)
|------------|-------------------|---------------------|--------------------------|---------------------------------------|----------------------------------------|-----------------------------
| ESCC       | ≥10               | 170                 | 123                      | 78                                    | 45                                     | 36.6                        |
| UC         | 186               | 184                 | 184                      | 146                                   | 18                                     | 11.1                        |
| GC/GEO     | ≥20               | 164                 | 164                      | 146                                   | 18                                     | 11.0                        |
| HNSCC      | ≥20               | 164                 | 164                      | 146                                   | 18                                     | 11.0                        |
| HNSC       | ≥20               | 164                 | 164                      | 146                                   | 18                                     | 11.0                        |

Conclusions PD-L1 IHC 22C3 pharmDx (Dako, USA) stains both TC and immune cells. Removal of the PD-L1 staining TC from the CPS algorithm reduces the number of specimens scored as positive for each indication’s respective diagnostic cut-off(s). Scoring only IC reduces the number of specimens scored as positive for each indication’s respective cutoff. http://dx.doi.org/10.1136/jitc-2020-SITC2020.0081

Abstract 82 SQ3370 consists of a local intratumoral injection of a prodrug-capturing biomaterial (named SQL70; shown in step 1) followed by 5 daily systemic infusions of SQP33, an attenuated prodrug of doxorubicin (step 2). Complementary chemical groups in the two components allow the local capture of the prodrug (step 3) and trigger release of active doxorubicin at the tumor site (step 4).

82 SQ3370 IS A NOVEL APPROACH THAT DECREASES ADVERSE DRUG EXPOSURE AND ACHIEVES ROBUST INJECTED AND NON-INJECTED ANTI-TUMOR RESPONSES

Sangeetha Srinivasan*, Nathan Yee, Kui Wu, Amir Mahmoodi, Michael Zakharian, Maxim Ruzzen, Jose Mejia Oneto, Shaoqi Inc, San Francisco, CA, USA; University at Albany, Albany, NY, USA

Background Cancer immunotherapies are dependent on endogenous biomarker expression and other biological factors that often result in varying response rates across tumor types and benefit only a subset of patients. Conversely, conventional cytotoxics, the first-line treatment against solid tumors, are effective in a large patient population, but lack specificity, and often result in dose-limiting systemic toxicity. Here, we present SQ3370, a modular approach that activates doxorubicin (Dox) directly at the tumor site with reduced toxicity and potentially activates an immune response against tumor. The technology is independent of biomarkers, enzymatic activity, pH or oxygen levels and is hence expected to be effective in a wider group of patients. SQ3370 consists of a local intratumoral injection of a prodrug-capturing biomaterial (SQL70) followed by 5 daily infusions of SQP33, an attenuated prodrug of Dox. Complementary click chemistry groups in both components allow active Dox release at the tumor site (figure 1). SQP33 prodrug is ~82-fold less cytotoxic than Dox in vitro. We safely administered SQ3370 in dogs at 8.95-times the veterinary clinical dose of Dox, thus widening the therapeutic window, and showed minimal side effects including cardotoxicity and immunosuppression. We hypothesize that releasing Dox at a local site with SQ3370 may also promote immune activation against the tumor. We evaluated this in a dual-tumor model of syngeneic MC38 tumors.

Methods Immunocompetent mice were inoculated with MC38 tumor cells in two subcutaneous flanks. One tumor was intratumorally injected with the biomaterial, SQL70. SQP33 prodrug, control Dox, or saline was administered intravenously as per treatment schedule (figure 2A). Tumors harvested from a subset of mice at 2 weeks were assessed for immune biomarkers.

Results In mice bearing two tumors, SQ3370 significantly increased overall survival and the anti-tumor response against injected tumors (figure 2B,C). Surprisingly, SQ3370 also induced regression of the non-injected tumors (figure 2D). Assessment of tumor-Infiltrating immune cells showed an increase in CD3+, CD4+, and CD8+ T cells and a decrease in CD3+ tumor infiltrating cells and a decrease in CD8+ T cells.

Abstract 82 Figure 1 SQ3370 Components and administration

Abstract 82 Figure 2 Systemic anti-tumor response and improved survival

Immunocompetent C57BL/6 mice were inoculated with mouse MC38 tumors. Tumors were implanted on Day 0. Treatments started on Day 7 with local injection of SQL70 biomaterial at ‘injected’ tumor, followed by systemic administration of saline, Dox, or SQP33 prodrug (A). Median overall survival (B) was significantly higher with SQ3370 as compared to Dox, as determined by Logrank test. Injected tumor response (C) was also significantly better with SQ3370, as assessed by corrected t tests, and non-injected tumors (D) showed a favorable response only with SQ3370. Injected-tumor growth curves show mean ± SEM, and growth curves for non-injected tumors show the percent change in tumor size from initial measurement. Curves stopped when a mouse died, or its tumor volume reached 2000 mm³.
in regulatory T cells in both injected and non-injected lesions (figure 3). The T-cell response correlated with the anti-tumor efficacy data, supporting the immune activation hypothesis.

Conclusions SQ3370 is a proof of concept example for a novel modular approach that addresses limitations of current immuno- and cytotoxic therapies for patients with solid tumors. Local release of Dox with SQ3370 expands the therapeutic window of Dox, minimizes toxicities and leads to a robust anti-tumor response that potentially also causes immune activation against the tumors.

Acknowledgements The authors would like to thank the National Institutes of Health (NIH), the National Science Foundation (NSF), and Y Combinator.

Ethics Approval This study, project number: SQI-FFS-ON-20181119_04 v4, was approved by the Institutional Animal Care and Use Committee (IACUC) of the vendor, following the guidance of Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), accreditation number 001516.

http://dx.doi.org/10.1136/jtc-2020-SITC2020.0082

Abstract 83 Figure 3 Tumor-infiltrating immune cells in both tumors. Tumor samples were stained with antibodies and analyzed by multicolor flow cytometry. Dead cells were excluded from analysis. Results show mean ± SEM (n = 3–5 per group) as a percentage of total or marker-gated (CD4 or CD8) cells obtained from the tumor sample. Statistical significance was assessed using a corrected t test.

Abstract 83 Figure 1 Kaplan-Meier survival estimates between groups with different ALC at the start date of pembrolizumab and at 6 weeks after initiation of pembrolizumab. There is a statistically significant difference in OS between patients with ALC < 1.4 and patients with ALC ≥ 1.4 at 6 weeks after initiation of pembrolizumab (p = 0.046), but not at the start of treatment (p = 0.095).

Abstract 83 Figure 2 Kaplan-Meier survival estimates between groups with different ANC/ALC ratio at the start date of pembrolizumab and at 6 weeks after initiation of pembrolizumab. There is a statistically significant difference in OS between patients with ANC/ALC < 5 and patients with ANC/ALC ≥ 5, both at the start date of pembrolizumab (p = 0.003) and at 6 weeks after initiation of pembrolizumab (p = 0.028).

Background Pembrolizumab is an anti-programmed cell death protein 1 (PD-1) antibody used for the treatment of advanced non-small cell lung carcinoma (NSCLC). Systemic inflammation has long been associated with poor outcomes in many types of solid tumors. Peripheral blood biomarkers such as absolute lymphocyte count (ALC) and absolute neutrophil count to absolute lymphocyte count ratio (ANC/ALC) serve as surrogate markers of inflammation. The aim of this study is to investigate ALC and ANC/ALC in patients with advanced NSCLC receiving pembrolizumab and determine if there is a correlation between these biomarkers and overall survival (OS).

Methods A total of 240 patients with advanced NSCLC treated with pembrolizumab at Northwell Health hospital centers were included. The ALC and ANC/ALC were examined at initiation of pembrolizumab and after 6 weeks on treatment. The prognostic role of these peripheral blood biomarkers on OS were examined with Kaplan-Meier curves and a multivariable cox regression analysis.

Results Of the 240 patients, the majority were male (52%), with a median age of 67 years (interquartile range [IQR] 59–73 years), had a diagnosis of adenocarcinoma (76%), with stage IV disease (82%). PD-L1 expression was ≥50% in 44% of the patients. The median time on treatment with pembrolizumab was 5.7 months [IQR: 2.7–12.5]. The median ALC and ANC/ALC were significantly lower at 6 weeks of pembrolizumab compared to the start date of treatment (1.38 vs. 1.4, p<0.001) and (3.6 vs. 4.6, p<0.001) respectively. An ALC greater than 1.4 was associated with an increased OS (figure 1), at 6 weeks after initiation of pembrolizumab (p=0.046), but not at the start of treatment (p=0.095). An ANC/ALC less than 5 was associated with improved OS (figure 2), both at initiation of pembrolizumab (p=0.003) and at 6 weeks after initiation of treatment (p = 0.028). Likewise, after adjusting for potential cofounders with a multivariate analysis (table 1), a baseline ANC/ALC of 5 or higher had a significantly increased risk of death (hazards ratio (HR)=1.84; 95% confidence interval (CI), 1.21–2.79; p=0.004), compared with patients with a lower ratio.

Conclusions High ALC at time of diagnosis as well as low ANC/ALC at baseline and at 6 weeks on treatment correlated with an increased OS in patients with advanced NSCLC treated with pembrolizumab. These findings represent a readily available predictive biomarker for oncologists and may help with risk stratification and strategizing treatment plans.

Abstract 83 THE PROGNOSTIC SIGNIFICANCE OF PERIPHERAL BLOOD BIOMARKERS IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER TREATED WITH PEMBROLIZUMAB: A CLINICAL STUDY

Kira MacDougall*, Muhammad Niaz, Jeff Hosny, Sylvester Hosny, Alexander Bershadskiy, Staten Island University Hospital, Staten Island, NY, USA

5 per group) as a percentage of total or marker-gated (CD4 or CD8) cells obtained from the tumor sample. Statistical significance was assessed using a corrected t test.
**Ethics Approval**  The study was approved by Zucker School of Medicine at Hofstra/Northwell at Staten Island University Hospital’s IRB #: 19–0922

**REFERENCE**

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0083

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**Quantifying Pharmacodynamic Biomarker Changes in Immuno-Oncology by Mass Spectrometry**

**Joseph Eckenrode, Omar Laterza, Michael Lassman. Merck and Co., Inc., Kenilworth, NJ, USA**

**Background**  Quantifying pharmacodynamic biomarker changes enables decision making and clinical trials in drug development. Pharmacodynamic biomarkers are used to determine the effects of treatment on disease. Mass spectrometry offers a quantitative, selective, and multiplex platform for pharmacodynamic protein biomarker analysis in clinical samples (e.g. blood and tumor) that is feasible across multiple sample conditions (e.g. fresh, frozen and formalin-fixed paraffin-embedded (FFPE)). To date, however, methodologies for targeted protein analysis by mass spectrometry (i.e. quantitative proteomics) are underdeveloped for application in immuno-oncology.

**Methods**  To address this, we sought to extract the immuno-oncology-associated T cell membrane proteins CD3, CD4 and CD8 from peripheral blood mononucleate cells (PBMC) and develop a multiplexed mass spectrometry method to quantify their expression. PBMC were isolated from whole blood and using detergent-based lysis buffers fractionated into a cytosolic and membrane protein lysate (figure 1). Analytical methods were then developed to detect proteotypic peptides of all three proteins (table 1 and figure 6) from the lysates by mass spectrometry. MS/MS method resulted in detection of endogenous CD3, CD4 and CD8 proteins from small volumes of whole blood (< 0.1 mL) and the analyte responses were linear over at least two orders of magnitude (figure 5).

**Results**  CD3, CD4 and CD8 were detected in the membrane protein fraction but not in the cytosolic protein fraction after whole-proteome tryptic digestion using a filter-aided sample preparation (or FASP) technique but with a signal-to-noise ratio of ≤ 2.0 (figure 2). Applying an additional immunoaffinity (IA) enrichment step with antibody-conjugated magnetic beads, prior to digestion, dramatically improved the analyte signal-to-noise ratios to > 100 (figure 3). Reverse-phase nanoflow liquid chromatography (LC) was used to separate all three analytes in multiplex over a 12-minute run prior to tandem mass analysis (MS/MS) (figure 4). Together, this IA-LC-MS/MS method resulted in detection of endogenous CD3, CD4 and CD8 proteins from small volumes of whole blood (< 0.1 mL) and the analyte responses were linear over at least two orders of magnitude (figure 5).

**Table 1**  Multivariable cox regression analysis for association of baseline peripheral blood biomarkers and overall survival.

Legend: HR, hazards ratio; CI, confidence interval; CNS, central nervous system, ANC/ALC, absolute neutrophil count to absolute lymphocyte count ratio; PDL-1, programmed death-1 ligand 1; ECOG, Eastern Cooperative Oncology Group performance scale.

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.00</td>
<td>0.97 – 1.02</td>
</tr>
<tr>
<td>CNS inv. (yes vs. no)</td>
<td>1.40</td>
<td>0.85 – 2.31</td>
</tr>
<tr>
<td>ANC/ALC &gt; 5 (yes vs. no)</td>
<td>1.84</td>
<td>1.21 – 2.79</td>
</tr>
<tr>
<td>PDL-1 expression &gt; 50% vs. ≤ 50%</td>
<td>0.54</td>
<td>0.35 – 0.83</td>
</tr>
<tr>
<td>ECOG (1-4 vs. 1-2)</td>
<td>2.58</td>
<td>2.58 – 1.67</td>
</tr>
</tbody>
</table>

**Figure 1**  Detergent-based protein extraction and fractionation of PBMC

**Figure 2**  Filter-aided sample preparation (FASP) for whole-proteome analysis

**Figure 3**  Immunoaffinity enrichment of proteins from PBMC lysates

**Figure 4**  Representative multiplex analysis from 1mL of whole blood
Conclusions This method was developed specifically to quantify pharmacodynamic changes in CD4 and CD8 T cell membrane expressions from clinically feasible samples (i.e. PBMC). This work, however, provides a foundation for developing methodologies to conduct quantitative proteomics applicable to immuno-oncology, which may be used to interrogate additional pharmacodynamic biomarkers.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0085

86 CO-DETECTION OF RNA AND PROTEIN IN FFPE TUMOR SAMPLES BY COMBINING RNASCPE IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY ASSAYS

Abstract 85 Figure 1

Conclusions We describe CVM and KS as novel metrics for measuring spatial heterogeneity of immune cells. Increased spatial heterogeneity of CD68+ TAMs and tumor cells was associated with worse OS in patients with metastatic ccRCC who received IT. These findings corroborate prior reports of TAMs eliciting an immunosuppressive effect on the tumor-immune microenvironment, and demonstrate the novel finding of a clinically significant effect of TAM spatial clustering on OS.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0085
interactions within the tumor and its microenvironment. Interrogation of complex cellular interactions within the tumor microenvironment (TME) requires a multi-omics approach where multiple RNA and protein targets can be visualized within the same tumor sample and be feasible in FFPE sample types. Simultaneous detection of RNA and protein can reveal cellular sources of secreted proteins, identify specific cell types, and visualize the spatial organization of cells within the tissue. Examination of RNA by in situ hybridization (ISH) and protein by immunohistochemistry (IHC) or immunofluorescence (IF) are widely used and accepted techniques for the detection of biomarkers in tumor samples. Given the similarities in workflow, co-detection of RNA and protein by combining ISH and IHC/IF in a single assay can be a powerful multi-omics solution for interrogating the complex tumor and its microenvironment.

Methods In this report we combined the single cell, single molecule RNA ISH technology known as RNAscope with IHC/IF to simultaneously detect RNA and protein in the same FFPE tumor section using both chromogenic and fluorescence detection methods.

Results We demonstrate co-localization of target mRNA and the corresponding protein in human cancer samples, visualize infiltration of immune cells into the TME, characterize the activation state of immune cells in the TME, identify single cell gene expression within cellular boundaries demarcated by IHC/IF, examine cell type-specific expression of multiple immune checkpoint markers, and distinguish endogenous T cells from activated CAR+ T cells. Overall, we show that codetection of RNA by the RNAscope ISH assay and protein by the IHC/IF assay in the same FFPE section is a feasible methodology. The combined RNAscope ISH-IHC/IF workflow is a powerful technique that can be used to study gene expression signatures at the RNA and protein level with spatial and single cell resolution.

Conclusions By leveraging the strength of the similar workflows of RNAscope ISH and IHC/IF assays, this methodology combines transcriptomics and proteomics in the same tissue section, providing a multi-omics approach for characterizing complex tissues and revealing cell type specific gene expression with spatial and single cell resolution.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0086

Abstract 87 Figure 1 RUNX1 expression in cancer progression of HNSCC. (A) Representative images of RUNX1 immunohistochemical staining between normal tissues and HNSCC tissues (scale bar 20μm). Insets (bottom) are lower magnification (15×) images of respective cores to show a more global view of individual samples. (B) The RUNX1 mRNA expression in tumor versus normal tissues from the TCGA database, which contains 31 normal samples and 91 HNSCC samples. (C) Immunoblotting analysis of RUNX1 expression in 3 pairs of HNSCC and non-tumor laryngeal tissues. (D) Quantitative and statistical analysis of the immunoblotting analysis. *P<0.05, **P<0.01

Abstract 87 Figure 2 Effect of RUNX1 on progression and the interrelationship between RUNX1 and OPN in HNSCC. (A) The migration ability of FaDu and SCC-9 cells transfected as above were assessed by wound-healing assay. Representative images were obtained at 0h and 24h (upper, magnification 40×) and quantified (bottom). (B) The migration and invasion ability detected by transwell assays. Representative images of FaDu and SCC-9 cells from migration and invasion assays experiment were obtained at 24h (upper, magnification 12×) and quantified (bottom). (C) Correlation analysis was performed between RUNX1 expression and OPN expression in HNSCC tissues (n = 29) and (D) in TCGA HNSCC database (n = 91). All P values are shown in the graphs. (E) Levels of nucleus OPN mRNA and (F) protein in the FaDu cells transfected with lentiviral vector encoding shRUNX1 or scrambled control were determined by real-time RT-PCR and immunoblotting analysis. (G) The predicted OPN promoter sequence bound by RUNX1 and their ChIP-PCR primers. (H) The binding of RUNX1 to predicted OPN promoter binding region was confirmed in FaDu using ChIP-qPCR and ChIP-PCR. IgG was used as the control. (I) Relative OPN activity was detected by luciferase assay in 293T cells co-transfected with RUNX1 and luciferase reporter. *P<0.01, **P<0.001, ****P<0.0001
Abstract 87 Figure 3  RUNX1-mediated HNSCC cell metastasis in MAPK pathway via stimulating OPN. (A) The migration ability of FaDu cells transfected as above were assessed by wound-healing assay. Representative images were obtained at 0h and 24h (magnification 40×). (B) The migration and invasion ability detected by transwell assays. Representative images of FaDu cells from migration and invasion assays experiment were obtained at 24h (magnification 12×). (C) Immunoblotting analysis for protein markers expression levels of the MAPK pathway in FaDu cells transfected as above. (D) The graph of tumor growth/volumes curve at the indicated time intervals (left). Tumor weights were quantified at the end of the experiment (right). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Results In our study, RUNX1 expression was increased with disease progression in patients with HNSCC (figure 1). The silencing of RUNX1 significantly decelerated the malignant progression of HNSCC cells, reduced Osteopontin (OPN) expression in vitro, and weakened the tumorigenicity of HNSCC cells in vivo (figure 2). Moreover, we demonstrated that RUNX1 activated the MAPK signaling by directly binding to the promoter of OPN in tumor progression and metastasis of HNSCC (figure 3).

Conclusions Our results may provide new insight into the mechanisms underlying the role of RUNX1 in tumor progression and metastasis and reveal the potential therapeutic target in HNSCC.

Ethics Approval The study was approved by the Ethics Board of BenQ Medical Center, the Affiliated BenQ Hospital of Nanjing Medical University.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0087

CELLULAR THERAPIES

89 FIRST-IN-HUMAN CAR T TARGETS CLEAVED MUC1, THE GROWTH FACTOR RECEPTOR FORM

Garima Kaushik*, Bhavna Verma, Amy Wesa. Champions Oncology, Rockville, MD, USA

Background The preclinical screening of immune-modulatory therapies suffers from the absence of models that recapitate in vivo heterogeneous tumor microenvironment (TME). 3D tumor organoid cultures provide a model that closely mimics in situ tumor architecture and is being aggressively used to evaluate therapeutic efficacy ex vivo. A vastly heterogeneous TME impacts patient treatment response, and there is a dearth of human tumor models (2D or 3D), that mimic in vivo diversity of TME, including infiltrating immune populations.

3D organoid cultures typically contain neoplastic epithelium; however, they fall short in representing tumor to tumor-infiltrating lymphocytes (TILs) interactions, limiting their ability to generate a clinically relevant response to immunotherapeutics. Addition of immune cells from unrelated donors to organoids can simulate that microenvironment but is complicated by T cell alloreactivity. Here we describe 3D patient-derived xenograft organoid (PDXO) co-cultures with matching autologous human TILs to recapitulate the tumor-specific immune response, leveraging confocal high content analysis and luminescence multiplex assays. This platform allows the evaluation and high throughput screening of novel immune targeting agents to determine impacts on patient-derived T cell function, T cell infiltration, and tumor cytotoxicity.

Methods Surgical resections from patients were used to generate patient-derived xenografts and tumor-infiltrating lymphocytes in parallel. PDX were resected and digested to establish PDXO. TILs and organoids from the same patient were fluorescently labeled and cultured together for four days to evaluate tumor infiltration and drug cytotoxicity in 3D cultures. CellInsight CX7 high content imaging platform was used to trace TILs and cancer cells and evaluate T cell infiltration and tumor cell killing in the presence and absence of immunomodulatory therapies.

Results PDXO were established to mimic in vivo tumor biology. Tumor-specific TILs were successfully expanded and characterized by flow cytometry. Co-culture resulted in TIL infiltration in organoids from day one in culture and increased over four days. Cytotoxicity and TIL infiltration were quantified using fluorescent dyes via high throughput imaging platform. Significantly enhanced TIL infiltration was observed in autologous co-cultures compared to non-autologous co-cultures. The established unique autologous PDXO immune organoid co-cultures could be used as an improved simulation of the modular activity of therapeutic agents in patient-specific T cells against their own tumors.

Conclusions Patient autologous TILs – PDXO co-culture platform is an advanced model for evaluating IO therapeutics with the tumor-specific immune microenvironment. The platform provides an opportunity for precision medicine and high throughput drug screening of immuno-modulatory therapies.

Ethics Approval The study was approved by Champions Oncology’s Institutional Animal Care and Use Committee (IACUC).

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88 DEVELOPMENT OF A 3D ORGANOID AUTOLOGOUS TIL CO-CULTURE PLATFORM FOR HIGH THROUGHPUT IMMUNO-ONCOLOGY STUDIES

Garima Kaushik*, Bhavna Verma, Amy Wesa. Champions Oncology, Rockville, MD, USA

Background The preclinical screening of immune-modulatory therapies suffers from the absence of models that recapitate in vivo heterogeneous tumor microenvironment (TME). 3D tumor organoid cultures provide a model that closely mimics in situ tumor architecture and is being aggressively used to evaluate therapeutic efficacy ex vivo. A vastly heterogeneous TME impacts patient treatment response, and there is a dearth of human tumor models (2D or 3D), that mimic in vivo diversity of TME, including infiltrating immune populations.

3D organoid cultures typically contain neoplastic epithelium; however, they fall short in representing tumor to tumor-infiltrating lymphocytes (TILs) interactions, limiting their ability to generate a clinically relevant response to immunotherapeutics. Addition of immune cells from unrelated donors to organoids can simulate that microenvironment but is complicated by T cell alloreactivity. Here we describe 3D patient-derived xenograft organoid (PDXO) co-cultures with matching autologous human TILs to recapitulate the tumor-specific immune response, leveraging confocal high content analysis and luminescence multiplex assays. This platform allows the evaluation and high throughput screening of novel immune targeting agents to determine impacts on patient-derived T cell function, T cell infiltration, and tumor cytotoxicity.

Methods Surgical resections from patients were used to generate patient-derived xenografts and tumor-infiltrating lymphocytes in parallel. PDX were resected and digested to establish PDXO. TILs and organoids from the same patient were fluorescently labeled and cultured together for four days to evaluate tumor infiltration and drug cytotoxicity in 3D cultures. CellInsight CX7 high content imaging platform was used to trace TILs and cancer cells and evaluate T cell infiltration and tumor cell killing in the presence and absence of immunomodulatory therapies.

Results PDXO were established to mimic in vivo tumor biology. Tumor-specific TILs were successfully expanded and characterized by flow cytometry. Co-culture resulted in TIL infiltration in organoids from day one in culture and increased over four days. Cytotoxicity and TIL infiltration were quantified using fluorescent dyes via high throughput imaging platform. Significantly enhanced TIL infiltration was observed in autologous co-cultures compared to non-autologous co-cultures. The established unique autologous PDXO immune organoid co-cultures could be used as an improved simulation of the modular activity of therapeutic agents in patient-specific T cells against their own tumors.

Conclusions Patient autologous TILs – PDXO co-culture platform is an advanced model for evaluating IO therapeutics with the tumor-specific immune microenvironment. The platform provides an opportunity for precision medicine and high throughput drug screening of immuno-modulatory therapies.

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89 FIRST-IN-HUMAN CAR T TARGETS CLEAVED MUC1, THE GROWTH FACTOR RECEPTOR FORM

Garima Kaushik*, Bhavna Verma, Amy Wesa. Champions Oncology, Rockville, MD, USA

Background The preclinical screening of immune-modulatory therapies suffers from the absence of models that recapitate in vivo heterogeneous tumor microenvironment (TME). 3D tumor organoid cultures provide a model that closely mimics in situ tumor architecture and is being aggressively used to evaluate therapeutic efficacy ex vivo. A vastly heterogeneous TME impacts patient treatment response, and there is a dearth of human tumor models (2D or 3D), that mimic in vivo diversity of TME, including infiltrating immune populations.

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Conclusions Patient autologous TILs – PDXO co-culture platform is an advanced model for evaluating IO therapeutics with the tumor-specific immune microenvironment. The platform provides an opportunity for precision medicine and high throughput drug screening of immuno-modulatory therapies.

Ethics Approval The study was approved by Champions Oncology’s Institutional Animal Care and Use Committee (IACUC).

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88 DEVELOPMENT OF A 3D ORGANOID AUTOLOGOUS TIL CO-CULTURE PLATFORM FOR HIGH THROUGHPUT IMMUNO-ONCOLOGY STUDIES

Garima Kaushik*, Bhavna Verma, Amy Wesa. Champions Oncology, Rockville, MD, USA

Background The preclinical screening of immune-modulatory therapies suffers from the absence of models that recapitate in vivo heterogeneous tumor microenvironment (TME). 3D tumor organoid cultures provide a model that closely mimics in situ tumor architecture and is being aggressively used to evaluate therapeutic efficacy ex vivo. A vastly heterogeneous TME impacts patient treatment response, and there is a dearth of human tumor models (2D or 3D), that mimic in vivo diversity of TME, including infiltrating immune populations.
Background T-cell based immunotherapies such as CAR-T, bispecific mAb, transgenic T cells and checkpoint blockade have profound efficacy in multiple tumor types but share a common limitation – target antigen (Ag) escape. One approach to address this limitation has been therapy directed at a parallel target (e.g. CD22 after CD19 loss), however, these lineage markers are frequently lost together. Here, we describe an alternate, broadly applicable, approach: potentiating fasL/fas-signaling to increase localized bystander killing of Ag tumor cells and thereby prevent Ag escape.

Methods We used a CRISPR/Cas9 library to screen for tumor expressed molecules that inhibit or facilitate T-cell killing. We then evaluated one candidate -fas- using murine transgenic T cells, murine and human CAR-T cells, bispecific mAb redirected PBMC, and tumoral RNAseq data from a large CAR-T clinical trial.

Results GFP-specific (JEDI) CD8 T cells were co-cultured with on-target (GFP+) and bystander (mCherry+) lymphoma cells that had been transfected with a CRISPR/Cas9 library; this screen revealed several tumor-expressed candidate molecules inhibiting or facilitating T-cell killing. Notably, we observed a marked dependence on fas for on-target tumor killing and then, surprisingly, an exquisite dependence on fas for localized bystander tumor killing. (figure 1). Because bystander tumor killing appeared critically fas-dependent, we hypothesized that potentiating fas-signaling might increase bystander killing. An in vitro screen of small molecules that modulate fas-pathway revealed several candidates, including inhibitors of histone deacetylases (HDAC), inhibitors of apoptosis proteins (IAP) and Bcl-2 family members in murine and human systems (figure 2). To validate these candidates, we demonstrated that HDACi increased GFP-specific T cell killing of both on-target and bystander lymphoma cells, in a completely fas-dependent manner (figure 3). Similarly, using a bispecific antibody-based system, we demonstrated increased, fas-dependent, T cell killing of both on-target and bystander human lymphoma cells with inhibitors of IAP and bcl-2 family members (e.g. MCL1).

Conclusions Preliminary results show that patients experienced robust CAR T cell expansion with CAR-positive T cells persisting at Day 60 post huMNC2-CAR44 T cell treatment. Possible signs of efficacy were measured.

Trial Registration NCT04020575

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Conclusions T-cell mediated tumor killing can be potentiated with fas pathway modulators. This augmentation improves both fas-dependent Ag+ and Ag− tumor cell death. Further studies of modulating the fas pathway alongside T-cell based immunotherapies are needed as potential treatments to prevent antigen escape and improve patient outcomes.

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Ethics Approval The studies were approved by The Mount Sinai Institutional Review Board.

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targeting Sirt2 rescues the metabolic fitness and effector functions of tumor-reactive T cells within the metabolically restricted tumor microenvironment

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Background The majority of cancer patients remain refractory to existing cancer immunotherapies. Despite the growing evidence that dysregulated metabolism contributes to the exhaustion of tumor-infiltrating T lymphocytes (TILs) and the loss of their effector functions within the metabolically restricted tumor microenvironment (TME), actionable targets to rescue metabolic fitness and anti-tumor activity of TILs remain elusive. Memory T (TM) cells and TILs rely on fatty acid catabolism to preserve their effector functions due to nutrient competition for glucose with tumor cells. Therefore, enhancing fatty acid catabolism of TILs represents an attractive strategy to increase the efficacy of immunotherapies. Sirt2 is an NAD+ dependent histone deacetylase. We previously showed that upregulation of Sirt2 in human TILs negatively correlates with response to TIL therapy in advanced non-small cell lung cancer (NSCLC) and Sirt2 deficiency leads to hyper-reactive T cells with superior antitumor activity.

Methods Sirt2 expression was analyzed by flow cytometry and Western blot. The role of Sirt2 in tumor immunity was studied using in vivo B16F10 tumor challenge models as well as ex vivo analysis including RNA-seqencing, CFSE proliferation assay, DAPI/AnnexinV staining, IFN-γ ELISpot assay, intracellular staining of effector molecules and LDH cytotoxicity assay on WT versus Sirt2KO T cells. Molecular partners of Sirt2 were identified using mass spectrometry (MS) and Co-immunoprecipitation analyses. The role of Sirt2 in T cell metabolism was investigated using Seahorse bioanalyzer and LC-MS/MS Metabolic profiling. AGK2, a Sirt2 selective inhibitor, was used for Sirt2 blockade in human T cells.

Results Sirt2 expression is upregulated during T cell activation, TM stage, and within the TME. Our molecular studies revealed that Sirt2 negatively impacts the acetylation status and the activity of the trifunctional protein, the key enzyme of fatty acid oxidation (FAO). Accordingly, Sirt2 deficiency enhanced FAO and metabolic fitness of activated T cells and mouse TILs isolated from B16F10 tumor nodules. As a consequence of enhanced FAO, Sirt2 deficient mice displayed increased accumulation of TM cells, which was associated with decreased apoptosis and increased survival after tumor challenge leading to superior tumor rejection. Most importantly, pharmacologic inhibition of Sirt2 in human TILs isolated from NSCLC patients enhanced their metabolic fitness and cytotoxic activity against their autologous tumor cells.

Conclusions Our findings indicate Sirt2 as a suppressor of T cell metabolism amenable to therapeutic targeting, and Sirt2 inhibition programs T cell metabolic fitness to optimally sustain their effector function within the hypoglycemic TME, thus, leading to an effective anti-tumor immune response.

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Abstracts

TAC receptors signal via the endogenous TCR, whereas CAR signaling is TCR-independent. Consistent with TCR-dependent signaling, ligation of TAC receptors resulted in the formation of conventional immunological synapses, whereas ligation of CARs produced unconventional synapses. Despite these functional differences, CARs and TAC receptors demonstrated a similar capacity to activate T cells against antigen-positive tumor cell targets. However, CAR-T cells displayed reactivity to antigen-negative cells, due to interaction with a cross-reactive antigen; TAC-T cells displayed no reactivity to antigen-negative cells.

Conclusions Tonic signaling in CAR-T cells reduces their activation threshold and increases their propensity to be activated by cross-reactive antigen. In contrast, TAC receptors do not deliver tonic signals, which increases the stringency of activation and reduces the likelihood of off-target responses. This feature of the TAC platform is advantageous to safeguard against the unexpected cross-reactivity that may occur when a new antigen-binding domain is deployed in vivo.

Ethics Approval Use of human materials was approved by the Hamilton Integrated Research Ethics Board (HiREB).

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INHIBITION OF AKT SIGNALING DURING EXPANSION OF TCR-ENGINEERED T-CELLS FROM PATIENT LEUKOCYTE MATERIAL GENERATES SPEAR T-CELLS WITH ENHANCED FUNCTIONAL POTENTIAL IN VITRO

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Background T-cells attributes for adoptive cell therapy of patients with advanced cancer can be optimized during ex vivo expansion culture. Autologous TCR-engineered T-cells targeting the MAGE-A4 antigen with Specific Peptide Enhanced Affinity Receptors (SPEAR T-cells) have shown promise in the clinic.1 The highly variable leukocyte material obtained from individual patients during apheresis can present a manufacturing challenge for autologous T-cell therapies. The degree of ex vivo expansion and the functional attributes of the expanded T-cell product impact therapeutic efficacy and can be suboptimal for some patient apheresis material. Both TCR and cytokine growth factor signals used for ex vivo T-cell expansion promote robust activation of AKT (Protein Kinase B) signaling, which drives T-cell activation, proliferation, and terminal differentiation. It is hypothesized that inhibition of AKT signaling during T-cell expansion may uncouple proliferation and terminal differentiation, leading to the generation of less differentiated T-cells that may have functional benefit in vivo.2 3

Methods We evaluated use of an AKT inhibitor during SPEAR T-cell manufacturing using leukocytes from healthy donors and patients with advanced solid cancers.

Results AKT inhibition resulted in the generation of a more consistent expansion and phenotype of the final T-cell product. This was observed using two SPEAR T-cell constructs, ADP-A2M4 and ADP-A2M4CD8. Ex vivo SPEAR T-cell expansion in the presence of an AKT inhibitor generated CD8+ T-cells that maintained a less differentiated phenotype (based on CCR7+CD45RA+ and CD62L+ expression). AKT inhibition was associated with enhanced antigen-specific responses of SPEAR T-cells in vitro, including effector cytokine production, target-cell killing, ability to proliferate in response to prolonged antigen-stimulation and maintenance of cytotoxic activity following antigen re-stimulation.

Conclusions We plan to introduce AKT inhibition into the GMP manufacturing process, and evaluate the efficacy of the resulting products in ongoing clinical studies.

Ethics Approval The experimental study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and was approved by local authorities. An independent ethics committee or institutional review board approved the clinical protocol at each participating center. All the patients provided written informed consent before study entry.

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DECTIBATINE GENE MODULATION SENSITIZES HUMAN NON–SMALL CELL LUNG CANCER (NSCLC) TO NY-ESO-1 TCR IMMUNOTHERAPY (LETETRESGENE AUTOLEUCEL; GSK3377794) IN VIVO

Dmitry Parkovsky, Ioanna Eleftheriadou, Anna Domogala, Sara Brett, Lea Patyask, Magdalena Kijewska, Gary Thripp, Jack Euesden, Jan Klajwijk, Katrina Soor, Miriam Damm, Mark D Hill, Miraila Georgoulis, Arman Shalabi, Cedrik Britten. GliarSmitkline, Collegeville, PA, USA

Background NY-ESO-1–specific T cells (letetresgene autoleucele [lete-cel] GSK3377794) are autologous CD4+ and CD8+ T cells transduced to express a high-affinity T-cell receptor (TCR) capable of recognizing NY-ESO-1 and LAGE-1a antigens in complex with human leukocyte antigen (HLA)-A*02. NY-ESO-1 (CTAG1B) and LAGE-1a (CTAG2) are tumor-associated antigens (TAA) that share the SLLMWITQC peptide bound to human leukocyte antigen HLA-A*02 and are expressed in various cancers. Emerging evidence suggests that TCR-engineered T cells targeting NY-ESO-1 hold promise for patients with solid tumors.1 Approximately 75% of synovial sarcomas can over-express NY-ESO-1 vs 12% of NSCLC;2 however, NSCLC expression of NY-ESO-1/LAGE1-a may have
therapeutic potential. A separate study using engineered T cells targeting NY-ESO-1 has shown a partial response in a patient with advanced lung adenocarcinoma. Decitabine (DAC) is a hypomethylating agent and potent inducer of TAA, including NY-ESO-1. We have reported in vitro use of DAC to selectively modulate TAA expression in TAA low-expressing tumor cell lines in order to enhance lete-cel therapy. The aim of this study was to assess enhancement of combination therapy with lete-cel and DAC in an in vivo NSCLC model.

Methods NOD scid gamma (NSG) mice were injected subcutaneously with the human NSCLC tumor cell line NCI-H1703. Upon engraftment, tumor-bearing mice were treated with a 5-day course of DAC or vehicle control followed by 2 days of rest. Lette-cel was infused on Day 8. RNA was isolated from tumor formalin-fixed paraffin-embedded blocks, and levels of NY-ESO-1 and LAGE-1a transcript were measured by RT-qPCR. Expression pattern of the NY-ESO-1 protein was assessed via immunohistochemistry. Efficacy was defined by changes in tumor volume and systemic IFN-g secretion.

Results Consistent with our previous in vitro studies, DAC treatment in vivo resulted in induction of NY-ESO-1 and LAGE-1a in NSCLC tumors. Lette-cel in combination with DAC significantly enhanced antitumor efficacy in vivo compared with lete-cel alone. This was associated with increased interferon-g secretion. Mice that received DAC treatment only did not show statistically significant tumor reduction compared with untreated mice.

Ethics Approval All animal studies were ethically reviewed and carried out in accordance with the Animals Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals. Human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an Institutional Review Board/Ethics Committee approved protocol.

Conclusions GSK is currently enrolling a Phase Ib/IIa, multi-arm, open-label pilot study (NCT03709706) of lete-cel as a monotherapy or in combination with pembrolizumab in HLA-A*02-positive patients with NSCLC whose tumors express NY-ESO-1/LAGE-1a. This work may support rationale for the use of DAC in combination with lete-cel to improve adoptive T-cell therapy by increasing levels of target antigens and anti-tumor effect in NSCLC.

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POLYFUNCTIONAL ACTIVITY OF GD3CAR T-CELLS AGAINST TUMORS IN TUBEROUS SCLEROSIS COMPLEX


Abstracts
levels in an array of malignancies including mesothelioma, ovary, non-small cell lung cancer, and pancreatic cancers and is an attractive target antigen for immune-based therapies. Early clinical evaluation of autologous MSLN-targeted chimeric antigen receptor (CAR)-T cell therapies for malignant pleural mesothelioma has shown promising acceptable safety\(^1\) and have recently evolved with incorporation of next-generation CAR co-stimulatory domains and armoring with intrinsic checkpoint inhibition via expression of a PD-1 dominant negative receptor (PD1DNR).\(^2\) Despite the promise that MSLN CAR-T therapies hold, manufacturing and commercial challenges using an autologous approach may prove difficult for widespread application. EBV T cells represent a unique, non-gene edited approach toward an off-the-shelf, allogeneic T cell platform. EBV-specific T cells are currently being evaluated in phase 3 trials [NCT03394365] and, to-date, have demonstrated a favorable safety profile including limited risks for GvHD and cytokine release syndrome.\(^3\) \(^4\) Clinical proof-of-principle studies for CAR transduced allogeneic EBV T cell therapies have also been associated with acceptable safety and durable response in association with CD19 targeting.\(^5\) Here we describe the first preclinical evaluation of ATA3271, a next-generation allogeneic CAR EBV T cell therapy targeting MSLN and incorporating PD1DNR, designed for the treatment of solid tumor indications.

**Methods** We generated allogeneic MSLN CAR+ EBV T cells (ATA3271) using retroviral transduction of EBV T cells. ATA3271 includes a novel 1XX CAR signaling domain, previously associated with improved signaling and decreased CAR-mediated exhaustion. It is also armored with PD1DNR to provide intrinsic checkpoint blockade and is designed to retain functional persistence.

**Results** In this study, we characterized ATA3271 both in vitro and in vivo, ATA3271 show stable and proportional CAR and PD1DNR expression. Functional studies show potent antitumor activity of ATA3271 against MSLN-expressing cell lines, including PD-L1-high expressors. In an orthotopic mouse model of pleural mesothelioma, ATA3271 demonstrates potent antitumor activity and significant survival benefit (100% survival exceeding 50 days vs. 25 day median for control), without evident toxicities. ATA3271 maintains persistence and retains central memory phenotype in vivo through end-of-study. Additionally, ATA3271 retains endogenous EBV TCR function and reduced alloreactivity in the context of HLA mismatched targets.

**Conclusions** Overall, ATA3271 shows potent anti-tumor activity without evidence of alloreactivity, both in vitro and in vivo, suggesting that allogeneic MSLN-CAR-engineered EBV T cells are a promising approach for the treatment of MSLN-positive cancers and warrant further clinical investigation.

**REFERENCES**


**Background** Adoptive immunotherapy using chimeric antigen receptor (CAR) is recently reported as one of the effective cancer therapy. Especially CAR-T cell therapy targeting CD19 antigen in B-cell tumors have shown impressive clinical results and CAR-T cell products targeting CD19 have already approved. However as the high relapse rate is still the problem and the clinical efficacy of CAR-T cell therapy for solid tumors is currently inadequate, further improvement of CAR design is required. It is known that the design of CAR construct affects the function of CAR-T cells. For example co-stimulatory domain such as CD28 and 4-1BB is used in the second generation CARs, CD28z-CAR-T cells show higher anti-tumor activity, whereas 4-1Bz-CAR-T cells demonstrate superior in vivo persistence. To enhance survival of T cells, several attempts had been made to optimize the signaling domains. Recently, we have developed the novel CARs incorporated GITR (glucocorticoid-induced tumor necrosis factor receptor family-related protein) intracellular domain for T cell survival prolongation and inhibition of regulatory T cells’ suppressive activity. It is also reported that the antigen-nonspecific activation of CAR-T cells (tonic signaling) is influenced by the CAR design, and excessive T cell activation leads exhaustion of CAR-T cells. Previously, we have found that the design of CAR, not only single chain variable fragments (scFvs), affects the strength of tonic signaling. Thus, the optimization of CAR construct is essential to induce antigen-specific response with minimal non-specific activation, which results in maximal efficacy.

**Methods** We have optimized the structure of anti-CEA-GITR-CAR targeting CEA antigen expressing solid tumor such as gastric or pancreatic cancer. We have constructed several CARs with the different composition such as hinge region, transmembrane domain, and the order of VL/VH in scFV region, and compared the tonic signaling and antigen-specific activity in CAR-T cells.

**Results** The property of CAR-T cells was largely affected by the CAR constructs, especially the hinge region. The CAR-T cells with CD80 hinge showed strong tonic signaling, the CAR-T cells with short hinge-CAR lost antigen specificity, and elimination of hinge region lowered the CAR expression level and antigen reactivity. Furthermore, GITR-CAR-T cells showed higher proportion of CCR7+CD45RA+ cells and lower expression of exhaustion markers (PD1, Tim3, and LAG3) compared to CD28z-CAR-T cells.

**Conclusions** Our CEA-GITR-CAR with the optimized scFV design and CD28-hinge demonstrated improved antigen-
specific response with reduced tonic signaling, potentially indicating that our novel CAR-T cells may show improved clinical efficacy on solid tumor.

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100 DRUG-REGULATABLE ENGINEERED T CELLS ELIMINATE CD33+ AND CD33AE2+ AML

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Background Bioengineered T cell treatments for acute myeloid leukemia (AML) are challenged by near universal expression of leukemia antigens on normal hematopoietic stem/progenitor cells. 1, 2 ‘on target/off tumor’ activity may cause myelosuppression while sustained antigen exposure can lead to T cell exhaustion. 3 In addition, splicing variants may allow antigen escape. We hypothesize that by using a novel CD33-C2-specific single domain VHH antibody as the antigen targeting domain in dimersizing agent-regulated immunoreceptor complex T cells (DARIC T cells), we will enable pharmacologically-controllable targeting of CD33, allowing eradication of leukemia expressing either of the major splice variants of CD33: i.e., full-length CD33 or CD33AE2.

Methods We engineered DARIC-expressing lentiviral vectors containing encoding separated CD33-C2-specific antigen binding and 41BB-CD3eta signaling chains that heterodimerize following addition of rapamycin via embedded FKBP12 and FRB* domains. 4 Peripheral blood mononuclear cells were stimulated with IL-2, anti-CD3, and anti-CD28 antibodies 24h prior to transduction with DARIC33 lentivectoral vector. Surface expression of antigen binding or signaling chains was assessed using biotinylated CD33, or antibodies to VHH-domains or FRB* respectively. Rapamycin-dependent in vitro activity was measured by IFNg release. To evaluate in vivo activity, NSG mice injected with 1 × 10^7 MOLM-14/luc cells were treated 5-7 days later with 1 × 10^7 DARIC33 T cells in the presence or absence of rapamycin and tumor progression followed by luciferase activity.

Results DARIC33+ T cells bound biotinylated-CD33, anti-VHH and anti-FRB* antibodies. Rapamycin addition increased expression of both signaling and antigen-recognition chains, suggesting augmented receptor stability in the presence of dimizing drug. In the presence of rapamycin, coculture of DARIC33 T cells with cell lines expressing either full length or CD33AE2 4 showed equivalent rapamycin-dependent activation, demonstrating DARIC33 responds to both splice variants. Titration experiments showed rapamycin-dependent activation with EC50 = 25pM. Negligible IFNg release was observed in the absence of drug. DARIC33 T cells significantly extended survival of AML-bearing mice, but only when treated with rapamycin. The DARIC33 T cells were activated in vivo by sub-immunosuppressive rapamycin dosing, as weekly or 0.1 mg/kg QOD dosing led to similar levels of tumor suppression.

Conclusions DARIC33 T cells appear to be potent antileukemic agents: they are activated by AML cell lines in vitro as demonstrated by cytokine release and cytotoxicity, and significantly extend survival in an aggressive xenograft model. Temporal control provided by the DARIC architecture promises to enhance safety and potentially efficacy of CAR T therapy for AML, for example by enabling hematopoietic recovery or providing T cell rest.

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101 ENGINEERING GAMMA/DELTA T CELLS WITH THE T-CELL ANTIGEN COUPLER RECEPTOR EFFECTIVELY INDUCES ANTIGEN-SPECIFIC TUMOR CYTOTOXICITY IN VITRO AND IN VIVO

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Background Engineered T cell therapies have revolutionized treatment of relapsed refractory haematological malignancies, however the cost of treatment for autologous products remains a significant challenge to their widespread use. The high cost is driven largely by the need for personalized manufacturing of autologous cell products. A non-conventional class of T cells, the gamma/delta T cell, can be safely transplanted into an unrelated recipient without inducing graft-versus-host disease, 1 making them an ideal candidate for mass-manufactured off-the-shelf T cell therapies. We have previously described a novel method of directing conventional alpha/beta T cells towards tumour targets by co-opting the T cell receptor using the T cell Antigen Coupler (TAC) receptor. 2 Here, we describe the use of TAC receptors to engineer antigen-specific reactivity into gamma/delta T cells, resulting in highly potent anti-tumor cytotoxicity.

Methods Engineered gamma/delta T cells were manufactured by activating PBMCs with Zoledronate and IL-2. The TAC transgene was introduced into T cells using either VSV-G pseudotype lentivirus or GALV-pseudotyped gamma-retrovirus vectors. Through optimization studies, we determined transduction was highest 24 hours post-activation for lentivirus and 72 hours post-activation for gamma-retrovirus vectors. Controls were fed with IL-2 supplemented media every 2 – 3 days and enriched on Day 14 to >99% gamma/delta T cell purity using CD4/CD8 magnetic-activated cell sorting depletion (Miltenyi Biotec).

Results Both methods of gene transfer tested for our pilot study yielded excellent gene transduction (40% - 70%). Using lentivirus-engineered gamma/delta T cells, we demonstrated that the TAC receptor re-directs gamma/delta T cells to attack tumors in an antigen-specific manner. The presence of the TAC receptor did not interfere with lysis of tumor cells via the natural tumor-reactive gamma/delta T cell receptors.

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Importantly, TAC-engineered gamma/delta T cells displayed robust cytotoxicity at very low effector:target ratios (<1) and caused regression of human tumor xenografts that were otherwise resistant to non-engineered gamma/delta T cells. Curiously, gamma/delta T cell manufacturing was sensitive to the quality of the lentivirus product, where products with low titers were associated with outgrowth of conventional alpha/beta T cells. Outgrowth of alpha/beta T cells was not observed with gamma-retroviruses. We are presently evaluating the anti-tumor activity of gamma-retrovirus-engineered gamma/delta T cells.

Conclusions Off-the-shelf engineered gamma/delta T cells represent a strategy to reduce manufacturing cost and may represent the next generation of engineered T cell therapies. TAC receptors provide a robust tool for directing gamma/delta T cells to attack tumors that are otherwise resistant to gamma/delta T cells and should be evaluated further.

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Ethics Approval The study was approved by McMaster’s Animal Research Ethics Board, AUP#19-02-10.

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102 CORD-BLOOD DERIVED NK CELLS, AND CAR-T CELLS, AN ATTRACTIVE IMPROVED IMMUNOTHERAPY TREATMENT TO BE CONSIDERED FOR HEMATOLOGICAL MALIGNANCIES

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Background Multiple myeloma (MM) remains an incurable hematological malignancy where a proportion of patients relapse or become refractory to current treatments. Administration of autologous T cells modified with a chimeric antigen receptor (CAR) against B cell maturation antigen (BCMA) has achieved high percentages of complete responses. Unfortunately, the lack of persistence of CART-BCMA cells in the patient leads to relapses. On the other side, cord-blood derived natural killer cells (CB-NK) is an off-the-shelf cellular immunotherapy option to treat cancer patients with high potential due to their anti-tumor activity. However, clinical results in patients up to date have been sub-optimal. Whereas CB-NK are innate immune cells and their anti-tumor activity is developed in a few hours, CART cells are adaptive immune cells and their activity develops at later time points. Moreover, we previously described that CB-NK secrete inflammatory proteins that promote the early formation of tumor-immune cell clusters bringing cells into close contact and thus, facilitating the anti-tumor activity of T cells. Therefore, we hypothesized that the addition of a small number of CB-NK to CART cells would improve the anti-tumor activity and increase the persistence of CART cells.

Methods T cells transduced with a humanized CAR against BCMA and CB-NK were employed at 1:0.5 (CART:CB-NK) ratio. Cytotoxicity assays, activation markers and immune-tumor cell cluster formation were evaluated by flow cytometry and fluorescence microscopy. In vivo models were performed in NSG mice.

Results The addition of CB-NK to CART cells demonstrated higher anti-MM efficacy at low E:T ratios during the first 24h and in long-term cytotoxicity assays, where the addition of CB-NK to CART cells achieved complete removal of tumor cells. Analysis of activation marker CD69 and CD107a degradation from 4h to 24h of co-culturing proved differences only at 4h, where CD69 and CD107a in CART cells were increased when CB-NK were present. Moreover, CB-NK accelerated an increased formation of CART-tumor cell clusters facilitating the removal of MM cells. Of note, CB-NK addition did not increase total TNFα and IFNγ production. Finally, an in vivo model of advanced MM with consecutive challenge to MM cells evidenced that the addition of CB-NK achieved the highest efficacy of the treatment.

Conclusions Our results suggest that the addition of ‘off-the-shelf’ CB-NK to CART cells leads to a faster and earlier immune response of CART cells with higher long-term maintenance of the anti-tumor response, suggesting this combinatorial therapy as an attractive immunotherapy option for MM patients.

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103 INCLUSION OF A DAP10 COSTIMULATORY DOMAIN ENHANCES ANTI-TUMOR EFFICACY OF CHIMERIC PD1-EXPRESSING T CELLS IN MULTIPLE TYPES OF SOLID TUMORS

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Background Adoptive transfer of T cells is a promising anti-tumor therapy for many cancers. To enhance tumor recognition by T cells, chimeric antigen receptors (CAR) consisting of signaling domains fused to receptors that recognize tumor antigens can be expressed in T cells. One receptor that is a prospective target for a new chimeric antigen receptor is PD1 because the ligands for the PD1 receptor are expressed on many cancer types. Therefore, we developed a murine chimeric PD1 receptor (chPD1) consisting of the PD1 receptor extracellular domain and the activation domain of CD3 zeta. In addition, current chimeric antigen receptor therapies utilize various costimulatory domains to enhance anti-tumor efficacy. Therefore, we also compared the inclusion of CD28, Dap10, 4-1BB, GITR, ICOS, or OX40 costimulatory domains in the chPD1 receptor to determine which costimulatory domain induced optimal anti-tumor immunity.

Methods To determine if this novel CAR could potentially target a wide variety of tumors, the anti-tumor efficacy of chPD1 T cells against murine lymphoma, melanoma, kidney, pancreatic, liver, colon, breast, ovarian, prostate, and bladder cancer cell lines was measured.

Results Of the eighteen cell lines tested, all expressed PD1 ligands on their cell surface, making them potential targets for chPD1 T cells. Regardless of the costimulatory domain in the CAR, all of the chPD1 T cells induced similar levels of T cell proliferation and tumor cell lysis. However, differences were observed in the cytokine secretion profiles depending on which costimulatory receptor was included in the CAR. While
most of the chPD1 T cell receptor combinations secreted both pro-inflammatory (IFNγ, TNFα, IL-2, GM-CSF, IL-17, and IL-21) and anti-inflammatory cytokines (IL-10), chPD1 T cells containing a Dap10 costimulatory domain secreted high levels of proinflammatory cytokines but did not secrete a significant amount of anti-inflammatory cytokines. Furthermore, T cells expressing chPD1 receptors with a Dap10 domain also had the strongest anti-tumor efficacy in vivo. ChPD1 T cells did not survive for longer than 14 days in vivo, however treatment with chPD1 T cells induced long-lived protective host-antitumor immune responses in tumor-bearing mice.

Conclusions Therefore, adoptive transfer of chPD1 T cells could be a novel therapeutic strategy to treat multiple types of cancer and inclusion of the Dap10 costimulatory domain in chimeric antigen receptors may induce a preferential cytokine profile for anti-tumor therapies.

Ethics Approval The study was approved by Longwood University’s IACUC.

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**BCMA-TARGETING CAR-T CELLS EXPANDED IN IL-15 HAVE AN IMPROVED PHENOTYPE FOR THERAPEUTIC USE COMPARED TO THOSE GROWN IN IL-2 OR IL-15/IL-7**

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Background Chimeric antigen receptor-T (CAR-T) cells that target B cell maturation antigen (BCMA-CARs) have emerged as a promising treatment for multiple myeloma (MM). Despite impressive initial responses to BCMA-CAR therapy in clinical trials, relapse is common, signifying a need to improve the in vivo efficacy and persistence of BCMA-CARs.1 The development of unfavourable differentiation or T cell dysfunction, such as exhaustion and senescence, during the ex vivo expansion of the BCMA-CARs could be limiting their therapeutic potential. For CD19-directed CARs, reduced dysfunction and differentiation and improved anti-tumour responses were achieved by expanding the cells with IL-15 instead of IL-2.2 Therefore, in this study, our aim was to determine whether expanding BCMA-CARs with IL-15 or IL-15/IL-7 instead of IL-2 alters their levels of exhaustion, senescence, differentiation and activity.

Methods T cells stimulated with anti-CD3/anti-CD28-coated beads were supplemented with IL-2, IL-15, IL-15 + IL-7 or no cytokine and transduced with AR12h, a BCMA-CAR with a 4-1BB co-stimulatory domain produced at our institution.3 Expanded BCMA-CARs were analysed by flow cytometry for markers of T cell dysfunction, or challenged with MM cell line ARP-1 and then tested for cytokine production, cytotoxic ability and activation signals.

Results BCMA-CARs cultured in IL-15 or IL-15/IL-7 expanded similarly to those grown in IL-2, with comparable CAR transduction efficiencies, CD4:CD8 ratios and proliferation rates. BCMA-CARs grown in IL-15 had low expression of exhaustion marker Lag-3 and high expression of the costimulatory molecule CD27, which is important for T cell survival and persistence, when compared to BCMA-CARs cultured in IL-2. Moreover, BCMA-CARs grown solely in IL-15 were less differentiated than those supplemented with IL-7, and had higher expression of stem cell memory marker CXCR3 within the naïve population than those expanded with IL-2. When challenged with MM cell line ARP-1, IL-15-grown BCMA-CARs upregulated activation marker CD69, exhibited strong cytotoxicity and robust production of IFNγ and IL-2. However, in comparison to BCMA-CARs expanded in IL-2 or IL-15/IL-7, those grown with IL-15 had lower mTORC1 activity and p38 MAPK phosphorylation when activated by ARP-1 cells, suggesting differential regulation of key pathways for T cell metabolism and senescence, respectively.

Conclusions To summarise, BCMA-CARs expanded with IL-15 alone exhibited the most favourable phenotype for therapeutic use compared those grown with IL-2 or IL-15/IL-7. Future experiments using murine MM models will be critical in understanding the in vivo benefits or drawbacks of culturing BCMA-CARs in IL-15 compared to IL-2 or IL-15/IL-7.

Ethics Approval Research involving human material was approved by the Ethical Committee of Clinical Research (Hospital Clinic, Barcelona). Peripheral blood T cells were obtained from healthy donors after informed consent in accordance with the Declaration of Helsinki.

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**A THIRD-GENERATION HUMAN GUCY2C-TARGETED CAR-T CELL FOR COLORECTAL CANCER IMMUNOTHERAPY**

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Background Colorectal cancer (CRC) presents a significant public health burden, responsible for the second most cancer-related deaths in the United States, with an increasing incidence in young adults observed globally.1,2 While the blockade of immune checkpoints received FDA approval as a CRC therapeutic, only patients with microsatellite instability, accounting for 15% of sporadic cases, demonstrate partial or complete responses.3 We present a third-generation chimeric antigen receptor (CAR)-T cell directed towards the extracellular domain of the mucosal antigen guanylyl cyclase C (GUCY2C), which is over-expressed in 80% of CRC cases, as a therapeutic alternative for late stage disease. Here, we demonstrate that human GUCY2C CAR-T cells can selectively kill GUCY2C-expressing colorectal cancer cells in vitro and produce inflammatory cytokines in response to antigenic stimulation.

Methods Peripheral blood mononuclear (PBMCs) cells were isolated from leukoreduction filters obtained from the Thomas Jefferson University Hospital Blood Donor Center (IRB #18D.495). Magnetic Activated Cell Sorting (MACS) technology was used to negatively select pan-T cells (Miltenyi Biotec), followed by activation and expansion using anti-CD3, anti-CD2, and anti-CD28 coated microbeads (Miltenyi Biotec) and supplemented with IL-7 and IL-15 (Biological Resources...
Increasing AMPK Activity in Human T Cells Enhances Memory Subset Formation Without Sacrificing in Vitro Expansion

Erica Braverman*, Andrea Dobbs, Darlene Monlish, Craig Byersdorfer. University of Pittsburgh, Pittsburgh, PA, USA

Background The ideal adoptive cell therapy consists of memory-like T cells with enhanced oxidative potential. However, current expansion protocols drive T cells towards terminal differentiation, decreasing the number of T cells fit for the in vivo environment. AMP-activated protein kinase (AMPK), whose activity increases in memory cells, is a key regulator of mitochondrial biogenesis and oxidative metabolism, making AMPK activation an attractive candidate to improve adoptive T cell function.

Methods To increase AMPK activity, AMPKγ, which controls the phosphorylation status of AMPKα and therefore activity of the AMPK complex, was cloned into a lentiviral plasmid downstream of the elongation factor 1α (EF1α) promoter and upstream of green fluorescent protein (GFP). An empty vector, containing GFP only, served as a negative control. Human T cells were transduced and expanded in vitro in the presence of IL-2. AMPK activity was assessed via immunoblot for phosphorylation of AMPKα on Thr172 and S555 on downstream target Unc-51-like kinase 1 (ULK1). Memory-marker expression and mitochondrial density (using Mitotracker Red) were analyzed by flow cytometry. Oxidative metabolism and spare respiratory capacity (SRC) were determined using the Seahorse Metabolic Analyzer. Fold changes of in vitro expansion were calculated by adjusting manual cell counts for GFP positivity and CD4+/CD8+ staining.

Results AMPKγ was efficiently transduced and expressed by human T cells, which significantly increased AMPK activity (AMPKα phosphorylation 1.93 ± 0.05 vs 0.6 ± 0.09, p<0.001, ULK1 phosphorylation 1.28 ± 0.11 vs 0.67 ± 0.08, p<0.01). AMPKγ-overexpressing T cells augmented expression of memory markers CD62L, CD27, and CCR7, with an increased yield of stem cell memory-like T cells marked by co-expression of CD45RA and CD62L (figure 1). Mitochondrial density, SRC, and maximal oxygen consumption rates were similarly increased in AMPKγ-transduced cells (figure 2A,B). Further, while enhanced memory cell production is often linked with reduced proliferation, T cells with increased AMPK activity maintained and even trended towards increased rates of expansion compared to empty-transduced controls (figure 3A), with a measurable increase in CD4+ T cell percentages by flow cytometry (figure 3B).

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Abstract 106 Figure 1 AMPK-transduced T cells increase expression of memory surface markers. Human T cells were transduced with AMPK-GFP or GFP-only control (Empty). Memory markers were assessed by flow cytometry on Days 7–14 of in vitro culture following expansion with IL-2. Plots are representative of 3 separate donors.
Abstract 106 Figure 2  AMPK-transduced T cells show enhanced mitochondrial density and SRC. (A) Human T cells transduced with AMPK-GFP or GFP-only (Empty) were stained with Mitotracker Red and fluorescence intensity compared between transduced cells and GFP-controls within the same culture to account for variability in Mitotracker dye staining. (B) AMPK and Empty transduced T cells were assessed via Seahorse Metabolic Analyzer using the Mito Stress Test. Results are representative of 3 separate donors. OCR = O2 consumption rate

Abstract 106 Figure 3  Proliferation is maintained in AMPK-transduced T cells, with enhanced recovery of CD4+ T cells. (A) Primary human T cells transduced with AMPK-GFP or GFP-only (Empty) were expanded in vitro in the presence of IL-2. Cells were manually counted and the ratio of day 7 to day 5 cell counts calculated to assess fold expansion over time. (B) At the same, CD4+ and CD8+ percentages were measured in GFP+ cells by flow cytometry

Conclusions  Increasing AMPK activity endows T cells with a variety of characteristics ideal for adoptive cell therapy, including increased memory-marker expression, enhanced SRC and oxidative metabolism, equivalent to augmented in vitro expansion, and improved CD4+ T cell yields. Further studies are ongoing to assess the activity and function of AMPK-transduced CAR-T cells both in vitro and in vivo.

Abstract 107  EFFECTS OF IL-2 AND IL-15 ON THE PROLIFERATIVE AND ANTITUMOR CAPACITIES OF ALLOGENIC CD20 CAR-ENGINEERED γδ T CELLS IN A 3D CELL LYMPHOMA SPHEROID ASSAY

Lu Bai, Kevin Nishimoto, Mustafa Turkuz, Marissa Herman, Jason Romero, Daulet Satpayev, Stewart Abbot, Sandra Hayes, Mary Brodey*, Adicet Bio, Inc., Menlo Park, CA, USA

Background  Autologous chimeric antigen receptor (CAR) T cells have been shown to be efficacious for the treatment of B cell malignancies; however, widespread adoption and application of CAR T cell products still face a number of challenges. To overcome these challenges, Adicet Bio is developing an allogeneic γδ T cell-based CAR T cell platform, which capitalizes on the intrinsic abilities of Vδ1 γδ T cells to recognize and kill transformed cells in an MHC-unrestricted manner, to migrate to epithelial tissues, and to function in hypoxic conditions. To gain a better understanding of the requirements for optimal intratumoral CAR Vδ1 γδ T cell activation, proliferation, and differentiation, we developed a three-dimensional (3D) tumor spheroid assay, in which tumor cells acquire the structural organization of a solid tumor and establish a microenvironment that has oxygen and nutrient gradients. Moreover, through the addition of cytokines and/or tumor stromal cell types, the spheroid microenvironment can be modified to reflect hot or cold tumors. Here, we report on the use of a 3D CD20+ Raji lymphoma spheroid assay to evaluate the effects of IL-2 and IL-15, positive regulators of T cell homeostasis and differentiation, on the proliferative and antitumor capacities of CD20 CAR Vδ1 γδ T cells.

Methods  Molecular, phenotypic, and functional profiling were performed to characterize the in vitro dynamics of the intra-spheroid CD20 CAR Vδ1 γδ T cell response to target antigen in the presence of IL-2, IL-15, or no added cytokine.

Results  When compared to no added cytokine, the addition of IL-2 or IL-15 enhanced CD20 CAR Vδ1 γδ T cell activation, proliferation, survival, and cytokine production in a dose-dependent manner but were only able to alter the kinetics of Raji cell killing at low effector to target ratios. Notably, differential gene expression analysis using NanoString nCounter® Technology confirmed the positive effects of IL-2 or IL-15 on CAR-activated Vδ1 γδ T cells as evidenced by the upregulation of genes involved in activation, cell cycle, mitochondrial biogenesis, cytotoxicity, and cytokine production.

Conclusions  Together, these results not only show that the addition of IL-2 or IL-15 can potentiate CD20 CAR Vδ1 γδ T cell activation, proliferation, survival, and differentiation into antitumor effectors but also highlight the utility of the 3D spheroid assay as a high throughput in vitro method for assessing and predicting CAR Vδ1 γδ T cell activation, proliferation, survival, and differentiation in hot and cold tumors.

107  EFFECTS OF IL-2 AND IL-15 ON THE PROLIFERATIVE AND ANTITUMOR CAPACITIES OF ALLOGENIC CD20 CAR-ENGINEERED γδ T CELLS IN A 3D CELL LYMPHOMA SPHEROID ASSAY

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108  MCY-M11, A CAR-PBMC CELL PRODUCT TRANSIENTLY EXPRESSING A MESOTHELIN TARGETED MRNA CAR, EXHIBITS DESIRABLE FUNCTIONAL AND IMMUNE PHENOTYPE ATTRIBUTED TO SUSTAINED ANTITUMOR IMMUNITY IN VITRO

Sashi Kasimsetty, Himavanth Gatla, Dhana Chinnasamy *, CARMA Cell Therapies™, Gaithersburg, MD, USA

Background  MCY-M11, an anti-mesothelin CAR (Meso-CAR) mRNA transfected PBMC cell product manufactured through <1 day-process is under clinical evaluation for the treatment of advanced ovarian cancer and peritoneal mesothelioma. In this in-vitro study, we characterized the phenotypic and functional status of immune cell populations in MCY-M11 and their possible role in antitumor immunity.

Methods  MCY-M11 cell product were generated using unmanipulated healthy donor PBMCs (n=5) by transfection of Meso-CAR mRNA using MaxCyte’s proprietary Flow Electroporation® system. Frozen MCY-M11 cell product was thawed and cultured for 18 hours, then co-cultured with hMSLNneg
or hMSLNpos human mesothelioma cell line, MSTO-211H, or stimulated with anti-CD3/anti-CD28 antibodies in vitro for 8 days. Distinct cell populations in MCY-M11 were evaluated for kinetics and duration of CAR expression, differentiation, activation, exhaustion, and their ability to secrete various immunomodulatory molecules during in vitro stimulation. Antigen-specific proliferation and cytokotoxicity of MCY-M11 against hMSLNpos tumor cells as well as their ability to mount long-term antitumor immunity through epitope spreading mechanisms were studied.

**Results** Individual cell populations in MCY-M11 exhibited a consistent but transient Meso-CAR expression persisting for about 7 days. Cell subsets in MCY-M11 acquired early signs of activation and differentiation within 18–24 hours post-culture, but only attained full activation and lineage-specific differentiation upon specific response to hMSLNpos tumor cells. hMSLN antigen experienced MCY-M11 retained significant fractions of Naive and Central Memory T cells and increased percentage of Effector Memory T cells along with increased expression of CD62L, CD27, and chemokine receptors (CCR5, CCR7, and CXCR3). MCY-M11 exhibited strong antigen-specific cytokotoxicity against hMSLNpos tumor cells with corresponding increase in activation and proliferation of CD4+ and CD8+ T cell subsets and displayed low or no acquisition of known exhaustion markers. NK cells also exhibited a functionally superior molecular signature exhibiting increased levels of NKG2D, NKp44, NKp46, FAS, and TRAIL. The Monocytes and B cells in MCY-M11 also acquired an activated, differentiated, and mature phenotype, expressing molecules required for antigen presentation (HLA-DR, HLA-ABC, and CD205) and T cell co-stimulation (CD80 and CD86) to mount a strong antitumor response. These phenotypic changes in cell subsets of MCY-M11 transpired with simultaneous secretion of potent immunostimulatory molecules and chemokines facilitating an extended antitumor response through epitope spreading.

**Conclusions** We demonstrated that MCY-M11 is a unique cell product possessing a complete built-in immune cellular machinery with favorable phenotype and enhanced functions specialized in mediating an effective and long-term antitumor response.

**Trial Registration** NCT03608618

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**109** DOMINANT-NEGATIVE TGFβ RECEPTOR 2 ENHANCES GPC3-TARGETING CAR-T CELL EFFICACY AGAINST HEPATOCELLULAR CARCINOMA

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**Background** Chimeric antigen receptors (CARs) are engineered synthetic receptors that reprogram T cell specificity and function against a given antigen. Autologous CAR-T cell therapy has demonstrated potent efficacy against various hematological malignancies, but has yielded limited success against solid cancers. MEDI7028 is a CAR that targets oncofetal antigen glypican-3 (GPC3), which is expressed in 70–90% of hepatocellular carcinoma (HCC), but not in normal liver tissue. Transforming growth factor β (TGFβ) secretion is increased in advanced HCC, which creates an immunosuppressive milieu and facilitates cancer progression and poor prognosis. We tested whether the anti-tumor efficacy of a GPC3 CAR-T can be enhanced with the co-expression of dominant-negative TGFβRII (TGFβRIIDN).

**Methods** Primary human T cells were lentivirally transduced to express GPC3 CAR both with and without TGFβRIIDN. Western blot and flow cytometry were performed on purified CAR-T cells to assess modulation of pathways and immune phenotypes driven by TGFβ in vitro. A xenograft model of human HCC cell line overexpressing TGFβ in immunodeficient mice was used to investigate the in vivo efficacy of TGFβRIIDN armored and unarmored CAR-T. Tumor infiltrating lymphocyte populations were analyzed by flow cytometry while serum cytokine levels were quantified with ELISA.

**Results** Armoring GPC3 CAR-T with TGFβRIIDN nearly abolished phospho-SMAD2/3 expression upon exposure to recombinant human TGFβ in vitro, indicating that the TGFβ signaling axis was successfully blocked by expression of the dominant-negative receptor. Additionally, expression of TGFβRIIDN suppressed TGFβ-driven CD103 upregulation, further demonstrating attenuation of the pathway by this armoring strategy. In vivo, the TGFβRIIDN armored CAR-T achieved superior tumor regression and delayed tumor regrowth compared to the unarmored CAR-T. The armored CAR-T cells infiltrated HCC tumors more abundantly than their unarmored counterparts, and were phenotypically less exhausted and less differentiated. In line with these observations, we detected significantly more interferon gamma (IFNγ) at peak response and decreased alpha-fetoprotein in the serum of mice treated with armored cells compared to mice receiving unarmored CAR-T, demonstrating in vivo functional superiority of TGFβRIIDN armored CAR-T therapy.

**Conclusions** Armoring GPC3 CAR-T with TGFβRIIDN abrogates the signaling of TGFβ in vitro and enhances the anti-tumor efficacy of GPC3 CAR-T against TGFβ-expressing HCC tumors in vivo, proving TGFβRIIDN to be an effective armoring strategy against TGFβ-expressing solid malignancies in pre-clinical models.

**Ethics Approval** The study was approved by AstraZeneca’s Ethics Board and Institutional Animal Care and Use Committee (IACUC).

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**110** IN-DEPTH CHARACTERIZATION OF VARIABILITY IN APHERESIS COLLECTIONS FROM NORMAL DONOR POPULATIONS FOR ALLOGENEIC CELL THERAPY

Shawn Fahl, Shawn Fahl, Shawn Fahl*. Discovery Life Sciences, Huntsville, AL, USA

**Background** The success of autologous CAR-T cell therapies has revolutionized and accelerated development in the cell therapy field. However, the requirement for patient-specific starting material for these therapies remains an impediment to establishing availability for all patients who could benefit, highlighting the need for a highly characterized normal donor pool to generate allogeneic cell therapy material.

**Methods** We have established a network of >2800 normal donors that have been genotyped at the HLA loci (6 digits) and stratified by reactivity to common human viruses, such as cytomegalovirus (CMV) and Epstein Barr Virus (EBV). Furthermore, cell collections from 35 randomly selected donors have been screened by flow cytometry for major immune cell subsets, including T cells, B cells, NK cells, and monocytes. The T cell compartment was further characterized by
expression of activation markers (CD25, PD1, CD69) and proliferative capacity in response to anti-CD3/CD28 stimulation.

**Results** N/A

### Abstract 110 Table 1

<table>
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<tr>
<th>Cell Type</th>
<th>T Cells</th>
<th>CD4+ T Cells</th>
<th>CD8+ T Cells</th>
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<th>NK Cells</th>
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<td>21.51%</td>
<td>32.68%</td>
<td>50.58%</td>
<td>38.47%</td>
<td>32.31%</td>
</tr>
</tbody>
</table>

**Conclusions** There was substantial variability (%CV 14.52%-50.58%, see table 1) in the percentage of each immune cell population across the donor pool, which would have effects on the relative success of downstream cell manufacturing. We are evaluating additional donors to identify specific sources of variability. Collectively, these data highlight the need for in-depth genotypic and phenotypic characterization of donor populations to ensure that the most robust material is selected for each type of cell therapy manufacturing.

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### Abstract 111

**HIGHLY EFFICIENT MULTIPLEXED BASE EDITING ENABLES DEVELOPMENT OF UNIVERSAL CD7-TARGETING CAR-T CELLS TO TREAT T-ALL**

Jason Gehrie*, Aaron Edwards, Ryan Murray, Angelica Messana, Lindsey Coholan, Henry Poulin, Melissa Le, Alden Ladd, Mark Nariong, Faith Musenge, Adam Camblin, Yeh-Chuin Poh, Sarah Smith, Giuseppe Ciaramella. Beam Therapeutics, Cambridge, MA, USA

**Background** Autologous CAR-T therapies have demonstrated remarkable efficacy in treating some hematologic cancers. However, generating bespoke cell therapies creates manufacturing challenges, inconsistent products, high cost of goods, and delays in treatment that are often incompatible with effective clinical management of patients. Strategies to create universally-compatible allogeneic CAR-T therapies have been developed as a solution to these challenges. Allogeneic CAR-Ts require mitigation of graft-versus-host-disease (GvHD), host rejection of CAR-Ts, and elimination of fratricide in instances where the target (e.g. CD7) is expressed on both malignant and healthy T-cells. Many allogeneic CAR-T approaches utilize DNA double strand break (DSB)-inducing nucleases to overcome these barriers. However, simultaneous induction of multiple DSBs results in unpredictable outcomes such as large-scale genomic rearrangements, megabase-scale deletions, and reduced cell proliferation. Here we leverage base editors (BEs), which are a novel class of gene editing reagents that enable programmable single-base changes in genomic DNA without forming DSBs, to create multiplex edited, fratricide-resistant, allogeneic CAR-T cells with no detectable genomic aberrations.

**Methods** T-cell acute lymphoblastic leukemia (T-ALL) is a disease with high and consistent expression of CD7 on malignant T cells, making CD7-targeting CAR-Ts (7CAR-Ts) an attractive therapeutic agent. We developed a GMP-compatible process to create 7CAR-Ts at clinical scale by isolating T cells from healthy human donors and electroporating the cells with base editor reagents, followed by transduction with a lentiviral vector encoding a second generation anti-CD7 CAR. 7CAR-Ts were characterized for their potency and specificity in vitro and in xenograft tumor models.

**Results** Simultaneous base editing at four genomic loci resulted in 7CAR-Ts that are edited with 80–98% efficiency at each target gene, with greatly diminished risk of GvHD, CAR-T rejection, fratricide, and immunosuppression. In contrast to nuclease editing, concurrent modification of four genomic loci using BEs produced no detectable genomic rearrangements, no observable change in cell expansion, and no activation of the DNA damage-induced p53 pathway. Base edited 7CAR-Ts demonstrate robust antigen-dependent cytokine release, potent in vitro cytotoxicity, and dose-dependent in vivo tumor control.

**Conclusions** Taken together, our approach addresses limitations in CAR-T manufacturing and demonstrates that multiplexed base editing is a feasible strategy for generating universally-compatible, fratricide-resistant 7CAR-T cells, which we are advancing towards clinical development for the treatment of T-ALL. More generally, this program demonstrates the potential for base editing to create highly-engineered cell therapies featuring at least four simultaneous edits which can confer a wide range of desirable therapeutic attributes.

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### Abstract 112

**RATIONAL DESIGN OF CHIMERIC ANTIGEN RECEPTOR T CELLS AGAINST GLYPICAN 3 DECOUPLES TOXICITY FROM THERAPEUTIC EFFICACY**

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**Background** Chimeric antigen receptor (CAR)-T therapy has yielded impressive clinical results in hematological malignancies and it is a promising approach for solid tumor treatment. However, toxicity, including on-target-off-tumor antigen binding, is a concern hampering its broader use.

**Methods** In selecting a lead CAR-T candidate against the oncofetal antigen glypican 3 (GPC3), we compared CAR bearing a low and high affinity single-chain variable fragment (scFv), binding to the same epitope and cross-reactive with murine GPC3. We characterized low and high affinity CAR-T cells immunophenotype and effector function in vitro, followed by in vivo efficacy and safety studies in hepatocellular carcinoma (HCC) xenograft models.

**Results** Compared to the high-affinity construct, the low-affinity CAR maintained cytotoxic function but did not show in vivo toxicity. High-affinity CAR-induced toxicity was caused by on-target-off-tumor binding, based on the evidence that high-affinity but not low-affinity CAR, were toxic in non-tumor bearing mice and accumulated in organs with low expression of GPC3. To add another layer of safety, we developed a mean to target and eliminate CAR-T cells using anti-TNFα antibody therapy post-CAR-T infusion. This antibody functioned by eliminating early antigen-activated CAR-T cells, but not all CAR-T cells, allowing a margin where the toxic response could be effectively decoupled from anti-tumor efficacy.

**Conclusions** Selecting a domain with higher off-rate improved the quality of the CAR-T cells by maintaining cytotoxic function while reducing cytokine production and activation upon antigen engagement. By exploring additional traits of the CAR-T cells post-activation, we further identified a mechanism whereby we could use approved therapeutics and apply them as an exogenous kill switch that would eliminate early activated CAR-T following antigen engagement in vivo. By
combining the reduced affinity CAR with this exogenous control mechanism, we provide evidence that we can modulate and control CAR-mediated toxicity.

Ethics Approval All animal experiments were conducted in a facility accredited by the Association for Assessment of Laboratory Animal Care (AALAC) under Institutional Animal Care and Use Committee (IACUC) guidelines and appropriate animal research approval.

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DEVELOPMENT OF T CELL-BASED IMMUNOTHERAPIES TO TARGET DORMANT DISSEMINATED BREAST CANCER CELLS

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Background A significant fraction of breast cancer survivors develop metastases years or even decades after initial diagnosis.1–3 Mounting evidence suggests these late recurrences arise from dormant disseminated tumor cells (DTCs).4–6 However, no therapy currently exists for targeting DTCs for the purpose of metastasis prevention. Immunotherapy represents a promising avenue to target dormant DTCs. Yet, a functional relationship between adaptive immunity and dormant DTCs has not been established.

Methods Here, we have utilized a bone marrow organotypic microvascular niche co-culture model and immunocompetent murine models of breast cancer dormancy to study the relationship between the adaptive immune response and dormant DTCs and to develop immunotherapies for the purpose of eliminating dormant DTCs and preventing breast cancer metastasis.

Results Our data suggest that breast cancer cells downregulate MHC class I antigen presentation upon dormancy induction, identifying one mechanism of immune evasion. Strikingly, outgrowing metastases re-express MHC I and presumably upregulate antigen presentation. These data suggest that MHC-dependent T cell-based immunotherapies may not effectively kill dormant DTCs, but that MHC-independent chimeric antigen receptor (CAR) T cells may be more applicable. Using the organotypic bone marrow microvascular niche co-culture system, we have shown that CAR T cells kill both proliferating and dormant tumor cells independent of tumor cell localization in the niche and independent of tumor cell cycle status. Further, we have established preclinical immunocompetent murine models of breast cancer dormancy to compare efficacy of engineered T cell receptor (TCR) and CAR T cells in eliminating dormant DTCs. From these models of breast cancer dormancy, we have found that CAR T cells eliminate both overt metastases and DTCs in the lung and bone marrow of mice. In contrast, preliminary data suggest that TCR T cells clear overt metastases but are less effective in clearing dormant disease, lending support that MHC I downregulation during dormancy may impact the efficacy of various T cell-based immunotherapies.

Conclusions Our findings identify CAR T cells as one potential immunotherapy to eradicate dormant disease, while simultaneously identifying both CAR and TCR T cells as effective treatments for the clearance of overt metastases. In sum, our findings lay the groundwork for developing adoptive cell therapies to eliminate dormant disease and prevent death from breast cancer metastasis.

REFERENCES

PRECLINICAL DEVELOPMENT OF A NOVEL iPSC-DERIVED CAR-MICA/B NK CELL IMMUNOTHERAPY TO OVERCOME SOLID TUMOR ESCAPE FROM NKG2D-MEDIATED MECHANISMS OF RECOGNITION AND KILLING

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Background MHC class I related proteins A (MICA) and B (MICB) are induced by cellular stress and transformation, and their expression has been reported for many cancer types. NKG2D, an activating receptor expressed on natural killer (NK) and T cells, targets the membrane-distal domains of MICA/B, activating a potent cytotoxic response. However, advanced cancer cells frequently evade immune cell recognition by proteolytic shedding of the α1 and α2 domains of MICA/B, which can significantly reduce NKG2D function and the cytolytic activity.

Methods Recent publications have shown that therapeutic antibodies targeting the membrane-proximal α3 domain inhibited MICA/B shedding, resulting in a substantial increase in the cell surface density of MICA/B and restoration of immune cell-mediated tumor immunity.1 We have developed a novel chimeric antigen receptor (CAR) targeting the conserved α3 domain of MICA/B (CAR-MICA/B). Additionally, utilizing our proprietary induced pluripotent stem cell (iPSC) product platform, we have developed multiplexed engineered, iPSC-derived CAR-MICA/B NK (iNK) cells for off-the-shelf cancer immunotherapy.

Results A screen of CAR spacer and ScFv orientations in primary T cells delineated MICA-specific in vitro activation and cytotoxicity as well as in vivo tumor control against MICA+ cancer cells. The novel CAR-MICA/B design was used to compare efficacy against NKG2D CAR T cells, an alternative MICA/B targeting strategy. CAR-MICA/B T cells showed superior cytotoxicity against melanoma, breast cancer, renal cell carcinoma, and lung cancer lines in vitro compared to primary NKG2D CAR T cells (p<0.01). Additionally, using an in vivo xenograft metastasis model, CAR-MICA/B T cells eliminated A2058 human melanoma metastases in the majority of the mice treated. In contrast, NKG2D CAR T cells were unable to control tumor growth or metastases. To translate CAR-MICA/B functionality into an off-the-shelf cancer immunotherapy, CAR-MICA/B was introduced into a clonal master engineered
**ENGINEERED T CELLS DIRECTED AT TUMORS WITH DEFINED ALLELIC LOSS**

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**Background**  Cell therapy, with all its promise as a powerful solid-tumor modality, is still hampered by the fundamental obstacle of cancer therapy: the acute shortage of truly tumor-specific targets. It is well known that an average tumor contains loss of heterozygosity (LOH) at an astonishing frequency: ~20% genome wide. These losses are irreversible and absolutely distinguish the cancer from normal cells.

**Methods**  We describe a novel approach to cancer immunotherapy that draws on LOH as a large, so far untapped source of cancer targets. To exploit such allelic losses, we focus on polymorphic loci and target the remaining allelic product of a locus that has LOH. We engineer T cells with a modular signal-integration circuit designed to be activated only by tumor cells that have lost expression of one specific allele on their surface.

**Results**  We use the HLA locus which undergoes LOH at a frequency of 13%, and the HLA-A*02 allele specifically, as proof of concept. We present a large body of quantitative in vitro data, along with in vivo data, that support the use of a synthetic signal-integration circuit called Tmod as a cancer therapy. We also describe Tmod’s mechanistic properties, including thorough structure/function analysis of its components.

**Conclusions**  LOH is a rich source of new targets, provided a system of sufficient power can be devised to exploit them. Our Tmod signal integration system confers on engineered T cells the capacity to discriminate effectively between normal and tumor cells that contain specific allelic losses.

**Ethics Approval**  The animal study was approved by Explora BioLabs’ Ethics Board, protocol number EB17-010-059.

**REFERENCE**


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**MULTI-ANTIGEN TARGETING OF HETEROGENOUS SOLID TUMORS USING CAR T CELLS SECRETING BI-SPECIFIC T-CELL ENGAGERS**

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**Background** Although CAR T cells have been shown to be effective and potent in treating several hematologic malignancies, engineered T-cell therapies have had limited success in addressing solid tumors. Unlike liquid tumors where uniformly expressed antigens are accessible and can be effectively targeted, tumor access and antigen heterogeneity are a significant barrier to the successful development of CAR-T cells in solid tumors.

**Methods**  Here we demonstrate that the combination of a bi-specific T-cell engager (BiTE) targeting EpCAM with a CAR T cell targeting HER2 enhances the in vitro and in vivo anti-tumor activity against heterogeneous solid tumors.

**Results**  We observed a dose-dependent enhancement of cytolytic activity when EpCAM-specific BiTEs were titrated alongside 4D5-based HER2-specific CAR T cells against HER2low tumors, enhancing maximal cytolysis by two-fold compared to CAR T cells alone (figure 1). Moreover, the escape of HER2-low tumor cells in mixed heterogeneous culture systems was circumvented by the combination of HER2-specific CAR T cells and EpCAM-specific BiTEs. The enhancement of efficacy was further demonstrated in an established HER2low MDA-MB-231 xenografts. HER2-specific CAR T cells were unable to contain Her2low tumors, whereas tumor growth was effectively controlled in mice receiving both EpCAM-specific BiTEs and HER2-specific CAR T cells.

**Abstract 116 Figure 1**  EpCAM specific BiTEs supplement CAR-T efficacy in vitro (A) HER2 and EpCAM expression of SKOV3, MDA-MB-231, and K562 tumor cells was assessed by flow cytometry. (B) HER2 specific CAR-T rapidly targeted and lysed HER2High SKOV3 tumor cells as measured via xCelligence RTCA assay. (C) SKOV3 were co-cultured with untransduced CD8+ T cells and the indicated concentrations of EpCAM BiTE and specific cytolysis was assessed. (D) MDA-MB-231 (HER2low) tumor cells were co-cultured with HER2 CAR-T ± EpCAM BiTE and specific cytolysis was determined.

**Conclusions**  Collectively, these data demonstrate that multi-antigen targeting mediated by BiTEs and CARs extends overall anti-tumor efficacy in preclinical models of heterogenous solid
RAPID POINT-OF-CARE SUBCUTANEOUS CAR-T FROM BLOOD DRAW TO INJECTION IN 4 HOURS WITH MODIFIED LV ENCODING CARS AND SYNTHETIC DRIVER ELEMENTS ENABLES EFFICIENT CAR-T EXPANSION AND TUMOR REGRESSION

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**Background** Adptive cellular therapy with chimeric antigen receptor (CAR)-T cells has demonstrated remarkable clinical activity in a number of hematologic malignancies, but product chain of custody, individualized manufacturing, preparative chemotheraphy, and patient management present technical and logistical hurdles to broader implementation.

**Methods** Lentiviral constructs for CARs (either CD19- or CD22-directed) co-expressed with a synthetic driver domain were identified from a >6 × 10^6 diversity combinatorial library of proliferative elements, transmembrane domains, leucine zippers, and an EGFR epitope screened for cellular expansion in a lymphoreplete model. Modified serum-free-lentiviral manufacturing process was developed to reduce complexity of CAR-T and to introduce CD3-activating elements into the viral envelope allowing activation and transduction of resting lymphocytes from peripheral blood.

**Results** Four-hour exposure of as little as 1 ml of blood to the CD3-directed CD19-targeted CAR encoding lentivirus followed by subcutaneous injection in NSG mice bearing CD19+/CD22+ Raji cells resulted in tumor regression (figure 1) and robust CAR-T cell expansion as determined by flow cytometry (figure 2) and qPCR (table 1), with peak levels >10,000 CAR-T cells/ml and less than three CAR copies per genome. In contrast, administration of the same products intravenously failed to support significant CAR-T expansion or control tumor growth (figure 3). Regression of established Raji tumors was also observed in NSG-(KbDb) (IA) animals following SC administration of CD19 or CD22 CARs with driver domains. CAR-T cells contracted in peripheral blood following tumor regression. Regression of Raji tumor from the initial median volume of 151 mm^3 throughout 40 days post subcutaneous administration of the LV transduced (at MOI 1 or 5) CD19-directed CAR T product (1M or 5M cells) in the NSG mice.

**Conclusions** We conclude that through a synthetic subcutaneous lymph node approach with modified lentiviruses and driver domains, rPOC SC may enable CAR-T generation with reduced complexity, while maintaining the ability of CAR-T cells to expand, persist and exert anti-tumor activity.

**Ethics Approval** All animal studies were IACUC approved.

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IL-6 IS CRITICAL FOR MEMORY RESPONSES ELICITED BY TH17 CELLS TO TUMORS

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**Background** Translation of novel T cell therapies is limited by cost and time-consuming protocols involving long-term T cell
expansion. We found that shortening ex vivo expansion of either TCR-specific murine Th17 cells or human CAR Th17 cells licenses the cell product to eradicate large tumors in low doses and generate long-lived memory against tumor.1 Therapeutic Th17 cells induce the systemic release of IL-6, IL-17, GM-CSF, and MCP-1 among other cytokines in tumor-bearing hosts, reminiscent of clinical cytokine release syndrome. As the toxicity of cytokine release is managed in patients through IL-6 blockade, we addressed the impact of IL-6 on efficacy and durability of Th17 cell therapy. We hypothesized that IL-6, induced by Th17 cells, was fueling the durable memory properties of this cell product.

Methods Th17 cells were expanded ex vivo using the TRP-1 transgenic mouse model in which CD4+ T cells express a TCR that recognizes tyrosinase-related protein 1 on melanoma. Naïve CD4+ T cells were polarized to the Th17 phenotype and infused into mice with B16F10 melanoma after a nonmyeloablative total body irradiation (5 Gy) preparative regimen.

Results IL-6 blockade, targeting either IL-6R or neutralization of the cytokine, did not significantly impact the primary immune response of adoptively transferred Th17 cells against tumor. However, administering IL-6 blockade acutely after Th17 transfer resulted in a higher incidence of tumor relapse upon secondary tumor challenge, thereby compromising long-lived antitumor immunity.1 Mounting a secondary response to tumor was dependent on CD4+ T cells, but not CD8+ T cells, persisting in the host. Mechanistically, IL-6 blockade reduced pSTAT3 and Bcl2 in transferred T cells but did not greatly impact the concentration of other systemic cytokines. As a small fraction of Tregs remain in the Th17 cell product ex vivo, we examined the engraftment of those Tregs after transfer. IL-6 was critical to suppress engraftment of FoxP3+ donor T cells from the CD4+ T cell product. Thus, IL-6 promoted robust tumor infiltration by donor effector over regulatory cells for early Th17 cells relative to cell products expanded longer durations ex vivo.1

Conclusions Overall, short-term expanded Th17 cells uniquely induced IL-6 unlike Th17 cells expanded longer ex vivo. IL-6 promoted Th17 survival, reduced engraftment of tumor-specific Tregs, and was critical to durable memory. This work may suggest that the universal strategy to inhibit IL-6 during cytokine release syndrome may come at the expense of long-term efficacy for specific cell therapy approaches.

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GUANYLYL CYCLASE C AS A TARGET FOR CAR-T CELL THERAPY IN A METASTATIC GASTRIC CANCER MODEL

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Background Gastric cancer is the sixth most common cancer and second-leading cause of cancer-related mortality worldwide.1 The heterogenous and genetically complex nature of this disease underlies the challenges in developing effective therapies for metastatic gastric cancer. In the majority of cases, stomach tumors evolve from intestinal metaplasia resulting in ectopic expression of the enterocyte differentiation antigen guanylyl cyclase C (GUCY2C) by ~50% of primary and metastatic tumors.

Methods mRNA-generated MUC1C CAR-T cells were evaluated for specificity and function by degranulation assay against various solid tumor and normal cells and cell lines. Autologous and allogeneic MUC1C CAR-T cells were produced using the piggyBac® DNA Modification System, a non-viral CAR-T manufacturing method that produces CAR-T products with an exceptionally high percentage of T stem cell memory (TSCM) cells. To produce allogeneic cells, multiplex editing of both TRBC and B2M was performed with the Cas-CLOVER™ Site-Specific Gene Editing System to reduce or eliminate GvHD and host versus graft alloreactivity, respectively.

Results Specific degranulation of transiently-expressing CAR+ T cells was observed against multiple tumor cells, with no observable activity against normal human primary cells. In assay of stable P-MUC1C-101 CAR-T cells, more than 95% expressed CAR, and were comprised of an exceptionally high-percentage of TSCM cells (CD45RA+CD62L+CD45RO-). In vitro, P-MUC1C-ALLO1 cells specifically proliferated, lysed, and secreted IFN-γ against MUC1+ breast and ovarian tumor cell lines. In breast cancer in vivo xenograft model, both unedited (MUC1C CAR-T) and edited (P-MUC1C-ALLO1) MUC1C CAR-T eliminated established, triple negative MDA.MB.468 tumor cells to undetectable levels, demonstrating the efficacy of the MUC1C CAR-T and the robustness of the allogeneic platform. In the OVCA3 xenograft model, intraperitoneally administered MUC1C CAR-T eliminated established tumor cells to levels below the limit of detection.

Conclusions P-MUC1C-ALLO1 is Poseida’s allogeneic CAR TSCM product that has a potential to treat multiple MUC1-expressing indications. P-MUC1C-ALLO1 displayed in vitro specificity for tumor vs normal cells, and in vivo efficacy against xenograft models of breast and ovarian cancer. We anticipate an IND filing and initiation of a Phase 1 clinical trial in 2021.

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metastatic gastric cancers.2–4 In the context of the efficacy of GUCY2C-directed chimeric antigen receptor (CAR)-T cells against metastatic colorectal cancer in animal models,5,6 we hypothesized that this adoptive cell therapy may be effective against metastatic gastric cancer.

**Methods** Here, we explored the efficacy of GUCY2C-directed CAR-T cells for gastric cancer in a patient derived xenograft (PDX) tumor model. Also, we interrogated translational GUCY2C biomarker assays using RT-qPCR, immunoblot analysis, and immunohistochemistry (IHC) for the intended purpose of identifying patients whose tumors express GUCY2C and could benefit from GUCY2C-directed CAR-T cell therapy.

**Results** GUCY2C-directed CAR-T cells significantly reduced subcutaneous T84 colorectal tumor growth, producing a 5-fold reduction in tumor volume, compared to control treated tumors. GUCY2C-directed CAR-T cells produced no response in tumors produced from the GUCY2C-deficient colorectal cancer cell line, SW480. Importantly, GUCY2C-directed CAR-T cells controlled gastric cancer PDX growth, maintaining a >12-fold reduction in tumor volume compared to control and in some cases produced complete tumor elimination. Furthermore, IHC based assays, indicate that antibodies developed in our laboratory may be suitable for development of a companion diagnostic for GUCY2C-directed CAR-T cells. Indeed, the commercial polyclonal antibody demonstrated robust, non-specific staining regardless of tissue type or GUCY2C mRNA profile, while novel monoclonal antibodies produced in our laboratory primarily detected protein localized to the membrane of glandular epithelial cells, demonstrating antigen specificity, and indicating their potential for further development in diagnostic companion assays to identify gastric cancer patients who may benefit from GUCY2C-directed CAR-T cell therapy.

**Conclusions** GUCY2C-directed CAR-T cells prevented the growth of, and at times eliminated, a subcutaneous gastric cancer PDX model. In the context of previously established safety in mouse models, additional studies defining the efficacy of GUCY2C-directed CAR-T cells in gastric cancer models may allow future translation of this therapy to patients with advanced gastric cancers. Concurrent development of a novel companion diagnostic IHC assay would permit identification of the ~50% of gastric cancer patients whose tumors express GUCY2C and could benefit from this therapy.

**Acknowledgements** This work was supported by a DeGregorio Family Foundation Award and by the Department of Defense Congressionally Directed Medical Research Programs (W81XWH-17-1-0299, W81XWH-191-0263, and W81XWH-19-1-0067) to AES. SAW is supported by the National Institutes of Health (NIH) (R01 CA204881, R01 CA206026, and P30 CA56036), the Defense Congressionally Directed Medical Research Program W81XWH-17-PRCRPT-TTSA, and Targeted Diagnostic & Therapeutics. SAW and AES were also supported by a grant from The Courtney Ann Diacont Memorial Foundation. SAW is the Samuel M.V. Hamilton Professor of Thomas Jefferson University. AZ was supported by NIH institutional award T32 GM008562 for Postdoctoral Training in Clinical Pharmacology. The authors thank the NCI Patient-Derived Models Repository for their support and resources to make this research possible. The authors also thank the Sidney Kimmel Cancer Center Translational Research & Pathology Core Facility, and the Office of Animal Resources at Thomas Jefferson University for their continued technical assistance and support in this research.

**Ethics Approval** This study was approved by the Thomas Jefferson University Institutional Review Board (#14.0204) and the Institutional Animal Care and Use Committee (Protocol #01529).

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and, upon binding, release a CD73-blocking scFv to inhibit the accumulation of extracellular ADO and mitigate immunosuppression of NK cells. Such localized response enhances specificity and reduces off-target effects of NK-based targeting. **Results** Primary NK cells were successfully electroporated to express our synthetic TIGIT-synNotch construct, as evidenced by increased expression levels of TIGIT (% and MFI) (figure 1). To evaluate the functionality of engineered NK cells against GBM targets, we tested the cytotoxicity of our engineered NK cells against a primary, patient-derived GBM cell line, GBM43. Overall, cytolytic function of engineered NK cells against GBM was significantly higher than that of non-engineered NK cells, with or without CD73 (10 ug/mL) and TIGIT (50 ug/mL) antibodies, for E:T ratios of 5:1 and 10:1 (figure 2), demonstrating the functional efficacy of our genetic construct. Further, engineered NK cells (T-PNK) expressed significantly higher levels of CD107a in response to GBM43 stimulation than non-engineered PNK at E:T ratios 2.5:1 and 10:1 (figure 3).

**Conclusions** Overall, we have shown that co-targeting CD155 and CD73 in a localized, responsive manner can dampen immunosuppression and significantly enhance the killing potential of engineered NK cells against aggressive patient-derived GBM tumors.

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**124 OPTIMIZING THE GENERATION FROM UMBILICAL CORD BLOOD OF ‘OFF-THE-SHELF’ CD19-CHIMERIC ANTIGEN RECEPTOR (CAR) EXPRESSING T CELLS**

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**Background** T lymphocytes expressing CD19-chimeric antigen receptor (CAR) showed the improvement of overall survival of patients with B-cell malignancies. Allogeneic CAR-T cells can overcome the limitation of the availability of patient’s lymphocytes, reducing the waiting time for the treatment and decreasing the cost of manufacturing. This study is aimed at the optimizing the generation of ‘off-the-shelf’ CAR-T cells utilizing Umbilical Cord Blood (UCB) to isolate T lymphocytes.

**Methods** UCBs have been collected at the time of childbirth from volunteer pregnant women at Sidra Medicine. Following the magnetic depletion of non-T cells, UCB-T lymphocytes were activated in vitro for 48 hr. by agonistic CD3/CD28 mAbs either conjugated to magnetic beads (Dynabeads) or to a colloidal polymeric nanomatrix (TranAct; Miltenyi Biotec). T cells generated in vitro were either i. untransduced (UT), or transduced with lentiviral encoding for ii. CD19-CD28z or iii. CD19-4-1BBz CARs. N=32 T cell cultures have been generated from fresh UCB (N=3) and, as control, from the peripheral blood lymphocytes of healthy donors (PBL; N=3) and used for deep phenotype analyses (28 markers) at different time points (Day +9 and Day+14) of the in vitro culture. Cytokines, perforin and granzyme B release (EliSpot or
FluoroSpot and cytotoxic activity (Delfia assay) have been assessed upon the co-culture with CD19+ or CD19- target cells.

Results Enrichment of CD4+CAR+ T cells, besides CD8 +CAR+, were observed in UCB-CAR- vs. PBL-CAR-T cells (40–59% of positive cells; as well as of CD45RA+ cells (40–60 vs. 20–30% of positive cells; p<0.05). The preferential selection of early stage of differentiation (CCR7+CD28 +CD27+CD137+CD62L+) for CAR-T cells isolated from both source of lymphocytes occurred. LAG3 and TIM-3 expressing T cells were found with higher frequency in UCB- vs. PBL-CAR-T cells, with superior association with CD4+ UCB-derived cells. CD19-CAR-T cells secreted IFN-g(300–400 N. spot/10 × 10⁴ T cells), regardless the co-stimulatory molecules (CD28z vs 4-1BBz), upon the engagement of CAR by CD19. A minority of IL-4 releasing T cells was found for few CAR-T cells activated with TransAct. IFN-gamma secreting CAR-T cells simultaneously released IL-2, Granzyme B and Perforin but not IL-5 and IL-17, thus belonging to TH-1/effector subset. The cytotoxic activity of these T cells against CD19+ target cells was also determined by europium release assay. Differential gene expression profile was determined in UCB-CAR-T vs. PBL-CAR-T cells bearing the different CARs following the co-culture with either CD19+ or CD19- target cells.

Conclusions The deep characterization of CD19-CAR-T cells contributed to validate the generation of anti-tumor ‘off-the-shelf’ CAR-T cells from UCB.

Ethics Approval The study was approved by Sidra Medicine Ethics Approval contributed to validate the generation of anti-tumor ‘off-the-shelf’ CAR-T cells from UCB.

Methods A combination of published and new data led us to test this hypothesis with current technology, including RNA hybridization in situ and further analysis of the clinical TCR's specificity to MAGE-A12 and other antigens.

Results We find that a key prediction of the MAGE-A12 toxicity hypothesis, the existence of rare, high-MAGE-A12-expressing cells in the brain, is not supported by the data. Our results imply that an alternative related peptide from the EPS8L2 protein is more likely responsible for the toxicity.

Therefore, it may be valuable to reconsider MAGE-A3 as an onco-testis antigen widely expressed in tumors and largely absent from normal adult tissues, our findings suggest that MAGE-A3 may deserve further consideration as a cancer target. We have identified CARs with selectivity profiles consistent with a cell therapeutic directed against HLA-A*02-positive, MAGE-A3-expressing cancers. The relative merits of TCRs and CARs for this target will be discussed.

REFERENCE


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Conclusions Given the qualities of MAGE-A3 as an onco-testis antigen widely expressed in tumors and largely absent from normal adult tissues, our findings suggest that MAGE-A3 may deserve further consideration as a cancer target. We have identified CARs with selectivity profiles consistent with a cell therapeutic directed against HLA-A*02-positive, MAGE-A3-expressing cancers. The relative merits of TCRs and CARs for this target will be discussed.

Abstract 126 Figure 1 Early-CAR-T protocol, including Naïve-T cells purification and expansion in IL-7 and IL-15 promotes the maintenance of a TSCM and TCM phenotype. A) Scheme of the 7-day production protocol for Early-CAR-T cells. B) Phenotype by FACS of the conventional CAR-T cells and the Early-CAR-T cells. Pooled data in triplicate for 6 donors. C) Phenotype by Mass cytometry comparing the Conventional-CAR-T cells vs Early-CAR-T cells vs Early-CD8-CAR-T cells. Data for one donor representative of 3 different donors.

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Background Chimeric antigen receptor (CAR-T) cells are a promising new therapy for patients with cancer. However, in contrast to their success in B cell malignancies, CAR-T cells targeting solid cancers have had limited success so far due to their poor proliferation and poor long-term persistence in vivo. To address this issue, we used naïve T cells to generate second-generation CAR-T cells recognizing the tumor antigen Lewis Y (LeY), termed ‘early’ CAR-T cells.

Methods Purified naïve T cells were activated by CD3/CD28 soluble tetrameric antibody complex, retrovirally transduced (LeY scFv-CD3z-CD28 CAR) and expanded in IL-7/IL-15. The early LeY CAR-T cell function was tested in vitro for cytotoxicity (Cr-release and degranulation), proliferation, and cytokine secretion by CBA, either de novo or following chronic stimulation for 1 month. Finally, early CAR-T cell persistence and anti-tumor efficacy was assessed in the OVCAR3-NSG model, in the presence or absence of anti-PD-1.

Results The early-CAR-T cells comprised stem cell memory-like (CD95+, CD62L+, CD45RA+) and central memory phenotype (CD95+, CD62L+, CD45RA-) T cells with increased expression of ICOS, Ki67, TCF7 and CD27 (Figure 1). The early-CAR-T cells retained potent antigen-specific cytotoxicity, and secreted significantly higher levels of cytokines (IFN-γ, TNF-α and IL-2) and increased proliferation compared to conventional CAR-T cells. Importantly, early-CAR-T cells had a significantly higher proliferative capacity after long-term chronic stimulation compared to conventional CAR-T cells (figure 2), and CD4+ CAR-T cells were critical for effective early CD8+ CAR-T cell proliferation capacity in vitro (figure 3). Early CAR-T cells had significantly better in vivo tumor control compared to conventional...
CAR-T cells (Figure 4), this was associated with increased CAR-T cell persistence. Because chronically stimulated early-LeY-CAR-T cells expressed PD-1 (figure 2), and OVCAR-3 cells expressed PD-L1 when co-cultured with LeY-CAR-T cells (figure 5), we combined early LeY-CAR-T cells with anti-PD-1 therapy and observed complete tumour regression in these mice. Interestingly, early LeY-CAR-T cell plus anti-PD-1 treatment also enhanced the percentage of circulating stem-cell memory like CAR-T cells in vivo (figure 5).

Conclusions
Our early CAR-T cells have better cytokine secretion and proliferation than conventional CAR-T cells. Early CAR-T cells also have superior anti-tumor efficacy in vivo, they have better persistence and maintain the circulating T cell memory pool. Importantly, low dose early-LeY-CAR-T cells combined with anti-PD1-treatment leads to complete clearance of LeY+ solid tumors in vivo. The early CAR-T cell production protocol is directly translatable for improving CAR-T cell efficacy in clinical trials for patients with solid tumors.

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Abstract 126 Figure 5 Anti-PD1 treatment enhances the efficacy of the Early-CAR-T cells. A) Upregulation of PD-1 on OVCAR3 when expanded in the supernatant from co-culture of OVCAR3 with LeY-CAR-T cells. B) Design of the in vivo experiment (n=7 mice per group). C) T-cell persistence, phenotype and anti-human IgG4 in peripheral blood were measured by FACS. D) Tumor kinetic of OVCAR-bearing NSG mice treated with Early-CAR-T cells or Early-CAR-T cells + Nivolumab

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Abstract 126 Figure 5 Anti-PD1 treatment enhances the efficacy of the Early-CAR-T cells. A) Upregulation of PD-1 on OVCAR3 when expanded in the supernatant from co-culture of OVCAR3 with LeY-CAR-T cells. B) Design of the in vivo experiment (n=7 mice per group). C) T-cell persistence, phenotype and anti-human IgG4 in peripheral blood were measured by FACS. D) Tumor kinetic of OVCAR-bearing NSG mice treated with Early-CAR-T cells or Early-CAR-T cells + Nivolumab

Background Natural killer (NK) cells are highly effective and fast-acting cytolytic cells capable of eradicating target cells with limited adverse effects such as cytokine release syndrome (CRS) or graft-versus-host disease. Chimeric antigen receptors (CARs)-engineered NK cells have been recently used against leukemia with encouraging clinical outcomes. The surface antigen CD19, expressed by B-lymphoblasts, represents an ideal CAR target against B cell acute lymphoblastic leukemia (B-ALL). We developed a highly potent CD19-directed CAR NK cell therapy, NKX019, with an extended in vivo half-life aimed at killing CD19-expressing target.

Methods NK cells isolated from healthy PBMCs were expanded in the presence of NKSTIM cells, IL-2, IL-12, IL-18 and transduced with both a CD19-targeted CAR construct and a membrane-bound form of IL-15 (mIL-15). Control (non-engineered) NK cells were produced in parallel. Cytotoxic activity of NKX019 against CD19+ B-ALL cell line (REH), pre-B ALL cell line (Nalm-6), allogeneic PBMCs was assessed using Incucyte® or flow cytometry. NSG mice bearing either Nalm-6.fluc (Nalm6) or REH.fluc (REH) tumor received different concentrations of NKX019 or control NK cells. In-life analysis of tumor-bearing and naïve NSG mice include: 1) bioluminescence imaging, 2) clinical observations, 3) serum cytokines and 4) CAR+ NK cell persistency.

Results
NKX019 showed enhanced cytolytic activity against REH and Nalm-6 tumor cells compared to control NK cells and CAR19+ T cells. The superiority of NKX019 over CAR19+ T cells was more pronounced at the earlier time point (24 hours) with near identical calculated EC50 observed at 72 hours for both cell types. Increased cytolytic activity of NKX019 was limited to CD19+ cells in bulk PBMCs. Consistent with our in vitro observations, NKX019 controlled Nalm-6 and REH tumor growth in doses as low as 2 × 106 cells/kg for up to 30 days with no apparent increase in cytokines commonly associated with CRS. Increased Nalm-6 tumor growth coincided with an apparent decrease in measurable NKX019 in the periphery. In tumour-naïve NSG mice, NKX019 was detectable in the blood for up to 9 weeks post-infusion consistent with its extended half-life.

Conclusions
NKX019 expresses mIL-15 and is produced in the presence of IL-12 and IL-18, resulting in enhanced in vitro expansion and longer in vivo half-life than non-engineered NK cells. NKX019 also exhibited advantages compared to CAR19+ T cells including faster cytotoxic kinetics and limited production of cytokines associated with CRS. A first-in-human trial of NKX019 in B cell malignancies is planned for 2021.

Ethics Approval
The animal procedures described in this abstract were conducted in accordance with Explora BioLabs Animal Care and Use Protocol approved by Explora BioLabs Institutional Animal Care and Use Committee.

REFERENCE
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DEVELOPMENT OF AN M1-POLARIZED, NON-VIRAL CHIMERIC ANTIGEN RECEPTOR MACROPHAGE (CAR-M) PLATFORM FOR CANCER IMMUNOTHERAPY

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Background We have previously developed CAR-M as a novel cell therapy approach for the treatment of solid tumors.1 CAR-M have the potential to overcome key challenges that cell therapies face in the solid tumor setting – tumor infiltration, immunosuppression, lymphocyte exclusion – and can induce epitope spreading to overcome target antigen heterogeneity. While macrophages transduced with the adenoviral vector Ad5f35 (Ad CAR-M) traffic to tumors, provide robust anti-tumor activity, and recruit and activate T cells, we sought to identify a robust non-viral method of macrophage engineering in order to reduce the cost of goods, manufacturing complexity, and potential immunogenicity associated with viral vectors.

Methods As innate immune cells, macrophages detect exogenous nucleic acids and respond with inflammatory and apoptotic programs. Thus, we sought to identify a means of mRNA delivery that avoids recognition by innate immune sensors. We screened a broad panel of mRNA encoding an anti-HER2 CAR comprising multiplexed 5'Cap and base modifications using an optimized and scalable electroporation approach and evaluated the impact of interferon-β priming on CAR-M phenotype and function.

Results We identified the optimal multiplexed mRNA modifications that led to maximal macrophage viability, transfection efficiency, intensity of CAR expression, and duration of expression. Non-viral HER2 CAR-M phagocytosed and killed human HER2+ tumor cells. Unlike Ad CAR-M, mRNA CAR-M were not skewed toward an M1 state by mRNA electroporation. Priming non-viral CAR-M with IFN-β induced a durable M1 phenotype, as shown by stable upregulation of numerous M1 markers and pathways. IFN-β priming significantly enhanced the anti-tumor activity of CAR but not control macrophages. IFN-β primed mRNA CAR-M were resistant to M2 conversion, maintaining an M1 phenotype despite challenge with various immunosuppressive factors, and converted bystander M2 macrophages toward M1. Interestingly, priming mRNA CAR-M with IFN-β significantly enhanced the persistence of CAR expression, overcoming the known issue of rapid mRNA turnover. RNA-seq analysis revealed that IFN-β priming affected pathways involved in increasing translation and decreasing RNA degradation in human macrophages.

Conclusions We have established a novel, optimized non-viral CAR-M platform based on chemically modified mRNA and IFN-β priming. IFN-β priming induced a durable M1 phenotype, improved CAR expression, improved CAR persistence, led to enhanced anti-tumor function, and rendered resistance to immunosuppressive factors. This novel platform is amenable to scale-up, GMP manufacturing, and represents an advance in the development of CAR-M.

REFERENCE

A NOVEL CAR CONDUCTING ANTIGEN-SPECIFIC JAK-STAT SIGNALS DEMONSTRATES SUPERIOR ANTITUMOR EFFECTS WITH MINIMAL UNDESIRED NON-SPECIFIC ACTIVATION

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Background Despite recent impressive successes in chimeric antigen receptor (CAR)-T cell therapy, there are still considerable clinical challenges. To improve T cell persistence and anti-tumor effect, which are critical for clinical responses, various efforts have been made to optimize the CAR design such as the inclusion of a costimulatory domain(s). It is known that non-specific activation of CAR-T cells is greatly influenced by the CAR design, and excessive T cell activation leads exhaustion of T cells and depletion of naïve/memory subsets important for durable clinical responses. Thus, the CAR construct needs to be optimized so that transduced T cells persist and induce potent antigen-specific response with reduced non-specific activation. For optimal T cell activation and proliferation, three signals including TCR (signal 1), co-stimulatory (signal 2), and cytokine (signal 3) signals, are essential. The conventional second and third generation CARs containing CD3ζ and a co-stimulatory domain such as a signal domain of CD28 and 4-1BB can conduct signal 1 and 2, but not signal 3. Recently, we have developed a new generation JAK-STAT CAR composed of a truncated cytoplasmic domain of the IL-2 receptor β chain and STAT3/5 binding motifs, CD28 co-stimulatory domain, and CD3ζ domain. The novel anti-CD19 JAK-STAT CAR-T cells showed antigen-specific activation of the JAK-STAT signaling pathway, enhanced proliferation, and limited terminal differentiation in vitro compared to second generation 28ζ CAR or 4-1BBζ CAR-transduced T cells. Furthermore, the anti-CD19 JAK-STAT CAR-T cells demonstrated superior in vivo persistence and antitumor effect in mouse models. In addition, we previously showed that a hinge region and the composition of a single chain variable fragment (scFv) such as the order of VH and VL regions critically influence not only antigen-dependent activation but also undesired antigen-independent activation known as tonic signaling.

Methods In this study, we have optimized the scFv design in 28ζ CAR and JAK-STAT CAR constructs to show superior antigen-specific activation and reduced tonic signaling for several targets (CD19, CD20, Mesothelin, and G2D). And we have evaluated the feature of JAK-STAT CAR-T cells compared to 28ζ CAR-T cells.

Results JAK-STAT CAR-T cells showed superior antigen-specific proliferation with less differentiated status, whereas 28ζ CAR-T cells showed antigen-independent proliferation and displayed higher exhaustion marker expression after repetitive stimulations.

Conclusions These results suggest that our JAK-STAT-CARs with enhanced antigen-specific response with minimized tonic signaling targeting various antigens has the potential to demonstrate improved clinical efficacy.

REFERENCES
Background The application of CRISPR-Cas9 for personalized medicine is potentially revolutionary for the treatment of several diseases including cancer. However, the bacterial origin of the Cas9 protein raises concerns about immunogenicity. Recent ELISA-based assays detected antibodies against Cas9 from Streptococcus pyogenes (SpCas9) and Staphylococcus aureus (SaCas9) in 5–10% of sera from 343 normal healthy individuals.1,2 SpCas9-specific memory CD8 T cell responses were not demonstrated in those individuals. To date, there are no conclusive studies assessing whether CRISPR-Cas9-modified CAR-T could raise CD8 T cell-mediated immunogenicity in humans. Refug e CAR-T cell platform employs an inducible, non-gene editing, nuclease deactivated Cas9 (dCas9) to modulate gene expression in response to external stimuli such as antigen-dependent CAR signaling to suppress PD-1 expression.

Methods In the present study, we analyzed two putative HLA-A*02:01 and two HLA-B*07:02-associated SpCas9 T cell epitopes. The candidate epitopes were derived from a prediction algorithm that incorporates T cell receptor contact residue hydrophobicity and HLA binding affinity. We engaged in-vitro sensitization (IVS) assay to identify immunogenic potential of dCas9 peptides.

Results Autologous IVS assay of T cells in two healthy donors PBMcs identified CD8-T cell responses after two rounds of stimulation against only one HLA-A*02:01-associated SpCas9 peptide (sequence NLIALSLGL) P1– while the other candidate epitopes did not elicit any response. Dextramer analysis demonstrated that 15% of CD8+ T cells were specific for P1 and ~11% of CD8 + cells produced INFγ upon challenge with P1-loaded T2 cells.

Conclusions Our in-vitro sensitization assay was able to demonstrate that dCas9 epitope P1 is immunogenic and may elicit adaptive immune response against gene edited CAR-T cells. Endogenous processing and presentation of P1 and other putative epitopes by Refuge CAR-T are currently being analyzed.

Acknowledgements Refuge Biotechnologies Inc. Menlo Park, California, 94025

Trial Registration N/A

Ethics Approval N/A

Consent N/A

REFERENCES


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131 COUPLED CAR® TECHNOLOGY STRENGTHENS ADOPTIVE T CELL THERAPY BY PROMOTING RAPID EXPANSION

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Background CAR T therapy has achieved remarkable results in the treatment of hematological tumors such as leukemia, lymphoma, and multiple myeloma. However, there remains challenges in treating solid tumors. These challenges include physical barriers, tumor microenvironment immunosuppression, tumor heterogeneity and target specificity. Especially, due to tumor microenvironmental barriers, CAR T cells are not effectively exposed to tumor antigens and cannot activate co-stimulation signals on CAR molecules, thus conventional CAR T cell therapy has thus far shown weak cell expansion in solid tumor patients, achieved little or no therapeutic responses. Here, we developed CAR T cells based on a novel CoupledCAR® technology to overcome the lack of persistence of solid tumor CAR T cells in vivo.

Methods We designed a ‘CoupledCAR’ lentivirus vector containing a single-chain variable fragment (scFv) targeting human TSHR. The lentivirus was produced by transfecting HEK-293T cells with ‘CoupledCAR’ lentiviral vectors and viral packaging plasmids. Patient’s CD3 T cells were cultured in X-VIVO medium containing 125U/mL interleukin-2 (IL-2), and transduced with ‘CoupledCAR’ lentivirus at certain MOI. Transduction efficiency and was evaluated at 7 to 9 days after ‘CoupledCAR’ lentivirus transduction, and quality controls for fungi, bacteria, mycoplasma, chlamydia, and endotoxin were performed. After infusion, serial peripheral blood samples were collected, and the expansion and the cytokine release of CART cells were detected by FACS and QPCR. The evaluation of response level for patients were performed at month 1, month 3, and month 6 by PET/CT.

Results We used prostatic acid phosphatase (PAP) as an exemplary CAR target for prostate cancer and demonstrated that our CoupledCAR® significantly enhanced the expansion of PAP CAR T cells in vitro and in vivo. Further, we observed that this expansion showed more memory-like phenotypes, and caused little exhaustion of PAP CAR T cells. Also, we find coupled solid tumor CAR T cells have stronger tumor killing ability. We demonstrated this simple expansion to enable the persistence of solid tumor CAR T cells and can be further applied to other kinds of T cell therapy like TCR T and TILs.

Conclusions We developed a novel platform technology (CoupledCAR®) that allows solid tumor CAR T cells to rapidly expand. This initial CAR T cell expansion enabled enhanced trafficking and infiltration of the tumor tissue whereby further cell expansion occurred and thereby achieved tumor clearance. We have carried clinical trials and obtained early promising clinical data. We will further verify the safety and efficacy of this technology in the treatment of different kinds of solid tumors in the clinic research.

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132 CAR MACROPHAGES (CAR-M) ELICIT A SYSTEMIC ANTI-TUMOR IMMUNE RESPONSE AND SYNERGIZE WITH PD1 BLOCKADE IN IMMUNOCOMPETENT MOUSE MODELS OF HER2+ SOLID TUMORS

Stefano Pietra*, Rashid Gabbasov, Linara Gabbitova, Yumi Ohtani, Michael Kichinsky. Carisma Therapeutics, Philadelphia, PA, USA

Background Despite the remarkable efficacy achieved by CAR-T therapy in hematologic malignancies, application in solid tumors has been challenging. We previously developed human CAR-M and demonstrated that adoptive cell transfer of CAR-M into xenograft models of human cancer controls tumor progression and improves overall survival [1]. Given that
CAR-M are professional antigen presenting cells, we developed an immunocompetent animal model to evaluate the potential for induction of a systemic anti-tumor immune response.

**Methods** Murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adenviral vector Ad5f35. CAR-M were phenotypically and functionally evaluated in vitro and in syngeneic models. To evaluate CAR-M efficacy in an immunocompetent animal model, BALB/c mice were engrafted with CT26-HER2+ tumors (single-tumor model) and were treated with intratumoral CAR-HER2 or untransduced (UTD) macrophages. To evaluate epitope spreading, we simultaneously engrafted BALB/c mice with CT26-HER2+ and CT26-Wt tumors on opposite flanks (dual-tumor model), and treated mice with CAR-M or controls into the CT26-HER2+ tumor only. Peripheral and tumor-infiltrating immune cells were phenotypically and functionally characterized.

**Results** In addition to efficient gene delivery, Ad5f35 transduction promoted a pro-inflammatory (M1) phenotype in murine macrophages. CAR-M, but not control UTD macrophages, phagocytosed HER2+ target cancer cells. Anti-HER2 CAR-M eradicated HER2+ murine CT26 colorectal and human AU565 breast cancer cells in a dose-dependent manner. CAR-M increased MHC-I and MHC-II expression on tumor cells and promoted tumor-associated antigen presentation and T cell activation. In vivo, CAR-M treatment led to tumor regression and improved overall survival in the CT26-HER2+ single-tumor model. In the dual-tumor model, CAR-M treatment cleared 75% of CT26-HER2+ tumors and inhibited the growth rate of contralateral CT26-WT tumors, demonstrating an abscopal effect. CAR-M treatment led to increased infiltration of intratumoral CD4+ and CD8+ T, NK, and dendritic cells – as well as an increase in T cell responsiveness to the CT26 MHC-I antigen gp70, indicating enhanced epitope spreading. Given the impact CAR-M had on endogenous T-cell immunity, we evaluated the combination of CAR-M and anti-PD1 in the CT26-HER2 model and found that the combination further enhanced tumor control and overall survival.

**Conclusions** These results demonstrate that CAR-M therapy induces epitope spreading via activation of endogenous T cells, orchestrating a systemic immune response against solid tumors. Moreover, our findings provide rationale for the combination of CAR-M with immune checkpoint inhibitors. The anti-HER2 CAR-M CT-0508 will be evaluated in an upcoming Phase I clinical trial.

**REFERENCE**


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**134 TUMOR-RESPONSIVE, MULTI-FUNCTIONAL GENETICALLY-ENGINEERED NATURAL KILLER CELLS FOR IMMUNOTHERAPY OF Glioblastoma**

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**Background** Treatment of solid tumors with cell therapeutics will require optimal T cell persistence, fitness, and trafficking. Heterogeneous solid tumors will also have to be attacked through multiple antigens simultaneously in order to prevent resistance linked to loss of antigen expression. Here we use chimeric antigen receptor (CAR) T cells that secrete bridging proteins that act as CAR-T engagers to create an optimal platform for attacking solid tumors in the CNS.

**Methods** Lentiviral vectors encoding an anti-CD19 CAR and secreted bridging proteins were created. The bridging proteins contained the CD19 extracellular domain, which is the target for the CAR, and anti-tumor antigen binding domains derived from antibodies (scFv and llama VH). The resulting anti-CD19 CAR T cells secrete the bridging proteins. These candidate cell therapeutics were evaluated for antigen binding and induction of antigen-specific cytotoxicity. An anti-CD19 CAR that secretes a CD19-anti-Her2 bridging protein has moved into development. Using the CD19-anti-Her2 bridging protein as a core module, we have begun evaluating a series of multi-antigen bridging proteins.

**Results** CAR-CD19 T cells that secrete bridging proteins have potent cytotoxic activity against single- and multi-antigen-positive cells. ALETA-002 is the lead candidate lentiviral vector construct encoding the anti-CD19 CAR domain and the CD19-anti-Her2 bridging protein, and has entered a GMP viral particle development campaign. This therapeutic will be systemically administered to Her2-positive breast cancer patients who are relapsing with CNS metastases. Next, multi-antigen bridging proteins encoding an anti-Her2 scFv and anti-B7H3, anti-B7H6 or anti-IL13Ra2 llama VH were assayed for potency. Lead candidates for development for the treatment of primary CNS malignancies were identified and are being manufactured at pilot-scale in 4-plasmid lentivirus production runs.

**Conclusions** The use of anti-CD19 CAR T cells that can expand off of the normal CD19-positive B cell pool enables tumor-antigen independent persistence, fitness and robust trafficking into the CNS. The use of small, modular bridging proteins allows us to leverage anti-CD19 CAR T cells and use these to attack solid tumor antigens that are present on CNS resident cancers and on CNS metastatic lesions. Novel cell therapeutics for the treatment of Her2-positive CNS metastases and heterogeneous primary CNS malignancies including glioblastoma and the pediatric gliomas have been developed.

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Abstract 134 Figure 1 Multifunctional genetically-engineered NK cells for immunotherapy of GBM. (A) Schematic representation of transgene representing the complete multi-functional construct: tumor-responsive anti-CD73 scFv-secreting dual-specific CAR targeting NKG2DL and GD2. (B) Schematic representation of tumor-responsive anti-CD73 scFv secreting dual-specific CARs. (C) Flow cytometry data showing the purity of isolated peripheral blood-derived NK (pNK) cells (CD56+CD3-) and GD2 expression on engineered pNK cells determined by flow cytometry after two rounds of lentiviral transduction. (D) NKG2D expression on engineered pNK cells determined by flow cytometry after two rounds of lentiviral transduction. (E) Expression of anti-CD73 scFv and anti-GD2 scFv on pNK cells determined by flow cytometry after two rounds of lentiviral transduction. (F) In vitro cytotoxicity of pNK and E-pNK cells against different GBM43 at indicated E/T ratios over 4 h. (G) Degranulation (% CD107) and IFN-γ production of pNK and E-pNK cells (% IFN-γ) after 4 h coculture with GBM43 cells (E/T ratio, 5:1). (H) In vitro cytotoxicity of pNK and E-pNK (following scCD73 scFv cleavage) cells against GBM43 cells at indicated E/T ratios over 4 h. (I) CD73 activity of GBM43 cells after incubation with cleaved scCD73 scFv following cleavage from uPA-treated E-pNK cells. (J) Tumor growth of individual treatment groups, including PBS, pNK cells and E-pNK cells. Tumor size was determined by caliper measurements. (K) Average tumor weight of the mice in each treatment group after necropsy on day 28 post-start of treatment. (L) Changes in body weight of the mice in each group during the treatment period. Note: the data shown in this study is for isolated pNK cells from one representative donor. Data are shown as mean ± SEM. *P < 0.05, **P < 0.01

pNK cells were evaluated against patient-derived GBM cells both in vitro and in vivo.

Results

We have designed and synthesized a multifunctional CAR construct that expresses an anti-CD73 scFv which is cleavable by GBM-associated proteases, and a dual CAR redirected against ligands for NKG2D and GBM-associated GD2 receptors (figure 1A-B). We have isolated primary NK cells (figure 1C) and genetically manipulated them to express NKG2D, anti-GD2 scFv and anti-CD73 scFv (figure 1D-E). EpNK cells showed a significantly higher in vitro antitumor activity towards GBM43 targets, patient-derived GBM cells, including increased percentage of tumor killing, degranulation and IFN-γ production (figure 1F-G). EpNK cells lacking the anti-CD73 scFv following uPA treatment displayed significantly decreased killing ability of target GBM43 cells after co-culture at E/T ratios of 2.5 and 5 for 4 h (figure 1H). In addition, after treatment with cleaved anti-CD73 scFv, GBM43 cells showed a significantly reduced ability to produce adenosine due to the inhibition of CD73 enzyme activity (figure 1I). Furthermore, EpNK cells showed potent anti-GBM activity in subcutaneously GBM43 xenografts (figure 1J-L). In vivo-adoptively transferred EpNK cells also showed superior intratumoral infiltration into GBM43 tumors when analyzed by IHC (data not shown).

Conclusions

We have generated EpNK cells showing improved antitumor activity against GBM through increased resistance to the immunosuppressive TME via adenosinergic CD73 blockade and the simultaneous ability to specifically target GBM cells via dual CARs. Based on these results, we are currently building the orthotopic GBM mouse model to further evaluate their in vivo therapeutic effects.

Acknowledgements

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Ethics Approval

Primary human NK (pNK) cells used in this study were obtained using Purdue University’s Institutional Review Board (IRB)-approved consent forms (IRB-approved protocol #1804020540).

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136  TARGETING MET WITH CHIMERIC ANTIGEN RECEPTOR T CELLS IN HEPATOCELLULAR CARCINOMA

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Background

Hepatocellular carcinoma (HCC) is the leading cause of cancer mortality worldwide. While HBV/HCV infection is the primary cause of HCC, overexpression of MET, the receptor of hepatocyte growth factor (HGF), occurs in 50% HCC patients, and is an indicator of poor prognosis. Although the multi-target MET tyrosine kinase inhibitor cabozantinib is FDA approved for treating advanced HCC, the long-term efficacy versus toxicity remains unknown. Our study is to develop specific MET-targeting chimeric antigen receptor T (CAR-T) cells for treating HCC with MET overexpression.

Methods

Based on a well-established anti-MET monoclonal antibody, we synthesized and cloned the single-chain variable fragment (ScFv) sequence into two retroviral based 2nd generation CAR vectors (MET-CAR,CD28,ζ and MET-CAR,4-1BB,ζ). A MET-CAR without CD3ζ domain (MET-CARA) served as a negative control. To produce MET-CAR-T cells, healthy PBMCs were stimulated with anti-CD3/CD28 antibodies in the presence of IL-7/IL-15 followed by transduction with MET-CAR viral particles. T cell transduction efficacy was...
determined using flow cytometry. HCC cell lines with variable MET expression from high/positive (MHCC97H, C3A, and JHH5) to MET low/negative (SNU398) were used to determine MET-specific CAR T cell specificity and effector function using MTS assay. We also collected media from the tumor-T cell co-cultures and determined IL-2 and IFNγ secretion using ELISA. Finally, real-time confocal imaging (24 h) was performed to record the progress of MET-CAR T cell mediated killing activity against MHCC97H/mCherry cells.

**Results** We show that both MET-CAR.CD28.ζ and MET-CAR.4-1BB.ξ - T cells significantly killed MHCC97H, C3A, and JHH5 cells in antigen dependent manner. MET-CAR T cell killing is MET dependent as we observed no killing of MET-negative SNU398 cells. In addition, MET-CAR.4-1BB.ξ and MET-CAR.CD28.ζ - T cells secreted IL-2 and IFNγ when co-cultured with MHCC97H, C3A, JHH5 cells, but not SNU398. Confocal imaging studies showed that both MET-specific CAR T cells migrated toward MHCC97H/mCherry cells, formed aggregations, and induced tumor cell death, while MET-CARA T cells failed to do so.

**Conclusions** Here we demonstrate that MET-CAR.4-1BB.ξ and MET-CAR.CD28.ζ - T cells specifically recognize and kill MET-positive HCC cells in vitro. While animal studies are required to validate the efficacy in vivo, our study has produced a novel therapeutic CAR T cell target for treating malignant HCC and other type of cancers with MET overexpression.

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**Ethics Approval** The study was approved by East Tennessee State University’s Ethics Board, approval number #0619.3s.

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**GENOMICS OF MULTIPLE MYELOMA INFLUENCES THE EXPRESSION OF CAR T-CELL TARGETS**

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**Background** Multiple Myeloma (MM) is an incurable disease, with a particularly poor prognosis for patients with refractory/relapsed MM or high-risk cytogenetics. Chimeric Antigen Receptor (CAR) T-cell therapy targeting BCMA can induce deep responses in highly pretreated RRMM; however, remissions are not sustained, and the majority of patients eventually relapse. We hypothesized that genomic determinants of MM play a role in dictating the expression of surface targets that can be of use for immune targeting.

**Methods** We analyzed the gene expression of 24 immunotherapeutic targets in a combined dataset of 1900 MM patients from three independent expression datasets obtained from the Multiple Myeloma Research Foundation CoMPass study and Gene Expression Omnibus. Given that CAR T-cell therapy may be especially important for patients with high-risk myeloma, we defined the expression of each target in high-risk MM patients by stratifying patients based on several genomic features impacting prognosis. Additionally, we conducted a gene co-expression network analysis and identified 30 gene modules highly correlated with 16 cell surface targets from our panel, further suggesting that genetic determinants of MM may shape a targetable cell surfaceome. In order to determine whether targeting any of these candidate antigens might cause major toxicity to normal cells, we utilized several repositories providing protein data to annotate their expression in several normal cell types.

**Results** We determined that a number of genomic factors could stratify the 24 targets into three general groups: 1) targets that show consistent overexpression in high-risk patients: IGF1R, ITGB7, GPRC5D and CD70, and are thus suitable for most high-risk patients; 2) targets that are down-regulated in patients with high-risk genomic features: CD200, CD19, CD40, CD1D and IGKC, perhaps playing a role in cancer immune escape; and 3) targets associated with one specific genetic abnormality, i.e. t(4;14): FUT3, SLAMF7, CD56, CD138 and BCMA, thus of use for precision CAR therapy in this high-risk patient subset.

**Conclusions** Our work provides a means of target selection for precision CAR therapy, by considering both patient genomic backgrounds and cancer cell surface profiles. Furthermore, our results provide a roadmap for immunotherapy of MM by unbiasedly comparing the expression of top MM cell surface targets in patient data and normal cells and suggest that the genetic landscape of MM may predict the expression of specific targets for precision immunotherapy. The quest for novel MM targets for immunotherapies remains open, and CAR target discovery driven by specific genetic events remains an active area of investigation.

**REFERENCE**


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**IN VIVO LOCALIZATION OF GENETICALLY ENGINEERED NATURAL KILLER CELLS AGAINST GliOBLASTOMA USING PET IMAGING**

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**Background** Glioblastoma (GBM) is a deadly brain malignancy with a dismal prognosis. While immunotherapy holds great promise for GBM treatment, most have failed due to a suppressive tumor microenvironment (TME). Antigen heterogeneity and adenosine signaling are two immunosuppressive mechanisms in GBM. The CD73-adenosine axis plays a multifaceted role in GBM pathogenesis and drives the dysfunction of NK cells in GBM TME.1,3 Our NKG2D-chimeric antigen receptor (CAR)-natural killer (NK) cells have shown anti-tumor activity when combined with CD73 blockade in vivo. To further extend the potency of these cells against GBM and address antigen heterogeneity in GBM, we combined the local blockade of CD73 with multi-antigen-targeting engineered NK cells. In order to improve treatment assessment, PET/MR imaging was employed to enable detailed, non-invasive assessment of tumor progression. Imaging assessment of adoptively-transferred CAR- NK cells was also developed to determine the fate of NK cell delivery to the tumor site over time.

**Methods** We generated multifunctional engineered NK (E-NK) cells that express an anti-CD73 scFv, which is cleavable by GBM-associated proteases, an NKG2D-CAR, as well as a GD2
Establishment of Canine CAR T Cell Treatment Model for Solid Tumor Immunotherapy Development

Shihong Zhang*, 1Karan Kohli, 1Brian Hayes, 1Cassandra Miller, 2Maxi Maeda-Whitaker, 1Brett Schroeder, 1Kraig Abrams, 3Bernard Seguin, 4Stephen Gottschalk, 5Peter Moore, 6Beverly Tork-Sarch, 7Seth Pollack. 1Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 2Canine Cancer Alliance, Seattle, WA, USA; 3Colorado State University, Fort Collins, CO, USA; 4St. Jude Children’s Research Hospital, Memphis, TN, USA; 5University of California, Davis, Davis, CA, USA

Abstract 139 Figure 1 PET imaging and gamma counting of the engineered NK cells

Figure 1 (A) Multifunctional, responsive CAR constructs; (B) In vitro killing activity against GBM43 cells after co-incubation with 89Zr labeled NK cells at an E:T ratio of 10 for 4 h with LDH assay (N=3); (C) & (D) In vivo PET imaging and ex vivo gamma counting with 89Zr at week 5 in 10 mice during 4 days, GBM intracranial implantation to NSG male mouse, 89Zr, 89Zr + NK cell, or 89Zr + E NK cell (7 × 106 cells with 500 µCi) was administered through intravenous injection, Qimage was used for the PET/MRI co-registration and analysis.

Conclusions We generated multifunctional E-NK cells which showed the improved killing of GBM cells using novel targeting approaches, including the blockade of CD73-mediated adenosinergic signaling. We also optimized E-NK cell radioscaling with 89Zr for GB10 therapy in vitro and in vivo fate mapping against a xenograft of patient-derived GBM.

Acknowledgements We gratefully acknowledge the Walther Oncology Embedding Program, Indiana University Simon Cancer Center, and In Vivo Therapeutics Core.

REFERENCES

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the CAR products following lymphodepletion was confirmed in two healthy dogs (figure 1B).

Results Canine solid tumors were confirmed to be B7H3 positive in almost all cases. Using the GALV-pseudotyped retrovirus system, transduction was efficient with up to 70% CAR+ cells. Post-transduction expansion was over 100 folds. B7H3 CAR transduced canine T cells were able to eliminate B7H3+ canine tumor spheroids effectively (figure 2). Safety of the CAR T cells (dose: 1 × 10^6/m2) were confirmed in both healthy animals following cyclophosphamide lymphodepletion. After week 6, cetuximab was given to the subjects to deplete EGFR+ cells. Subject 2 experienced fever after CAR T cell administration. Both dogs showed elevated serum ALP and ALT levels and returned to normal (figure 3). No other treatment-related adverse events were observed. Information of the CAR T cell products can be found in table 1.

Conclusions We demonstrated that, similar to human cancers, B7H3 is a target in canine solid tumors. We successfully generated canine B7H3 specific CAR T cell products that are highly efficient at killing canine 3D tumor spheroids using a production protocol that closely models human CAR T cell production procedure and confirmed the safety in vivo. We plan to test and optimize various approaches to enhance CAR T cell efficacy for solid tumor treatment both in vitro and in canine sarcoma patients.

Ethics Approval The study was approved by Fred Hutchinson Cancer Research Center's Institutional Animal Care and Use Committee (IACUC), approval number PROTO201900860.

Abstract 139 Table 1 Infused CAR T cell product information

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>16</td>
<td>15.4</td>
</tr>
<tr>
<td>Infused cells (x10^6)</td>
<td>641</td>
<td>625</td>
</tr>
<tr>
<td>Production start (x10^6)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Production end (x10^6)</td>
<td>2729</td>
<td>6560</td>
</tr>
<tr>
<td>Transduction efficiency</td>
<td>32.9%</td>
<td>47.6%</td>
</tr>
</tbody>
</table>

Both subjects are adult male beagle mix.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0139

140 ADOPTIVE TRANSFER OF T CELLS SURFACE-TETHERED WITH IL-12 PROMOTE ANTIGEN SPREADING FOR ENHANCED ANTI-TUMOR EFFICACY

1Ditte Jaehger*, 2Kate Stokes, 1Hólafriedur Hallíðórsdóttir, 2Alvin Pratama, 2Gulzar Ahmad, 1Jonathan Nardozi, 1Katharine Sackton, 2Douglas Jones, 1Thomas Andresen. 1Technical University of Denmark, Kgs. Lyngby, Denmark; 2Repertoire Immune Medicines, Cambridge, MA, USA

Background Acquired resistance is a major limiting factor for durable T cell therapies in solid tumors. Antigen escape pathways such as insufficient antigen coverage or loss of target antigen remain major resistance mechanisms that need to be addressed in order to expand the field of T cell therapies. Interleukin-12 (IL-12) is a potent stimulator of innate and adaptive immune cells that holds strong potential for cancer immunotherapy, but its clinical utility has been limited by high systemic toxicities. We have previously shown that tethering an IL-12 immunocytokine to the surface of T cells prior to adoptive cell transfer (ACT) safely improves anti-tumor efficacy by promoting T cell function specifically in the tumor. Here, we demonstrate that cell-tethered IL-12 delivers adjuvant activity that leads to priming and expansion of bystander, tumor-specific T cells, and thereby counteract common immune escape pathways.

Methods Adjuvant activity of IL12-tethered pmel T cells, reactive towards the gp100 antigen of B16 tumors, was evaluated in the B16-OVA syngeneic mouse model. Notably, adoptive transfer of IL12-tethered pmel T cells, but not pmel T cells alone, resulted in proliferation of endogenous tumor infiltrating lymphocytes. To assess whether this reflected tumor-specific T cell responses, we used dextramer staining against non-targeted, tumor-specific antigens and found that both abundance and activation increased following cell-tethered IL-12 treatment. Encouraged by these findings, the OT-1 model was used to track epitope spreading to tumor-specific naïve T cells. Following treatment with IL-12-tethered PMEL T cells, we tracked the proliferation and tumor engraftment of labelled, naïve OT-I T cells, which are reactive towards the non-targeted OVA antigen.

Results Cell-tethered-IL12, but neither ACT nor ACT and systemically administered IL-12, induced proliferation and engraftment OT-I T cells in tumor-draining lymph nodes (dLNs) and tumors of B16-OVA-bearing mice. This effect was antigen-dependent as the OT-I T cells were not primed in B16.F10 (OVA antigen-negative) tumors. Mechanistically, this priming was associated with IL-12-induced increases in
activation and tdLN infiltration of cross-presenting dendritic cells (cDC1) as well as increased presentation of the SiN-FEKL epitope of OVA specifically on this subset of dendritic cells.

**Conclusions** Together, our findings suggest that tethering IL-12 to tumor-specific T cells prior to adoptive transfer promotes epitope spreading through the combination of tumor cell-killing induced by the ACT therapy and IL-12-induced activation of cDC1 in the tdLN. This adjuvant activity from T cell-tethered IL-12 holds promise for overcoming antigen escape pathways that limit the efficacy of antigen-specific T cells against heterogeneous tumors

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141 PBMCE-BASED CANCER VACCINES GENERATED WITH MICROFLUIDICS SQUEEZING DEMONSTRATE SYNERGISTIC AND DURABLE TUMOR REDUCTION IN COMBINATION WITH PD1 CHECKPOINT AND FAP TARGETED IL-2 VARIANTS

1Matthew Booty, 1Adam Stockmann, 1Olivia Pyor, 1Melissa Myint, 2Christine Trumpfheller, 3Valeria Nicolini, 1Christian Klein, 2Laura Codari, 1Pablo Umana, 1Ammon Sharei, 1Howard Bernstein, 1Katherine Seidl, 1Scott Loughead*. 1SQZ Biotecnologies, Watertown, MA, USA; 2Roche (pRED), Schlieren, Switzerland

**Background** We engineered unfractionated peripheral blood mononuclear cells (PBMCs) to function as antigen presenting cells (APCs) that generate potent CD8+ T cell responses. We investigated the combined efficacy of PBMC-based cancer vaccine with targeted interleukin 2 variants (IL2v); anti-Programmed Cell Death Protein 1 (muPD1-IL2v) and anti-Fibroblast Activation Protein (muFAP-IL2v).

**Methods** We generated PBMC-based cancer vaccine with microfluidic cell engineering system (Cell Squeeze®), which facilitates direct cytosolic antigen delivery and enables cell subsets within PBMCs to function as APCs. The immunocytokines used contain IL2v fused with antibody counterparts that enable targeting to tumor-associated stroma or immune cells (aFAP and aPD1, respectively) with modified FcR binding. The IL2v moiety, compared with wild-type IL-2, has abolished binding to IL-2Ra (CD25) resulting in IL-2Rβg binding only, thus fully maintaining activity on NK and CD8+ T cells, while avoiding Treg activity and CD25 mediated toxicity.

**Results** In the murine TC-1 HPV tumor model, SQZ-PBMC-based vaccines show efficacy as monotherapy (1e6 cells administered iv on day 14 post-tumor implant), while SQZ combination therapy with targeted immunocytokines, muPD1-IL2v and muFAP-IL2v (2 mg/kg or 1 mg/kg, respectively, administered iv on days 21, 28, and 35 post-tumor implant) significantly delayed tumor growth and improved survival in murine TC-1 HPV tumor model. Median survival of combination treated groups remained undefined at day 84 post-tumor implant, while the monotherapy treated groups had calculated median survival times of 36.5, 42, and 70 days for the muFAP-IL2v, muPD1-IL2v, and SQZ monotherapy groups, respectively. Following initial tumor clearance, tumor-free mice (2/12 animals for SQZ monotherapy; 8/12 animals for SQZ with muFAP-IL2v; 11/11 animals for SQZ with muPD1-IL2v) were all re-challenged at day 84 and all remained tumor free at least 7 weeks post re-challenge, suggesting the generation of anti-tumor memory response. In a mechanistic study, SQZ-PBMCs in combination with muPD1-IL2v resulted in increased expansion of intra-tumoral, antigen-specific CD8+ T cells compared with separate administration of either therapy (~3.6-fold over SQZ alone; ~2000-fold over muPD1-IL2v alone; per mg of tumor). Combination therapy also resulted in improved IFNγ and TNFα cytokine production by SQZ-elicited CD8+ T cells (~1.7-fold and ~9-fold, respectively, over SQZ monotherapy).

**Conclusions** Monotherapy with SQZ-PBMC-based cancer vaccines can drive anti-tumor responses in murine systems. These responses are enhanced by combined administration of targeted immunocytokines. Monotherapy with SQZ-PBMC-HPV is currently under clinical evaluation for HPV16+ tumor indications. These preclinical data support the combination of SQZ-PBMC with FAP-IL2v or PD1-IL2v targeted immunocytokine as promising cancer immunotherapies.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0141

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142 CONTEXTUAL REPROGRAMMING OF CAR T CELLS FOR THE TREATMENT OF HER2-EXPRESSING CANCERS

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**Background** Combining checkpoint inhibition (CPI) to adoptive cell therapy (ACT) is a promising strategy to prevent chimeric antigen receptor (CAR)-engineered T cell exhaustion and improve outcomes. However, cumulative toxicities and costs limit this approach. Here, we apply a conditional, antigen-dependent non-editing CRISPR interference-(CRISPRi) modulation circuit that we originally described in yeast and eukaryotes1,2-3 (RB-340-1) to promote CAR T resilience to checkpoint suppression extending in vivo persistence and effectiveness.

**Methods** RB-340-1 is an CAR T cell product engineered via synthetic biology approaches to express a combination of molecules to prevent CAR T cell exhaustion and improve solid tumor treatment outcomes. The components include two constructs. The first construct encodes an anti-HER2 (4DS) CAR single chain variable fragment (scFv), with CD28 and CD3ζ co-stimulatory domains linked to a tobacco etch virus (TEV) protease and a programmed cell death protein 1 (PD1) promoter region-targeting single guide RNA (PD1sg). The second construct encodes a protein, linker for activation of T cells (LAT), complexed to nuclease-deactivated/dead Cas9 (dCas9)-Kruppel-associated box (Krab) via a TEV-cleavable linker. Activation of CAR brings CAR-TEV in close proximity to the LAT-dCas9-Krab complex releasing the enzyme for nuclear localization to the PD1 regulatory region to conditionally and reversibly suppress its expression. RB-340-1 was compared in vitro and in vivo against conventional and control (cRB-340-1, lacking PD1sg) HER2 CAR T cells in combination with CPI with Atezolizumab (5 mg/Kg administered intravenously twice a week).

**Results** RB-340-1 consistently induced higher production of homeostatic cytokines such as IL-2 resulting in significantly enhanced proliferation in vitro (figure 1a). Our in vivo data showed significantly enhanced suppression of growth of HER2 + FADU oropharyngeal cancer xenografts upon intra-tumoral (figure 1b) and systemic administration (figure 1c) and prolonged persistence of CAR T cells in vivo.

A86

Abstract 142 Figure 1  Rb-340-1 performance in vitro and in vivo.

Conclusions  Intrinsic conditional regulation of checkpoint expression in CAR T cells provides a simplified approach to combination therapies that limits systemic toxicities and reduces costs. Since the expression of multiple genes can be simultaneously controlled by CRISPRi, broader applications can be envisioned in the future.

Ethics Approval  Not Applicable

Consent  Not applicable

REFERENCES

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144  RTX-240, AN ALLOGENEIC ENGINEERED RED BLOOD CELL EXPRESSING 4–1BBL AND IL-15TP, PROMOTES NK CELL FUNCTIONALITY IN VITRO AND IN VIVO

Anne-Sophie Dugas*, Shannon McAdel, Enping Hong, Arjun Bollampalli, Maegan Hoover, Sneha Pawar, Vital Amin, Kangjian Qiao, Christopher Ta, Laurence Turka, Thomas Wickham, Siran Elloul, Rubius Therapeutics, Cambridge, MA, USA

Background  Agonist antibodies and recombinant cytokines have had limited success in the clinic due to three factors: severe toxicity leading to a narrow therapeutic index, the diminished activity of an agonistic antibody compared with natural ligand, and the lack of multiple signals needed to effectively activate most cell types. To address these limitations, Rubius Therapeutics has developed RTX-240, an allogeneic cellular therapy using red blood cells genetically engineered to express 4-1BBL and IL-15/IL-15Ra fusion (IL-15TP) in their natural conformation on the cell surface. RTX-240 is designed to recapitulate human biology by broadly stimulating adaptive and innate immunity to generate an anti-tumor response and provide improved safety due to the restricted biodistribution of red blood cells to the vasculature. Here we demonstrate that RTX-240 is highly active in preclinical models.

Methods  PBMCs or NK cells were treated with RTX-240 in vitro. mRBC-240 was used for in vivo studies.

Results  Treatment of either PBMCs or isolated NK cells with RTX-240 induced a dose-dependent increase in NK cell activation, proliferation and functionality. These effects were further enhanced with increased 4-1BBL and IL-15TP expression on the surface of RTX-240. NK cell counts, Nkp44 and Trail expression were increased 150, 4.6 and 6-fold over media control, respectively. Activation of NK cells with RTX-240, followed by incubation with K562 targets enhanced NK cell cytotoxicity (1.3-2.8 over control), that was accompanied by increased NK cell activation (CD69) and degranulation (CD107a) (3.1-fold and 1.9-fold, respectively). RTX-240-activated NK cells showed higher frequency of CD56dim/CD16+ NK cells, which have been reported to induce natural and ADCC-dependent cytotoxicity. Correspondingly, RTX-240 promoted enhanced ADCC-induced killing of Raji cells when combined with anti-CD20 mAb (1.4-fold over control). Intravenous administration of mRBC-240 to a B16F10 intravenous lung metastases model led to NK cell expansion on Day 4 (3.8-fold over control). These NK cells were cytotoxic (Granzyme B+) and highly proliferative (Ki67+) (1.4-fold and 18.8-fold over control, respectively). Treatment with mRBC-240 increased the frequency of terminally differentiated NK cells (NK1.1+/-CD11b+/-CD27/-KLRG1+) in the tumor (2.1-fold increase over control). Terminally differentiated NK cells are highly cytotoxic and their frequency in the tumor was strongly correlated with efficacy in this model (p=0.0001).

Conclusions  Taken together, these data indicate that RTX-240 promotes NK cell activity and functionality in preclinical models. RTX-240 has now entered a first-in-human Phase 1 trial for the treatment of patients with relapsed/refractory or locally advanced solid tumors, with a planned arm evaluating RTX-240 in relapsed/refractory acute myeloid leukemia.

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145  PRECLINICAL DEVELOPMENT OF EDIT-201, A MULTIPLEXED CRISPR-CAS12A GENE EDITED HEALTHY DONOR DERIVED NK CELLS DEMONSTRATING IMPROVED PERSISTENCE AND RESISTANCE TO THE TUMOR MICROENVIRONMENT

Karrie Wong*, Steven Sexton, Kelly Donahue, Lucy Prem Antony, Kevin Wasko, Jared Nasser, Glenn Leary, Amanda Pfautz, Owen Porth, William Pierce, Patricia Sousa, Sean Scott, Aaron Wilson, Kai-Hsin Chang, John Zuris, Christopher Wilson, Richard Morgan, Christopher Borges. Editas Medicine, Cambridge, MA, USA

Background  Natural killer (NK) cells distinguish tumor from healthy tissue via multiple mechanisms, including recognition of stress ligands and loss of MHC class I expression. However, effector function of allogeneic NK cells can be diminished by the lack of functional persistence, as well as tumor-intrinsic immunosuppressive mechanisms, such as production of TGF-β. We developed a next-generation allogeneic NK cell therapy using CRISPR-Cas12a gene editing to enhance NK cell function through knockout of the CISH and TGFBR2 genes. We hypothesized that knockout of CISH, a negative regulator of IL-2/IL-15 signaling, would improve NK cell effector function, while knockout of the TGF-β receptor gene,
Conclusions

and an increase in median survival time. T-allo10 cells were currently being evaluated in a phase I clinical trial in patients with hematological malignancies undergoing allo-HSCT (NCT03198234). Herein, we aimed to confirm that Tr1 cells are the active ingredient responsible for the T-allo10 suppressive function, and reveal the underlying molecular signatures to elucidate the mechanisms of Tr1 cell-mediated suppression.

Methods

T-allo10 cells were generated in a co-culture of healthy host or patient tolerogenic dendritic cells (DC-10) with allogeneic healthy donor CD4+ T cells, then tested for Tr1 phenotype, anergy, suppression and cytokine production. Sorted T-allo10-derived Tr1 cells and non-Tr1 cells, as well as control effector T cells (Teff) and parental CD4+ T cells, were analyzed by TCR- and RNA-seq. Protein expression for key differentially expressed genes were validated, and the functional roles for IL-10, CTLA-4 and PD-1 in T-allo10-mediated suppression were confirmed in a suppression assay.

Results

We show that the T-allo10 cell product is: i) enriched for Tr1 cells, ii) anergic in response to alloantigen re-challenge, but not to non-specific stimuli or 3rd party antigens, and iii) suppresses host-reactive T cells, but not T cell responses to other antigens. Furthermore, T-allo10-derived, isolated Tr1 cells had a restricted TCR repertoire, suggesting they clonally expand in response to alloantigens. T-allo10-derived Tr1 cells have a distinct signature compared to non-Tr1 cells, and, in addition to IL-10, express high levels of CTLA-4 and PD-1 (but not FOXP3). Notably, blockade of CTLA-4 and the PD-1 pathway completely abolishes T-allo10-mediated suppression of T cell responses.

Conclusions

Our data shows that Tr1 cells are the active, suppressive, and antigen-specific ingredient of T-allo10 cells. Furthermore, while the role of IL-10 in Tr1 cell-mediated suppression is well known, we uncover that Tr1 suppress in addition through CTLA-4 and PD-1. Collectively, these intriguing findings underscore the importance of CTLA-4 and PD-1 pathways in conferring cell-mediated immunological tolerance. Further, they define the key characteristics and modes of action of antigen-specific Tr1 cells, providing crucial information for the ongoing T-all10 trial and future design of novel Tr1 cell-based therapies.

Ethics Approval

The patient study was approved by Administrative Panels on Human Subjects in Medical Research, Stanford University, Tallo10 eProtocol # 38734. Healthy donor samples were purchased as deidentified human blood products from the Stanford Blood Center, and are thus exempt.
proliferation, cytotoxicity, and persistence in vivo. To enhance an NK-specific GVT effect, we propose blocking the poliovirus receptor CD155 checkpoint molecule, which is overexpressed on osteosarcoma and can engage both activating and inhibitory receptors on NK cells. The impact of CD155 blockade on GVT and graft-versus-host-disease (GVHD) is unknown.

Methods NK cells from C57BL/6 (B6) mice were expanded with recombinant IL-15/IL-15R and analyzed by flow cytometry. Cytotoxicity assays were performed with IL-15 expanded B6 NK cells and mKate2-expressing K7M2 murine osteosarcoma at a 1:1 ratio with blockade of CD155 and CD155 ligands. To test efficacy of NK cell infusion and CD155 blockade after allogeneic BMT, BALB/c mice were lethally irradiated, transplanted with allogeneic B6 bone marrow, and challenged with luciferase-expressing K7M2 on day 0. At day 7, mice received IL-15 expanded B6 NK cells intravenously with either anti-IgG control or anti-CD155 antibody intraperitoneally and IL-2 subcutaneously on days 7 and 11. Mice were monitored for tumor growth by bioluminescence, and toxicity by GVHD using weight loss and clinical scores.

Results Compared to unexpanded murine NK cells, IL-15 expanded NK cells (n = 6) show increased expression of NKG2D (65.33% ± 10.77%; p = 0.0077; 1030 ± 177.0 MFI; p = 0.0101) and an increased ratio of the CD155 activating (CD226) to inhibitory (TIGIT) ligand expression (11.71% ± 4.121; p = 0.0362). In cytotoxicity assays with IL-15 expanded allogeneic murine NK cells (n = 3 replicates), CD155 blockade enhances K7M2 osteosarcoma lysis (60.62% ± 3.19%; p = 0.0189) compared to IgG control (29.01% ± 7.668%). CD226 blockade decreased tumor killing (10.62% ± 8.51%; p = 0.0053) compared to CD155 blockade. In vivo allogeneic murine NK cell infusion and anti-CD155 antibody treatment after allogeneic BMT decreased tumor area under the curve by 44.3% compared to IgG control, without exacerbating GVHD.

Conclusions These findings demonstrate that blockade of CD155 enhances an allogeneic NK cell-specific GVT effect for osteosarcoma treatment without exacerbating GVHD. CD155 blockade has the potential to improve usage of allogeneic BMT and NK cell adoptive immunotherapy as a combination treatment for osteosarcoma, and perhaps other pediatric sarcomas.

Acknowledgements This work was supported by grants from the National Institute of General Medical Sciences/National Institute of Health T32 GM008692 and Training in Cancer Biology Training Grant under the National Institute of General Medical Sciences/NIH T32 GM008692 and Training in Cancer Biology Training Grant. The impact of CD155 blockade on GVT and graft-versus-host-disease (GVHD) is unknown.

Methods NK cells from C57BL/6 (B6) mice were expanded with recombinant IL-15/IL-15R and analyzed by flow cytometry. Cytotoxicity assays were performed with IL-15 expanded B6 NK cells and mKate2-expressing K7M2 murine osteosarcoma at a 1:1 ratio with blockade of CD155 and CD155 ligands. To test efficacy of NK cell infusion and CD155 blockade after allogeneic BMT, BALB/c mice were lethally irradiated, transplanted with allogeneic B6 bone marrow, and challenged with luciferase-expressing K7M2 on day 0. At day 7, mice received IL-15 expanded B6 NK cells intravenously with either anti-IgG control or anti-CD155 antibody intraperitoneally and IL-2 subcutaneously on days 7 and 11. Mice were monitored for tumor growth by bioluminescence, and toxicity by GVHD using weight loss and clinical scores.

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Conclusions These findings demonstrate that blockade of CD155 enhances an allogeneic NK cell-specific GVT effect for osteosarcoma treatment without exacerbating GVHD. CD155 blockade has the potential to improve usage of allogeneic BMT and NK cell adoptive immunotherapy as a combination treatment for osteosarcoma, and perhaps other pediatric sarcomas.
Background In recent years, the FDA has approved engineered autologous T cell therapies with remarkable efficacy against hematological cancers. In addition, non-engineered tumor infiltrating lymphocyte (TIL) therapies have shown unprecedented benefit against solid tumors in early clinical trials. Despite their success, TIL products have limitations including the need for specialized surgery to obtain sterile tumor for T cells, low neoantigen breadth, and the potential for T cells that may be pro-tumor, exhausted, or not tumorspecific. These limitations may hinder efficacy and accessibility for certain patients. We have developed an autologous, peripheral blood-derived non-engineered T cell therapy, GEN-011, that embraces the advantages of TIL while improving on their limitations by targeting true tumor-specific neoantigens identified by the ATLAS™ bioassay and avoiding potentially pro-tumor Inhibigans™.

Methods Peripheral blood mononuclear cells and a tumor biopsy are collected from each subject; tumor DNA is sequenced by WES. The ATLAS bioassay is used to individually screen each tumor mutation with the patient’s own T cells to identify neoantigen targets of pre-existing CD4+ and/or CD8+ T cell responses. The robust clinical scale manufacturing process, PLANET™, expands the patient’s peripheral blood T cells on ATLAS-identified stimulatory neoantigens.

Results The PLANET process produces GEN-011 drug products (DP) containing billions of antigen-specific, cytolytic T cells. Development and engineering runs using peripheral blood T cells from cancer patients and healthy donors resulted in DPs containing >97% T cells, >90% of which were central and effector memory phenotypes. A median 334-fold increase in antigen-specific T cells was observed in GEN-011 DPs over their starting frequency in peripheral blood with up to 67% of cells upregulating activation markers upon antigen recognition. Additionally, DP T cells secrete up to 50,000 pg/mL of IFN-gamma in response to antigen stimulation. In cancer patient samples, DPs respond to up to 89% of intended neoantigen targets compared to <10% reported recently for TIL products.

Conclusions GEN-011 is an autologous, neoantigen-specific T cell product, with key advantages over TIL therapy. First, GEN-011 has an unparalleled breadth of neoantigen coverage, targeting up to 30 relevant neoantigens with non-exhausted CD4+ and CD8+ memory T cells to overcome non-tumor specific ‘passenger’ T cells. Second, GEN-011 avoids pro-tumor Inhibigans that may be detrimental to clinical responses. Third, GEN-011 does not require extra surgery or viable tumor for manufacturing. In conclusion, GEN-011 is a first-in-class transformational T cell therapy candidate with characteristics that should improve accessibility and efficacy for patients with solid tumors.

Ethics Approval Informed consent was obtained from all individuals providing samples for this study.

REFERENCES
Background Metastatic castration-resistant prostate cancer (mCRPC) is a lethal, heterogeneous disease that has been largely resistant to immunotherapy. The lack of efficacy is due, in part, to the immunosuppressive tumor microenvironment and new therapeutic strategies for mCRPC must stimulate an antitumor response in the immunologically ‘cold’ tumors. Combination therapies that target both the tumor stroma and cancer cells could overcome the limitations of current immunotherapies and are demonstrated to be effective in multiple cancer models.\(^1,2\) NK cells are being explored as cell therapies and targeting NK cells to solid tumors can be improved by engineering the effector cells to express CD64, a high-affinity Fc receptor for human IgG. CD64 can capture soluble antibodies with 30–100x higher affinity than CD16A and mediates cell killing when antibody is bound.\(^3\) This docking platform allows for switchable targeting elements to redirect NK cells to multiple tumor antigens and facilitates the development of combination cell therapies.

Methods NK-92MI\(^{CD64}\) cell therapy was evaluated in combination with antibodies targeting the prostate tumor antigen tumor-associated calcium signal transducer 2 (TROP2) and the cancer-associated fibroblast (CAF) marker fibroblast activation protein alpha (FAP). Antibodies were bound to CD64 and effector cells (1:1 aTROP2 and aFAP mAb) were co-cultured with prostate cancer and CAF target cells (1:1 DU145 and hPrCSC-44 cells). Killing effect was measured using the DEL-FIA Cell Cytotoxicity assay and IFN-\(\gamma\) production was assessed by flow cytometry. Tumor-bearing NSG mice (DU145 and hPrCSC-44 cells; 100–200 mm\(^3\); \(N=4/\)group) received adoptive transfer of NK-92MI\(^{CD64}\) cells with or without bound antibodies (1x10\(^7\) cells; 1:1 aTROP2 and aFAP mAb) or saline (s.c.; 1x/wk for 4 wks). Therapeutic efficacy was evaluated by measuring tumor volumes.

Results IFN-\(\gamma\) production was increased with the addition of TROP2- or FAP-targeted antibodies. Cytotoxicity of the combination therapy was two-fold higher than either monotherapy (ANOVA \(P=0.012\); figure 1) and six-fold higher than NK-92MI\(^{CD64}\) cells alone (ANOVA \(P=0.0018\); figure 1). The killing effect was lost when the antibodies were switched to an iso type control, indicating that the targeting mechanism is antigen dependent. Robust antitumor activity was demonstrated in vivo and the combination therapy significantly reduced tumor growth by 78% compared to the saline control (ANOVA \(P=0.004\); figure 2).

Conclusions Our study suggests that NK-92MI\(^{CD64}\) cell therapy with antibodies targeting the tumor stroma and malignant cells is effective in a prostate cancer model. Validation of this combination therapy presents a new approach for treating mCRPC and could improve antitumor response.

Ethics Approval The study was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC) approval number 1708A-35052.

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Abstract 151 Figure 1 TROP2- and FAP-targeted antibodies bound to CD64 demonstrate enhanced antitumor effect compared to monotherapy or NK cells alone.

**Abstract 151 Figure 2** Adoptive NK-92MI\(^{CD64}\) cell transfer in combination with TROP2- and FAP-targeted antibodies reduces tumor growth in mice.
included T cells reactive to a p53 mutation in an autologous manner for the treatment of patients with metastatic epithelial cancers (n=12). Except for the two patients who exhibited an objective response (RECIST), most of the patients did not respond to the therapy, possibly due to low frequencies of anti-mutant p53 cells in the infusion product, exhausted phenotype, and/or poor persistence (table 2). To overcome these barriers to TIL treatment, we retrovirally transduced autologous peripheral blood T cells to express an allogeneic anti-mutant p53 TCR. We engineered the HLA-A*02:01-restricted anti-p53 R175H TCR into patient 4349’s lymphocytes (transduction efficiency of 64%) and saw less expression of exhaustion markers relative to the TIL infusion products (table 2). This patient with metastatic breast cancer was refractory to the six prior chemotherapy regimens. After the transfer of 5.3e10 cells, the patient experienced an objective partial response, showing regression by 55% of skin and mediastinal lesions for 7 months. The persistence of the infused T cells was higher than the other patients who received the TIL treatment (table 2).

Conclusions The library of anti-mutant p53 TCRs we have generated can potentially be used to treat ~6% of all cancer patients. We are pursuing the adoptive transfer of TILs against mutant p53 naturally occurring in the tumor or TCR-engineered cells using ‘off-the-shelf’ receptors against mutant p53.

Ethics Approval This study was approved by the Institutional Review Board (IRB) of the NCI, and the approval numbers are as follows: Protocol 10-C-0166 (TIL treatment); Protocol 18-C-0049 (allogeneic TCR engineered T cell therapy)

REFERENCE

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153 NEO-PTC-01 (BNT-221), AN AUTOLOGOUS NEOANTIGEN-SPECIFIC T-CELL PRODUCT FOR ADOPTIVE CELL THERAPY OF METASTATIC MELANOMA


Background Neoantigens are tumor-specific antigens that are important in the anti-tumor immune response. These antigens are not subject to central immune tolerance and are therefore potentially more immunogenic than tumor-associated antigens. NEO-STIM™, our propriety ex vivo induction process, was developed to generate T-cell products specific to these neoantigens from the peripheral blood of patient. Here, we present the results of a proof of concept, pre-clinical study with multiple successful process engineering runs generating a neoantigen-specific T-cell product (NEO-PTC-01) using leukaphereses from metastatic melanoma patients. These products contain specific T-cell responses targeting multiple neoantigens from each individual patient’s tumor.

Methods Patient-specific neoantigens were predicted using our RECON™ bioinformatics platform. Predicted high-quality neoantigens were utilized in our ex vivo stimulation protocol, NEO-STIM, in the process engineering runs of NEO-PTC-01. NEO-STIM is used to prime, activate and expand memory and de novo T-cell responses from both the CD4+ and the CD8+ compartment. High throughput flow cytometric analysis was performed to characterize the specificity and functionality (cytokine production and cytolytic capacity) of the induced T-cell responses.
Results Here we present the successful induction of 4–5 CD8+ and 4–7 CD4+ T-cell responses per patient, generated using peripheral blood mononuclear cells from multiple melanoma patients during these successful process engineering runs. We then extensively characterized these T-cell responses and demonstrate that these responses are functional, specific and have cytolytic capacity. Moreover, the induced T cells can recognize autologous tumor.

Conclusions NEO-STEM is a novel platform that generates ex vivo T-cell responses to high-quality neoantigen targets. NEO-PTC-01, the neoantigen-specific T cell product generated from this process, is a potent adoptive cell therapy targeting multiple immunogenic neoantigens in patients with metastatic melanoma.

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154 MARROW-INFLTRATING LYMPHOYTES (MILS): A NOVEL ADAPTOIVE IMMUNOTHERAPY FOR HEMATOLOGICAL AND SOLID TUMORS

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Background Marrow infiltrating lymphocytes (MILs™) are the product of activating and expanding bone marrow T cells.1 The bone marrow is a specialized niche in the immune system enriched for antigen-experienced, memory T cells. In patients with multiple myeloma and other hematological malignancies that relapse post-transplant, MILs have been shown to contain tumor antigen-specific T cells and adoptive cell therapy (ACT) using MILs has demonstrated antitumor activity.2 3 The bone marrow has been shown to harbor tumor-antigen specific T cells in patients with melanoma,4 5 glioblastoma,6 7 breast,8 7 non-small-cell lung9 and pancreatic cancers.10 Here, we sought to determine if tumor-specific MILs could be expanded from the bone marrow of patients with a range of different solid tumors.

Methods Bone marrow and blood samples were collected from patients with advanced and metastatic cancers. To date, samples have been collected from a minimum of four patients with non-small cell lung cancer (NSCLC), prostate cancer, head and neck cancer, glioblastoma, and breast cancer. Samples from patients with multiple myeloma were used as a reference control. Using a 10-day proprietary process, MILs and peripheral blood lymphocytes (PBLs) were activated and expanded from patient bone marrow and blood samples, respectively. T cell lineage-specific markers (CD3, CD4 and CD8) were characterized by flow cytometry prior and post-expansion. Tumor-specific T cells were quantitated in expanded MILs and PBLs using a previously described cytokine-secretion assay [2]. Briefly, autologous antigen-presenting cells (APCs) were pulsed with lysates from allogeneic cancer cell lines and co-cultured with activated MILs or PBLs. APCs pulsed with irrelevant mismatched cancer cell line lysates or media alone were used as negative controls. Tumor-specific T cells were defined as the IFN-gamma-producing population by flow cytometry.

Results MILs were successfully expanded from all patient bone marrow samples tested, regardless of tumor type. Cytokine-producing tumor-specific CD4+ and CD8+ T cells were detected in each of the expanded MILs. In contrast, tumor-specific T cells were not detected in any of the matched activated and expanded PBLs.

Conclusions MILs have been successfully grown for all solid tumor types evaluated, including NSCLC, prostate, head and neck, glioblastoma and breast cancer. Clinical studies have been completed in patients with multiple myeloma and other hematological cancers.2 3 A Phase IIa trial to evaluate MILs in combination with a checkpoint inhibitor is underway in patients with anti-CD1/PDL1-refractory NSCLC (ClinicalTrials.gov Identifier: NCT04069936). The preclinical data presented herein demonstrate that expanding MILs is feasible. MILs-based therapies hold therapeutic promise across a wide range of tumor indications.

Ethics Approval This study was approved by each participating institution’s IRB.

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155 IPSC-DERIVED NK CELLS MEDIATE ROBUST ANTI-TUMOR ACTIVITY AGAINST GLIOBLASTOMA

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Background Gliomas represent the most common brain tumors within the central nervous system, with glioblastoma being the most aggressive type.1 Conventional treatment combines several approaches including surgery, chemotherapy, and radiation.2 However, the prognosis for glioblastoma remains unfavorable, with only 5% of patients surviving more than 5 years post-diagnosis.3 Thus, new treatment approaches are urgently needed. Natural killer (NK) cells directly lyse malignantly transformed or virally infected cells and secrete inflammatory cytokines that polarize cytotoxic immunity. Allogeneic
NK cell adoptive transfer has shown clinical benefit in patients with advanced cancer. However, limitations of this approach include relatively low numbers of donor NK cells that can be isolated during an apheresis and variability in the quality of NK cells between donors. To overcome these limitations, we have developed a GMP manufacturing strategy to mass produce NK cells from induced pluripotent stem cells (iPSCs) as an approach to off-the-shelf cancer immunotherapy. We refer to these cells as ‘iNK’ (iPSC-derived NK) cells. Here, we provide preclinical data demonstrating the efficacy of iNK cells for immunotherapy against glioblastoma.

Abstract 155 Figure 1 Engineered iNK cells exhibit highly effective antitumor function in a xenogeneic model of glioblastoma. (A) Schematic of the experimental design to test iNK cell function against glioblastoma in vivo. (B) Kaplan Meier plots showing survival for groups of mice that received either vehicle alone or iNK cells after tumor engraftment (n=5 mice/group)

Methods We generated iNK cells using previously published methods. iNK cells were used as effectors against an array of patient-derived glioblastoma lines in 2-dimensional live imaging IncuCyte assays where iNK cell-mediated killing was observed over the course of 48 hours. To investigate iNK cell infiltration and cytotoxicity in a more physiological context that accounts for the 3-dimensional architecture of the tumor, we also performed live imaging IncuCyte assays using iNK cell-mediated killing and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood 2020;105:3051-3057.

156 DISCOVERY OF TSC-100: A NATURAL HA-1-SPECIFIC TCR TO TREAT LEUKEMIA FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION THERAPY

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Background Approximately 30–40% of AML patients relapse following allogeneic hematopoietic stem cell transplantation therapy, leaving them with very few treatment options. Rare patients that naturally develop an HA-1-specific graft-versus-leukemia T cell response, however, show substantially lower relapse rates. A recently discovered TCR (TSC-100) targets a highly restricted minor histocompatibility antigen, making it an ideal candidate for TCR immunotherapy for liquid tumors.
Methods We developed a high-throughput TCR discovery platform that enables rapid cloning of antigen-specific TCRs from healthy donors. We then used this platform to screen 178.3 million naïve CD8+ T cells from six unique HA-1-(VLDDLLLEA, genotype RS_1801284 G/G) donors, identifying 329 HA-1-specific TCRs. We tested each TCR for expression and the ability to kill HA-1+ target cells, using a previously published, clinical-stage HA-1-specific TCR as a benchmark for these studies.6 In parallel, we tested TCR constant region modifications to promote expression and proper pairing of exogenous TCR alpha and beta chains and designed a lentiviral vector to co-deliver CD8 coreceptors as well as a CD34 enrichment tag to enable purification of engineered T cells. The top 11 candidates were cloned into our optimized backbone and evaluated for cytotoxicity, cytokine production, and T cell proliferation using a panel of HLA-A*02:01+ HA-1+ cell lines. Finally, the top two TCRs were evaluated for allo-reactivity and off-target cross-reactivity using our proprietary genome-wide T-Scan platform.

Results The TCR discovery and evaluation platform described here identified 329 HA-1-specific TCRs from a total of 178.3 million naïve T cells, and TSC-100 as the most active TCR. Defined mutations in the constant region of TSC-100 enhanced its surface expression while decreasing expression of endogenous TCRs, and co-introduction of CD8 enabled efficient engagement and function of engineered CD4 cells. Overall, TSC-100 exhibited comparable activity to a clinical-stage benchmark TCR when challenged with cell lines expressing moderate to high levels of HA-1, and superior activity when incubated with cell lines expressing low levels of both HA-1 and MHC-I.6 In addition, TSC-100 exhibited no detectable allo-reactivity to 108 different HLA types tested, and minimal off-target effects when challenged with a genome-wide library expressing peptides derived from human proteins.

Conclusions TSC-100 exhibits comparable or superior activity to a clinical-stage therapeutic TCR, with minimal allo-reactivity or off-target effects. Based on these results, TSC-100 has been advanced to IND-enabling activities to prepare for first-in-human testing in 2021.

Ethics Approval All clinical samples used in the study were collected by STEMCELL Technologies, StemExpress and HemaCare using their IRB approved protocols.

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Background Adaptive cell therapy (ACT) with antigen-specific CD8+ T cells is a promising approach for treating patients with various solid malignancies including melanoma. In vivo expansion of adoptively transferred T cells is one of the major determinants of successful ACT. On the other hand, a frequently overlooked consideration is that the host antigen-presenting cells affect the antitumor efficacy of ACT. Accumulating evidence suggests that tumor-residing Batf3-dependent conventional type I dendritic cells (cDC1s) play an important role in trafficking of adoptively transferred T cells into the tumor by producing chemokines such as CXCL10, and improve antitumor efficacy of ACT. However, a role of cDC1s in expansion of adoptively transferred T cells remains unclear.

Methods We utilized Pmel-1 T cell receptor transgenic T cells in the B16 melanoma model to investigate the role of cDC1s in expansion of adoptively transferred tumor-specific T cells.

Results While adoptive transfer of in vitro-activated Pmel-1 T cells with vaccination of cognate antigen, hgp100, agonistic anti-CD40 monoclonal antibody (mAb), and Toll-like receptor 7 (TLR7) agonist delayed the tumor growth and survival in wild type C57BL/6 mice (WT), antitumor efficacy of ACT was completely abrogated in Batf3-/- mice. Flow cytometric analysis of peripheral blood showed expanded adoptively transferred Pmel-1 T cells was significantly compromised in WT mice but not in in Batf3-/- mice. Mechanistically, loss-of-function studies using mixed bone marrow chimera reconstituted with Batf3-/- and CD40-/- (Batf3-/-/CD40-/-), Batf3-/- and CD70-/- (Batf3-/-/CD70-/-), or Batf3-/- and CD80/86-/- (Batf3-/-/CD80/86-/-) revealed CD40-CD70 axis but not CD80/86 signaling in host cDC1s plays an important role in expansion of adoptively transferred T cells. Accordingly, overall survival of Batf3-/-/CD70-/- mixed chimeric was significantly shorter than that of Batf3-/-/WT mice, while survival of Batf3-/-/CD80/86-/- mice was similar to that of Batf3-/-/WT mice. Furthermore, induction of cDC1s by administration of Fms-like tyrosine kinase 3 receptor ligand (gain-of-function) demonstrated significantly enhanced in vivo expansion of adoptively transferred Pmel-1 T cells associated with improved tumor control and survival.

Conclusions These findings elucidate a role of host cDC1s in expansion of adoptively transferred in vivo restimulated tumor-specific T cells, and identify CD40 and CD70 as key molecules.

In vivo expansion of adoptively transferred tumor-specific T cells, and identify CD40 and CD70 as key molecules.

Background Durable responses have been observed with adoptive T cell therapy (ACT) in some patients. However, current
ADOPTIVELY TRANSFERRED CD8+ T CELLS THAT TARGET NEOANTIGEN PERSIST AND REGRESS MELANOMAS TO A GREATER EXTENT THAN THOSE THAT TARGET SELF/TUMOR-ANTIGEN

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Background In patients treated with immunotherapy, mechanisms underlying why some respond to or fail treatment are not fully understood. Higher tumor mutational burden is often correlated with better responses, because the immune system reacts more strongly against mutated antigens (versus self antigens) due to a higher affinity interaction with the T cell receptor. In adoptive T cell transfer therapy (ACT), engraftment and persistence of the T cells are critical to prolonged antitumor responses. It remains unclear whether the affinity of the interaction between tumor antigen and TCR alone impacts the engraftment and persistence of tumor-specific T cells post ACT.

Methods To simulate this clinical scenario in mice, we used two different melanoma models: 1) B16F10 expressing a higher affinity peptide (hgp100), which represents a neoantigen-expressing tumor, and 2) Pmel-1 CD8+ T cells expressing a TCR that recognizes gp100 were adoptively transferred into mice bearing B16F10 melanoma.

Results We posited that the function and persistence of adoptively transferred pmel-1 T cells would be increased in mice with neoantigen- compared to self- antigen expressing tumors. Indeed, we found that pmel-1 were less exhausted as well as engrafted and persisted far better in mice bearing tumors expressing neoantigens. Moreover, these large subcutaneous hot tumors shrank post ACT treatment and the animals survived long-term. Beneficial outcome was correlated with the appearance of vitiligo. Importantly, these cured mice were protected when rechallenged with a secondary tumor even after an intravenous rechallenge, implicating this ACT treatment mediates durable memory responses.

Conclusions Herein, we underscore how tumor antigen affinity can drastically change T cell fate. Future work will concentrate in exploring in depth the correlation of less differentiated cytotoxic T cells treating neo/self-antigen expressing melanomas mimicking a clinical setting.

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than traditional T cells in the host. We explored the characteristics of CpG-expanded T cells and found that T cells generated from a CpG culture incur a unique proteomic and cell surface signature phenotype. Of all the cell types present in the starting culture (CD4+ T cells, NK cells, B cells, DCs, macrophages), B cells were the only cell type critical to achieve a more potent T cell therapy with CpG. In a direct comparison of CpG class A (targeting DCs) and CpG class B (targeting B cells), only the B cell-activating CpG improved cell therapy. Finally, we found that B cells alone could improve purified CD8+ T cells for ACT when the co-culture was activated with CpG, indicating that B cells become potent APCs in this context.

Conclusions Collectively, our findings indicate a novel way to use TLR agonists to improve ACT and reveal a critical role for B cells in the expansion of potent anti-tumor CD8+ T cells. Translating these findings to ACT therapies could provide dramatic improvements in patients with late stage malignancies.

Acknowledgements Proteomic analysis was performed at the Mass Spectrometry Facility, a University Shared Research Resource at the Medical University of South Carolina, using instrumentation acquired through the NIH shared instrumentation grant program (S10 OD010731-Orbitrap Elite Mass Spectrometer or Orbitrap Fusion Lumos ETD/UVD MS (S10 OD025126).

Trial Registration NA

Ethics Approval All animal procedures were approved by the Institutional Animal Care & Use Committee of the Medical University of South Carolina, protocol number 0488.

Consent NA

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161 DEVELOPMENT OF A CD8 CO-RECEPTOR INDEPENDENT T CELL RECEPTOR SPECIFIC FOR TUMOR-ASSOCIATED ANTIGEN MAGE-A4 FOR NEXT GENERATION T CELL-BASED IMMUNOTHERAPY

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Background The cancer-testis antigen MAGE-A4 is an attractive target for T cell-based immunotherapy, especially for indications with unmet clinical need like non-small-cell lung carcinoma or triple-negative breast cancer. Overcoming high tumor burden using adoptive transfer of T cells modified to express a transgenic T cell receptor (TCR) demands optimal recognition of the corresponding target on tumor cells by the TCR-modified T cells (TCR-Ts). Here we describe the isolation and pre-clinical characterization of high avidity TCR-Ts expressing a human leucocyte antigen (HLA)-A*02:01-restricted MAGE-A4-specific TCR that is fully functional in T cells irrespective of CD4 or CD8 co-receptor expression.

Methods An unbiased CD137-based sorting approach was first used to identify an immunogenic MAGE-A4-derived candidate epitope that was properly processed and presented on HLA-A2 molecules encoded by the HLA-A*02:01 allele. To isolate high avidity T cells via subsequent multimer sorting, an in vitro priming approach using HLA-A2-negative donors (allogeic-HLA-restricted priming approach) was conducted to bypass central tolerance to this self-antigen. Pre-clinical parameters of safety and activity were assessed in a comprehensive set of in vitro and in vivo studies of the lead TCR candidate derived from a selected T cell clone.

Results A TCR recognizing the MAGE-A4-derived decapeptide GVYDREHTV was isolated from primed T cells of a non-tolerant HLA-A2-negative donor. The respective TCR-T cell product bbT485, expressing the lead TCR in T cells from healthy donors, was demonstrated pre-clinically to have a favorable safety profile and superior in vivo potency compared to TCR-Ts made using a TCR derived from an HLA-A2-positive donor bearing a tolerized T cell repertoire to self-antigens. The natural high avidity allogeneic (allo)-derived TCR was found to be CD8 co-receptor-independent, allowing effector functions to be elicited in transgenic CD4+ T helper cells. These CD4+ TCR-T cells not only supported an anti-tumor response by direct killing of MAGE-A4-positive tumor cells, but also upregulated hallmarks associated with helper function, such as CD154 expression and release of key cytokines upon tumor-specific stimulation.

Conclusions The extensive pre-clinical assessment of safety and in vivo potency of this non-mutated high avidity, CD8 co-receptor-independent, MAGE-A4-specific HLA-A2 restricted TCR provide the basis for its use in clinical TCR-T immunotherapy studies. The ability of this co-receptor-independent TCR to activate all transduced T cells (irrespective of CD4 or CD8 expression) could potentially provide enhanced cellular responses in the clinical setting through the induction of functionally diverse T cell subsets that goes beyond what is currently tested in the clinic.

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163 NICE: NEOANTIGEN-CYTOKINE-CHEMOKINE MULTIFUNCTIONAL ENGAGER FOR NK CELL IMMUNOTHERAPY OF SOLID TUMORS

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Background The effectiveness of natural killer cell-based immunotherapy against solid tumors is limited by the lack of specific antigens and the immunosuppressive tumor microenvironment. To improve the clinical efficacy and specificity of NK cell therapy, we are designing, developing, and characterizing a new generation of multi-specific killer engagers, which consists of a neoantigen-targeting moiety, together with cytokine and chemokine-producing domains.

Methods Targeting a neoantigen-an antigen formed specifically in response to tumor genome mutations-enables substantially enhanced tumor specificity to be achieved. We evaluated the responsiveness of NK cells to Wilms Tumor 1 (WT1) antigen in GBM by synthesizing an antibody that is able to recognize the WT1/HLA complex. Incorporation of cytokine (namely IL-2, IL-15, and IL-21)-essential for the maturation, persistence, and pre-clinical characterization of high avidity TCR-Ts expressing a human leucocyte antigen (HLA)-A*02:01-restricted MAGE-A4-specific TCR that is fully functional in T cells irrespective of CD4 or CD8 co-receptor expression.

Methods An unbiased CD137-based sorting approach was first used to identify an immunogenic MAGE-A4-derived candidate epitope that was properly processed and presented on HLA-A2 molecules encoded by the HLA-A*02:01 allele. To isolate high avidity T cells via subsequent multimer sorting, an in vitro priming approach using HLA-A2-negative donors (allogeic-HLA-restricted priming approach) was conducted to bypass central tolerance to this self-antigen. Pre-clinical parameters of safety and activity were assessed in a comprehensive set of in vitro and in vivo studies of the lead TCR candidate derived from a selected T cell clone.

Results A TCR recognizing the MAGE-A4-derived decapeptide GVYDREHTV was isolated from primed T cells of a non-tolerant HLA-A2-negative donor. The respective TCR-T cell product bbT485, expressing the lead TCR in T cells from healthy donors, was demonstrated pre-clinically to have a favorable safety profile and superior in vivo potency compared to TCR-Ts made using a TCR derived from an HLA-A2-positive donor bearing a tolerized T cell repertoire to self-antigens. The natural high avidity allogeneic (allo)-derived TCR was found to be CD8 co-receptor-independent, allowing effector functions to be elicited in transgenic CD4+ T helper cells. These CD4+ TCR-T cells not only supported an anti-tumor response by direct killing of MAGE-A4-positive tumor cells, but also upregulated hallmarks associated with helper function, such as CD154 expression and release of key cytokines upon tumor-specific stimulation.

Conclusions The extensive pre-clinical assessment of safety and in vivo potency of this non-mutated high avidity, CD8 co-receptor-independent, MAGE-A4-specific HLA-A2 restricted TCR provide the basis for its use in clinical TCR-T immunotherapy studies. The ability of this co-receptor-independent TCR to activate all transduced T cells (irrespective of CD4 or CD8 expression) could potentially provide enhanced cellular responses in the clinical setting through the induction of functionally diverse T cell subsets that goes beyond what is currently tested in the clinic.

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construct further supports NK cell recruitment and may stimulate the recruitment of other immune cells. NK activation through the tri-specific engager is achieved through NKp46-mediated signaling. We are investigating the ability of the tri-functional engager to support and enhance NK cell-mediated cytotoxicity against GBM in vitro and in patient-derived GBM xenografts in vivo.

Results
We hypothesize that taking advantage of our multifunctional engager, NK cells will exhibit, at once, superior persistence, infiltration and antitumor activity, simultaneously addressing three of the main limitations to the use of NK cells in immunotherapy of GBM and other solid tumors.

Conclusions
N/A
Acknowledgements
N/A
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Abstracts

164 AGENT-797, A NOVEL ALLOGENIC AND ‘OFF-THE SHELF’ iNKT CELL THERAPY PROMOTES EFFECTIVE TUMOR KILLING
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Background
Harnessing both the innate and adaptive immune system could increase the efficiency of current cancer immunotherapies and promote durable anti-tumor immunity. Invariant natural killer T (iNKT) cells are innate-like lymphocytes that bridge innate and adaptive immune responses and promote anti-cancer immunity. iNKT cells are activated and respond rapidly via multiple signals such as recognition of lipid antigens through the invariant T cell receptor (TCR), pro-inflammatory cytokines or recognition of stress ligands. Here we describe, AgenT-797, a novel, allogeneic and ‘off-the-shelf’ iNKT cell therapy, designed to promote effective anti-cancer immunity against a wide range of malignancies.

Methods
iNKT cells isolated from healthy donors were expanded by stimulation of the invariant TCR with alpha-GalCer and cytokines using the AgenTus manufacturing protocol. The phenotype and functional activity of the expanded unmodified iNKT cells, AgenT-797, were characterized by flow cytometry. The cytotoxic potential of AgenT-797 was assessed in tumor co-culture assays against CD1d-expressing cancer cell lines. To further direct anti-tumor responses, iNKT cells were engineered to express Chimeric Antigen Receptors (CARs), and the cytotoxic potential assessed against antigen-expressing cancer cells.

Results
iNKT cells were rapidly expanded up to $2 \times 10^{10}$ cells in 30 days, with over 99% purity. Expanded, unmodified iNKT cells, AgenT-797, were found to secrete both Th1 (IFNy, TNFa, GM-CSF) and Th2 (IL4, IL13) type cytokines. After rapid expansion, AgenT-797, retained their inherent cytotoxic capacity against CD1d-expressing tumor cell lines. Further, killing of tumor target cells, in vitro, was mediated through their endogenous invariant TCR or engineered CAR receptor.

Conclusions
AgenT-797 is an ‘off-the-shelf’ and allogenic cell therapy with effective cancer killing properties. Strategies to engineer iNKT cells using CAR technology further enhance the tumor killing potential of iNKT therapy.

165 ACTIVATING ANTIGEN CARRIERS GENERATED WITH MICROFLUIDICS CELL SQUEEZING DRIVE EFFECTIVE ANTI-TUMOR RESPONSES
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Background
Activation of T cell responses is essential for effective tumor clearance, however generating targeted, effective antigen presentation to stimulate T cell response remains challenging. We can harness the natural process of red blood cell (RBC) clearance from the body to activate the antigen-specific immune responses. Using the Cell Squeeze® microfluidics platform, we generate activating antigen carriers (AACs) from RBCs to drive antigen presentation and T cell activation in human and murine models.

Methods
We loaded proteins or synthetic long peptide antigens together with adjuvants into murine or human RBCs with Cell Squeeze® (SQZ’ing) to generate AACs and investigated the effects of SQZ’ing on the RBC membrane. Following intravenous AAC injection into mice, we measured AAC clearance kinetics and characterized the site and cell type of AAC uptake. We investigated the regulation of activation markers on phagocytes that engulf AACs, clearance of endogenous RBCs in mice treated with AACs, and the effect of boosting with AACs on endogenous T cell responses. To determine the ability of AACs to control subcutaneously implanted tumors, we measured tumor growth rates in mice therapeutically treated and boosted with AACs. Finally, we observed in vitro uptake of human AACs loaded with adjuvant and resultant maturation of monocyte-derived dendritic cells (MODCs) to qualify adjuvant delivery. Peptide antigen delivery to human AACs was measured with flow cytometry and fluorescence microscopy.

Results
We demonstrated that SQZ’ing effectively loads AACs without reducing CD47 expression. When administered into a mouse, AACs were cleared from circulation within one hour and were engulfed by professional phagocytes in both the spleen and liver. In vivo, AACs upregulated activation markers on macrophages and DCs, and administration of AACs does not affect clearance or half-life of endogenous RBCs. Therapeutic AAC administration to mice strongly impedes tumor growth and extends survival; the anti-tumor responses correlate with >10x increase in antigen-specific CD8+ tumor-infiltrating lymphocytes compared to untreated mice. Boosting enhances endogenous T cell responses and boosting at early time points in the tumor model enhances low dose vaccinations. In an in vitro human system, we demonstrated that human AACs can be loaded with peptide antigen and adjuvant such that upon engulfment, AACs stimulated MODC maturation.

Conclusions
In summary, these results indicate that AACs loaded with antigen and adjuvant can effectively drive antigen presentation and prime a potent anti-tumor response in mice. These data support the further study of SQZ AACs as an immunotherapy for cancer treatment.

Ethics Approval
All methods were performed in accordance with the relevant guidelines and regulations. Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at SQZ Biotechnologies, using the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the Office of Laboratory Animal Welfare. All activities were also
conducted in accordance with Public Health Service (PHS) Policy on Humane Use and Care of Laboratory Animals.

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**166** MUCOSAL-ASSOCIATED IN Variant T-CELLS (MAIT) IN Pancreatic Cancer

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**Background** Immunotherapy has changed the standard of care for multiple cancers; however, its efficacy is limited. Chemotherapy and radiation had little effect in pancreatic ductal adenocarcinoma (PDAC) outcome1 in patients with metastatic disease, hence the urgency for new effective courses of treatment. Increasing evidence suggests mucosal-associated invariant T-cells (MAIT) play a role in anti-cancer T-cell responses, by recognizing transformed cells or bacterial products. MAIT respond towards microbial antigens and vitamin derivatives, produce pro-inflammatory cytokines2 3 and have been found present in primary and metastatic cancer lesions.3 4 Long-term survival PDAC patients present a unique microbiome pattern. In contrast, some microbial species may promote oncogenesis.5 The focus of this project is the characterization of MAIT as immune effector cells in PDAC specimens.

**Methods** We performed a retrospective analysis of long-term survivors (LTS) and short-term survivors (STS) patients with pancreatic cancer associating clinical endpoints with the presence of MAIT infiltration in the tumor tissue using immunofluorescence staining for MR1 (MHC class I-related gene, a receptor) sequencing which allows to back-trace TCRs directed against cancer cells can serve as viable blueprints to engage with MR1 on PDAC recognizing tumor-associated targets or microbial products that elicit IFN-γ production. This allows to explore MAIT TCRs for adoptive therapies or distinct microbial species that drive clinically relevant responses.

**Conclusions** MAIT cells may exhibit anti-tumor properties, based on cytokine production and cellular marker activation. TCRs directed against cancer cells can serve as viable blueprints to engage with MR1 on PDAC recognizing tumor-associated targets or microbial products that elicit IFN-γ production. This allows to explore MAIT TCRs for adoptive therapies or distinct microbial species that drive clinically relevant responses.

**Acknowledgements** The authors would like to thank to Champalimaud Foundation Biobank and Vivarium Facility at Champalimaud Foundation.

**Ethics Approval** This study was approved by the Champalimaud Foundation Ethics Committee and by Ethics Research Committee of NOVA Medical School of NOVA University of Lisbon.

**Consent** For each patient, written informed consent and approval by the Ethical Committee of the Champalimaud Foundation will be obtained. The study will be in compliance with the Declaration of Helsinki.

**REFERENCES**

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**167** B-CELL-BASED VACCINATION ELICIT POTENT IMMUNITY AGAINST Glioblastoma

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**Background** Despite the tremendous effort in basic, translational and clinical research, the standard-of-care of patients with glioblastoma (GBM) has been virtually unchanged for the past two decades, aside from tumor-treating fields. GBM is one of the immunologically ‘coldest tumors’ where T-cell exclusion is at its maximum, and myeloid infiltration predominates. This is due to profound immunosuppression, the metabolically hostile microenvironment, and the low mutational burden of these tumors. Together, these barriers have hindered the development of effective immunotherapies. With the goal of exploring ways to boost anti-GBM immunity, we developed a B-cell-based vaccine (BVax) that consists of 4-1BBL+ B cells activated with CD40 agonism and IFNgamma stimulation.

**Methods** Studies on B-cell-driven inflammation have identified a subset of B cells expressing the co-stimulatory marker 4-1BBL (or CD137L) capable of enhancing CD8+ T-cell anti-tumor cytotoxicity. Such activation was achieved through multiple mechanisms, including antigen presentation, T-cell co-stimulation (4-1BBL and CD86), and cytokine production (TNFalpha). Thus, 4-1BBL+ B cells could be utilized to boost anti-tumor CD8+ T-cell response. In order to stabilize their antigen presentation function in-vivo and avoid potential immunosuppressive functions, we activated 4-1BBL+ B cells using CD40 and IFNgamma receptor (IFNgR) ligation (designated as BVax, figure 1A), both of which were effective to enhance B-cell-mediated antigen presentation (figure 1B-E). In the present study, we explored the ability of BVax to inhibit GBM growth by promoting tumor-specific CD8+ T-cell immunity and production of tumor-reactive antibodies. BVax’s therapeutic effectiveness was examined both alone and in combination with radiation and checkpoint blockade.

**Results** BVax migrate to key secondary lymphoid organs and are proficient at antigen cross-presentation (figure 2A), which
Abstract 167 Figure 1  BVax generation and its effector function as APC

BVax generation. (A) BVax are generated from 4-1BBL-expressing B cells obtained from glioma-bearing mice secondary lymphoid organs or GBM patient peripheral blood. After magnetic isolation, B cells are activated with B-cell survival factor BAFF, CD40 agonist and IFNγamma. Cells are pulse with tumor lysate before intravenous injection. BVax effector function as APC. (B) BVax were tested for their ability to uptake AlexaFluor488-conjugated OVA [BVax (OVA)], and (C) present SIINFEKL peptide via MHC class I (H-2Kb). Surface transport of the H-2Kb + SIINFEKL complex was inhibited using Brefeldin A (BFA). Shown, a representative experiment of 3 independent experiments. Bars represent 15 mm (fluorescence images) and 50 mm (fluorescence and bright light image). (D) BNaive, BNaive + IFNγ, BVax and dendritic cells (DCs) were pulse with OVA and tested for their ability to promote OT-I CD8+ T-cell activation assessed by cell proliferation (expansion index, X-axis) and intracellular expression of GzmB (Y-axis). (E) OT-I CD8+ T cells cultured with BNaive, BVax pulse with OVA and isotype control, BVax (OVA) + IC, or with MHC class I blocking Ab, BVax (OVA) + anti-H2Kb, and tested for their cellular expansion. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Abstract 167 Figure 2  BVax therapeutic effect

BVax promote survival and activation of CD8+ T cells. (A) Rag1 deficient mice were challenged intracranially with CT2A cells. Nine days after, mice received intravenously and concomitantly both Cell Tracker red CMPTX BVax (red cells) and CellTracker green CMFDA-labeled CD8+ T cells (green). BVax and CD8+ T-cell splenic localization were analyzed by fluorescent microscopy. Bars represent 100 um (left image, 20x magnification) and 50 um (right image, 63x magnification). (B) B-cell deficient (B KO) mice were challenged intracranially with GL261-OVA. Nine days after mice received intravenously BNaive or BVax pulsed with OVA protein. A group of BVax (OVA) was pretreated with pertussis toxin (PTX) before injection (n=4 micelgroup). Seven days after the cell adoptive transfer, SIINFEKL-specific CD8+ T cells were analyzed in the tumor-bearing brains by flow cytometry using SIINFEKL-tetramer. (C) B KO mice were challenged intracranially with CT2A cells. Nine days after mice received intravenously BNaive and BVax pulsed with CT2A tumor lysates pretreated with or without PTX (n=5 micelgroup). Seven days after the cell adoptive transfer, CD8+ T cells were evaluated for the intracellular expression of GzmB and IFNγ in the tumor-bearing brain, blood, and dCLN. BVax potentiate the therapeutic effect of combined RT+CD8+ T+PD-L1 blockade. (D) Irradiated CT2A-bearing mice received vehicle (Mock, black line), 3 injections of anti-PD-L1 (dotted black line), 3 injections of CD8+ T cells and anti-PD-L1 (gray line), or 3 injections of BVax + CD8+ T cells, and anti-PD-L1 (pink dotted line). A non-irradiated group was kept as control (No RT, dashed black line). Seventy-five days after tumor challenge (arrow), surviving mice were re-challenged with CT2A cells in the left hemisphere, opposite of the initial tumor injection site. (E) Long-term survivors (LTS) were sacrificed and checked for the presence of tumor mass using H&E staining. Non-tumor-bearing but skull drilled (no tumor) and age-matched CT2A-bearing mice (Control) were used as controls. Sections were performed as depicted in the cartoons. For LTS treated with BVax and CD8+ T cells (LTS-BVax+CD8), brains were sectioned both in the right hemisphere (1st site of injection, LTS-BVax+CD8 R) and left hemisphere (2nd site of injection – rechallenge, LTS-BVax+CD8 L). H&E sections images are representative of 3 LTS-BVax+CD8, 2 control and 1 no tumor brains. Bars represent 5 mm. (F) The same brains utilized in (E) were used to stain for infiltrating CD8+ T cells. Representative images of one LTS-R section where the choroid plexus, site of injection, the pons (arrows) and the cerebellum (arrows) are magnified. For the top image, bars represents 2.5 mm. For magnified images bars represent 100 um. (G) Gently dissected brains from no tumor (n=2), control (n=3), LTS that only received CD8+ T cells and PD-L1 blockade (LTS-CD8, n=4) and LTS-BVax+CD8 (n=5) mice were analyzed for lymphocytes phenotype. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Ethics Approval  All human samples (tumor, peripheral blood, and frozen tissue) were collected by the Nervous System Tumor Bank at Northwestern University (NSTB) under the institutional review board (IRB) protocol N° STU000202003. All animal experimentation protocols are approved by the Institutional Animal Care and Use Committee (IACUC) under protocol N° IS00002459 at Northwestern University.
Abstract 167 Figure 3  GBM patient-derived BVax

GBM patient-derived BVax promote anti-tumor CD8+ T cells. (A) Paired fresh peripheral blood and tumor were collected from newly diagnosed GBM patients (n=4). BVax were generated and pulsed with tumor lysates and co-cultured with autologous eFluor450-labeled CD8+ T cells. CD8+ T-cell activation was assessed by cell proliferation (eFluor450 fluorescence dilution measured as expansion index) and intracellular expression of GzmB. (B and C) Paired samples from primary GBM IDH WT (case NU 02120, B) and recurrent GBM IDH WT (NU02265, C). BVax-activated autologous CD8+ T cells were obtained as shown in (A) and tested for their ability to kill autologous glioma cells. Cell killing measurement were taken periodically for 12.5 hours using the IncuCyte S3 Live Cell Analysis System. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Abstract 167 Figure 4  BVax produce anti-tumor IgG

BVax produce tumor-reactive antibodies with therapeutic effect. (A) Schema of BVax-derived serum immunoglobulin (Ig) obtainment. BNaive, BAct and BVax-derived IgG were tested for IgG subtype (top) and their reactivity to B KO tumor-bearing-brains (bottom). Top: Diagram representing the distribution of different Ig subtypes from serum antibodies derived from BNaive, BAct and BVax. Ig subtype measurement of serum samples was performed by ELISA, and mean total Ig concentration is shown in the bottom of the diagram (mg/ml). The experiment was performed in 7 mice/group. Bottom: B-cell subsets IgG reactivity was measured by immunofluorescence. Serum samples were incubated on tumor-bearing brains sections from B KO. Binding IgG was detected using anti-mouse IgG Cy5 (red) secondary antibody. Nuclei was detected using DAPI (blue), and myeloid cells were evaluated by using anti-mouse CD11b AF488 antibody (green). Bars represent 100 mm. Shown, a representative experiment of serum obtainment in 4 mice/group, performed twice independently. (B) BNaive, BAct and BVax were generated from GL261 overexpressing ovalbumin (GL261-OVA) tumor-bearing mice. B cells were allowed to produce antibodies in GL261-OVA-bearing B KO. Serum samples were collected and IgG were purified and tested for their reactivity against OVA peptide SIINFEKL by ELISA. Semi-quantitative measurement is shown as optical density (O.D). Serum from B-cell deficient mice and C57BL/6 SIINFEKL-immunized mice were used as negative and positive control respectively (n=4/group). (C) Purified IgG were tested for their therapeutic effect in the CT2A model. IgG were delivered intracranially for 3 consecutive days (12.5 mg/mouse/injection). Untreated mice (black line) were used as controls. Experimental groups received either BNaive-derived IgG (BNaive IgG, blue line) or BVax-derived IgG (BVax IgG, pink line). The experiment was performed using n=10 micegroup. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Acknowledgements In conclusion, BVax tackles GBM immunosurveillance escape by using both cellular (CD8+ T-cell activation) and humoral (anti-tumor antibody production) immunity. Our study provides an efficient alternative to current immunotherapeutic approaches that can be readily translated to the clinic.

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168 A NOVEL PROSTATE-RESTRICTED TUMOR-ASSOCIATED ANTIGEN: A POTENTIAL THERAPEUTIC TARGET

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Background Prostate cancer is the second leading cause of cancer related death in men in the United States, mainly due to disease progression to metastatic castration-resistant prostate cancer (mCRPC). Although immunological treatment with the FDA-approved vaccine sipuleucel-T extends survival for 2–4 months by targeting the prostate-restricted antigen PAP, the identification of more immunogenic tumor-associated antigens (TAAs) continues to be an unmet need.

Methods We evaluated the differential expression profile of the subset of epithelial cells reported to give rise to CRPC from mice following an androgen deprivation/repletion cycle. The expression levels of a set of androgen-responsive genes was further evaluated in prostate, brain, colon, liver, lung, and skin normal tissues from murine and human databases. The expression of a novel prostate-restricted TAA was then analyzed in primary tumors across all human cancer types in The Cancer Genome Atlas (TCGA). Finally, the immunogenicity of this novel prostate-restricted TAA was evaluated in vitro by autologous co-culture assays with cells from healthy donors and in vivo by antibody profiling (PhIP-Seq) in the sera of a cohort of prostate cancer patients treated with AR blockade alone or in combination with the cell-based vaccine GVAX.

Results Here, we discovered a set of androgen-responsive genes exclusively expressed by the putative cell-of-origin for prostate cancer. We confirmed prostate-restricted enrichment of these androgen-responsive genes in normal tissues from murine and human databases. Among these prostate-restricted genes, we identified PAP, PSA, and a novel non-mutated TAA. This novel TAA was confirmed to be expressed in prostate cancer. Furthermore, its expression was associated with survival in patients with primary prostate cancer. Interestingly, we found that pro-inflammatory activated TBET+ EM CD8 and CD4 T cells were expanded by moDCs pulsed with our novel TAA to a greater extent than moDCs pulsed with either PAP or PSA were used. An IgG antibody response to this novel prostate-restricted TAA was detected in 30% of vaccinated patients, while fewer than 8% of vaccinated patients developed antibody responses to PSA or PSMA.

Conclusions Taken together, these results suggest we have found a novel immunogenic prostate-restricted TAA that represents a promising therapeutic target for treating mCRPC.

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**169 MICROFLUIDICS CELL SQUEEZING ENABLES HUMAN PBMCs AS DRIVERS OF ANTIGEN-SPECIFIC CD8+ T RESPONSES ACROSS BROAD RANGE OF ANTIGENS FOR DIVERSE CLINICAL APPLICATIONS**

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**Background** Antigen-specific CD8+ T cell activity is critical for mounting an effective immune response in a wide range of indications, including immune-oncology and infectious diseases.

**Methods** To elicit antigen-specific CD8+ T cell activity, we used microfluidics cell squeezing (Cell Squeeze®) to deliver antigens directly to the cytosol of antigen presenting cells (APCs). Direct cytosolic delivery bypasses the need for cross-presentation and efficiently loads antigen into the major histocompatibility complex class I (MHC-I) pathway. The Cell Squeeze® platform is generally agnostic to cell type and material. Therefore, not only does microfluidic squeezing enable cell subsets within human peripheral blood mononuclear cells (PBMCs) to function as unconventional APCs, but it also enables us to efficiently investigate a wide range of antigens including whole protein, peptides, and mRNA. This 'plug and play' nature of the platform allows for broad application in multiple disease areas.

**Results** In human cells, we demonstrated that microfluidic squeezing of PBMCs enables effective delivery to the major cell subsets including T cells, B cells, NK cells and monocytes. Delivery of CMV and HPV16 synthetic long peptides (SLPs) resulted in robust in vitro responses of both CD8+ T cell clones and patient-derived memory populations. To broaden the impact of our PBMC-based cell therapy approach, we investigated several other antigens relevant to other disease areas. Additional materials we delivered via squeezing and demonstrated antigen presentation include neoantigens, M1 Influenza mRNA, and pp65 SLP. Cell Squeeze® platform is simple to use and amenable to scale up. We demonstrated that delivery and viability for research scale process (~2 × 10^6 cells) is equivalent to delivery and viability of PBMCs processed at manufacturing scale (~1 × 10^9 cells).

**Conclusions** Microfluidic cell squeezing of human PBMCs with antigenic material can be tailored to produce APCs that drive robust CD8+ T cell response against targets across multiple disease areas and has been scaled up for clinical use. SQZ-PBMC-HPV are currently under clinical evaluation for treatment of HPV16+ tumors.

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**170 MICROFLUIDICS CELL SQUEEZING ENABLES POTENT CELLULAR VACCINES IN MURINE MODELS THROUGH DIRECT CYTOSOLIC LOADING AND DIRECT CD8 T CELL PRIMING**

Emrah Ozay*, Matthew Booty, Katarina Blagovic, David Soto, Olivia Pryor, Adam Stockmann, Disha Subramanya, Kelly Volk, ArmonSharei, Howard Bernstein, Dfene Yarar, Katherine Seidl, Scott Loughhead. SQZ Biotechnologies, Watertown, MA, USA

**Background** The presentation of sufficient antigen on major histocompatibility complex class I (MHC-I) is essential to prime CD8+ T cells.

**Methods** To achieve efficient MHC-I presentation, we used microfluidics cell squeezing (Cell Squeeze®) to deliver antigens directly to the cytosol of antigen presenting cells (APCs), bypassing the need for cross-presentation. In addition to facilitating priming by professional APCs, this approach enables lymphocytic subsets within peripheral blood mononuclear cells (PBMCs) to function as unconventional APCs in mouse pre-clinical models.

**Results** We demonstrated that microfluidic cell squeezing delivers cargo to major cell populations within splenocytes (T cells, B cells, NK cells, and monocytes) and that protein, peptide, or mRNA antigens are rapidly processed and presented. In vivo, squeezed splenocytes directly presented antigen to CD8+ T cells. In the TC-1 tumor model for HPV+ cancers, squeezed splenocytes completely protect mice when administered prophylactically, protecting 15/15 animals from primary challenge and 11/15 animals from tumor re-challenge. Following therapeutic administration, squeezed splenocytes significantly improved median survival time to 56 days from 28 days, as observed with untreated controls. Immunization can also be combined with chemotherapy to further enhance therapeutic efficacy, improving median survival to over 100 days compared to 81 days with SQZ monotherapy or 32 days with chemotherapy alone. When tumor infiltrating lymphocytes (TILs) were analyzed following therapeutic immunization, squeezed splenocyte immunization elicited a significant influx of antigen specific CD8+ T cells: with SQZ treatment, ~87% of tumor-infiltrating CD8 T cells were antigen-specific, as measured by an E7-tetramer stain, while only ~33.6% and ~1.15% of infiltrating CD8 T cells were specific for E7 with subcutaneous peptide vaccination and no treatment, respectively.

**Conclusions** Through the direct cytosolic delivery of antigen, we have engineered unfractionated PBMCs to function as potent APCs. This strategy generates potent antigen-specific CD8+ T cell responses in mouse models. Taken together, these findings support the potential of SQZ-PBMCs as an effective antigen-specific vaccination strategy against cancer. SQZ-PBMC-HPV is currently under clinical evaluation for HPV16+ tumor indications.

**Ethics Approval** All methods were performed in accordance with relevant guidelines and regulations; Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at SQZ Biotechnologies, using the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the Office of Laboratory Animal Welfare. All activities were also conducted in accordance with Public Health Service (PHS) Policy on Humane Use and Care of Laboratory Animals.

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**171 PRECLINICAL STUDIES SUPPORT THERAPEUTIC APPLICATION OF THE LEUKEMIC CELL-BASED CANCER RELAPSE VACCINE DCP-001 IN OVARIAN CANCER**

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**Background** Ovarian cancer (OC) is the gynecological malignancy with the highest mortality due to the late diagnosis of
Increasing activation of human tumor-reactive T cells (CD39+CD103+CD8+) by gene silencing of DCP-001 with self-delivering RNAi Intasyl(TM)

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Background Tumor infiltrating lymphocyte (TIL) therapy has proven effective for patients with stage IV melanoma, however there are critical issues that can limit the efficacy of standard TIL therapy across a broad range of different malignancies. We and others have shown that some tumor types contain a low percentage of tumor-specific T cells. We hypothesize that most of the patients that do not respond to TIL therapy are likely receiving a low percentage of tumor-reactive T cells and therefore a high percentage of non-therapeutic bystander T cells. We have developed a streamlined method that expands a highly enriched fraction of tumor-reactive T cells contained within the CD39+CD103+CD8+ TIL in greater than 90% of patient samples from a wide variety of malignancies (melanoma, colon cancer, head and neck cancer, etc.). This TIL product displays a broad repertoire of tumor-specific TCRs. The expanded CD39/CD103 TIL can kill autologous tumors in vitro, but the possibility remains that they could revert to a suppressed or exhausted state when they reach the tumor microenvironment upon transfer back into patients. To mitigate the suppressive effects of the tumor microenvironment we have evaluated Phio Pharmaceutical’s self-delivering RNAi INTASYL(TM) platform to silence PD-1 in the expanded TIL product.

Methods The TIL product was treated during the rapid expansion phase of the protocol with either nontargeting control compounds or PD-1 targeting INTASYL(TM) compounds. PD-1 protein levels and TIL functionality were assessed via flow cytometry and cytokine bead array.

Results Silencing of PD-1 expression in the expanded TIL product was obtained by adding the self-delivering RNAi compounds to the cell culture media, without needing transfection media, delivery formulations or electroporation. The RNAi-treated TIL product showed increased IFN-?? and Granzyme B expression.

Conclusions These data highlight a promising combination to improve the activity of tumor-reactive TIL in future human clinical trials.

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we describe the development and careful optimization of an in vivo mouse CRISPR-screening platform to identify knock-out targets in primary T cells, with the goal of increasing T cell abundance and persistence in tumors with different TMEs. Using a mouse retroviral system to express single-guide RNA (sgRNA) libraries in T cells from Cas9 transgenic mice, we performed in vivo screens in syngeneic, fully immune-competent mouse tumor models. 

**Results** We identified both known and potential novel regulators of T cell activation and persistence. Importantly, we have discovered knock-out targets that accumulate in multiple, distinct TMEs and other targets that are TME-specific. The use of sub-genomic-focused libraries allowed us to rapidly screen in multiple tumor model systems and reproducibly identify hits across individual mice. 

**Conclusions** We have developed a fully optimized an in vivo genetic screen, which could be a rich source for target discovery, and can enable identification of functional regulators of T cells for rapid incorporation into CRISPR-engineered T cell therapies for different solid TMEs.

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**Abstracts**

**An Ex Vivo Tumoroid Model of Fresh Patient Tumors (3D-ACT) to Assess Efficacy of Cellular Therapy in Immun-oncology**

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**Background** Adoptive T cell therapy (ACT) strategies have achieved substantial advances in the treatment of malignant tumors. Some of the unique challenges posed to ACT by solid tumors include locating target cells, as well as entering and surviving the complex tumor microenvironment. To develop better ACT applications and identify combination therapies to enhance tumor cell killing efficacy of ACT it is imperative to develop preclinical platforms that recapitulate the complexity of patient tumor microenvironment (TME). The goal of this study was to develop an integrated confocal-based high-throughput, high-content real time imaging platform to assess immunogenic tumor cell killing (TCK) activity of ACT applications such as CAR-T and TCR using fresh patient tumor samples.

**Methods** All patient tumor samples were obtained with patient consent and relevant IRB approval. For the confocal imaging platform, unpropagated 3D tumoroids with intact TME measuring 150 micron in size were prepared from fresh tumor samples of renal cell carcinoma (RCC), colorectal carcinoma (CRC) and non-small cell lung cancer (NSCLC) using proprietary technology developed at Nilogen Oncosystems. Engineered T-cells were labeled with different fluorescent cell tracker dyes to monitor cell migration and locations within tumoroids by confocal analysis. Comprehensive flow cytometry analysis was performed to corroborate confocal imaging findings from TCK and multiplex cytokine release assays used to assess changes in the TME.

**Results** Our studies demonstrated that the confocal-based high-content real time imaging platform described here, combined with a custom image analysis algorithm, allowed for monitoring of treatment-mediated tumor cell killing with structural and functional analysis of engineered T-cells in intact 3D tumoroids. The penetration rate of CAR-T and TCR cells into tumoroids as well as associated tumor cell death varied significantly between different tumor types. Flow cytometry analysis allowed for monitoring of the activation status and viability of engineered T-cells, and treatment-mediated changes in tumor resident immune cell populations.

**Conclusions** Our data indicated that the immunosuppressive tumor microenvironment may have implications for the application of ACT. Use of the ex vivo platform described here (3D-ACT) may aid in the validation of combinatorial therapies that block or deplete suppressive factors present within the TME, allowing these therapies to overcome mechanisms associated with dysfunction in CAR-T and TCR cell applications.

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**A Fas-4-1BB immunomodulatory fusion protein converts a pro-death to a pro-survival signal, enhancing T cell function and efficacy of adoptive cell therapy in murine models of AML and Pancreatic Cancer**

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**Background** Adoptive cell therapy (ACT) with genetically-modified T cells has shown impressive results against some hematologic cancers, but limited efficacy against tumors with restrictive tumor microenvironments (TMEs). FasL is a particular obstacle for ACT; it is expressed in many tumors and TMEs, including AML, ovarian and pancreatic cancers, and upregulated on activated T cells, where it can mediate activation-induced cell death (AICD).

**Methods** We engineered T cells to boost function with novel immunomodulatory fusion proteins (IFPs) that combine an inhibitory ectodomain with a costimulatory endodomain. Like current checkpoint-blocking therapies, IFPs can abrogate an inhibitory signal, but also provide an often absent costimulatory signal. Additionally, IFP-driven signals are delivered only to the T cells concurrently engineered to be tumor-specific, thereby avoiding systemic T cell activation. For FasL-expressing TMEs, we developed an IFP that replaces the Fas intracellular tail with costimulatory 4-1BB. We tested the Fas-4-1BB IFP in primary human T cells and in immunocompetent murine models of leukemia and pancreatic cancer.

**Results** Fas-4-1BB IFP expression enhanced primary human T cell function and enhanced lysis of Panc1 pancreatic tumor cells. When delivered with a mesothelin-specific TCR, Fas-4-1BB T cells prolonged survival in a murine AML model. When delivered with a mesothelin-specific TCR, Fas-4-1BB T cells prolonged survival in the autochthonous KPC pancreatic cancer model, increasing median survival to 65 from 37 days (with TCR-only, *P=0.0042). Single-cell RNA sequencing revealed differences in the endogenous tumor-infiltrating immune cells, included changes in cell frequency and programming.
Conclusions We developed an engineering approach to enhance the in vivo persistence and antitumor efficacy of transferred T cells. Our targeted, two-hit strategy uses a single fusion protein to overcome a death signal prevalent in the TME of many cancers and on activated T cells, and to provide a pro-survival costimulatory signal to T cells. Our results suggest that this fusion protein can increase T cell function when combined with murine or human TCRs, and can significantly improve therapeutic efficacy in liquid and solid tumors, supporting clinical translation.

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176 EVALUATING THE SAFETY OF TUMOR TREATING FIELDS (TTFIELDS) APPLICATION TO THE TORSO – IN Vivo STUDIES
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Background Tumor Treating Fields (TTFields) are a noninvasive, antineoplastic treatment delivered locoregionally to tumor bed via low intensity (1–3 V/cm), intermediate frequency (100–500 kHz), alternating electric fields. This treatment modality has been shown to be cytotoxic to rapidly dividing cells, with highest efficacy demonstrated at different optimal frequencies depending on tumor cell-type. TTFields therapy is FDA-approved for the treatment of newly diagnosed and recurrent glioblastoma (GBM), with the overall tolerable safety profile (EF-11 and EF-14 clinical trials) attributed to the low rate of mitotic events in normal, quiescent brain cells. Further evaluation of the safety profile of TTFields is needed for treating cancer in different body regions where there are high rates of cellular proliferation, i.e. torso. Many solid malignant tumors may reside in the torso region – mesothelioma and non-small cell lung carcinoma (NSCLC) in the thoracic segment; pancreatic cancer, hepatocellular carcinoma, and gastric cancer in the abdomen; and ovarian cancer in the pelvis. Hence, we investigated the safety of delivering TTFields to the torso of healthy rats at conditions previously deemed effective for treating the aforementioned cancer cell types.

Methods TTFields were applied using the Novo-TTF100L system at frequencies of 150 or 200 kHz and intensities of 1–2 V/cm RMS to torsos of Sprague Dawley (SD) female rats for a duration of 2 weeks. Throughout treatment, animals underwent daily clinical examinations. Blood samples and comparative histological evaluation of major internal organs were performed at treatment cessation.

Results No significant differences were observed for the TTFields treated groups in comparison to control groups for the following parameters: activity level, food and water intake, stools, motor neurological status, respiration, weight, complete blood count, blood biochemistry, and pathological findings.

Conclusions These results demonstrate the safety of 150 and 200 kHz TTFields when delivered to torsos of healthy rats, where there are normal tissues with high cellular proliferation rates. Overall, TTFields delivery to the torso demonstrated safety and feasibility for the treatment of thoracic and other abdominal and pelvic cancers. TTFields are currently being investigated in clinical studies for the treatment of solid tumors located in the torso, including locally advanced pancreatic cancer (PANOVA-3 Study, NCT03377491), ovarian cancer (INNOVATE-3 Study, NCT03940196), lung cancer (LUNAR Study, NCT02973789), hepatocellular carcinoma (HEPANOVA Study, NCT03606590) and gastric cancer.
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A SEVERE CYTOKINE RELEASE SYNDROME WITH RESPIRATORY FAILURE IN RECURRENT MESOTHELIOMA INDUCED BY EPCAM CAR-T CELLS INFUSION: A CASE REPORT
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Background With the development and maturity of chimeric antigen receptor T (CAR-T) cells therapy-related technologies, the application of CAR-T therapy has progressed from blood tumors to solid tumors, and its potential risks and side effects have been more widely recognized. As the most common complication of CAR-T therapy, cytokine release syndrome (CRS) is an inflammatory syndrome caused by the activation and proliferation of T-cell and the increased levels of multiple cytokines. Epithelial cell adhesion molecule (EpCAM) is over-expressed in a variety of tumors and has been used as one of the targets of CAR-T therapy. Case reports of severe CRS due to the use of anti-EpCMA CAR-T cell therapy are very rare.

Methods A 45-year-old malignant mesothelioma woman with EpCAM-positive whose disease progressed after chemotherapy was enrolled into our study (ChiCTR2000030274). The patient received a total of 1.8×107 autologous T cells which contained sequences encoding single-chain variable fragments (scFv) specific for EpCAM after cyclophosphamide lymphodepletion. After the infusion of CAR-T cells, the patient developed typical CRS reactions such as fever, hypoxemia, pulmonary edema, and elevated inflammatory factors. The patient’s condition did not improve after the use of anti-inflammatory and antipyretic drugs. After administration of tocilizumab (4 mg/kg, day 6 and day 17) combined with glucocorticoid (40 mg q12h, decreasing gradually), the patient’s general condition gradually improved, and chest computed tomography (CT) showed that pulmonary edema was absorbed.

Results The patient’s CRS was successfully eliminated after the use of IL-6 inhibitor tocilizumab combined with glucocorticoid.

Conclusions Although EpCAM CAR-T is safe in general, serious complications still happen possibly requiring close monitoring and timely treatment. Our findings suggest that tocilizumab combined with glucocorticoid can be an effective therapeutic method for severe CRS caused by CAR-T cells therapy in solid tumor.
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Checkpoint blockade therapy

**IMMUNOTHERAPY TRIALS LACK A BIOMARKER FOR INCLUSION: IMPLICATIONS FOR DRUG DEVELOPMENT**

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**Background** Next-generation sequencing and other biomarkers have demonstrated the capability to identify potentially pathogenic molecular aberrancies. Immune checkpoint inhibitors (ICI) have benefited patients in almost every oncologic histology. Combining these innovations has transformed how oncologists treat previously untreatable diseases.

**Methods** The 413 trials from clinicaltrials.gov website were reviewed using the terms ‘nivolumab’ or ‘pembrolizumab’ between January 1, 2019 and December 31, 2019. Additionally, all 33 interventional therapeutic trials for ‘glioblastoma multiforme’ and 79 for ‘pancreatic cancer’ that were either recruiting, not yet recruiting, or active not recruiting trials between January 1, 2019 and December 31, 2019 were analyzed.

**Results** In total of 413 trials, 57,853 were planned for enrollment with 37 (8.96%) trials requiring a biomarker for entry (n = 5,602 [9.7%]). Overall, there were 41 trials with single-agent immunotherapy planned to enroll 6222 patients and of those trials 7 (17.1%) required a biomarker for enrollment (n = 285 [4.6%]). There were 193 trials with >2 immunotherapies combined planned to enroll 21,360 patients and of those trials 17 (8.8%) required a biomarker for enrollment (n = 1254 [5.9%]). There were 69 trials with immunotherapy and chemotherapy combined planned to enroll 12,354 patients and of those trials 3 (4.3%) required a biomarker for enrollment (n = 83 [0.67%]). There were 58 trials with immunotherapy and targeted therapy combined planned to enroll 11,967 patients and of those trials 6 (10.3%) required a biomarker for enrollment (n = 3244 [27.1%]). There were 52 trials with other immunotherapy combinations (e.g. vaccine) planned to enroll 5950 patients and of those trials 4 (7.7%) required a biomarker for enrollment (736 [12.4%]). Within pancreatic cancer, 31 trials were planned to use immunotherapy (monotherapy, combination, with chemotherapy, with targeted therapy) including 4493 patients total; 5 (16%) of those trials required biomarkers enrolling 309 (7%) patients. Within glioblastoma multiforme, 13 trials were planned to use immunotherapy (monotherapy, combination, with chemotherapy, with targeted therapy) including 730 patients total; 1 (8%) of those trials required biomarkers enrolling 304 patients including 272 NSCLC, 24 UC and 8 RCC subjects were evaluated. 54 patients underwent a mini

**Conclusions** For immunotherapy-based trials in 2019, <10% of patients expected to be enrolled would be selected by a biomarker for inclusion. Precision oncology continues to struggle in the era of ICI with an all-comers approach to patient selection and trial initiation. Selecting patients for trials based on biomarkers may help better identify responders to ICI.

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**THE EFFECT OF PACKED RED BLOOD CELL TRANSFUSIONS ON THE CLINICAL EFFICACY OF IMMUNOTHERAPY**

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**Background** Transfusions of packed red blood cells (PRBC) have been postulated to be immunosuppressive, an effect known as transfusion-related immunomodulation (TRIM). TRIM is thought to be a result of the immunosuppressive and pro-inflammatory effects of residual leukocytes, apoptotic cells, inflammatory mediators, micro particles and free hemoglobin. Prior studies have shown a negative association between perioperative PRBC transfusions and overall mortality in multiple malignancies. To date there are no studies addressing the impact of transfusions on survival in patients undergoing treatment with checkpoint inhibitor (CPI) immunotherapy. We conducted a retrospective study to investigate the clinical outcomes associated with PRBC transfusions in patients with non-small cell lung cancer (NSCLC), urothelial carcinoma (UC) and renal cell carcinoma (RCC) who received immunotherapy for advanced/metastatic disease.

**Methods** From January 2010 - June 2019, patients at Fox Chase Cancer Center who received a PRBC transfusion within 120 days of treatment with a CPI and with advanced NSCLC, UC and RCC were included. Patient demographics including age, sex, ethnicity, race, tobacco use and ECOG performance status (PS) were abstracted. We also assessed previous chemotherapy, radiation and targeted therapy utilization among all patients. The primary endpoints were progression free survival (PFS) and overall survival (OS) in those who have and have not received PRBCs. We then evaluated PFS and OS via a cox proportional hazards model that was adjusted for cancer type, age, PS, previous therapies and tobacco use.

**Results** 304 patients including 272 NSCLC, 24 UC and 8 RCC subjects were evaluated. 54 patients underwent a minimum of one PRBC transfusion during the pre-specified time period. Both median PFS (8.2 months versus 3.9) and overall

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Abstract Figure 1 Progression free survival. Y refers to transfused population. N refers to non transfused population.
survival (26.1 months versus 13.8) were shorter in patients who underwent transfusion. After multivariable adjustment, the negative associations between transfusion and PFS (HR: 1.53, p=0.03) and overall survival (HR: 1.40, p=0.09) were preserved (figure 1–2). A sub-analysis of the NSCLC patients was conducted and shorter PFS (HR:1.58, p=0.03) and overall survival (HR:1.56, P=0.03) were again seen in the transfusion cohort (figure 3–4).

**Conclusions** PRBC transfusions led to an inferior PFS and OS in advanced cancer patients receiving checkpoint inhibitors even after adjustments for multiple prognostic variables. These results suggest a possible attenuation of the effectiveness of immunotherapy as a result of the immunosuppressive effects of PRBC transfusions. The findings require prospective and mechanistic confirmation as inherent bias may exist in this retrospective analysis.

**Ethics Approval** This study was approved the institutional review board at Fox Chase Cancer Center, approval number 19-9006.

**Consent** N/A

**REFERENCES**
181 ANALYSIS OF THERAPEUTIC EFFECT AND SAFETY OF PD-1 INHIBITORS IN CLINICAL TREATMENT OF ORAL AND MAXILLOFACIAL MALIGNANT TUMORS

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Background As the sixth largest cancer in the world, the recurrence rate and metastasis rate of oral cancer are relatively high. Although the incidence of oral cancer has decreased in the past decade, the overall survival rate has only increased by 5%. The 5-year survival rate of early oral squamous cell carcinoma is only 50% to 60%. PD-1 inhibitors can bind to programmed death molecule 1 (PD-1) and block its binding to programmed death molecule ligand 1 (PD-L1), so as to restore immune function and achieve anti-tumor effect.

Methods 33 patients with oral malignant tumors were selected from the Department of Maxillofacial surgery of the first affiliated Hospital of Zhengzhou University from August 2019 to June 2020. Among them, 8 patients were only treated with PD-1 inhibitor Camrelizumab injection combined with the targeted drug apatinib. 25 patients were treated with PD-1 inhibitor Camrelizumab injection combined with targeted drug apatinib after the operation. The dose of PD-1 inhibitor was 200 mg by intravenous infusion every three weeks, and the dose of apatinib was daily 500 mg orally. The duration of treatment with PD-1 inhibitors combined with apatinib ranged from 1 month to 10 months. The survival status and related immune adverse reactions of patients after one year of treatment were followed up and evaluated.

Results For 33 patients enrolled in the study, after excluding the cessation of PD-1 inhibitor treatment due to a variety of reasons, the overall disease control rate was 80.0%, of which 3 patients developed further and died. Other relevant data need to be further tracked because they have not reached the end point of observation. Among the 33 patients, 5 patients had immune-related adverse reactions (15.2%), including 2 cases of skin rash, 1 case of skin capillary hyperplasia and 2 cases of other adverse reactions.

Conclusions The patients with Oral malignant tumor treated with PD-1 inhibitor Camrelizumab injection combined with targeted drug apatinib or postoperative adjuvant therapy can effectively control tumor development, improve the survival of patients, and help to improve the stability of postoperative efficacy.

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182 HIGHLY POTENT FULLY HUMAN ANTI-VISTA ANTIBODIES – A NEW TARGET CHECKPOINT INHIBITOR AGAINST IMMUNOSUPPRESSIVE MYELOID CELLS


Background Activation (VISTA/PD-1H) is a B7 family ligand expressed on V-domain Immunoglobulin Suppressor of T cell differentiation of MDSC as well as their immunosuppressive function. Anti-VISTA antibodies mediate single-agent antitumor effects in syngeneic tumor models in wild-type mice and show enhanced activity in combination with anti-PD1 and anti-CTLA-4 treatment. Candidate anti-VISTA antibodies have also been evaluated in exploratory tolerability and PK studies in cynomolgus monkey. These studies demonstrated that multiple weekly doses of antibodies are well-tolerated with appropriate PK for lead selection and optimization.

Methods Kineta has analyzed 107 fully human ScFv antibodies directed against VISTA.

Results Our lead candidates exhibit high potencies in the sub-nanomolar range and are also characterized by a long kDIs. They specifically target human and cynomolgus monkey VISTA on a singular unique epitope. In a Staphylococcus Enterotoxin B T-cell activation assay, Kineta’s anti-VISTA antibodies robustly induce IFNγ secretion. They also promote strong maturation of Antigen Presenting Cells with an increase of CD80 and HLA-DR surface expression as well as CXCL10 secretion. The mechanism of action is mediated in part by NK cells. We demonstrated that myeloid cells acquire a high level of VISTA expression during MDSC or M2 differentiation in vitro and that Kineta’s anti-VISTA antibodies prevent the differentiation of MDSC as well as their immunosuppressive activity against T cells. Anti-VISTA antibodies mediate single-agent antitumor effects in syngeneic tumor models in wild-type mice and show enhanced activity in combination with anti-PD1 and anti-CTLA-4 treatment. Candidate anti-VISTA antibodies have also been evaluated in exploratory tolerability and PK studies in cynomolgus monkey. These studies demonstrated that multiple weekly doses of antibodies are well-tolerated with appropriate PK for lead selection and optimization.

Conclusions Our results strongly favor further characterization and continued development of selected lead antibodies for the potential treatment of colder, less immunogenic tumors.

Ethics Approval Study approved by the Institutional Animal Care and Use Committee PHS Assurance # D16-00885 and D16-00114

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183 OVERCOMING IMMUNOTHERAPY RESISTANCE IN T CELL-INFLAMED LUNG CANCER

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Background Tumor infiltrating T cells (TIL) are highly correlated with response to checkpoint blockade immunotherapy (CBT) in melanoma. However, in non-small cell lung cancer (NSCLC), 61% of patients have TIL, but only 32% respond to CBT. It is unknown how these T cell-inflamed tumors are resistant to CBT. Understanding and overcoming this resistance would greatly increase the number of cancer patients who benefit from CBT.

Methods To understand lung-specific anti-tumor immune responses, a NSCLC cell line derived from an autochthonous murine lung cancer (KP cell line) was transplanted into syngeneic C57BL/6 mice subcutaneously or intravenously. To study antigen-specific responses, the KP cell line was engineered with SIY and 2C TCR transgenic T cells, which are specific for SIY, were adoptively transferred into tumor-bearing animals.

Results Intra-tumor KP tumors responded to CBT (aCTLA-4 and aPD-L1) with significant tumor regression while lung KP tumors were CBT resistant. Immunohistochemistry found that CD8+ TIL in lung lesions had blunted effector molecule expression that correlated with a lack of IL-2 signaling. Adoptive transfer of naïve, tumor-reactive 2C T cells resulted in...
equally robust T cell proliferation in both the inguinal and mediastinal lymph nodes (LN). However, RNA sequencing of adoptively transferred 2C T cells isolated 3-days after transfer from draining LN identified that T cells activated in the mediastinal LN had reduced levels of IL-2 signaling and blunted effector functions early during priming. Flow cytometry confirmed that T cells primed in the mediastinal LNs did not express CD25, GZMB, or IFN-γ, while T cells in inguinal LNs upregulated all three of these effector molecules. Delivery of IL-2 and IL-12 during priming was sufficient to restore effector molecule expression on 2C T cells in mediastinal LNs. Analysis of patient data identified that a subset of lung cancer patients showed a sizable population of CD8+ TIL with low IL-2 signaling and low expression of effector molecules, including common targets of CBT.

Conclusions Immunotherapy resistance in T cell-inflamed tumors is due to defective CD8+ T cell effector differentiation. IL-2-based therapies could enhance differentiation of functional CD8+ effector T cells and could turn immunotherapy-resistant tumors to immunotherapy-sensitive tumors. This is the first mechanistic study providing evidence for a distinct type of T cell dysfunction resistant to current CBT.

Ethics Approval This study was approved by MIT’s Committee on Animal Care, protocol number 0220-006-23.

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TWO TYPES OF ANTI-TIGIT ANTIBODIES WITH DISTINCT BINDING EPITOPE AND FUNCTIONAL ACTIVITIES

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Background TIGIT is an inhibitory receptor mainly expressed on natural killer (NK) cells, CD8+ T cells, CD4+ T cells and Treg cells. TIGIT competes with CD226 for binding with CD155. In cancers, CD155 has been reported to up-regulate on natural killer (NK) cells, CD8+ T cells, CD4+ T cells and Treg cells. TIGIT competes with CD226 for binding with CD155. In cancers, CD155 has been reported to up-regulate on tumor cells, and TIGIT was found to increase on TILs. Activation of TIGIT/CD155 pathway would mediate immunosuppression in tumor; while blockade of TIGIT promotes anti-tumor immune response.

Methods AK126 and AK113 are two humanized anti-human TIGIT monoclonal antibodies developed by Akesobio. Binding activity of AK126 and AK113 to human TIGIT, and competitive binding activity with CD155 and CD112, were performed by using ELISA, Fortebio, and FACS assays. Cross-reactivity with cynomolgus monkey TIGIT and epitope binning were also tested by ELISA assay. In-vitro assay to investigate the activity to promote IL-2 secretion was performed in mixed-culture of Jurkat-TIGIT cells and THP-1 cells.

Results AK126 and AK113 could specifically bind to human TIGIT with competitive affinity and effectively blocked the binding of human CD155 and CD112 to human TIGIT. X-ray crystal structure of TIGIT and PVR revealed the C’-C” loop and FG loop regions of TIGIT are the main PVR interaction regions. The only amino acid residue differences in these regions between human and monkey TIGIT are 70C and 73D. AK126 binds to both human and monkey TIGIT, AK113 binds only to monkey TIGIT. This suggests that these residues are required for AK113 binding to human TIGIT, but not required for AK126. Interestingly, results from cell-based assays indicated that AK126 and AK113 showed significantly different activity to induce IL-2 secretion in mixed-culture of Jurkat-TIGIT cells and THP-1 cells (figure 1A and B), in which AK126 had a comparable capacity of activity to 22G2, a leading TIGIT mAb developed by another company, to induce IL-2 secretion, while, AK113 showed a significantly higher capacity than 22G2 and AK126.

Abstract 184 Figure 1

Anti-TIGIT Antibodies Rescues IL-2 Production In Vitro T-Cell Activity Assay in a dose dependent manner. Jurkat-TigIT cells (Jurkat cells engineered to over-express human TIGIT) were co-cultured with THP-1 cells, and stimulated with plate-bound anti-CD3 mAb in the presence of TIGIT ligand CD155 (A) or CD112 (B) with anti-TIGIT antibodies. After incubated for 48h at 37°C and 5.0% CO2, IL-2 levels were assessed in culture supernatants by ELISA. Data shown as mean with SEM for n = 2.

Conclusions We discovered two distinct types of TIGIT antibodies with differences in both epitope binding and functional activity. The mechanism of action and clinical significance of these antibodies require further investigation.

REFERENCES

CAMRELIZUMAB MONOTHERAPY OR COMBINATION THERAPY IN PATIENTS WITH RECURRENT OR METASTATIC CERVICAL AND ENDOMETRIAL CARCINOMA: A RETROSPECTIVE STUDY

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Background Patients with recurrent or metastatic cervical and endometrial carcinoma have poor prognosis and few treatment options. Blocking the interaction between PD-1 and its ligands is a promising treatment strategy. Camrelizumab is a humanised anti-programmed death-1 (anti PD-1) antibody. This study aimed to assess the anti-tumour activity and safety of camrelizumab in patients with recurrent or metastatic cervical and endometrial carcinoma.

Methods We performed a retrospective analysis for recurrent or metastatic cervical and endometrial carcinoma patients. Eligible patients were aged 28–73 years with an Eastern Cooperative Oncology Group performance status of 0 or 2. Patients received camrelizumab alone(200 mg iv d1 q2w)or in combination with chemoadiatherapy/chemotherapy. The primary endpoint was objective response (ORR). The secondary endpoints included disease control rate (DCR), median progression-free survival (mPFS) and safety.

Results A total of 21 patients were enrolled between September 20, 2019, and July 8, 2020. 18 patients were evaluated for efficacy and 21 patients were available for safety analysis.
For 18 evaluated patients, the ORR and DCR was 50% (9/18) and 83.3% (15/18), respectively. In addition, 4 patients received camrelizumab monotherapy with the ORR of 0% (0/4) and DCR of 25% (1/4), and 14 patients received camrelizumab combination therapy with the ORR of 64.3% (9/14) and DCR of 100% (14/14). 16 of 21 patients were still receiving the treatment, the median PFS was not yet achieved. Exploratory analysis showed that patients with reactive cutaneous capillary endothelial proliferation (RCCEP) had the higher objective response rate than those without RCCEP (57.1% vs 45.5%). Treatment-related adverse events occurred in 47.6% (10/21) of patients, and the most common adverse events were RCCEP (33.3%), rash (14.3%), dry skin (9.5%). Treatment-related grade 3 adverse events occurred in 4.8% (1/21) of patients.

Conclusions Camrelizumab showed antitumour activity in recurrent or metastatic cervical and endometrial carcinoma with manageable toxicities. Camrelizumab combination therapy had better efficacy compared with monotherapy. RCCEP occurrence was positively associated with outcomes of camrelizumab. Further studies are needed to verify this data.

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186 DISTINCT IMMUNE SIGNATURES PREDICTING CLINICAL RESPONSE TO PD-1 BLOCKADE THERAPY IN GYNECOLOGICAL CANCERS REVEALED BY HIGH-DIMENSIONAL IMMUNE PROFILING

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Background Although immune checkpoint blockade revolutionized cancer therapy, response rates have been mixed in gynecological malignancies. While uterine endometrial cancer with high microsatellite instability (MSI-H) and high tumor mutational burden (TMB) respond robustly to checkpoint blockade, high-grade serous ovarian cancer (HGSOC) with low TMB respond modestly. Currently, there has been no known immune signature or T cell phenotype that predicts clinical response in gynecological tumors.

Methods To dissect the immune landscape and T cell phenotypes in gynecological cancer patients receiving PD-1 blockade, we used high-dimensional cytometry (flow cytometry and mass cytometry (CyTOF)). We performed longitudinal deep immune profiling of PBMC from patients with recurrent uterine endometrial cancer receiving single-arm nivolumab, and HGSOC patients receiving neoadjuvant nivolumab plus platinum-based chemotherapy prior to debulking surgery.

Results Chemotherapy-resistant MSI-H uterine cancer patients treated with nivolumab had a proliferative T cell response 2–4 weeks post PD-1 blockade, consistent with responses seen in high TMB melanoma and lung cancer. The responding Ki67+ CD8 T cell population was largely CD45RAloCD27hi or CD45RAloCD27lo and highly expressed PD1, CTLA-4, and CD39, consistent with the phenotype of exhausted T cells (TEx). These exhausted-like cells are enriched in responders, whereas early expansion Tregs are enriched in non-responders. Unlike patients with uterine endometrial cancer, patients with TMBlo ovarian cancer did not have a clear proliferative CD8 T cell response after neoadjuvant nivolumab plus chemotherapy treatment, suggesting systemic immune suppression. At baseline, ovarian without recurrence have more terminally differentiated effector-like CD8 T cells, and patients with recurrence have more naive-like cells. Thus, both high and low TMB gynecological tumors have distinct immune landscapes associated with clinical response. Additionally, in MSI-H uterine endometrial cancer patients, the length of time between the prior chemotherapy and the initiation of immunotherapy was negatively correlated with T cell reinvigoration post immunotherapy and clinical response. This suggests the importance of optimize therapeutic timing to maximize the therapeutic efficacy when combining immunotherapy and chemotherapy.

Conclusions Collectively, our immune profiling revealed the distinct immune signatures associated with clinical response to PD-1 blockade in gynecological cancers. Our results also suggest that TMBhi inflamed versus TMBlo cold tumor microenvironment, and timing of chemo/immunotherapy could impact differentiation and functions of T cells.

Ethics Approval The study was approved by MSKCC Ethics Board, approval number 17–180 and 17–182.

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187 REAL-WORLD TREATMENT PATTERNS AND CLINICAL PREDICTORS OF OVERALL SURVIVAL AMONG ANTI-PD-1 EXPOSED ADVANCED MELANOMA PATIENTS WITH DOCUMENTED EVIDENCE OF DISEASE PROGRESSION

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Background Immuno-oncology (I-O) plays a major role in the treatment of advanced melanoma (aMel); however, resistance to therapy remains an important clinical problem. This study examined treatment patterns and overall survival (OS) for aMel patients who progressed on anti-programmed death ligand 1 (anti-PD-1) therapy in a real-world clinical setting.

Methods A retrospective database study of Flatiron electronic medical records (EMR) was conducted with 304 aMel patients who progressed on first or second line anti PD-1 (baseline) therapy with pembrolizumab or nivolumab and received subsequent (index) therapy with ≥3 months of potential follow-up. Patients who discontinued treatment for reasons other than progression (primarily toxicity) were excluded. The primary outcome was OS, defined using EMR data linked to external mortality sources (e.g. Social Security Death Index). OS analysis was stratified by several factors (e.g. age, ECOG, BRAF, LDH, type of index therapy, and best overall response [BOR] to baseline anti-PD-1 therapy). BOR defined as response, stable disease, or disease progression was based on clinician assessment following radiographic imaging. Descriptive and log-rank test statistics for OS were used.

Results Among patients receiving index therapy (n = 304), 50% received I-O (n = 91/151 combination therapy), 36% received BRAFi/MEKi (n = 102/109 combination therapy) and 14% received other therapies (n = 34/44 chemotherapy). Median (range) age was 67 (23–85) years, with 65% male, 62% ECOG ≤1, 33% elevated LDH, and 51% with BRAF mutations. Most patients received baseline anti-PD1 monotherapy (77%) as first line therapy. Median OS (95%CI) was 7.2 (6.4, 8.8) months, with a significant OS association with ECOG ≤1 (p < 0.001), normal LDH (p < 0.001), and BRAFi/MEKi (p = 0.02), with higher median OS of 9 vs 5 months, 11 vs 6 months, and 11 vs 7 and 6 months, respectively, compared to
patients with ECOG ≥2, elevated LDH, and treated with I-O and other therapies. For a subgroup of index therapy patients with a BOR assessment to baseline anti-PD-1 therapy (n=237), there was a significant association (p<0.01) of OS with BOR to baseline therapy, with higher median OS for those with an initial response (12 months) or stable disease (14 months) compared to a BOR of disease progression (6 months). There was also a significant OS association with BOR to baseline anti-PD-1 therapy for the subgroups receiving I-O therapy (n=119/237, p<0.01) and other therapies (n=37/237, p=0.01).

Conclusions Suboptimal OS in patients who progress on anti-PD-1 therapy in a real-world clinical setting, with predictors of enhanced survival, highlights the need for further research to inform optimal treatment strategies.

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188 NOVEL ANTI-SIRPALPHA ANTIBODIES WITH DIFFERENTIATED CHARACTERISTICS AS PROMISING CANCER THERAPEUTICS

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Background Therapeutically targeting tumor myeloid cells has emerged as a novel and complementary strategy to existing cancer immunotherapy approaches. The interaction of tumor expressed CD47 with SIRP alpha (signal regulatory protein-alpha, SIRPA) on macrophages, dendritic cells and neutrophils inhibits key immune effector mechanisms. Targeting SIRPα-CD47 represents a novel approach to enhance anti-tumor immunity by augmenting or reactivating critical tumor clearance mechanisms.

H5F9, an antibody against CD47, has shown promising therapeutic activities in patients with MSD, AML and NHL. However, agents targeting CD47 present hematological toxicities and present a huge antigen sink leading to not achieving an optimum therapeutic window. Our approach is to target SIRP alpha, the receptor of CD47 and focus therapeutic targeting to relevant mechanisms related to phagocytosis and myeloid cell activation and at the same time avoid undesired effects of blocking CD47. SIRP gamma, a very close relative of SIRP alpha is expressed on T cells and also binds to CD47. It has been shown that blockade of SIRP gamma-CD47 interaction inhibits T cell proliferation and blocks transendothelial T cell migration. Hence, our aim is to generate SIRP alpha selective antibodies that do not cross-react with SIRP gamma and have minimal impact on T cell functions.

Methods Using Apexigen’s APXiMAB™ proprietary antibody discovery platform, we have generated two novel anti-SIRP alpha antibodies (APX701 & APX702) with differentiated properties as compared to other approaches targeting the CD47/SIRP alpha axis. We have used ELISA, FACS based cell binding and blocking assays, and functional assays including in vitro phagocytosis and antibody-dependent cell phagocytosis (ADCP) in combination with tumor-opsonizing antibody to select APX701 & APX702.

Results Our novel preclinical-stage APX701 & APX702 antibodies have demonstrated the following attributes: high binding affinity to human SIRP alpha (APX701 Kd = 0.95nM, APX702 Kd = 0.88nM), no binding to SIRPA gamma, efficient blockade of SIRP alpha binding to CD47 (APX701 IC50 = 1.04nM, APX702 IC50 = 0.80nM), potent macrophage mediated phagocytosis, enhancement of ADCP mediated by tumor-opsonizing antibody and favorable developability CMC profiles. In comparison with the benchmark antibody OSE-172, APX701 & APX702 showed potent phagocytosis activity and ADCP enhancement in all donors tested while OSE-172 induced phagocytosis in only 50% of the donors. This may result from the fact that APX701 and APX702 bind to all major SIRP alpha variants (V1, V2 & V8; covering ~92% population) while OSE 172 only binds to SIRPalpha V1 (~50% population).

Conclusions APX701 and APX702 demonstrate differentiated anti-SIRPaLpha activities by enhancing myeloid cell-mediated anti-tumor immunity and reactivating critical tumor clearance mechanisms within the tumor microenvironment.

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189 A CLEAR INCREASE IN TILS AND MODEST TUMOR GROWTH INHIBITION BY PEMBROLIZUMAB IN PROSTATE CANCER TUMORS GROWING IN BONE OF CD34+ ENGRAFTED NOG MICE

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Background The recent KEYNOTE-199 trial raises hope for new treatment options for prostate cancer patients with the encouraging results of checkpoint inhibitor activity in a subset of prostate cancer patients, also including patients with bone-predominant disease. However, the patient subset that benefited from the treatment was small, needing identification predictive biomarkers. Proper preclinical models can help in the biomarker quest as well as in the search and selection of the best possible combination partners for further clinical trials.

Methods In this study the bone-metastatic disease was modeled by intratibial inoculation of LNCaP human prostate cancer cells to male CIEA NOG® (NOG) mice and NOG mice engrafted with human CD34+ hematopoietic stem cells (huNOG, Taconic Biosciences). Tumor growth was followed by serum PSA measurements and tumor-induced bone changes by X-ray images. At study week 4, the PSA positive mice were stratified to two groups (n=10) treated with IgG4 isotype control or pembrolizumab (5 mg/kg, i.p., Q5D) until the end of the study. Tumor-induced bone changes were followed by X-ray 4, 8 and 10 weeks after inoculation. The study was terminated 10 weeks after inoculation and tumors were processed for histological and immunohistochemical (IHC) analysis of tumor infiltrating lymphocytes (TILs). Changes in blood cell counts were assessed by flow cytometry and hematology.

Results At sacrifice, tumor-induced bone changes were observed in all mice, and there was no difference between the groups. Even though the PSA was not significantly lower in the pembrolizumab-treated group, the average histological tumorous surface was lower. In flow cytometry of peripheral blood, increases in the portions of CD3+ leukocytes and double positive CD4+CD8+ cells were observed, but no differences were found in CD4+ nor CD8+ T-cells. However, CD8+ T-cells were radically increased within the tumor as analyzed by IHC.
Conclusions The model successfully mimicked the prevalent clinical situation, where clear responses in PSA or target lesions are not observed. However, a dramatic increase of cytotoxic T-cells in the tumor was observed, revealing the effects of pembrolizumab in a model of prostate cancer growth in bone of huNOG mice. The model presents a suitable platform for studying combination partners with pembrolizumab, that would boost or unlock the anti-tumor activity of the increased TILs.

Ethics Approval This study was approved by the National Animal Experiment Board in Finland; license number ESAV1-2331-04 10 07-2017.

REFERENCE

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190 TIMING OF STEROID DOSES AND RESPONSE RATES TO IMMUNE-CHECKPOINT INHIBITORS IN METASTATIC CANCER

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Background Corticosteroids (CS) are the mainstream of immune-related adverse effect (irAE) management, as well as for other indications in cancer treatment. Previous studies evaluating whether CS affect immune checkpoint inhibitor (CPI) efficacy compared patients receiving steroids vs. no steroids. This comparison may be confounded by different rates of irAEs, which are known to be associated with higher response rates to CPIs. Preclinically CS have been shown to diminish naive T-cell proliferation and differentiation, though there is a paucity of clinical data evaluating how the timing of concomitant CS affects CPI efficacy.

Methods We retrospectively collected data from patients treated with CPIs alone, who received CS during their CPI treatment at a single institution. Patients were allocated into two cohorts based on timing of initiation of CS (> 2 months vs. < 2 months after initiating CPI). Patient characteristics, irAEs, cancer type, treatment type, treatment response/progression per RECIST v1.1, and survival data were collected. Kaplan Meier and Cox proportional hazard regression methods were used to estimate hazard ratios (HR) for the primary endpoint of progression free survival (PFS) along with overall survival (OS).

Results We identified 247 patients with metastatic cancer who received CS concurrently with CPIs alone. The majority of patients had non-small cell lung cancer (n=98), followed by renal cell carcinoma (n=43), and melanoma (n=30). 242 patients were on PD-1 inhibitor monotherapy, while 45 patients received CPI in combination with anti-CTLA-4 ipilimumab (table 1). The median time on steroids for all patients was 1.8 months. After adjusting for differences in rates of treatment type, tumor type, brain metastases and irAEs, patients who were treated with CS > 2 months after starting CPI had a statistically significant longer progression free survival (PFS) [HR of 0.33, p≤0.0001], and overall survival (OS) [HR of 0.36, p≤0.0001] than those who received steroids < 2 months after starting CPI. Rates of irAEs in each group were not significantly different (p = 0.15). Objective response rate (ORR) for patients on CS > 2 months was 39.8%, vs. ORR for patients <2 months was 14.7% (p-value = <0.001).

Abstract 190 Table 1 Cancer subtypes and drug types in the study population (n=247)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>&lt;2 months</th>
<th>≥2 months</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer subtypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>45 (45.92)</td>
<td>53 (54.08)</td>
<td>0.0095*</td>
</tr>
<tr>
<td>RCC</td>
<td>22 (51.16)</td>
<td>21 (48.84)</td>
<td></td>
</tr>
<tr>
<td>Melanomas</td>
<td>19 (63.33)</td>
<td>11 (36.67)</td>
<td></td>
</tr>
<tr>
<td>Urothelial</td>
<td>8 (57.14)</td>
<td>6 (42.86)</td>
<td></td>
</tr>
<tr>
<td>HCC</td>
<td>4 (40.00)</td>
<td>6 (60.00)</td>
<td></td>
</tr>
<tr>
<td>Small cell</td>
<td>15 (88.24)</td>
<td>2 (11.76)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>16 (45.71)</td>
<td>19 (54.29)</td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nivolumab</td>
<td>94 (55.95)</td>
<td>74 (44.05)</td>
<td>0.0531</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>24 (59.34)</td>
<td>37 (60.66)</td>
<td></td>
</tr>
<tr>
<td>Alezolizumab</td>
<td>9 (69.23)</td>
<td>4 (30.77)</td>
<td></td>
</tr>
<tr>
<td>Durvalumab</td>
<td>1 (25.00)</td>
<td>3 (75.00)</td>
<td></td>
</tr>
<tr>
<td>Ipi</td>
<td>96 (49.23)</td>
<td>99 (50.77)</td>
<td>0.0348</td>
</tr>
<tr>
<td>Ipi not received</td>
<td>30 (66.67)</td>
<td>15 (33.33)</td>
<td></td>
</tr>
</tbody>
</table>

1Statistically significant at 0.05

Conclusions After adjusting for possible confounding factors such as rates of irAEs, our results suggest that early use of steroids during CPI treatment significantly hinders CPI efficacy. These data need to be validated prospectively. Future studies should focus on the immune mechanisms by which CS affect T-cell function early in CPI treatment course.

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191 ASSOCIATION OF IMMUNE RELATED ADVERSE EVENTS WITH THE EFFICACY OF IMMUNE CHECKPOINT INHIBITORS IN METASTATIC RENAL CELL CARCINOMA

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Background Immune checkpoint inhibitors (ICI) are first-line therapy for tumors including metastatic renal cell carcinoma (mRCC). Use of ICI is complicated by diverse immune-related adverse events (irAEs), which can add significant morbidity but are also associated with improved efficacy of therapy. Risk factors for development of irAE are still poorly understood. We hypothesized that patients with mRCC treated with ICI as first-line therapy have higher rates of developing irAE’s than patients previously treated with other therapies.

Methods We conducted a single-institution, retrospective medical record review of patients with mRCC treated with immune-checkpoint inhibitors from March 2011 through April 15, 2020. We identified therapy duration, and presence, severity, and treatment of adverse events. We defined overall survival as time elapsed from date of diagnosis until death or until completion of study. We classified severity of adverse events according to CTCAE guidelines. Statistical methods included univariate
Cox proportional hazards and logistic regression models, and Kaplan-Meier curves were plotted for subgroups.

Results A total of 64 unique charts were reviewed. 18 patients (28%) of patients were treated with ICI as first-line therapy. 28 patients (44%) experienced immune-related adverse events with a total of 40 irAE’s identified. Most irAE were grade I-II (78%), with 7 (17%) grade III and 1 (2.4%) grade IV irAE’s. Most common sites were skin (29%), thyroid (20%) and gastrointestinal (15%). Patients with irAE had increased survival compared to those who did not have irAE (median survival not reached, vs 139 weeks, p=0.0004) (figure 1). This finding remained after excluding patients who had only experienced dermatologic irAE (median survival not reached in non-derm irAE subgroup, vs 144 weeks for dermatologic or no irAE, p=0.011) (figure 2). Patients treated with ICI as first line therapy had greater rates of developing irAE (72%) than those who had prior therapies (32%) (OR 5.4; p = 0.006). There was no association between histology type and rate of irAE.

Conclusions The development of irAE’s in patients with mRCC treated with ICI is associated with longer survival. This study joins the growing body of evidence showing that presence of irAE’s is associated with increased treatment efficacy. Use of ICI as first-line therapy is associated with higher risk of irAE. Given growing use of ICI as first-line therapy, further study to predict onset and severity of irAE’s is required.

Acknowledgements Hong Wang, PhD, for statistical support.

Ethics Approval This study was approved by the University of Pittsburgh Institutional Review Board. Approval number STUDY19100386.

REFERENCES

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192 'IMMUNE-BRAIN': A CASE SERIES OF COGNITIVE DYSFUNCTION/DECLINE IN CANCER PATIENTS ON IMMUNOTHERAPY
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Background Immunotherapy agents are now the standard of care for many types of malignancies and use of checkpoint inhibitor immunotherapy (IO) is widespread across oncology. Cognitive dysfunction/decline (CD) is a well-known side effect of conventional chemotherapy (i.e. ‘chemo-brain’), but the neuro-cognitive impact of checkpoint inhibitor immunotherapy (IO) is not well described, despite the known potential for inflammatory neurotoxicities and encephalitis. Though high grade neurologic events are reported in <1% of patients receiving IO, less severe or possibly transient neurocognitive effects that do not lead to formal neuropsychological evaluation are probably under-reported. Combination of IO with other anti-cancer modalities like cytotoxic chemotherapy and radiation could theoretically compound neurotoxicity through neuroinflammation.

Methods From January 2015-December 2018 at University of Vermont Medical Center, we retrospectively identified cancer patients who received at least one infusion of IO and had a concurrent diagnosis of CD on the problem list, medical history, or billing codes. We used the search terms: cognitive impairment, mild cognitive impairment, neurodegenerative cognitive impairment, memory change/memory deficit/memory difficulty/memory impairment, altered mental status, or encephalopathy to define cognitive impairment. Though high grade neurologic events are reported in <1% of patients receiving IO, less severe or possibly transient neurocognitive effects that do not lead to formal neuropsychological evaluation are probably under-reported. Combination of IO with other anti-cancer modalities like cytotoxic chemotherapy and radiation could theoretically compound neurotoxicity through neuroinflammation.

Results We identified 55 patients and excluded 16 for CD before IO started, 23 with toxic/metabolic causes (including...
stroke, sepsis, medications, seizures), 4 for primary central nervous system malignancy, and 6 for CD related to new or worsening brain metastases. Six had CD possibly related to IO (32.7%), compared to all other lung cancers (44.9%), X2 (1, N = 329) = 4.4, p = 0.037. Conversely, patients with lung cancer types other than squamous cell carcinoma were more likely to develop thyroid irAEs, OR = 1.68 (95% CI: 1.03 – 2.73).

Conclusions Thyroid irAEs occurred significantly less frequently in patients with squamous cell carcinoma than those with other lung cancer types. This analysis may allow clinicians to better identify patients more likely to develop thyroid dysfunction based on lung cancer type.

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194 A MULTICENTER CHARACTERIZATION OF CHRONIC TOxicoITYs FOLLOWing ADJUVANT ANti-PD-1 THERAPY FOR HIGH RISK RESECTED MELANOMA

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Background Anti-programmed death-1 (anti-PD-1) therapies have improved long-term survival across many advanced cancers. However, chronic immune-related adverse events (irAEs) are not well-defined. We sought to determine the incidence, time-course, spectrum, and predictors of chronic irAEs arising from adjuvant anti-PD-1.

Methods In this retrospective cohort, we analyzed patients from 8 academic medical centers with stage III-IV melanoma treated with anti-PD-1 in the adjuvant setting. Acute and chronic (persisting at least 3 months after therapy cessation) irAEs were characterized by type, time-course, management, and incidence.

Results Among 387 patients, most were male (60.7%) with a median age of 63 years, had cutaneous primaries (85.8%), BRAF/NRAS WT (51.2%), and resected stage IIIb (33.1%) or IIIc (39.5%) melanomas. Median overall survival and relapse-free survival (RFS) were not reached. 359 patients (93.0%) were alive at median follow-up of 529 days. Patients with acute irAEs were more commonly dermatitis/pruritus (25.8%), thyroiditis/hypothyroid (16.3%), arthralgias (10.6%), colitis/diarrhea (9.8%) and required glucocorticoids in 109 patients...
(28.2%). Of these, 167 patients (43.2%) developed chronic irAEs; 82 (49.1%) were symptomatic, 55 (32.9%) required glucocorticoids, and most were grade 1–2 (96.4%). Endocrinopathies (73/88, 83.0%) arthritis (22/45, 48.9%), xerostomia (9/17, 52.9%), neurotoxicities (8/8, 100.0%), and ocular events (5/5, 63.0%) were likely to become chronic events. In contrast, colitis (6/44, 13.6%), hepatitis (4/25, 16.0%), pneumonitis (6/18, 33.3%) were less likely to become chronic. Overall, the most common chronic irAEs were hypothyroidism (14.0%), dermatitis/pruritus (6.6%) arthralgias (5.7%), adrenal insufficiency (3.1%), and xerostomia (2.3%). Age (p=0.67), gender (p=0.31), time of onset of acute irAEs (p=0.95), and initial need for glucocorticoids (p=0.15) were not associated with chronicity. Only 24 (14.4%) of chronic irAEs ultimately resolved during the median 529-day follow-up. In particular, endocrinopathies (100%) arthralgias (100%) ocular events (100%), xerostomia (88.9%), and cutaneous events (89.5%) had high rates of persistence at last follow-up.

Conclusions Chronic irAEs to anti-PD-1 were more common than previously recognized and frequently persisted even with prolonged follow-up, although most were low-grade. The risks of chronic toxic effects should be integrated into treatment decision making.

Ethics Approval This study was approved by the Vanderbilt Institutional Review Board.

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195 AVELUMAB INTERNALIZATION AND LYOSOMAL DEGRADATION BY CIRCULATING IMMUNE CELLS IN HUMAN IS MEDIATED BY BOTH FC GAMMA RECEPTOR (FCGR) AND PD-L1 BINDING

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Background Receptor-mediated endocytosis results in antibody recycling (via endosomes) or degradation (in lysosomes). Avelumab is a human anti-PD-L1 antibody, with wild type (WT) IgG1 isotype and effector function, approved for treating certain cancers. Here, we report the mechanism of avelumab internalization and association with pharmacokinetic (PK) properties.

Methods A flow cytometry-based antibody internalization assay using pH-sensitive fluorescent dye was applied to directly monitor antibody internalization and lysosomal degradation in healthy donor blood. Avelumab, a WT IgG1 with full FcγR binding capability, its FcγR binding-deficient variant (N297A amino acid substitution), and a PD-L1 binding-deficient R99K variant were compared. Internalization of avelumab/variants was also compared with another anti-PD-L1 antibody with an amino acid sequence identical to atezolizumab (IgG1 with N297A substitution) and its WT IgG1 Fc-restored variant. In vivo PK studies in cynomolgus monkeys were performed after a single intravenous (IV) bolus injection of 5 mg/kg. Serum concentrations were measured by immunoassay.

Results Compared with avelumab, the FcγR binding-deficient N297A variant showed significantly reduced internalization. PD-L1 binding-deficient R99K variant showed a reduced internalization ratio as well, although to a lesser extent, particularly in granulocytes. These data indicate that both FcγR and PD-L1 binding contribute to avelumab internalization, with FcγR binding playing the major role. To test this hypothesis, we compared the internalization of avelumab and its N297A variant with an internally generated antibody that has the same Fab domain as atezolizumab (containing N297A replacement) and its WT IgG1 variant. The two WT IgG1 antibodies showed clearly different internalization ratios, indicating that the PD-L1 binding epitope may influence either their internalization or fate after internalization. However, N297A variants of both antibodies showed strong reduction in internalization, indicating the main receptor mediating the internalization is FcγR. Similar results were observed using whole blood from cynomolgus monkeys. Conducting the internalization experiment in the presence of competing soluble FcγRs, showed soluble CD64 significantly reduced internalization of avelumab. Serum concentration profiles after IV dosing in cynomolgus monkeys showed the R99K variant had the longest half-life, followed closely by the N297A variant. In comparison, avelumab showed the shortest half-life in vivo.

Conclusions These findings indicate that the major mechanism of avelumab internalization by circulating immune cells in human blood is through FcγR binding, in synergy with PD-L1 binding, and suggest that these mechanisms have a major impact on antibody PK properties. These results will support optimization of future therapeutic antibody development.

Ethics Approval The study was conducted according to the principles of the Declaration of Helsinki. All volunteers provided written informed consent. Protocol approval was obtained from independent review boards or ethics committees at each site.

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196 CHECKPOINT BLOCKADE THERAPY FOR BRAIN-METASTATIC NON-SMALL CELL LUNG CANCER: A COMPARATIVE EFFECTIVENESS ANALYSIS OF NATIONAL DATA

Nayan Lamba1, Bryan Iorgulescu*. 1Harvard Medical School, Boston, MA, USA; 2Dana-Farber Cancer Institute, Boston, MA, USA

Background Management of advanced non-small cell lung carcinoma (NSCLC) has been transformed by PD-1/PD-L1 immune checkpoint inhibitors (ICI), with FDA approvals in 2015 (second-line) and 2016 (first-line). Despite ~40% of NSCLC patients developing brain metastases, these patients were disproportionately excluded from the pioneering ICI trials. Thus herein we evaluate the overall survival (OS) associated with ICI in NSCLC brain metastases nationally.

Methods Patients newly-diagnosed with stage 4 NSCLC, including brain metastases, from 2010–2016 were identified from the National Cancer Database (comprising >70% of all newly-diagnosed cancers in the U.S.) Landmark survival analysis was used to address immortal time bias. Post-approval, median time from diagnosis to ICI was 58 days, and this timepoint was selected for all landmark survival analyses (OS estimated by Kaplan-Meier technique, and compared by log-rank test and multivariable Cox regression) and for multivariable logistic regression to identify predictors of ICI utilization.

Results 50,858 patients presented with advanced NSCLC that involved the brain: representing 27.6% of all newly-diagnosed stage 4 cases. Following initial FDA approvals in 2015, ICI use in brain metastasis patients rose from 7.2% in 2015 to 12.7% in 2016. OS for NSCLC brain metastasis patients
diagnosed post-approval (i.e. 2015, median 6.3 months, 95% [confidence interval] CI: 6.0–6.6) was substantially better than those diagnosed pre-approval (median 5.5 months, 95%CI: 5.4–5.7, p<0.001) and, in fact, than those diagnosed in 2014 (median 5.9 months, 95%CI: 5.6–6.1, p=0.002). Among patients diagnosed post-approval (in 2015, n=7,431), ICI receipt demonstrated substantially improved OS in landmark survival analyses (median 13.8 months, 95%CI: 12.2–15.1; vs. 8.5 months, 95%CI: 8.3–8.9, p<0.001) – benefits which persisted in multivariable landmark survival analyses (hazard ratio [HR] 0.83, 95%CI: 0.71–0.96, p=0.02), independent of patient characteristics, other therapies, and extracranial disease. For patients diagnosed post-approval, who reached the landmark timepoint, ICI receipt was independent of patient demographics, socioeconomic status, and hospital type—with the exception of Medicaid-insured patients, who were less likely than privately insured patients to receive ICI (OR 0.77, 95% CI: 0.60–0.97, p=0.03).

Conclusions Nationally, the use of ICI for NSCLC brain metastasis patients is increasing, generally without significant socioeconomic barriers. Brain metastasis patients diagnosed in the post-approval second-line ICI era (2015) demonstrated significantly better OS than patients diagnosed pre-approval and even than patients diagnosed only in 2014. ICI was associated with a >60% relative increase in median OS. Together our findings from a real-world population demonstrate that the dramatic OS benefits of ICIs for advanced NSCLC also extended to brain metastasis patients.

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Survival Outcomes and Toxicity Among Patients Treated with Concomitant Radiotherapy and Immunotherapy for Advanced Melanoma: Two Faces of the Abscopal Effect?

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Background Combined treatment with radiotherapy (RT) and checkpoint inhibition (CPI) can theoretically increase both treatment response and toxicity. We recently reported a high rate of immune-mediated adverse events (irAEs) among patients with advanced melanoma and Merkel cell carcinoma (MCC) treated with concomitant RT and CPI. We now present survival data from the same cohort.

Methods The original study population consisted of 30 patients with advanced melanoma and 5 with MCC who underwent RT within 30 days of CPI; eligible patients were identified via an institutional retrospective registry. Information on the development of new irAEs diagnosed within 3 months of RT initiation was collected. Overall survival (OS) was calculated by the Kaplan-Meier method. Outcomes of patients who did or did not develop new irAEs after RT were compared via the log-rank test. To limit heterogeneity, the survival analysis was restricted to patients with melanoma.

Results Of the 30 patients with melanoma included in the survival analysis, 25 had died and 5 remained alive when data were censored in August 2020. Median follow-up was 18 months. Treatment with concomitant RT and CPI constituted first-line therapy for most patients (21/30); 8 patients had received one previous line of treatment and 1 patient had progressed on multiple regimens. Thirteen patients (43.3%) experienced at least one new irAE following RT in the context of concomitant CPI. Patients who experienced new irAEs post-RT demonstrated longer median OS of 25 months (95% confidence interval [CI]: 8.6 - 41.4 months) in comparison to a median OS of 11 months for patients who did not develop post-RT irAEs (95% CI: 0.0 – 24.4 months). In the post-RT irAE group, 1-year and 2-year OS (69.2% and 53.8%, respectively) were higher compared to patients without irAEs (47.1% and 23.5%, respectively). These differences in survival did not reach statistical significance within this limited cohort size (figure 1; p = 0.076).

Conclusions The use of concomitant RT and CPI was associated with an elevated rate of new irAEs. Patients who developed new irAEs following RT experienced a substantial absolute increase in median OS of 14 months, an observation from a limited cohort which warrants further investigation. These data support prior reports of increased OS among patients experiencing irAEs and may suggest that RT and CPI in combination can meaningfully potentiate immune response in certain clinical contexts.

Ethics Approval The study was approved by the Cleveland Clinic Foundation Institutional Review Board, approval number 18–1225

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Combination Intratumoral Treatment with Intasyl™ Self-Delivering RNAi Targeting TIGIT and PD-1/PD-L1 Improves Tumor Control Compared to Monotherapy in a CT26 Model of Murine Colorectal Cancer

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Background Despite clinical successes of immune checkpoint blockade (ICB) antibodies blocking the inhibitory receptors CTLA-4, PD-1, or PD-L1, substantial challenges remain. Many patients do not respond, and ICB treatment is associated with serious immune-related adverse effects (irAEs) which are exacerbated by combination therapies. TIGIT blockade has been demonstrated to provide tumor control in pre-clinical studies,
sparking ongoing clinical trials, including those targeting TIGIT in combination with anti-PD-1 or anti-PD-L1. The INTASYL™ platform is a self-delivering RNAi technology that (1) provides efficient delivery into target cells bypassing the need for specialized formulations, mechanical perturbation, or drug delivery systems; and (2) specifically and durably silence target gene expression when administered intratumorally (IT), providing in vivo tumor control. IT administration restricts pharmacokinetics to the tumor; an attractive strategy for mitigating ICB-mediated systemic irAEs. Additionally, using INTASYL™, multiple targets can be silenced in combination. Here we demonstrate the in vivo efficacy of INTASYL specifically targeting TIGIT (PH-804), PD-1 (PH-762), PD-L1 (PH-790) alone or in combination in a CT26 model of murine colorectal carcinoma.

Methods To assess silencing activity, activated human pan-T cells were incubated in vitro with INTASYL compounds either alone or in combination and mRNA silencing was determined by qRT-PCR and protein silencing by flow cytometry. To assess in vivo tumor efficacy CT-26 cells were implanted subcutaneously into BALB/c mice. INTASYL compounds were administered IT at 1 mg/dose on Days 1, 3, 7, and 10 either as single agents (mPH-804, mPH-762, mPH-790) or in combination (mPH-804 + mPH-762 or mPH-804 + mPH-790). Controls consisted of PBS (vehicle; (IT)), and anti-TIGIT, anti-PD-1, or anti-PD-L1 antibodies (0.2 mg/dose) administered via intraperitoneal injection (IP). Tumor volumes and body weight were recorded throughout the study. Tumors were taken at the end of the study for analysis.

Results Single and combination knockdown of target molecules was validated at the mRNA level (=90%) by qRT-PCR and at the protein level (=80%) in activated human pan-T cells. In vivo, combination treatment with mPH-804 + mPH-762 or mPH-790 improved tumor control compared to individual monotherapies providing evidence of potential synergy. All treatments were well tolerated.

Conclusions n/a

Acknowledgements We demonstrate the potential of INTASYL-mediated combination therapy targeting TIGIT and PD-1/PD-L1. These findings indicate that combination of TIGIT + PD-1/PD-L1 silencing improves tumor control compared to monotherapy. As INTASYL IT is efficacious and may mitigate irAEs caused by antibody ICB, INTASYL combination therapies including PH-804, PH-762 and PH-790 warrant further investigation in patients.

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199 ENHANCED IMMUNE RESPONSES IN HUMAN BREAST AND COLON CANCER FOLLOWING CHECKPOINT THERAPY IN A CD34+ STEM CELL HUMANIZED NCG (HUCD34NCG) MOUSE MODEL

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Background Breast and colon cancer rank second and third, respectively, in world-wide prevalence of malignancies and present a large unmet medical need. The correlation between lymphocyte infiltration into the tumor microenvironment and efficacy of anti-cancer immunotherapies has been established. Therefore, relevant and cost-saving pre-clinical models are needed for developing new treatment approaches to predominant human tumor types. HuCD34NCG mice facilitate studying human immune responses in vivo elicited by experimental therapeutic antibodies. We characterized growth kinetics and human immune responses to checkpoint blockade in human breast and colon tumor-bearing HuCD34NCG mice. Aging, non tumor-bearing HuCD34NCG mice were also monitored for indicators of spontaneous hematopoietic cancer formation.

Methods HSC engraftment was quality controlled prior to inoculating HuCD34NCG mice with either colon adenocarcinoma (COLO 205) or triple negative breast cancer (MDA-MB-436) cells (both purchased from American Type Culture Collection, Manassas, VA). Mice were randomized into treatment groups based on tumor size, and checkpoint inhibitor antibodies were dosed twice weekly (anti-human PD-1, BioXcell clone: RMP1-14 or Keytruda; anti-human CTLA-4, BioXcell clone: BN13; and combination therapy). Body weights, general health status and survival were monitored. Peripheral blood (PB) and selected tissues were analyzed for the presence and composition of human immune cells by acoustic focusing flow cytometry. Non tumor-bearing aged HuCD34NCG mice (27 weeks post-engraftment) were sampled biweekly over ten weeks for lymphoma immunophenotyping.

Results Both tumor-bearing models showed significant anti-hPD-1 and anti-hCTLA-4 responses, but combination therapy only enhanced growth reduction significantly in MDA-MB-436 tumors. Flow cytometric analysis identified viable human leukocytes in tumor and spleen at study termination. These tumor-infiltrating lymphocytes (TIL) and splenocytes from surviving COLO 205 and MDA-MB-436 mice consisted of a total T-cell phenotype (CD3+/−), CD4+, CD8+ and Treg subsets. Additionally, myeloid cells (CD11b+ or CD11c+) and M1/M2 macrophages were detected within these infiltrates. Splenic and tumor-infiltrating T-cells readily secreted human cytokines (IFN-γ, IL-2, TNF-α) and granzyme B upon ex vivo activation exhibiting multifunctional and cytotoxic capabilities in all treatment groups. Baseline murine and human cytokine levels were distinguished in plasma from aging, non tumor-bearing HuCD34NCGs. Their phenotypes also showed no conclusive indicators of abnormal blood cells developing or graft failure.

Conclusions Breast and colon tumor cell-line derived models were established in HuCD34NCG mice. Standard checkpoint inhibitor treatment promoted human T-cell infiltration into tumor microenvironments inhibiting growth. These results demonstrate that HuCD34NCG are a robust and relevant host for various human cell xenotransplants to advance preclinical immuno-oncology drug development.

Ethics Approval Animal studies were executed in compliance with local Charles River IACUC guidelines, IACUC number I-033.

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200 NBTXR3 NANOPARTICLE WITH IMMUNORADIATION IMPROVES SURVIVAL AND GENERATES LONG-TERM ANTI-TUMOR MEMORY IN AN ANTI-PD1 RESISTANT MURINE LUNG CANCER MODEL

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Background Although treatment with high-dose (HD) radiation (XRT) and NBTXR3 on primary tumors in combination with
systemic anti-PD1 was able to significantly improve abscopal effect in 344SQR murine metastatic lung cancer model, most of the mice eventually died due to the growth of secondary tumors. Therefore, we intended to use HD-XRT plus NBTXR3 injection into primary tumors and low-dose (LD) radiation on secondary tumors plus dual-agent immunotherapy (IT) of anti-PD1 and anti-CTLA-4 to achieve complete control of both the primary and secondary tumors in mice.

Methods Five groups of 8 mice each were inoculated subcutaneously with $5 \times 10^4$ anti-PD1-resistant 344SQR cells in each hind leg, 3 days apart, to establish ‘primary’ (right) and ‘secondary’ (left) tumors. All mice in treatment groups received intraperitoneal anti-PD1 and anti-CTLA-4 on days 4, 7, 10, and 13, and continuing anti-PD1 treatment on days 20, 27, 34, 41, and 49 and 12 Gy x3 (HD-XRT) to the primary tumors on days 7, 8 and 9. Primary tumors in groups 3 and 5 also received intratumoral NBTXR3 on day 6. Secondary tumors in groups 4 and 5 were also irradiated with 1Gy x2 (LD-XRT) on days 12 and 13. Experimental groups were designated as 1=Control, 2=HD+IT, 3=NBTXR3+HD+IT, 4=HD+LD+IT, and 5=NBTXR3+HD+LD+IT. The secondary tumors were analyzed by flow cytometry and Nanostring. On day 178, the survivor mice were rechallenged with $5 \times 10^4$ 344SQR cells on the right flank and the tumor growth was monitored for an additional 36 days.

Results All mice in all the groups except NBTXR3+HD+LD+IT died due to the growth of either the primary tumor or the secondary tumor by day 36. Both the primary and the secondary tumors in 4 mice of NBTXR3+HD+LD+IT group were completely eliminated. No tumor growth was observed in these mice after rechallenged with 344SQR cells. Flow cytometry data demonstrated that only the mice in the groups with NBTXR3 had significantly more CD8+ T cell infiltration in the secondary tumor collected on day 16 than the control. Both flow cytometry and Nanostring data showed that only the mice in NBTXR3+HD+LD+IT had a significantly higher CD8+ T cell/Treg cell ratio than the control.

Conclusions The combination of NBTXR3 plus high and low dose radiation with immunotherapy effectively controlled the growth of both primary and secondary tumors, significantly extended the survival, generating long-term antitumor memory. This combination therapy induced immune-mediated control of the secondary tumor at both genetic and cellular levels.

Acknowledgements This work was supported by Cancer Center Support (Core) Grant CA016672 to The University of Texas MD Anderson Cancer Center; the Goodwin family research fund; the family of M. Adnan Hamed and the Orr Family Foundation to MD Anderson Cancer Center’s Thoracic Radiation Oncology program; an MD Anderson Knowledge Gap award; Nanobiotix.

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201 CARBOPLATIN, PACLITAXEL AND PEMBROLIZUMAB FOR THE FIRST LINE TREATMENT OF RECURRENT AND/OR METASTATIC HEAD AND NECK SQAMOUS CELL CARCINOMA

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Background A recent phase III study (Keynote-048) demonstrated survival benefit of platinum, 5-FU and pembrolizumab in 1st line treatment of recurrent and/or metastatic head and neck squamous cell carcinoma (RM-HNSCC). However, administration of 5-FU has been a challenge for logistics and toxicities. As platinum, paclitaxel and pembrolizumab has been shown to be an effective and safe treatment for non-small cell lung cancer, we hypothesized that carboplatin, paclitaxel and pembrolizumab would be safe and effective for 1st line treatment of RM-HNSCC.

Methods We performed a retrospective study of RM-HNSCC patients who received carboplatin, paclitaxel and pembrolizumab for first line systemic therapy, treated between December 2015 and January 2020 at the University of California, San Francisco. Patients who received at least 1 cycle of treatment with pre-treatment and post-treatment images were included in the analyses. Response to the treatment was assessed using RECIST criteria version 1.1. We also estimated overall survival and progression free survival using Kaplan-Meier method.

Results Nine patients who received carboplatin, paclitaxel and pembrolizumab as first line systemic therapy for RM-HNSCC were identified. Two patients had HPV positive oropharyngeal SCC, the other patients unknown primary SCC in head and neck (2), oral cavity SCC (2), laryngeal SCC (1), hypopharyngeal SCC (1) and SCC of orbit (1). There were 1 complete response (CR, 11%), 6 partial responses (PR, 55%), 1 stable disease (SD, 11%) and 1 progressive disease (PD, 11%). Overall response rate (ORR) was 78%, and median progression free survival and median overall survival have not reached with median follow-ups of 6 months and 8 months, respectively. Two patients discontinued chemotherapy after 1 cycle for grade 4 acute kidney injury and grade 4 anaphylaxis, yet achieved CR and PR, respectively.

Conclusions The retrospective analysis suggests that first line carboplatin, paclitaxel and pembrolizumab for RM-HNSCC is an active regimen and can be considered in place of platinum, 5-FU and pembrolizumab, which merits further investigation.

Ethics Approval The study was approved by UCSF’s Institutional Review Board, approval number 19-29363.

REFERENCES


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Methods BxPC-3, PANC-1, and MIA-PaCa2 were incubated alone or in combination with Tinzaparin (T) and/or Nab-Paclitaxel (A) and/or Gemcitabine (G) and/or Nivolumab (NI), Pembrolizumab (PE) and/or Ipilimumab (IPI). The effect of these regimens on various signaling pathways controlling proliferation and apoptosis was identified in vitro through Western blot. Cell viability was measured with MTT assay. NOD/SCID mice will be used to generate xenografts with the PANC-1 cell line. Human peripheral blood mononuclear cells (PBMCs) from healthy donors will be injected to give mice a human-like immune system.2

Results In a triple combinatorial scheme, NEPE+IPI+T, the protein levels of VEGFR2 were decreased (0.1 to 0.7 folds) in a dose-dependent way in mtkRAS PC cell lines (PANC1 and MIAPACA2). The number of PANC-1 cells was decreased around 40% in a triple combinatorial scheme of T+IPI+(NI or PE) after 48 hours. The triple combination of Gemcitabine + Nab-paclitaxel + Tinzaparin leads to a decrease in tumor size relative to control by 51% and relative to Nab-P + G by 15%. The combination of chemotherapy, immunotherapy, and Tinzaparin leads to a reduction in tumor size compared to control by up to 60%. Tinzaparin contributes an additional 20% Preliminary data show that the quadruple therapeutic regimen increases the percentage of CD8+ cells from 5% to 27% and decreases Tregs' percentage from 9.5% to 4% (in TILs).

Conclusions In vitro experiments show a decrease in the cell viability of PC cell lines and a reduction in the protein levels of VEGFR2 in mtkRAS cell lines. In vivo experiments with NOD/SCID mice and humanized NOD/SCID mice show a significant reduction in tumor volume in the combination therapy regimens with Tinzaparin. Possible mechanisms for these effects include an increase in CD8+ cells, a decrease in Tregs, a reduction in VEGFR-2 expression, and an increase in cancer cell apoptosis. This synergistic strategy can create new avenues for the treatment of patients with pancreatic cancer, achieving a better clinical outcome and greater survival.

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http://dx.doi.org/10.1136/ijt-o-2020-SITC2020.0202

203 PRECLINICAL CHARACTERIZATION AND DEVELOPMENT OF MG1124, A NOVEL IMMUNE CHECKPOINT INHIBITOR TARGETING CEACAM1 FOR NSCLC PATIENTS

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Background CEACAM1 is the only member of CEACAM family which is expressed on lymphocytes such as T cells and NK cells that mediate suppression of inflammatory T cell response. It is known that CEACAM1-CEACAM1 homophilic interaction induces downregulation of ZAP70 phosphorylation in response to T cell receptor (TCR) stimulation. There is a wealth of research demonstrating the correlation between CEACAM1 expression and cancer progression, in a wide range of indications. We developed a fully human monoclonal antibody (mAb) MG1124 that specifically binds to CEACAM1 but not to other CEA family members, thereby exerting anti-tumor effect via triggering immune response.

Methods T cell activation of MG1124 was determined by an NFAT-luciferase reporter assay with CEACAM1 overexpressing Jurkat stable cells. In vitro efficacy of MG1124 was examined using an NK cell- or cytotoxic T cell-mediated tumor cell killing assay. The anti-tumor efficacy of MG1124 alone or in combination was studied in a humanized mouse model. As MG1124 binds to monkey CEACAM1 with high affinity, pharmacokinetics assessment of MG1124 was performed in cynomolgus monkeys.

Results An anti-CEACAM1 antibody MG1124 bound to CEACAM1 but not to other CEA family members. MG1124 blocked CEACAM1 homophilic interaction by binding to the N domain of CEACAM1. Especially the homophilic interaction induced downregulation of ZAP70 phosphorylation in response to TCR stimulation in a CEACAM1 overexpressing Jurkat stable cell line, which was rescued by MG1124 resulting in augmentation of NFAT activity and IL-2 expression. NK cell or cytotoxic T cell-mediated tumor lysis was increased by MG1124 in a CEACAM1 expression-dependent manner. MG1124 inhibited tumor growth in CEACAM1 expressing NSCLC DXD humanized mouse models. In an NSCLC PDX humanized mouse model, MG1124 dose-dependently inhibited tumor growth as monotherapy. Moreover, MG1124 showed synergistic anti-cancer activity with pembrolizumab in NSCLC hupPDX models. Pharmacokinetic (PK) analysis in cynomolgus monkeys showed that the half-life (T1/2) of MG1124 was estimated to range from 14 to 17 days, and the peak plasma concentration (Cmax) and overall exposure (AUC) were found to be generally dose proportional. Following this PK study, a toxicity study in cynomolgus monkeys is ongoing.

Conclusions MG1124, a novel anti-CEACAM1 mAb, blocked CEACAM1-mediated negative regulation and restored NK or cytotoxic T cell activities. MG1124 showed effective anti-tumor activity in vivo mouse models and its combination with PD-1 blockade further enhanced treatment efficacy. The data presented herein support further advancement of MG1124 towards clinical development. MG1124 is a potential therapeutic candidate for immune checkpoint blockade in cancer therapy.

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204 THE ROLE OF IMMUNE CHECKPOINT INHIBITOR AS A SINGLE AGENT OR COMBINATION THERAPY IN ADVANCED THYROID CANCER

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Background There is a high unmet need for effective systemic treatment for patients with metastatic radioactive iodine
refractory (RAI-R) differentiated thyroid cancer (DTC) and anaplastic thyroid cancer (ATC). Immunotherapy may be used as an alternative option for those without targetable mutations or have become resistant to targeted therapy. Here we review the clinical trials and retrospective studies and discuss the potential role of immune checkpoint inhibitors (ICIs) in advanced thyroid cancer.

Methods The details of pertinent clinical trials were obtained from clinicaltrials.gov (NIH) using search terms including ‘thyroid cancer’ and ‘immunologic.’ The NCT numbers and search terms were used to search for published results on databases such as PubMed, American Association of Cancer Research, and American Society of Clinical Oncology. The efficacy outcome measures were determined using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.

Results In RAI-R DTC, responses to three different regimens have been reported: pembrolizumab, nivolumab plus ipilimumab, and pembrolizumab plus lenvatinib. No CR was reported, and the overall response rates (ORRs) varied from 9% (pembrolizumab monotherapy and nivolumab plus ipilimumab) to 64% (pembrolizumab plus lenvatinib) (figure 1a).1,4 In ATC, four studies have reported favorable outcomes in the context of dabrafenib and trametinib.5 The efficacy of spartalizumab, a PD1-inhibitor, was evaluated in a phase I/II trial, rendering an ORR of 19%, with 3 CRs (7%) and 5 PRs (12%) [6]. The study of nivolumab plus ipilimumab reported an ORR of 30% in ATC, with a near CR and two without clear evidence of disease at 13 and 26 months.2 A trial that tested the combination of atezolizumab, vemurafenib, and cobimetinib in BRAFV600E-mutated patients reported an ORR of 59%.7 A retrospective study reported an ORR of 60% after adding pembrolizumab at the time of progression on lenvatinib8 (figure 1b). There are 25 ongoing trials evaluating the efficacy of ICIs in different types of thyroid cancer. Three trials are testing pembrolizumab as monotherapy, three trials are assessing ICI combination therapy, and six trials are testing the efficacy of various ICI and tyrosine kinase inhibitor (TKI) combinations (figure 2).

Conclusions The recent trials and a retrospective study have reported favorable outcomes in ATC, suggesting ICIs have a potential role in treating patients with ATC. In particular, dual ICIs or combination of TKI and ICI can be developed as treatment options for ATC. Further large scale randomized prospective studies are required to establish ICIs as standard of care.

REFERENCES

Abstract 204 Figure 1 Comparison of responses in different regimens
*A The study population consisted only of BRAFV600E-positive patients
**Retrospective study
Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; Uneval, unevaluable; PEM, pembrolizumab; IPI, ipilimumab; NIVO, nivolumab; LENV, lenvatinib; ATEZO, atezolizumab; VEM, vemurafenib; COBI, cobimetinib; DAB, dabrafenib; TRAME, trametinib.

Abstract 204 Figure 2 Comparison of responses in different regimens
A. Number of checkpoint inhibition trials for various thyroid cancer histologies. B. Landscape of combination checkpoint inhibition agents. Abbreviations: DTC, differentiated thyroid cancer; ATC, anaplastic thyroid cancer, MTC, medullary thyroid cancer; NIVO, nivolumab; IPI, ipilimumab; LENV, lenvatinib; ATEZO, atezolizumab; VEM, vemurafenib; COBI, cobimetinib; DAB, dabrafenib; TRAME, trametinib; PEM, pembrolizumab; DOXY, doxycycline; SBRT: Stereotactic radiation therapy.
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205 PD-1/PD-L1/CTLA-4 INHIBITOR THERAPY FOLLOWING PROGRESSION ON A DIFFERENT PD-1/PD-L1 INHIBITOR: A CASE SERIES

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Background There are increasing numbers of immune checkpoint inhibitors (CPI) targeting the PD-1/PDL-1 and CTLA-4 pathways, which are approved in a wide variety of tumor types. A case series has previously described the sequential use of first line CPI, followed by second line CPI in renal cell carcinoma and melanoma patients, and both patient populations had progressive disease. There is still a lack of data on the safety and efficacy of challenging a patient who has previously progressed on a CPI with a different class of CPI, in other tumor types.

Methods We retrospectively collected data from patients treated with a CPI, who were subsequently challenged with another CPI, at a single institution. Induction criteria included patients with renal cell carcinoma and melanoma. Patient characteristics, immune-related adverse effects (irAEs), cancer type, tumor proportion score if available, treatment type, treatment response/progression per RECIST v1.1, and survival data were collected.

Results We identified 11 patients with various pathologies who received sequential CPI after progressing on first line CPI (table 1). Cancer types included non-small cell lung cancer (n=5), head and neck cancer (n=2), urethelial carcinoma (n=1), Merkel cell carcinoma (n=1), poorly differentiated carcinoma (n=1), and hepatocellular carcinoma (n=1). The tumor proportion score was available in 6 patients. Out of these patients, all were metastatic at the time of second line CPI. First line CPIs were all PD(L)-1 inhibitors, second line CPIs were all PD(L)-1 inhibitors except for one patient who received a CTLA-4 inhibitor in combination with a PD-1 inhibitor. Out of these patients, 3 patients who were trialed with second line CPI had stable disease, 5 patients had progression of disease, 1 patient had an irAE leading to discontinuation of CPI, and 2 patients died from adverse events unrelated to CPI. Out of 3 patients with stable disease on second line CPI, 2 patients had stable disease for over 2 years, and 1 patient had progressive disease for over 1 year.

Conclusions Despite concerns that sequential immunotherapy may not be efficacious, 3 out of 11 patients did significantly benefit with the long-term stable disease. We need further large-scale prospective studies and research to know more about tumor characteristics, the mechanism of resistance in immuno-oncology to help us identify patients who would benefit from sequential immunotherapy.

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206 AN IMMUNE-COMPETENT TUMOR ORGANOID PLATFORM TO TEST NOVEL IMMUNE CHECKPOINT COMBINATIONS TARGETING THE RECEPTOR CD47 IN TRIPLE NEGATIVE BREAST CANCER

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Background Immune checkpoint blockade therapy targeting PD-L1 has recently been approved for metastatic triple negative breast cancer (TNBC) patients. However, a 7% response rate calls for better models and strategies to stimulate TN tumor immunogenicity to increase patient response. Overexpression of the receptor CD47 impairs innate and adaptive tumor immunosurveillance when engaged to its counter receptor SIRPα or ligand thrombospondin-1. Co-expression of CD47 and PD-L1 is implicated in disease progression in TNBC patients. We examined through murine models and tumor organoid platforms whether targeting CD47 sensitizes TNBC tumors to PD-L1 therapy, focusing on the modulation of cellular bioenergetics as a potential mechanism and potentially predict response.

Methods The effects of targeting CD47 and PD-L1 were examined through orthotopic syngenic 4T1 and EMT-6 TNBC murine models. Due to predicting patient therapeutic response challenges, tumor organoid platforms investigated mechanisms of tumor sensitization to anti-PD-L1 by targeting CD47. Organoids were constructed by embedding murine TNBC tumor tissue and AH1 CD8+ T cells in a specialized ECM mimicking hydrogel. Immunohistochemistry was performed on organoid, human and murine TNBC tumor tissue. Cellular bioenergetics was analyzed through Seahorse® bioanalyzer.

Results Staining of human TNBC biopsies found elevated CD47 expression, signifying a potential therapeutic target. Targeting CD47 or in combination with anti-PD-L1 resulted in decreased tumor volume and weight in a TNBC murine model. The decrease in tumor burden was correlated with
SMALL MOLECULE INHIBITORS OF SEC61 COTRANSLATIONAL TRANLOCATION REGULATE THE PHAGOCYTOSIS CHECKPOINT MOLECULE CD47

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Background Many tumor cells escape immune cell clearance by overexpressing CD47, a multi-pass transmembrane protein, which binds signal regulatory protein α (SIRPα) on macrophages leading to decreased phagocytic activity. Blockade of CD47/SIRPα interactions enhances macrophage phagocytosis and is being targeted with antibody-based drugs, some of which are used in combination therapies in clinical trials. A novel method to target CD47 is through the inhibition of cotranslational translocation of transmembrane proteins. Immediately after exiting the ribosome, signal sequences that are unique to each protein are directed through the Sec61 channel into the ER for extracellular expression. Several Sec61-targeting compounds have been identified to suppress translocation in a signal sequence-specific manner. We previously described Sec61 inhibitors capable of selectively targeting immune checkpoint proteins and enhancing T cell function. Here, we demonstrate the blockade of CD47 expression on tumor cells and enhancement of macrophage phagocytosis with small molecule inhibitors of Sec61.

Methods Sec61-dependent expression of target proteins was assayed using HEK293 cells overexpressing constructs comprised of signal sequences fused to a luciferase reporter. Stimulated PBMCs or tumor cells were incubated with Sec61 inhibitors, and surface expression of checkpoint molecules were examined by flow cytometry. Necrotic and apoptotic cells were assessed by Annexin V and 7AAD labeling. Human CD14+ monocytes were differentiated to M1- or M2-type macrophages. Jurkat or SKBR3 cells were incubated with Sec61 inhibitors, labeled with a pH sensitive dye and co-cultured with macrophages to assess phagocytosis.

Results We identified Sec61 inhibitors that block select immune checkpoint proteins. Compounds demonstrated either selective or multi-target profiles in transient transfection screens, which was supported by decreased protein expression on activated T cells. KZR-9275 targeted multiple checkpoint molecules, including PD-1, LAG-3 and CD73, along with a potent inhibition of the CD47 signal sequence reporter. CD47 surface expression was decreased on Jurkat and SKBR3 cells following 72 hours of compound treatment. KZR-9275 treatment of SKBR3 cells induced a minor increase in apoptotic cells, which was not detected in Jurkat cells. Increased macrophage phagocytosis, especially with M2-type macrophages, was observed when Jurkat or SKBR3 cells were pre-treated with KZR-9275.

Conclusions Our findings demonstrate that Sec61 inhibitors can block the expression of CD47, a phagocytosis checkpoint protein, on tumor cells and subsequently modulate macrophage phagocytic activity. Small molecule inhibitors of Sec61 provide an opportunity to target multiple checkpoint proteins on various cell populations. Future in vivo tumor models will assess the efficacy of Sec61 inhibitors to provide combination-like therapy.

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Groups of 16 mice received each agent as monotherapy or in combinations. Sequencing of combination administration was also varied: Group 4 started treatment on the same day; Group 5 received E7777 2 days prior to start of anti-PD-1; Group 6 received anti-PD-1 first. Tumor growth was compared across all groups. In survival studies, mice were treated for 3 weeks and observed with twice weekly tumor measurements. In other experiments, tumors, tumor-draining lymph nodes, and spleens were examined by IHC and by flow cytometry of immune cells from dissociated tissues at defined points, for immune biomarkers.

**Results** Figure 1 shows additive benefit from the E7777 + anti-PD-1 combinations over either monotherapy. Most importantly, figure 2 and table 1 show significantly enhanced overall survival from a 3 week course of combinations compared to either agent alone (p<0.005) or to vehicle controls (p<0.000001). There was no clear distinction among different sequencing regimens. Benefit correlated with enhanced CD8: Treg ratios in tumors.

**Abstract 208 Table 1** Calculated median survival

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Median Survival (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle Control</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>E7777</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>α-PD-1</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>E7777 + α-PD-1</td>
<td>19.5</td>
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<tr>
<td>5</td>
<td>α-PD-1</td>
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</table>

**Conclusions** Depletion of Tregs by E7777 significantly increased anti-tumor activity and durably extended overall survival compared to treatment with anti-PD-1 alone in syngeneic solid tumor models. Clinical studies of a combination of the two agents are planned.

**Ethics Approval** All studies were conducted at Crown Bio, and were approved by the Crown Bio IACUC.

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**Abstract 209** PRECLINICAL MECHANISTIC AND CLINICAL EVALUATION OF THE CORTICOSTEROID DEXAMETHASONE’S DETRIMENTAL EFFECTS ON IMMUNE CHECKPOINT BLOCKADE IN GliOBLASTOMA CANCER

**Background** Increasing data indicate that corticosteroids can exert a detrimental effect on immunotherapy for oncology patients. Dexamethasone, a uniquely potent corticosteroid, is frequently administered to brain tumor patients to decrease tumor-associated edema, but limited data exist describing how dexamethasone affects the immune system systemically and intratumorally in glioblastoma patients – particularly in the context of immunotherapy.

**Methods** We evaluated the dose-dependent effects of dexamethasone when administered with PD-1 blockade and/or radiotherapy on survival and tumor response in immunocompetent C57BL/6 mice with syngeneic GL261 and CT-2A glioblastoma tumors. The immune microenvironment was comprehensively profiled using flow cytometry analysis. Clinically, the effect of dexamethasone on survival was evaluated in 181 IDH-wildtype glioblastoma patients treated with PD-(L)1 blockade, with adjustment for relevant prognostic factors using multivariable Cox regression.

**Results** Despite the inherent responsiveness of GL261 to immune checkpoint blockade, concurrent dexamethasone administration with anti-PD-1 therapy reduced survival in a dose-dependent manner (figure 1). Concurrent dexamethasone...
also abrogated survival following anti-PD-1 with or without radiotherapy in immunoresistant CT-2A models (figure 2). Dexamethasone decreased T lymphocyte numbers (figure 3) by increasing apoptosis (figure 4), in addition to decreasing lymphocyte functional capacity (figure 3/C/D). Myeloid and NK cell populations were also generally reduced by dexamethasone (figure 3). Thus, dexamethasone appears to negatively affect overall survival; 95CI, 95% confidence interval; NR, not reached.

***p<0.001; Dex, dexamethasone; BLI, bioluminescence imaging; OS, overall survival; 95CI, 95% confidence interval; NR, not reached.

Abstract Figure 1 Concurrent dexamethasone reduces the survival benefit of anti-PD-1 therapy in GL261-luc2 glioblastoma mouse models. Kaplan-Meier OS estimates are depicted, with comparison by logrank test and Cox regression. (A) To assess concurrent dexamethasone’s effect on a dose-intensive schedule of anti-PD-1 with or without RT in GL261-luc2 mice (n=8/group), anti-PD-1 was administered IP via a loading dose (500 μg) followed by 5 additional doses (250 μg/dose) at 3-day intervals. RT was administered in 2 Gy fractions/day for 5 days beginning on day 6. Dexamethasone was delivered IP daily from days 6–27 at 10 mg/kg. (B) For GL261-luc2 mice (n=8/group), anti-PD-1 (αPD1) was administered IP via an abbreviated dosing schedule every 3 days beginning on day 6 for a total of 4 doses (250 μg/dose). (C) For CT-2A-luc mice (n=8–16/group), anti-PD-1 was administered IP via a loading dose (500 μg) followed by 7 additional doses (250 μg/dose) at 3-day intervals. RT was administered in 2 Gy fractions/day for 5 days beginning on day 6. Dexamethasone was delivered IP daily from days 6–27 at 10 mg/kg *p<0.05; **p<0.01; ***p<0.001; Dex, dexamethasone; 95CI, 95% confidence interval; NR, not reached.

Abstract Figure 2 Concurrent dexamethasone decreases the OS benefit of anti-PD-1 plus RT in syngeneic GL261-luc2 and CT-2A-luc glioblastoma mouse models. Kaplan-Meier OS estimates are depicted, with comparison by logrank test and Cox regression. (A) To assess concurrent dexamethasone’s effect on a dose-intensive schedule of anti-PD-1 with or without RT in GL261-luc2 mice (n=8/group), anti-PD-1 was administered IP via a loading dose (500 μg) followed by 5 additional doses (250 μg/dose) at 3-day intervals. RT was administered in 2 Gy fractions/day for 5 days beginning on day 6. Dexamethasone was delivered IP daily from days 6–27 at 10 mg/kg. (B) For GL261-luc2 mice (n=8/group), anti-PD-1 (αPD1) was administered IP via an abbreviated dosing schedule every 3 days beginning on day 6 for a total of 4 doses (250 μg/dose). (C) For CT-2A-luc mice (n=8–16/group), anti-PD-1 was administered IP via a loading dose (500 μg) followed by 7 additional doses (250 μg/dose) at 3-day intervals. RT was administered in 2 Gy fractions/day for 5 days beginning on day 6. Dexamethasone was delivered IP daily from days 6–27 at 10 mg/kg *p<0.05; **p<0.01; ***p<0.001; Dex, dexamethasone; 95CI, 95% confidence interval; NR, not reached.

Abstract Figure 3 Concurrent dexamethasone negatively affects intratumoral and systemic adaptive and innate immune cell populations in the GL261-luc2 glioblastoma mouse model. (A) Experimental schema. Tissue was collected at day 16 of a dose-intensive regimen of anti-PD-1, in which anti-PD-1 (αPD1) was administered IP beginning on day 6 (500 μg loading dose) followed by 3 additional doses (250 μg) at 3-day intervals, with dexamethasone (10 mg/kg) administered IP on days 6–16. Tissue (n=4–8/group) was harvested on day 16 and analyzed by flow cytometry. Immune cell counts were evaluated by multiple linear regression, normalized to the corresponding IgG control group’s mean count (displayed as dashed gray line), and displayed as mean ± SE. (B) Differences in CD45+ leukocytes and CD45+ CD3- lymphocytes, including CD4+ and CD8+ T cells between treatment groups. (C) Percentage of splenic IFNγ+ CD4+ and CD8+ lymphocytes by treatment group. (D) Change in the number of early activated CD69+ T cells by site for each treatment group. Additionally, differences between treatment groups in innate immune cells including (E) myeloid cells (CD45hi CD11bhi), macrophages (Ly6Cint Ly6G-), monocytes (Ly6Chi Ly6G-), and microglia (in the brain, CD45lo CD11bhi), (F) dendritic cells (DCs; CD45+ CD11c+) and NK cells (CD45+ CD3- NK1.1+), as well as (G) activated (CD80+ CD86+) myeloid cells and DCs, PD-L1+ myeloid cells, and K67+ NK cells were analyzed cLN, cervical lymph node; Dex, dexamethasone; ns, not significant, p>0.05; *p<0.05; **p<0.01; ***p<0.001.
Abstract 209 Figure 4  Concurrent dexamethasone increases apoptosis of CD4+ and CD8+ T cells in the GL261-luc2 glioblastoma mouse model. (A) Late apoptosis was evaluated by 7-AAD+ and annexin-V+ staining in non-tumor-bearing mouse spleens (n=3/group) either 1 hour after the first dexamethasone dose or 1 hour after the sixth daily dexamethasone dose. Apoptosis differences were tested by two-way ANOVA with post-test correction. Cell counts normalized to the corresponding IgG control group’s mean count (B) and percent (C) of proliferating CD4+ and CD8+ T cells were evaluated by Ki67 staining, using the same dosing schema and analyses as figure 3 (n=4–8/group) cLN, cervical lymph node; Dex, dexamethasone; hr, hour; ns, not significant, p>0.05; *p<0.05; **p<0.01; ***p<0.001

Abstract 209 Figure 5  Baseline dexamethasone is associated with decreased OS among glioblastoma patients receiving anti-PD-(L)1 therapy, irrespective of dexamethasone dose. Kaplan-Meier OS estimates for 181 IDH-wildtype glioblastoma patients treated with anti-PD-(L)1 therapy, who were either on ≥2 mg (dashed gray line), <2 mg (dashed black line), or no (solid black line) baseline dexamethasone are depicted; including both (A) unadjusted analyses (n=181) and (B) analyses adjusted (by a Cox regression model; n=163) for relevant prognostic factors including disease setting (newly-diagnosed vs. recurrent), patient age, MGMT promoter methylation, KPS and tumor volume prior to anti-PD-(L)1 initiation, and extent of resection *p<0.01; ***p<0.001; Dex, dexamethasone ; mos, months

Abstract 209 Table 1  Multivariable Cox regression analysis of the effect of baseline dexamethasone on overall survival in glioblastoma patients treated with anti-PD-(L)1.

<table>
<thead>
<tr>
<th>Dexamethasone at aPD-(L)1 baseline</th>
<th>n</th>
<th>Multivariable Cox regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>105</td>
<td>Referent</td>
</tr>
<tr>
<td>&lt;2mg Dex</td>
<td>25</td>
<td>2.16 (1.30-3.60) 0.003</td>
</tr>
<tr>
<td>≥2mg Dex</td>
<td>33</td>
<td>1.97 (1.23-3.16) 0.005</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td></td>
<td></td>
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<tr>
<td>&lt;45</td>
<td>27</td>
<td>Referent</td>
</tr>
<tr>
<td>45-54</td>
<td>41</td>
<td>1.37 (0.75-2.52) 0.31</td>
</tr>
<tr>
<td>55-64</td>
<td>58</td>
<td>1.95 (1.10-3.45) 0.02</td>
</tr>
<tr>
<td>≥65</td>
<td>37</td>
<td>2.19 (1.16-4.14) 0.02</td>
</tr>
<tr>
<td>Disease setting</td>
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<td></td>
</tr>
<tr>
<td>Newly-diagnosed</td>
<td>43</td>
<td>0.45 (0.29-0.70) &lt;0.001</td>
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<tr>
<td>KPS at aPD-(L)1 baseline</td>
<td></td>
<td></td>
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<tr>
<td>≤70</td>
<td>29</td>
<td>0.89 (0.52-1.53) 0.68</td>
</tr>
<tr>
<td>&gt;70</td>
<td>55</td>
<td>Referent</td>
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<tr>
<td>MGMT promoter status</td>
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<tr>
<td>Unmethylated</td>
<td>93</td>
<td>0.48 (0.32-0.72) &lt;0.001</td>
</tr>
<tr>
<td>Methylated</td>
<td>56</td>
<td>Referent</td>
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<tr>
<td>Partially methylated</td>
<td>14</td>
<td>1.54 (0.80-2.95) 0.19</td>
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<td>Tumor volume at aPD-(L)1 baseline</td>
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<tr>
<td>Lowest tertile</td>
<td>40</td>
<td>0.71 (0.43-1.18) 0.18</td>
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<tr>
<td>Middle tertile</td>
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<td>Referent</td>
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<tr>
<td>Highest tertile</td>
<td>45</td>
<td>1.30 (0.81-2.09) 0.28</td>
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<td>GTR prior to aPD-(L)1</td>
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<tr>
<td>No</td>
<td>88</td>
<td>Referent</td>
</tr>
<tr>
<td>Yes</td>
<td>75</td>
<td>0.82 (0.57-1.38) 0.29</td>
</tr>
</tbody>
</table>
both adaptive and innate immune responses. As a clinical correlate, a retrospective analysis of 181 consecutive IDH-wildtype glioblastoma patients treated with PD-(L)1 blockade revealed poorer survival among those on baseline dexamethasone. Upon multivariable adjustment by relevant prognostic factors, baseline dexamethasone administration was the strongest predictor of poor survival, regardless of dose (referent no dexamethasone; <2 mg HR 2.16, 95%CI: 1.30–3.68, p=0.003; ≥2 mg HR 1.97, 95%CI: 1.23–3.16, p=0.005; table 1 and figure 5).

Conclusions We demonstrate that concurrent dexamethasone administration, even at a low dose, limits the therapeutic benefit of anti-PD-1 therapy both in mouse glioblastoma models and in a retrospective cohort of 181 IDH-wildtype glioblastoma patients. Mechanistically, dexamethasone decreased intratumoral T cells and systemic levels of T cells, natural killer cells, and myeloid cells, while qualitatively impairing lymphocyte function. The mechanism of T cell depletion included induction of apoptosis. These findings indicate that dexamethasone hinders both adaptive and innate immune responses, intratumorally and systemically, and that its administration should be carefully assessed among glioblastoma patients undergoing second-generation immunotherapy clinical trials. Our findings also have ramifications for brain metastasis patients where immune checkpoint inhibitors are part of standard-of-care management.

Acknowledgements We thank Min Wu for assistance in generating CT-2A luciferase-transduced cells, and Drs. Geoffrey Young, Lei Qin, Xin Chen, and Jing Li for assistance in evaluation of patients' radiographic imaging.

Ethics Approval Approved under DFCI Institutional Review Board protocol 10-417.

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REGULATION OF TIM-3 BY PHOSPHATIDYLSERINE

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Background Immune checkpoint blockade has proven effective in targeting exhausted T-cells to reactivate the immune system against cancer. However, the majority of patients fail to respond to currently available therapies, which primarily target PD-1. Thus, a key challenge for checkpoint blockade therapy is to identify and understand new therapeutic targets. Another immune checkpoint receptor is TIM-3, which is to identify and understand new therapeutic targets. Another chimeric receptor, TIM-3 mutants, changes in receptor expression, and a functional TIM-3 antibody, we show that preventing the interaction between TIM-3 and PS blocks TIM-3 activity. These data suggest that blocking the PS-TIM-3 interaction is a key mechanism for functional antibodies targeting TIM-3. Ultimately, this work supports the development and use of clinical antibodies that block the interaction of TIM-3 with PS and provides new mechanistic insight into how TIM-3 modulates TCR signaling.

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REFERENCES

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TARGETING BTN2A1 MODULATES ANTI-TUMOR ACTIVITY OF VG9VD2 T CELLS

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Background Vg9Vd2 T constitute the predominant subset among gd T cells in peripheral blood. Their infiltration into malignant tissues is associated with a favorable prognosis. Their anti-tumor activity is triggered by intracellular accumulation of organic phosphoantigens (pAgs) due to tumorigenesis. Recently, BTN2A1 was shown to bind to the Vg9TcR chain allowing immune synapse between cancer and Vg9Vd2T cells, thus initiating the anti-tumoral response. In this study, we generated monoclonal antibodies against BTN2A1 and evaluated their ability to modulate γδT cell cytotoxicity.

Methods Anti-BTN2A1 mAbs were generated by mouse immunization. Their effect on Vg9Vd2 T cell degranulation, secretion of IFNγ/TNF-α, and target cell killing as depicted by caspase 3/7 cleavage, were tested in co-cultures with Daudi, HL-60 cell lines and primary acute myelocytic leukemia (AML) blasts with or without zolodronate or the anti-BTN3A signaling, suggesting a threshold of receptor expression needed to modulate T-cell signaling, similar to what has recently been reported for PD-1.1 However, chimeric receptors that maintained the TIM-3 cytoplasmic tail but were unable to bind PS failed to enhance T-cell signaling like the full-length TIM-3 receptor. Cells expressing mutant TIM-3, which displayed reduced PS binding as quantified by SPR, also displayed reduced T-cell signaling compared to cells expressing wild-type TIM-3. Importantly, treatment of TIM-3-expressing cells with a functional TIM-3 antibody that blocks PS binding also reduced T-cell signaling compared with untreated TIM-3-expressing cells.

Conclusions Our results support a role for PS as a ligand capable of modulating TIM-3 activity. Using chimeric receptors, TIM-3 mutants, changes in receptor expression, and a functional TIM-3 antibody, we show that preventing the interaction between TIM-3 and PS blocks TIM-3 activity. These data suggest that blocking the PS-TIM-3 interaction is a key mechanism for functional antibodies targeting TIM-3. Ultimately, this work supports the development and use of clinical antibodies that block the interaction of TIM-3 with PS and provides new mechanistic insight into how TIM-3 modulates TCR signaling.
mAb 20.1. These readouts were measured by flow cytometry. Endometrial cancer spheroids were used to assess the ability of the anti-BTN2A1 antagonistic mAb to inhibit Vg9Vd2 T cell killing of cancers cells.

**Results** We generated 7 anti-BTN2A1 mAbs and tested their effect on Vg9Vd2 T cell degranulation against Daudi cells with or without zoledronate. Six out of 7 anti-BTN2A1 mAbs significantly inhibited basal Vg9Vd2 T cell degranulation against Daudi up to 17-fold, and 5 of them were able to inhibit Vg9Vd2 T cell degranulation against Daudi in presence of zoledronate. Consistently, anti-BTN2A1 mAbs abrogated zoledronate and anti-BTN3A 20.1-induced apoptosis with different efficiencies. The level of apoptosis inhibition after zoledronate and 20.1 treatment were correlated. Anti-BTN2A1 7.48 mAb was the clone with the highest inhibitory potential. Increasing concentrations of 7.48 abrogated not only Vg9Vd2 T cell degranulation (IC50 = 0.033 ± 0.0003 μg/mL) but also TNFα (IC50 = 0.03 ± 0.06 μg/mL) and IFNγ (IC50 = 0.015 ± 0.004 μg/mL) secretion against Daudi cells in presence of pAg. The ability of anti-BTN2A1 antibodies to inhibit Vg9Vd2 induced tumor cell apoptosis was also shown in 3D endometrial cancer spheroids. In co-cultures of Vg9Vd2 T cells with primary AML blasts, the anti-BTN2A1 7.48 inhibited Vg9Vd2 T cell degranulation as well as TNFα, IFNγ production and killing of AML blasts.

**Conclusions** Antagonist antibodies to BTN2A1 highlighted its critical role in Vg9Vd2 anti-tumor responses. BTN2A1 is involved in Vg9Vd2 T cell anti-tumoral activity and can constitute an interesting therapeutic target for gdT cell response immunomodulation in cancer or immune diseases treatment.

**Ethics Approval** The research was approved by the relevant institutional review boards (ethic committee and ANSM, HEMATO-BIO IPC 2013-015, Ref ANSM 131368B-11, Sponsor Institut Paoli Calmettes N° ID RCB 2013-A01437-38).

**Consent** Informed consent was obtained from all donors in accordance with the 121 Declaration of Helsinki.

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**Abstracts**

212 **CLEC-1 IS A NOVEL MYELOID IMMUNE CHECKPOINT FOR CANCER IMMUNOTHERAPY LIMITING TUMOR CELLS PHAGOCYTOSIS AND SYNERGIZING WITH TUMOR-TARGETED ANTIBODIES**

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**Background** Myeloid cells represent one of the most abundant immune cell types in solid tumors that impede myeloid phagocytosis by triggering ‘don’t eat me’ and ‘don’t find me’ signals. Recent literature demonstrates that C-type lectin receptors (CLRs) normally constrain immune cell-mediated tissue damage by suppressing myeloid cell activation and then promote tumor immune evasion. We previously identified the orphan (CLRs) CLEC-1 as over-expressed in situation of established immune tolerance and reported that CLEC-1 expression by dendritic cells (DCs) and macrophages is enhanced by TGFβ and tempers downstream T cells responses. Furthermore, we reported that CLEC-1 is highly expressed by myeloid cells purified from human tumor micro-environment significantly more expressed by suppressive macrophages.

**Methods** As DCs and macrophages are professional phagocytes of dying/dead cell, we evaluated whether CLEC-1 could be a receptor of damaged cells in the phagocytosis.

**Results** We found that CLEC-1 fusion protein, binds specifically to late apoptotic and secondary necrotic healthy or tumor cells induced by chemotherapy, radiation (UV, X-ray) or culture stress conditions. Importantly, we observed in vivo that CLEC-1 deficient mice, but not wild-type, eradicate MC38 colorectal tumors in combination with cytotoxic and immunogenic chemotherapy (eg. Cyclophosphamide). We then generated, screened and identified different anti-human Clec-1 agonist monoclonal antibodies (mAbs) with the capacity to block the CLEC-1/CLEC-1L interaction. We discovered that various agonist CLEC-1 mAbs, but not non-agonist CLEC-1 control mAbs, increase the phagocytosis of CLEC-1L-positive human tumor cells by human CLEC-1 expressing TGFβ-polarized DCs or macrophages. Indeed, TGFβ-polarized DCs phagocytosed more efficiently Rituximab (anti-CD20 mAb)-opsonized Burkitt lymphoma cells (Raji) as well as bare NSCLC cells (A549) when CLEC-1 is antagonized by antibodies. Furthermore, macrophages more productively engulfed Rituximab-opsonized Raji cells as well in the context of CLEC-1 blockade (2–3 fold increase). Moreover, Cetuximab opsonized colon carcinoma cells (DLD-1; EGFR+) and Trastuzumab opsonized mammary carcinoma cells (SK-BR-3; Her2+) were likewise more phagocytosed by CLEC-1 blocked macrophages.

**Conclusions** Altogether, these data indicate illustrate that CLEC-1 broadly inhibits tumor-cell phagocytosis and synergized with tumor-targeted cytotoxic monoclonal antibodies in both solid and hematological tumors.

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Next, we analyzed signaling molecules activated in CD11b cells pulsed with PD-L1 \(\pm\) CD200AR-L, followed up with in vitro and in vivo effects of CD200AR-L on the expression of PD-1/PD-L1 and CTLA-4. Finally, we analyzed the ability of the CD200AR-L to surmount the suppressive effects of PD-L1.

**Results** Our studies demonstrate that the inhibitory CD200R1 and PD-1 mediate immune checkpoint signaling activities through SHIP1 protein. Moreover, CD200AR-L overpowers the suppressive effects of CD200 and PD-L1, which are both shed by tumors, by downregulating the inhibitory CD200R1 and PD-1 on both antigen-presenting cells (APC) and T-cells (figure 1). In addition, CD200AR-L downregulates PD-1 on APCs and inhibits the upregulation of PD-L1 and CTLA4.

**Conclusions** These studies led to the discovery that this novel peptide modulates the CD200, PD-1/PD-L1 and CTLA-4 pathways, providing the basis for the translatable development of a novel CD200 peptide inhibitor for clinical use against multiple tumors, including gliomas. These studies led to the FDA approval for the first in human peptide checkpoint inhibitor to initiate a phase I single center, open-label, dose-escalation clinical trial in adult patients with recurrent glioblastoma, to be followed by a clinical trial for children with recurrent malignant brain tumors.

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**THE EFFECT OF ANTI-PD-1 THERAPY ON MEDIAN OVERALL SURVIVAL AND PROGRESSION FREE SURVIVAL IN GIOBLASTOMA MULTIFORME PATIENTS WITH CERTAIN TUMOR MARKERS**

Awais Paracha*, 1 Jian Campian, 1 Saint Louis University Medical School, Saint Louis, MO, USA; 2 Washington University, Saint Louis, MO, USA

**Background** Almost 1 in 6 malignant brain cancers are Glioblastoma Multiforme, relative to most other brain cancers it is the most aggressive and prevalent by the numbers. 1 Even with the best treatment options median Overall Survival(OS) remains morbid at 14.6 months and Progression Free Survival (PFS) remains 6.9 months. 2 Telomerase Reverse Transcriptase promoter mutations, 3 Isocitrate Dehydrogenase(IDH) mutations, 4 and Tumor Mutation Burden(TMB) 5 are three prominent tumor markers that are known to be associated with better PFS and OS; markers like these in the presence of new therapies maybe prove crucial to the development of novel therapies. Immunotherapy has been dubbed a ‘game changer’ in certain hematological and solid malignancies. Specifically, PD1 is a glycoprotein that is a strong negative regulator of the immune system, by blocking this glycoprotein Anti-PD-1 agents harness a strong response by the immune system to fight a malignancy. 6 In conjunction with these new found tumor markers, Anti-PD-1 agents maybe the solution that could dramatically improve OS and PFS in these patients.

**Methods** The goal of this study was to retrospectively analyze patients' charts who had received Anti-PD-1 therapy and had TERT promoter mutations, IDH mutations, different TMBs, and other markers and to compare their OS and PFS outcomes with conventional therapies and their response to immunotherapy.

**Results** Upon analyzing the data the presence of a TERT promoter 124C>T mutation, IDH wildtype, and lower TMB gave much better OS and PFS after treatment in patients on Anti-PD1 therapy.

**Conclusions** Although this was a small study, these results certainly can be used to examine larger subsets of patients with these markers receiving immunotherapy because they had definitely better outcomes as compared to status quo treatment options.

**Ethics Approval** The study was approved by Washington University Ethics Board, approval number 201111001.

**REFERENCES**


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**215**

AO-176, A HIGHLY DIFFERENTIATED CLINICAL STAGE ANTI-CD47 ANTIBODY, PREFERENTIALLY BINDS TUMOR VERSUS NORMAL CELL CD47 WHEN COMPLEXED TO \(\beta 1\) INTEGRIN

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**Background** Overexpression of CD47 by tumor cells exploits an immune checkpoint preventing tumor recognition and destruction by innate immune cells. Binding of tumor CD47 to SIRP\(\alpha\) on macrophages and dendritic cells triggers a ‘don’t eat me’ signal that inhibits phagocytosis and allows escape from innate immune surveillance. Blockade of the CD47/ SIRP\(\alpha\) axis, however, enables immune recognition and phagocytic clearance of tumor cells. We have developed a clinical stage CD47 targeting antibody AO-176 that is highly differentiated among agents in this class. AO-176 not only blocks the
CD47/SIRPα interaction and induces phagocytosis of tumor cells, but it also has a direct killing mechanism (via PCDIII) and induction of immunogenic cell death, leveraged by preferential binding to tumor versus normal cell CD47.

Methods CD47 and β1 integrin expression and localization were evaluated using a combination of flow cytometry, western blotting, confocal microscopy and immunohistochemistry.

Results Previously, we described that the preferential binding of AO-176 to tumor versus normal cells was due to its interaction with CD47 molecules that were pre-complexed to β1 integrin. This finding was particularly important and suggestive of why AO-176 does not bind red blood cells since they do not express β1 integrin. We have extended these findings to show that β1 integrin as well as CD47 are also expressed at lower levels in normal versus tumor cells, and that solid and hematologic tumor cells overexpress both CD47 and β1 integrin which correlate with poor prognosis in cancer. In addition, we show that AO-176 is able to bind and occupy CD47/β1 integrin complexes to a greater extent at acidic versus physiologic pH such as would be found in tumor microenvironments, an observation that also contributes to the enhanced targeting of AO-176 to tumor cells. Taken together, these findings add further insight into the preferential binding of AO-176 to tumor versus normal cells.

Conclusions The context dependent binding of AO-176 to CD47, when complexed to β1 integrin, is unique among CD47 axis targeting agents and together with its direct killing mechanism of action offers a potentially better safety profile and opportunity for a therapeutic advantage. AO-176 is currently being evaluated in Phase 1 clinical trials for the treatment of patients with select solid tumors (NCT03834948) and multiple myeloma (NCT04445701). Trial Registration NCT03834948, NCT04445701.

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### Abstracts

#### 216 ANTI-TUMOR ACTIVITY OF IOSH2 BY BLOCKING LILRB2 RECEPTOR SIGNALLING

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**Background** The human leukocyte immunoglobulin-like receptor family B (LILRB) acts as check point blockade of the innate immune system by inhibiting leukocyte activation through SHP phosphatase recruitment. Some of the physiological ligands include classical HLA class I molecules, including beta-2-microglobulin (B2M) free open conformers (OC). Natural HLA-OC expression is known from autoimmune disease leading to immune activation by pleiotropic effects since they bind to LILRB and KIR family members reducing Treg and MDSC numbers and increased effector T-cell and NK-cell activation, respectively. We have generated an IgG4-HLA-57 open conformer (OC) molecule (iosH2) with high affinity for LILRB molecules and demonstrate its anti-cancer activity in vitro and in vivo.

**Methods** iosH2 was produced by transient gene expression in CHO cells and purified by standard chromatography. Affinity of iosH2 binding was quantified by ELISA and SPR analysis. HLA-G mediated signaling and competition was assessed using functional cell lines. Effect of iosH2 on activation of SHP1/2 was assessed using Western Blot. Functional assays including in vitro polarization and phagocytosis potential of primary macrophages was assessed by flow cytometry in the presence of iosH2 or isotype control. Effect of iosH2 on T cell activation was evaluated in co-cultures of cancer and T cells. Mouse models were used to assess in vivo activity.

**Results** iosH2 binds to LILRB2 with high affinity and blocks the activation of HLA-G. In addition, iosH2 blocks receptor-mediated activation of SHP1/2. iosH2 promotes a shift from M2 to M1 macrophages with enhanced tumor cell phagocytosis in vitro. iosH2 enhances activation and killing potential of T cells in cancer cells and T cells co-culture assay. iosH2 exerts therapeutic efficacy in mouse transgenic (melanoma) and different syngeneic tumor models (e.g. pancreatic, colon and breast cancer) as monotherapy. Moreover, it acts synergistically in vivo with PD1 blocking antibodies achieving long-term tumor control. Ex vivo tumor sample analysis demonstrates a significant reduction of MDSC and Tregs and a shift towards an activated inflammatory M1 macrophage phenotype. Loss of MDSC functionality was paralleled by enhanced CD8+ T cell expansion and activity.

**Conclusions** iosH2 binds to LILRB2 with high affinity, restores immune cell function in vitro and demonstrates anti-tumor activity in different in vivo mouse models. In addition, it acts synergistically in vivo with PD1. iosH2 is a first-in-class OC therapeutic with robust anti-tumor activity by promoting key components of the innate immune system. Clinical development is under way and phase I trial in preparation.

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#### 217 EVALUATING BIOMARKERS OF JTX-8064 (ANTI-LILRB2/ILT4 MONOCLONAL ANTIBODY) IN AN EX VIVO HUMAN TUMOR HISTOCULTURE SYSTEM TO INFORM CLINICAL DEVELOPMENT

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**Background** Leukocyte immunoglobulin-like receptor B2 (LILRB2; ILT4) is an immunoinhibitory protein expressed on the surface of myeloid cells that has been increasingly recognized as a therapeutic target of interest in immuno-oncology (IO). Upon binding its ligands, MHC I molecules (e.g. HLA-G/HLA-A), LILRB2 inhibits myeloid cell activation and promotes an M2-like (anti-inflammatory) state. LILRB2 was the first target prioritized from a macrophage discovery effort leading to the development of JTX-8064, a humanized monoclonal antibody that specifically binds to and antagonizes LILRB2. JTX-8064 has been shown to induce an M1-like (pro-inflammatory; anti-tumor) functional state in macrophages. Rodents do not express LILRB proteins limiting their usefulness as a model for preclinical study of JTX-8064. To overcome this limitation, we conducted an ex vivo human tumor histoculture study to assess the pharmacodynamic effects of LILRB2 antagonism. Protein and/or gene expression analysis of matched tumor samples enabled the discovery of predictive biomarkers associated with the induction of specific pharmacodynamic signatures in ex vivo cultured human tumors in response to JTX-8064. Finally, tumor types were identified that had a high prevalence of these predictive biomarkers suggesting they may be priority indications for JTX-8064 therapy.

**Methods** More than 100 fresh treatment-naïve human tumor samples obtained post-surgery from kidney, lung, and head and neck cancer were treated with JTX-8064 or isotype
A PRECLINICAL STUDY OF IMC-002, A FULLY HUMAN THERAPEUTIC ANTIBODY SAFELY TARGETING CD47 IN CANCER

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Background Immunotherapy with immune checkpoint inhibitors such as PD-(L)1 and CTLA-4 blockier has become an important part of cancer treatment. For the cancers resistant to these drugs, however, many other therapeutic targets are being tested to modulate the tumor microenvironment (TME) toward anti-cancer immunity. Due to the functional flexibility, macrophages play an essential role in orchestrating tissue immunity including TME. CD47 is one of the key targets that modulate macrophages, which is often overexpressed on cancer cells. When it binds to its receptor, SIRPα, it gives a ‘don’t-eat-me’ signal and inhibits phagocytosis of cancer cells by macrophages. IMC-002 is a fully human IgG4 monoclonal antibody targeting human CD47, which has been engineered to minimize hematological toxicities such as anemia which is a class effect of the CD47-targeting antibodies. The first-in-human (FIH) study of IMC-002 is ongoing in the US sites. The purpose of the study is to assess the safety and tolerability of IMC-002 and determine the recommended Phase 2 dose (RP2D) of IMC-002 in subjects with metastatic or locally advanced solid tumors and relapsed or refractory lymphomas.

Ethics Approval All experimental procedures were performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the contract research organizations.

REFERENCES

LONG-TERM CLINICAL OUTCOMES ASSOCIATED WITH SEQUENTIAL TREATMENT OF BRAF MUTANT ADVANCED MELANOMA PATIENTS

Ahmad Tarhini*, David McDermott, Apoorva Ambavane, Agnes Benedict, Cho-Han Lee, Corey Ritches, Brian Stivala, Meredith Reegan, Michael Atkins. Moffitt Comprehensive Cancer Center and Research Institute, Tampa, FL, USA; Harvard Medical School; Beth Israel Deaconess Medical Center, Boston, MA, USA; Evidera, London, MD, UK; Bristol Myers Squibb, Princeton, NJ, USA; Harvard Medical School and Dana-Farber Cancer Institute, Boston, MA, USA; Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, USA

Background Patients with BRAF mutant advanced melanoma can be treated sequentially with immunotherapies (IO) and BRAF+MEK inhibitors. We evaluated the clinical outcomes associated with various treatment sequences for BRAF mutant advanced melanoma based on the 5-year follow-up data from clinical trials.

Methods In the absence of head-to-head trial data, a matching-adjusted indirect comparison (MAIC) was conducted for IO vs. BRAF+MEK inhibitors, using the longest follow-up available in the published literature. Multivariate risk equations

...
were developed to predict time-to-event outcomes based on patient-level data from pooled CheckMate-067 &-069 trials. Risk equations were inserted into a discrete event simulation to estimate the average life-years (LYs) and quality-adjusted life-years (QALYs) that can be gained with various treatment sequences or trial conduct and the absence of data on 2L combinations. Limitations of the study are the reliance on published information for BRAF+MEK, which could lead to biased results due to unmeasured differences in the patient populations or trial conduct and the absence of data on 2L combination IO. Anti-PD-1+anti-CTLA-4 as second line option has not been included because of a lack of clinical evidence. Findings from this analysis will require validation in ongoing prospective randomized clinical trials.

Conclusions In this sequencing model with 5-year data from randomized clinical trials, initiating 1L treatment with IO provided prolonged survival compared to initiating 1L treatment with BRAF+MEK. Time spent in TFI represents a significant proportion of survival time for patients on IO initiating sequences. Limitations of the study are the reliance on published information for BRAF+MEK, which could lead to biases due to unmeasured differences in the patient populations or trial conduct and the absence of data on 2L combination IO. Anti-PD-1+anti-CTLA-4 as second line option has not been included because of a lack of clinical evidence. Findings from this analysis will require validation in ongoing prospective randomized clinical trials.

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220 REAL-WORLD OUTCOMES OF PATIENTS WITH RESECTED STAGE IIIA MELANOMA TREATED WITH ADJUVANT NIVOLUMAB

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Background Nivolumab is approved in the US and EU for the adjuvant treatment of resected stage III-IV melanoma based on results from the CheckMate-238 clinical trial. However, the trial did not enroll any patients with Stage IIIA disease per the American Joint Committee on Cancer (AJCC) 7th edition criteria and included a limited number of patients with stage IIIA disease per the AJCC 8th edition. Recognizing the need for real-world data to assess outcomes of patients with resected stage IIIA melanoma treated with adjuvant nivolumab, a non-interventional study was conducted to investigate treatment patterns and outcomes among patients receiving adjuvant nivolumab within the US community practice setting.

Methods A retrospective analysis of the US Oncology Network’s iKnowMed medical data was conducted to examine patients with resected stage IIIA melanoma treated with adjuvant nivolumab between 01-Jan-2018 and 31-Dec-2019 with a follow-up period through 31-Mar-2020. Patients were followed for up to 27 months after their sentinel lymph node biopsy. Baseline demographic/clinical characteristics and treatment patterns were examined descriptively. Duration of treatment (DOT) and overall survival were analyzed using the Kaplan-Meier method.

Results A total of 58 patients with stage IIIA melanoma treated with adjuvant nivolumab were identified. Median age was 57.8 years (range 21.5–93.5), 62.1% were male, and 75.9% were Caucasian. Among patients with a documented Eastern Cooperative Oncology Group (ECOG) performance status (51.7%), all had an ECOG score of 0 or 1. Median follow-up time was 12.6 months (range 0.3–25.1). Median DOT was 10.6 months (range 6.8–12.0). Overall survival rates at 12 and 24 months were 97.7% (95% CI 84.6–99.7) and 92.2% (95% CI 69.6–98.2), respectively.

Conclusions This real-world analysis of patients with stage IIIA melanoma treated with adjuvant nivolumab showed that a large proportion of patients were alive at the end of the study period, suggesting these patients have a favorable prognosis. Further investigation and follow-up is warranted to assess clinically relevant outcomes among patients with resected stage IIIA melanoma.

Ethics Approval The study was approved by US Oncology Inc’s Institutional Review Board, approval number 20-020E-2020-0224-01.

REFERENCES

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221 POOR PERFORMANCE STATUS NEGATIVELY AFFECTS SURVIVAL BENEFIT OF IMMUNOTHERAPY IN NON-SMALL CELL LUNG CANCER

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Background Immunotherapy has shown survival benefit as both frontline and subsequent therapy in multiple cancers. However, its efficacy in patients with poor performance status is unknown since they are excluded from the clinical trials. We conducted a retrospective study to investigate the effect of poor performance status (PS) on survival in patients with non-small cell lung cancer (NSCLC) who received immunotherapy as a subsequent line of treatment.
Methods We reviewed the medical records of 341 patients with NSCLC receiving immunotherapy as between July 2013 and June 2018. Progression-free survival and overall survival was calculated using Kaplan-Meier curve.

Results The average age of patients was 66 years (range: 39–90 years), with a male predominance (57%). Majority of the patients were Caucasian (87%), followed by African-American (12%), and Asian (1%). Most of the patients were former smoker (72%), followed by current smoker (19%) and never smoker (7%). Adenocarcinoma and squamous cell carcinoma was diagnosed in 206 (60%) patients and 112 (33%) patients, respectively. The ECOG-PS was 0, 1, 2 and 3 in 46 (13%), 175 (51%), 86 (25%) and 34 (10%), respectively. Four different immunotherapies were used, namely atezolizumab in 10 (3%), durvalumab in 34 (10%), nivolumab in 152 (44%) and pembrolizumab in 144 (42%) patients. Average number of cycles of atezolizumab received by the patient was 6 (range 2–22 cycles), durvalumab 15 (range 1–29 cycles), nivolumab 11 (range 1–112 cycles), and pembrolizumab 12 (range 1–52 cycles). Patients were grouped in good performance status (ECOG 0–1) and poor performance status (ECOG ≥2). The median progression free survival (PFS) was 7 months (95% CI 6.3–8.2) in patients with good PS and 3 months (95% CI 1.8–4.6) in patients with poor performance status (p<0.001). The median overall survival (OS) for patients with good performance status was 30 months (95% CI 16.6–42.3) and 4 months (95% CI 3.2–8.1) in patients with poor PS (figure 1). Adverse effects were recorded in a total of 83 (24%) patients, 18 (5%) patients had ECOG-PS 0, 50 (14%) patients had ECOG-PS 1, 18 (4%) patients had ECOG-PS 2 and 3 (1%) patients had ECOG-PS of 3. Most common adverse effects were pneumonitis (28%), diarrhea (8%) and hypothyroidism (8%).

Conclusions Our data suggests that while the patients with poor PS tolerated the immunotherapy. However, poor PS was associated with significantly lower PFS and OS. Further studies are required to evaluate the effect of PS on survival in frontline immunotherapy.

Acknowledgements We thank Dr. Saqib Abbasi for helpful discussions.

REFERENCES

N/A

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222 INCREASED PD-L1 TUMOR EXPRESSION CORRELATES WITH HIGH RATE OF RESPONSE TO PD-1 INHIBITORS IN PATIENTS WITH UNRESECTABLE, RECURRENT, AND METASTATIC CUTANEOUS SQUAMOUS CELL CARCINOMA

Nate Bowers*, Kimberly Burcher, Jess Savas, Phillip Williford, Laura Doerfler, Hafiz Shabbir Patwa, Joshua Waltonen, Christopher Sullivan, James Brown, Mercedes Porosnicu. Wake Forest, Winston Salem, NC, USA

Background PD-1 inhibitors were approved for locally advanced and metastatic cutaneous squamous cell carcinoma (CSCC) in 2019. The identification of tumor characteristics that predict potential responders to immune checkpoint inhibitors (ICI) is an area of ongoing research. Here we present a series of consecutive patients with locally advanced, recurrent, or metastatic CSCC treated with PD-1 inhibitors and analyze tumor and blood genomics as well as PD-L1 expression with the aim of correlating with treatment response.

Methods We analyzed cases of CSCC treated with single agent PD-1 inhibitors in the last 2 years at Wake Forest. Demographic and outcome data were collected. Tumor tissue, whenever available, was tested for PD-L1, TMB, MSI, and genetic mutations. Blood was tested for circulating tumor at the beginning of treatment and at the time of maximum response.

Results Fourteen patients with CSCC treated with PD-1 ICI were included in this study. Six had locally advanced disease, seven had recurrent locally advanced disease, and one had metastatic disease. Four patients received treatment for >12 months and all had complete response (CR). Five patients had 6–12 months of treatment and all had near CR (pending imaging studies and ctDNA to confirm). Three patients had <6 months of treatment and had partial response (PR). Two of the patients had progressive disease, although one with possible pseudoprogression based on review of post-treatment surgical pathology specimen. Treatment was well tolerated with no immune related side-effects except one case of grade I hypothyroidism. Eleven patients had sufficient tumor tissue for genomic and PD-L1 testing. Initial blood genomic testing was performed in 12 of 13 patients and in follow up in patients who achieved maximum response. Patients with CR had PD-L1 of at least 30%. The additional tested patients had PD-L1 above 10%. The most frequently mutated gene was TP53 present in tumor in all tested patients and in blood in 6 patients, followed by NOTCH1/2 detected in the tumor of 10 of 11 patients tested. TMB was intermediate/high in tested patients except in the only patient who presented clear tumor progression.

Conclusions Treatment of locally advanced, recurrent, and metastatic CSCC with ICI led to a dramatic change in the management and prognosis of CSCC. Our series of patients with CSCC had a higher than reported rate of response. This corresponded with high TP53 alterations, NOTCH 1/2 alterations, high/intermediate TMB, and high level of expression of PD-L1. PD-L1 rates were higher than previously published.1 2
223 RACIAL DIFFERENCES IN OUTCOMES FOR METASTATIC RENAL CELL CARCINOMA (MRCC) PATIENTS MANAGED ON IMMUNE-CHECKPOINT INHIBITOR (ICI) THERAPY

T Anders Olsen*, 1Dylan Martini, 2Subir Goyal, 3Lauren Yantorni, 1Greta Russler, 1Sarah Caulfield, 1Jamie Goldman, 1Bassel Nazha, 1Wayne Harris, 1Viraj Master, 1Omer Kucuk, 1Bradley Carthon, 1Mehmet Bilen. 1Emory University School of Medicine, Atlanta, GA, USA; 2Emory University, Atlanta, GA, USA; 3Winship Cancer Institute of Emory University, Atlanta, GA, USA

Background Immune checkpoint inhibitors (ICIs) have increased in prevalence for the treatment of metastatic clear-cell renal cell carcinoma (mccRCC) in recent years given their efficacy and favorable toxicity profile. However, there has been insufficient investigation in the literature of how clinical outcomes differ on the basis of race. In this paper, we investigated differences in clinical outcomes between African American (AA) and Caucasian mRCC patients treated with ICI therapy.

Methods We performed a retrospective study of 198 patients with mRCC who received ICI at the Emory Winship Cancer Institute from 2015–2020. Clinical outcomes were measured by overall survival (OS), progression-free survival (PFS), and clinical benefit (CB). OS and PFS were calculated from ICI-initiation to date of death and radiographic or clinical progression, respectively. CB was defined as a best radiographic response of complete response, partial response, or stable disease maintained for at least 6 months per response evaluation criteria in solid tumors version 1.1. The association of self-identified race with OS and PFS was generally modeled by Cox proportional hazards model. Univariable and multivariable logistic regression models were used for binary outcomes of CB. The univariate association of immune-related adverse events (irAEs) and non-clear-cell RCC (ncRCC) with race was assessed using Chi-square test.

Results Our cohort was made up of 38 AA (19%) and 160 Caucasian (81%) patients. Most of the patients were diagnosed with ccRCC (78%) and more than half received PD-1 monotherapy (57%). Most patients were international mRCC database consortium (IMDC) intermediate (57%) or poor-risk (25%) groups. AA patients displayed significantly shorter PFS (HR=1.52, 95% CI: 1.01–2.3, p=0.045) and trended towards decreased CB (OR=0.51, 95% CI: 0.22–1.17, p=0.111) in MVA (table 1). There was no difference in OS (HR=1.09, 95% CI: 0.61–1.95, p=0.778) between the two racial groups in MVA (table 1). On Kaplan-Meier method, AA patients had shorter median OS (17 vs 25 months, p=0.3676) and median PFS (3.1 vs 4.4 months, p=0.0676) relative to Caucasian patients (figure 1). Additionally, AA patients more commonly had ncRCC compared to Caucasian patients (41.7% vs 17.5% nccRCC, p=0.002). AA patients also trended towards a lower incidence of irAEs compared to Caucasian patients in UVA (23.7% vs 35.8%, p=0.153).

Conclusions In this group of mRCC patients treated with ICI, African American patients had significantly shorter PFS compared to Caucasian patients. These findings suggest race could play a role in the management of late-stage mRCC. Larger, prospective studies are needed to validate these findings.

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Trial Registration Not applicable.

Ethics Approval This retrospective study was approved by the Emory University Institutional Review Board.

Consent Not applicable.

REFERENCES

Not applicable.

224 OUTCOMES OF STAGE IV MELANOMA IN THE ERA OF IMMUNOTHERAPY: A NATIONAL CANCER DATABASE (NCDB) ANALYSIS

Tamara Sussman*, Wei Wei, Pauline Funchain, Brian Gastman. Cleveland Clinic Foundation, Cleveland, OH, USA

Background Immunotherapy (IO) has revolutionized the treatment landscape for metastatic melanoma and is now the mainstay of treatment since the approval of ipilimumab in 2011 and anti-PD-1 therapies (nivolumab and pembrolizumab) in 2015. The majority of data stems from trials that have
specific inclusion criteria, and often exclude important populations. In this analysis, we present the first real-world evidence of outcomes for stage IV patients with cutaneous melanoma receiving IO from 2015–2016 and factors associated with receipt of IO, and compare these outcomes with patients receiving IO (likely interferon and interleukin-2) from 2004–2010, and IO (addition of ipilimumab) from 2011–2014.

Methods NCDB was analyzed to identify stage IV patients with melanoma from 2004–2014. Overall, patients treated with and without IO were propensity matched on age, gender, and stage. Overall survival (OS) analysis was done by Kaplan-Meier and Cox proportional hazard models; log-rank test was used for between-group OS comparisons.

Results A total of 21,696 patients with stage IV melanoma were analyzed from 2004–2016. Patients were categorized into receipt of IO or not during time periods from 2004–2010, 2011–2014, and 2015–2016. Patients treated with and without IO were propensity matched on age, gender, and stage. Overall survival (OS) analysis was done by Kaplan-Meier and Cox proportional hazard models; log-rank test was used for between-group OS comparisons.

Abstract 224 Figure 1  OS in patients with melanoma treated with IO

Among patients who received IO, 2-year OS significantly improved by 2015 (40% [95%CI, 37–42%] for both 2004–2010 and 2011–2014 vs. 48% [95%CI, 44–51%] in 2015; p=0.01) (figure 1). In the overall cohort, younger patients (<60 years), female gender, private insurance, no comorbidities, and treatment at academic/research centers were associated with better OS (p<0.0001 for all). Receipt of radiation therapy and lack of surgery were both associated with worse OS (p<0.0001 for both). Race and area of residence (metro/rural/urban) were not associated with differences in OS (p=0.09 and p=0.07, respectively). In 2015–2016, receipt of IO was associated with younger age (<60 years), lack of comorbidities, private insurance, higher median income (= $38,000), residence in metro area, and treatment at academic/research centers (p<0.0001 for all) (table 1).

Conclusions Survival was improved in stage IV patients with melanoma receiving IO, especially in 2015, with the approvals of pembrolizumab and nivolumab. Our findings are consistent with recent trials, like KEYNOTE 006 and CheckMate 067 where 2-year OS for anti-PD-1 therapy was 55% and 60%, respectively.1, 2 Significant socioeconomic factors may impact receipt of IO and survival.

REFERENCES

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Abstract 224 Table 1  Factors associated with receipt of IO for patients diagnosed in 2015

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<th>Factor</th>
<th>No Immunootherapy (N=1003) (%)</th>
<th>Immunotherapy (N=907) (%)</th>
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<td>Age</td>
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<td>270 (31)</td>
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<tr>
<td></td>
<td>≥60 years</td>
<td>1313 (71)</td>
<td>737 (94)</td>
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<tr>
<td>Gender</td>
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<td>577 (35)</td>
<td>315 (35)</td>
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<tr>
<td></td>
<td>Male</td>
<td>1036 (65)</td>
<td>652 (65)</td>
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<td>Ethnicity</td>
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<td>Asian</td>
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<td></td>
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CHECKPOINT BLOCKADE HASTENS A SWITCH FROM AN NKT DOMINANT, TNF-ALPHA-DRIVEN TO A CD4+/CD8+ IFN-GAMMA-DRIVEN IMMUNE RESPONSE WITHIN MC-38 TUMOR-INFLTRATING LYMPHOCYTES

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Background It is incompletely understood which populations of tumor-infiltrating lymphocytes (TIL) respond to checkpoint blockade (CB) and when. Recent studies in murine MC-38 colon carcinoma demonstrate CD4+ T cells are among the most prominent responders,1 but these studies were undertaken late in tumor growth, weeks after CB blockade was initiated. Here, we profile how the landscape of CB-responding TIL change between early and late MC-38 tumor growth, and uncover a novel switch that occurs between natural killer T (NKT) and conventional CD4/CD8 T cell responses.

Methods We treated C57BL/6 mice bearing subcutaneous MC-38 tumors with anti-PD-1 and/or anti-CTLA-4 antibodies, and analyzed TIL 11 or 21 days later using a 23-paramter flow cytometry panel that includes three markers of effector function: TNF-alpha, IFN-gamma, and CD107a. We then investigated paired major populations, including NKT (NKT) and conventional CD4/CD8 T cell responses.

Results We identified 37 TIL populations in MC-38 tumors with anti-PD-1 and/or anti-CTLA-4 antibodies, and analyzed TIL 11 or 21 days later using a 23-paramter flow cytometry panel that includes three markers of effector function: TNF-alpha, IFN-gamma, and CD107a. We then investigated paired major populations, including NKT (NKT) and conventional CD4/CD8 T cell responses.

Results Our analysis identified 37 TIL populations in MC-38 tumors, representing CD4+ or CD8+ T cells, natural killer (NK), and NK cell. The distribution and effector function among TIL shift dramatically between early and late MC-38 growth. At 11 days, the immune response is dominated by...
TNF-alpha-producing NKT, which represent 53.5 ± 3.7% of all TIL. These are accompanied by modest frequencies of CD4+ and CD8+ TIL, producing low levels of IFN-gamma. After 21 days, NKT populations are reduced to 15.2 ± 1.5%, giving way to increased NK, CD4+, and CD8+ TIL, with increased IFN-gamma production. CB hastens this switch, markedly reducing NKT to less than 20% of all TIL, downregulating TNF-alpha production across NKT and CD4+ T cell subpopulations, increasing CD4+ and CD8+ TIL frequencies, and significantly up-regulating IFN-gamma production at 11 days. CD107a expression patterns suggest degranulation is most associated with NK and NKT TIL (figure 1). NKT displayed no CD1d-restricted cytotoxicity against MC-38 ex vivo. However, CD1d overexpression on MC-38 significantly delayed tumor growth in vivo, suggesting early NKT activity may indirectly suppress tumor progression, but by what precise mechanism(s) is currently unknown.

Conclusions Despite evidence of an indirect benefit of early NKT activity, CB hastens a switch from TNF-alpha-driven NKT involvement toward a IFN-gamma-driven CD4+ and CD8+ T cell response in subcutaneous MC-38 tumors. These results corroborate earlier findings that CD4+ TIL are a major CB-responding population, and introduce a NKT/T cell switch that may be a key feature of the CB response in certain tumors.

Ethics Approval Animal experiments in this study were performed according to protocols approved by the University of South Florida’s institutional animal care and use committee (IACUC) committee, number R ISO0005710.

REFERENCE
http://dx.doi.org/10.1136/jitc-2020-SITC2020.0226

227 USING 3D SPHEROID CULTURES TOWARDS PERSONALIZED EX VIVO PROFILING OF IMMUNE CHECKPOINT INHIBITOR EFFICACY IN MELANOMA AND NON-SMALL CELL LUNG CANCER

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Background Immune checkpoint inhibitors (ICIs) have shifted the cancer treatment paradigm. Cancers such as melanoma and non-small cell lung cancer (NSCLC) demonstrate high tumor mutational burden and tumor neoantigen expression which renders them more responsive to checkpoint inhibitor blockade compared to other malignancies. Yet, 40–65% of metastatic melanoma patients do not have an initial response to ICI therapy\(^1\) and in NSCLC, PD-L1 expression, often a prerequisite for ICI treatment, does not definitively associate with ICI clinical response\(^2\). Mechanisms of resistance often result from aberrant interactions between tumor and immune cells. Development of pre-clinical models that mimic the complex interplay between cells within the tumor microenvironment in a patient-specific manner are critical for accurate ex vivo profiling of ICIs. To improve immunotherapy predictive testing, we present a 3D spheroid culture system for testing personalized ICI efficacy.

Methods Cell lines co-cultured with T-cells from healthy donor peripheral blood mononuclear cells were used to optimize assay conditions and confirm ICI binding to target proteins. For ex vivo testing, primary melanoma or NSCLC tumor tissue from treatment naïve patients was dissociated and cultured as 3D spheroids using autologous immune cells to profile ICI target expression and sensitivity to treatment. ICI enhanced T-cell killing of tumor cells was quantified via lactate dehydrogenase release. Changes in IFNy were used as a metric for ICI induced immune cell activation. Ratios and activation status of T-cell subsets was determined using flow cytometry. Fluorescent imaging was used to confirm the mechanism of tumor cell killing.

Results ICI binding to target proteins was measured across six ICIs, and no significant differences in concentration-dependent site occupancy within drug target classes was observed. However, differences in drug induced cytotoxicity across different tumor samples was detected even within the same drug target class. The immune composition of tumor samples that responded to ICIs displayed increased T-cell activation and increased IFNy production. Furthermore, changes in PD-L1 and MHC-class I expression were detected which reflected ICI response. Finally, T-cell-dependent induction of tumor cell
apo-}

cel recovery was confirmed to be the observed mechanism of
cytotoxicity within the 3D spheroid system.

Conclusions This work demonstrates that differences in ICI
induced cytotoxicity can accurately be detected using our ex
vivo 3D spheroid platform. The differences in therapy sensi-
tivity can be related back to cell composition and function to
potentially predict patient-specific drug response. Future corre-
lation to patient clinical outcomes will be necessary for true
clinical applications.

Acknowledgements N/A

Ethics Approval N/A

Trial Registration N/A

Ethics Approval Tissue for this study was procured from com-
mercial vendors who maintain strict ethical compliance, includ-
ing fully de-identified materials and stringent IRB and Ethics
Committee compliance.

Consent N/A

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228 RADIOLeGICAL DYNAMICS AND RESISTANCE TYPES IN
PATIENTS WITH ADVANCED MELANOMA TREATED
WITH ANTI-PD-1 MONOTHERAPY

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Background The SITC Immuneonology Resistance Taskforce recently de-

fined primary and secondary resistance to anti-PD-1 therapy, yet there is little data that compares these two sce-
narios. In particular, detailed radiological dynamics of the di-

fferent resistance types remain undescribed.

Methods We performed independent single-blind reevaluations of available radiological image data on a retrospectively
assembled cohort of advanced melanoma patients (n=254, median follow-up 31.3 months, figure 1) treated with anti-
PD-1 monotherapy initiated between Sept 2009 and Aug 2018 at both Massachusetts General Hospital and Peking Uni-
versity Cancer Hospital. Radiological characteristics and timing at multiple crucial radiological landmarks were analyzed and
correlated with each other and with survival. As per the SITC
Taskforce, primary resistance was defined as a best response of
stable disease (SD) lasting less than six months or disease
progression (PD), secondary as PD following an initial partial
or complete response (PR/CR) or SD lasting 6 months or
greater.

Results The most dramatic tumor reduction occurred within
the first 3 months after anti-PD-1 initiation. A subpopula-
tion of patients who had SD (28.6%, all with tumor shrinkage)
experienced further tumor reduction and upgraded to CR/PR and 11.1% of patients with initial PR
upgraded to CR. No patients without tumor shrinkage at the initial evaluation ultimately responded. Baseline tumor
burden, response depth, timing of maximal response and

PD pattern demonstrated great variation and were signifi-
cantly correlated with each other and with survival. In mul-
tivariate analyses, deeper response depth was independently
associated with a less widespread progression pattern, less
involved organs, smaller target lesion size and slower tumor
growth rate (all P<0.001) at PD, and longer post progression sur-
vival (PPS) (P=0.005). Compared to primary resistance, sec-
dary resistance was correlated with less broad progression
pattern, less tumor burden and slower tumor growth (all
P<0.001). Patients with secondary resistance were more likely to
receive further local/regional therapy (46.5% vs. 30.9%,
P=0.07) rather than systemic therapy (27.9% vs. 56.9%,
P<0.001), yet had a significantly longer PPS (HR 0.503, 95%
CI, 0.288–0.879, P=0.02). Median PPS was not reached (95%
CI, 11.8 months to not reached) for patients with secondary
resistance and was 10.3 months (95% CI, 7.7–16.1) for patients
with primary resistance (figure 2).

Conclusions Radiological dynamics were heterogeneous, yet
significantly correlated with survival. Patterns of progression and
PPS of the SITC Immunotherapy Resistance Taskforce
defined primary and secondary resistance are different. This
distinction may be important for the design of clinical trials
targeting a PD-1 resistant population.

Abstract 228 Figure 1

MGH, Massachusetts General Hospital. PUCH, Peking University Cancer Hospital

Abstract 228 Figure 2

PPS of patients with primary resistance was significantly longer than those developed secondary resistance
(P=0.008), with median PPS of 10.3 months (95% CI, 7.7 to 16.1) and
not reached (95% CI, 11.8 to not reached), respectively

Ethics Approval This study has been conducted in compliance
with local Institutional Review Board policies.
DISCOVERY OF GANGLIOSIDE GM2 ACTIVATOR AS A SINGLE CELL PIK3 GENE EXPRESSION PATTERNS


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229 DISCOVERY OF GANGLIOSIDE GM2 ACTIVATOR AS A NOVEL PROTEOMIC BIOMARKER ASSOCIATED WITH RESPONSE TO TREATMENT IN FIRST-LINE MELANOMA SUBJECTS TREATED WITH PD-1 IMMUNOTHERAPY

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Background: Immune checkpoint inhibitors (ICI) have greatly improved the treatment options for patients with metastatic melanoma. Yet, a large percentage of melanoma patients do not respond to ICIs, there is a need for biomarkers that can predict patients’ clinical benefit thereby identifying the patient population most likely to respond. Here, we apply unbiased discovery proteomics to deeply characterize global tumor proteomes and associate proteins and pathways at baseline with clinical response to anti-PD-1 immunotherapy.

Methods: Unbiased, data-independent acquisition (DIA) mass spectrometry was used to analyze Formalin Fixed Paraffin Embedded (FFPE) tumor tissue from subjects with stage IIIc-IV melanoma which were resected prior to initiation of first-line anti-PD-1 therapy. The selected samples represent two distinct clinical subgroups; those who received clinical benefit (CR or PR by RECIST criteria or OS >1 year with SD by RECIST criteria, n = 13), and those with no clinical benefit (PD by RECIST criteria or OS <1 year with SD by RECIST criteria n = 9). Previously, the sample cohort had been analyzed by a 2-hour LC-MS/MS gradient setup operated in DIA mode. The selected samples were analyzed with a longer gradient of 4-hours which enabled the quantification of 1’000 more proteins and enabled an updated analysis with a deeper level of characterization.

Results: 8’548 proteins were quantified across all samples, with 7’416 quantified on average per sample. Univariate statistical testing between groups identified 285 proteins that were significantly regulated in subjects who received clinical benefit. Through partial least squares discriminant analysis (PLS-DA) a set of 25 proteins was identified that describe the variance between the two sample groups. Ganglioside GM2 activator (GM2A) and other members of its interaction network such as HEBB, HRNR and CPPED1 were identified to be upregulated in the non-responder group.

Conclusions: Global profiling of the baseline tumor proteome provides a unique characterization of melanoma tumor biology. A signature of 25 protein markers was identified as a driver of separation between responder and non-responder patients to PD-1 blockade. Among the protein markers, GM2A and its interactors, were previously shown to perturb T cell function, which might explain their enrichment in the non-responder group and provide an attractive target for improving patient response to immunotherapy.

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230 SINGLE CELL PIK3 GENE EXPRESSION PATTERNS SUPPORT DUVELISIB (PIK3-DELTA, GAMMA INHIBITOR) TREATMENT OF MELANOMA AND OTHER TUMORS AFTER CHECKPOINT INHIBITOR THERAPY

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Background: Duvelisib, an FDA-approved oral phosphoinositide 3-kinase (PI3K)-δ,γ inhibitor, targets tumor cells of B/T cell malignancies, but may modulate non-malignant immune cells in the tumor microenvironment (TME) of many cancers. PI3K-δ and PI3K-γ downmodulate immunosuppressive Tregs and myeloid cells in solid tumors.1,2 We used single-cell RNA analysis of PIK3CD and PIK3CG to explore resistance mechanisms to checkpoint inhibitors (CPI).

Methods: Single-cell melanoma (SKCM) RNAseq datasets: GSE120575;4 CD45+ cells from 48 CPI responders and non-responder tumors, and GSE115978;5 33 treatment-naïve and CPI-progressing (resistant) tumors. Cancer cells and CD45+ TME subpopulations, specified by gene expression signatures and tSNE plots, had PI3K gene expressions profiled. Differential gene expression (DE) was gated in MAST/Seurat. Fishers test Odds Ratio (OR) was calculated for ‘high’ expression.

Results: PIK3CD expression is higher in SKCM than most cancers (10.8 median RSEM log 2). By single-cell analysis, PIK3CD (> 0.3 log2 TPM) occurs in 68.2% of cancer cells, with PIK3CB, PIK3CA, and PIK3CG expressed in 32.3%, 12.0%, and 7.2% respectively. PIK3CD-high cancer cells (>4 log2 TPM) have a 711-gene DE gene signature mostly related to immune processes. A higher proportion of cancer cells in CPI resistant tumors express PIK3CD, than untreated tumors (OR 2.02, 95% CI 1.65–2.48, p=3.04 × 10−12), as do PIK3CD+PIK3CG-expressing cancer cells (OR 2.14, 95% CI 1.47–3.13, p=4.2 × 10−5). Additionally, in PI3K-δ or PI3K-γ high melanoma cell lines duvelisib inhibited proliferation, p-AKT and c-myc.7 PIK3CD and PIK3CG are prominently expressed in many SKCM CD45+ TME (84.5% and 31.7% CD45+ respectively). PIK3CD (>0.3 log2 TPM) occurs in a high fraction of T (85.7%), CD8+ T (86.3%), CD4+ T (86.9%), B (78.5%), macrophages (88%), and NK (85%). PIK3CG is highest in B, dendritic, cycling lymphocytes and plasma cells. Strikingly, a significantly higher proportion of PIK3CD+ cells occur in resistant tumors compared to untreated for all CD45+ cells, (OR 1.64, 95% CI 1.40–1.94, p=4.79 × 10−10), CD8+ T (OR 2.15, 95% CI 1.61–2.86, p=6.5 × 10−8), and an exhausted C8+ T subpopulation (OR 3.17, 95% CI 1.89–5.37, p=2.95 × 10−6). PIK3CD +PIK3CG-expressing CD45+ cells are significantly increased in CPI-resistant tumors (OR 1.22, 95% CI 1.07–1.39, p=0.002).

Conclusions: These findings support a mechanism where CPI therapies may contribute to modulation of PI3K expression in cancer cells and the immune TME. The PI3K-δ,γ inhibitor duvelisib is being investigated in combination with CPI and evaluated in the context of CPI resistance in clinical trials: pembrolizumab (HNSC, NCT04193293), and nivolumab (Richter’s Syndrome, NCT03892044).

REFERENCES


Abstract 231 Table 1 Antibodies detected in the serum or plasma of patients with metastatic melanoma treated with ICI therapy. Treatment response indicates best overall response according to RECIST v1.1. Post-treatment blood collections were drawn during or after ICI therapy.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Tumor-specific antigen</th>
<th>Tumor-specific antibody specificity</th>
<th>Reactivation antibody specificity</th>
<th>% of patients with response</th>
<th>% of patients with partial response</th>
<th>% of patients with complete response</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY-ESO-1</td>
<td>NY-ESO-1</td>
<td>NY-ESO-1</td>
<td>NY-ESO-1</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
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<tr>
<td>SSX2</td>
<td>SSX2</td>
<td>SSX2</td>
<td>SSX2</td>
<td>100%</td>
<td>100%</td>
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<tr>
<td>MAGEA10</td>
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</table>

Conclusions Our comprehensive screening platform detected circulating antibodies specific to multiple melanoma-associated cancer antigens in patients receiving clinical benefit from ICI. Expanded investigations of the evolution of antibody production over the course of ICI therapy, associated with tumor response to treatment and development of IRAEs, are warranted.

Acknowledgements This study was supported by the Johns Hopkins Bloomberg-Kimmel Institute for Cancer Immunotherapy, and NIH P30-AR070254.

Ethics Approval This study was approved by the Johns Hopkins Institutional Review Board, approval #NA_00090257.

Abstract 232 The epithelial-to-mesenchymal transition (EMT) contributes to immunosuppression in breast carcinomas and regulates their response to immune checkpoint blockade

Background Immune checkpoint blockade (ICB) has generated some dramatic responses in certain types of human tumors, most notably, melanomas. However, the response of breast tumors has been largely limited. We have previously demonstrated that the residence of breast cancer cells in the epithelial or mesenchymal phenotypic states can itself be used as an important determinant of the success or failure of ICB. Specifically, we have shown that while epithelial tumors are sensitive
to anti-CTLA4, mesenchymal tumors are highly resistant. Most strikingly, in tumors arising from a mixture of both cell types, a minority population (10%) of mesenchymal cells can cross-protect the vast majority (90%) of their epithelial neighbors from immune attack. However, the mechanisms underlying such cross-protection remain elusive. This is particularly important as most human breast cancers contain minority populations of such mesenchymal cells which can protect the tumor as a whole from immune attack.

Methods Using a combination of transcriptomic and CRISPR/Cas9 approaches, we first identified that mesenchymal carcinoma cells express high levels of CD73, an ecto-enzyme that catalyzes the production of adenosine. Additionally, we used digital spatial profiling to determine whether CD73 expression differs across distinct epithelial and mesenchymal sectors in mixed tumors.

Results Abrogation of CD73 from mesenchymal carcinoma cells prevented the assembly of an immunosuppressive tumor microenvironment and resulted instead in increased numbers of tumor-infiltrating lymphocytes and cross-presenting dendritic cells. Most strikingly, abrogation of CD73 sensitized previously refractory mesenchymal tumors completely to ICB. In the context of mixed tumors comprised of both epithelial and mesenchymal carcinoma cells, gradients in expression of CD73 were observed corresponding to the epithelial or mesenchymal sectors of these mixed tumors. Importantly, mixed tumors in which the minority population of mesenchymal carcinoma cells lacked the expression of CD73, were also sensitized partially to ICB. Thus, these mesenchymal carcinoma cells knocked out for CD73 could no longer protect their epithelial neighbors from immune attack.

Conclusions Taken together, our work suggests that mesenchymal carcinoma cells exert immune-suppressive effects which are also prominent in heterogeneous tumors. Furthermore, targeting the adenosinergic signaling pathway in mesenchymal carcinoma cells can potentiate the efficacy of ICB.

REFERENCE

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233 GENE EXPRESSION ANALYSIS OF IMMUNE CHECKPOINT THERAPY IN MOUSE TUMOR MODELS REVEALS SIMILARITIES AND DIFFERENCES IN IMMUNE CELL POPULATIONS AND FUNCTIONAL PROCESSES THAT REFLECT RESPONSE TO TREATMENT

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Background Experimental therapies that target the immune system have expanded greatly in recent years due to the success of immune checkpoint inhibitory antibodies such as ipilimumab and pembrolizumab. Preclinical development of these novel immune-oncology drugs requires the availability of well characterized mouse models to evaluate therapeutic mode of action, efficacy, and safety. Syngeneic mouse tumor models provide robust systems in which to evaluate novel immune-oncology therapies. Efficacy in these models can be measured by tumor volume changes in subcutaneous implants or by impacts on survival for orthotopic implants. Mode of action can be assessed by identifying changes in the tumor microenvironment following dosing. Multiple analytical methods can be used to track changes in immune populations and activation status from flow cytometry to immunohistochemistry to gene expression analysis.

Methods We endeavored to characterize the functional tumor microenvironment changes for two syngeneic models following treatment with anti-PD-1 and anti-CTLA-4 antibodies. The syngeneic models used for the study were both adenocarcinomas, MC38 and CT26. Mice bearing subcutaneous tumors were dosed intraperitoneally with either vehicle alone, anti-CTLA-4, anti-PD-1, or a combination of the two immune checkpoint inhibitors on days 1, 4, and 8. Tumors were harvested on day 9 and assessed for gene expression by microarray analysis. The gene expression results were evaluated for the relationship between treatment regimen and tumor volume change by expression level association, functional set enrichment analysis, and immune cell population gene set variation analysis.

Results For each of the tumor models, >10,000 genes were found to be significantly differentially expressed. Functional set enrichment analysis showed notable changes in cell cycle and mitotic markers as well as immune response markers in MC38. In contrast, CT26 showed principally changes in immune response markers. Immune cell population set analysis revealed differential impacts on numerous immune cell populations between the models which correlate with therapy induced changes in tumor growth. These include expected changes in CD8+ T-cell populations for both models but also differential changes in other populations including CD56dim NK cells, eosinophils and B cells in MC38 and neutrophils in CT26. We also conducted a genomic analysis by whole exome sequencing. Both tumor models have relatively high tumor mutational burden; CT26 TMB = 377 MB and MC38 TMB = 69/MB. These data show the value of robust bioinformatics analysis of gene expression data sets to provide insights into the mode of action and model responses to investigational immune-oncology drugs.

Conclusions N/A

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235 ANTIGEN PRESENTATION PATHWAYS PRIME MELANOMA PATIENTS FOR MORE DURABLE RESPONSE TO ANTI-PD-1 CHECKPOINT BLOCKADE THERAPY

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Background Favorable outcomes utilizing anti–PD-1 based immune therapies for unresectable melanoma patients are hypothesized to be dependent on antigen processing and presentation mechanisms. The present study utilizes multomics to examine the contribution of antigen presentation pathways in metastatic melanoma tumors either responsive or resistant to anti-PD-1 therapy.

Methods To unveil the mechanisms that predispose unresectable, stage III/IV melanoma patients to respond to anti–PD-1-based therapies, we conducted expression proteomics as well as employed the mRNA immune oncology panel (HTG Molecular Diagnostics, Inc., Tucson, AZ). Formalin-fixed, paraffin-embedded tissues collected from 27 patients prior to anti-PD-1 therapy (previously consented and enrolled in the
Moffitt Cancer Center Total Cancer Care (TCC) protocol) were utilized in this study. For the proteomics analysis, we examined 19 FFPE samples, whereas the targeted mRNA analysis utilized 25 FFPE samples with 17 samples analyzed using both omics approaches. Robust mass spectrometry analysis used a pooled sample to optimize the number of detected peptides. The melanoma patients were selected from the database based on whether they had progression free survival (PFS) greater than 1 year (n=15) or PFS less than 6 months (n=12).

Results We identified more than 250 transcript/protein candidates that demonstrated differential expression between poor and good responders following anti-PD-1 therapy. Utilizing MetaCore software and subsequent downstream analyses of expression profiles for a knowledge-based curation of pathways and protein networks, we illustrated both the enrichment of Gene Ontology (GO) terms and specific antigen processing/presentation proteins. Of the top 10 GO processes, 7 were related to antigen processing/presentation and Major HLA compatibility Complex (MHC) presentation. Antigen processing/presentation and cytokine production/signaling via IFN-γ-mediated signaling through NF-kB and the JAK/STAT pathway interaction with INOS were mechanistic candidates of response to anti-PD-1 therapy.

Conclusions These comparative analyses illustrated the importance of antigen processing/presentation pathways mediated by both MHC class I and II in activating the immune system to initiate and maintain the immune-based response to anti-PD-1 therapy in metastatic melanoma patients. The current study also demonstrated the value of proteogenomics in defining mechanisms of response and resistance to anti-PD-1 therapy.

Trial Registration N.A.

Ethics Approval The procurement of FFPE samples was approved under IRB-approved protocol (MCC 18583, Advarra) from patients who had received a biopsy within 1 year prior to the start of anti-PD-1 therapy. Biopsy samples were obtained from the institutional biobank (Total Cancer Cancer). The radiology reads (performed by T.R.) from this research were funded by a parent research project to Moffitt Cancer Center by Navigate BioPharma to J.G. and J.M.

Consent N.A.

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236 EVALUATION OF PD-L1 EXPRESSION IN PRIMARY LUNG TUMOR AND METASTATIC LYMPH NODES IN THE PRESENCE OF IMMUNE CELLS

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Background Immunotherapies against programmed death ligand-1 (PD-L1) have been established as an effective treatment for a subset of lung cancer patients. Even though it is critical for a successful therapy to know prevalent PD-L1 expression patterns in all affected tissues, information on matching lymph node metastases and immune cells is particularly limited. The purpose of this study was thus to evaluate comparative PD-L1 expression profiles in those tissues.

Methods FDA-approved IHC assays for PD-L1 (Dako 22C3) were performed on a lung tissue array (LC814A, US Biomax) according to manufacturer’s instructions. Histopathological analysis by H-scoring was performed to determine the rate and intensity of positive tumor and immune cell staining for each of the 80 cores. The H score was calculated as follows:

Abstracts

Abstract 236 Figure 1 PD-L1 Staining in adenocarcinoma

A total of up to 300 cells were assessed, per specimen, at 40x high-power magnification (typically over 7–10 fields). A staining level of 0–3 was then assigned to each cell, to designate the intensity of specific positive membranous-to-cytoplasmic staining. The H score was subsequently calculated as % cells staining at level 1 (x1) +% cells staining at level 2 (x2) +% cells staining at level 3 (x3) = total H score per sample. This resulted in a maximum possible H score of 300.

Results Of the 16 adenocarcinoma tumor samples with a valid staining, 7 (44%) showed positive PD-L1 staining for tumor cells and 10 (63%) for primary immune cells. Importantly, 9 matching metastatic lymph node samples out of the 16 samples (56%) showed an increased PD-L1 H score compared to primary tumors for both tumor cells and immune cells (figure 1). Of the 15 squamous cell carcinoma samples with a valid staining, 11 (73%) showed detectable PD-L1 expression levels in the primary tumor and 12 (80%) in the primary immune cells, while 7 (47%) and 9 (60%) showed lower scores in matching metastatic lymph node tumor cells and their immune cells, respectively (figure 2). Very low or no expression of PD-L1 was detected in small cell lung cancer, as to be expected from previous studies.

Conclusions Squamous cell carcinomas and adenocarcinomas display significant heterogeneity with regard to PD-L1 expression in associated lymph node metastases. While the reasons for this frequent discordant PD-L1 expression pattern involving both tumor and immune cells need to be investigated further, our findings may help guide the proper interpretation of PD-L1 companion diagnostic test results and subsequent therapeutic decisions.

Acknowledgements The views in this Abstract have not been formally disseminated by the U.S. Food and Drug Administration and should not be construed to represent any agency determination or policy.

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Abstract 236 Figure 2 PD-L1 Staining in squamous cell carcinoma
IN VITRO POTENCY ASSAYS FOR IMMUNE CHECKPOINT BLOCKADE USING HUMAN PRIMARY CELLS, MURINE HUGEMM IMMUNE CELLS AND PATIENT-DERIVED TUMOR ORGANOIDS

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Background The demand of evaluating potency of immune checkpoint modulators is steadily growing for immune-oncology drug development.

Methods We aimed to establish a platform to assess the effects of immune checkpoint blockade using human primary immune cells, humanized murine primary immune cells, and co-cultures of tumor cells or patient-derived tumor organoids with immune cells.

Results First, we validated the potency of immune checkpoint blockade, such as anti-PD-1 antibodies, using mixed lymphocyte reaction (MLR) assay and T cell activation assay by in vitro stimulation. Secondly, we introduced tumor cell lines into co-culture system with immune cells and validate the potency assay by measuring cytokine production and tumor cell killing by allogenic T cells. Thirdly we used huGEMM mouse-derived immune cells to replace human primary immune cells in potency assays. HuGEMM mice express engineered human immune checkpoint targets on immune cells and they can serve as an excellent resource of primary immune cells to test the drug candidates targeting human checkpoints in vitro. Last, we developed a patient-derived tumor organoid co-culture system with immune cells. We profiled the expression of immune inhibitory molecules on the tumor organoids and assessed the potency of immune checkpoint inhibitors.

Conclusions In summary, we have established an extensive in vitro platform to evaluate the potency of the next generation of immune checkpoint inhibitors.

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MOLECULAR DISSECTION OF TUMOR-IMMUNE MICROENVIRONMENT FACTORS ASSOCIATED WITH RESPONSE TO CHECKPOINT INHIBITOR THERAPY IN NON-SMALL CELL LUNG CANCER PATIENTS USING NANOSTRING DIGITAL SPATIAL PROFILING

Omar Jabado *, Suzana Couto, Jordan Blum, Patrick Franken, Patricia Coutinho de Souza, Maria June-Kunkel, Nora Pencheva, Brandon Higgs, Kate Sasser, Mark Fereshteh. Genmab, Princeton, NJ, USA

Background Understanding the dynamics of immune cells in the lung tumor microenvironment following immune checkpoint inhibitor (ICI) therapy is important for developing therapies tailored to patients with progressive disease. We sought to characterize protein and mRNA biomarkers in the tumor and stromal microenvironment in such patients with the Nanostring Digital Spatial Profiling (DSP) platform. DSP technology allows highly multiplexed profiling of proteins and RNA in a spatially resolved manner.

Methods FFPE non-small lung adenocarcinoma biopsies from 18 patients were sourced commercially (Capital Biosciences, MD). Patients had surgical resection of tumors then adjuvant chemotherapy. Upon progression, patients received monotherapy ICI (nivolumab or pembrolizumab). Once progressed on ICI, biopsies were performed and patients were then treated with platinum-doublet or single agent chemotherapy and followed until progression and/or death. Best overall response (BOR) and progression free survival (PFS) was available for ICI. FFPE tumors were sectioned and stained with anti-Pan-Cytokeratin, anti-PDL1 and anti-4-1BB (CD137) using standard immunofluorescence techniques. Twelve circular regions of ~400 µm in diameter containing tumor (PanCK+) and stromal (PanCK-) areas were selected per patient (figure 1). The technology uses a photocleavable DNA barcode strategy to multiplex antibodies and RNA in-situ hybridization probes. The GeoMX instrument was used to generate counts for 58 proteins and 84 RNAs on serial sections. Data normalization, linear modeling and survival analysis was conducted in R.

Results Lymphoid and myeloid markers were more abundant in stroma, indicating the microenvironment is diverse and confirming the DSP platform can segment adjacent cells. High levels of PDL1 protein in the tumor were correlated with T cell markers in the stroma (CD3, CD8, ICOS, IDO, OX40L) and inversely correlated with granulocytic (CD66b) and angiogenesis markers (CD34). We focused outcomes analysis on ICI response (9 PD/9 PR). OX40L protein was higher in patients with progression.
Decr2 Loss Promotes Resistance of Tumor Cells to Immunotherapy by Affecting CD8+ T Cell-Regulated Tumor Ferroptosis

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Background Checkpoint blockade therapies have transformed the landscape of cancer care. Durable clinical responses have been observed in a subset of patients. However, many patients do not respond, and understanding the mechanisms that determine tumor resistant to checkpoint blockade drugs could potentially benefit more patients. Ferroptosis is a relatively newly described form of regulated cell death distinct from apoptosis and necroptosis. Recently, T cell–promoted tumor ferroptosis was shown to be an anti-tumor mechanism and targeting this pathway could be a potential therapeutic approach.

Methods To identify genes critical to immunotherapy resistance, B16.SIY cells were transduced with a genome-scale gRNA lentivirus to generate loss of function mutants. In vitro–primed CD8+ T cells isolated from 2C/Rag2−/− TCR transgenic mice specific for the SIY antigen were co-cultured with transduced B16.SIY tumor cells. Resistant mutants were identified by sequencing the gRNAs of survival clones. The gene encoding Decr2, a peroxisomal 2,4-dienoyl-CoA reductase, was identified. To investigate the role of Decr2 in tumor growth and immune responses in vivo, the Decr2 knock-down or Decr2 overexpressed tumors were transplanted into B6 mice and the mice were subsequently treated with anti-PD-L1 antibody. The tumor microenvironments were analyzed by flow cytometry. To understand the resistance mechanism of Decr2 knock-down tumors, RNA-seq was performed and analyzed. The CD8+ T cell mediated tumor ferroptosis in vitro and in vivo was analyzed for lipid reactive oxygen species.

Results Decr2 mutants were relatively resistant to CD8+ T cell killing in vitro. Consistent with this resistance to CD8+ T cell killing, Decr2 knock-down tumors showed minimal response to anti-PDL1 therapy in vivo. RNA-seq analysis of Decr2 knock-down B16.SIY tumors revealed upregulation of ferroptosis-related genes, including slc7a11. Further mechanistic studies showed that Decr2 knock-down tumors displayed defects in ferroptosis in vitro and in vivo.

Conclusions Decr2-deficient tumors were relatively resistant to CD8+ T cell killing in vitro and anti-PD-L1 immunotherapy in vivo by modulating CD8+ T cell-induced ferroptosis.

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Background Recent studies report of liver metastases (LM) as a poor prognostic factor in patients treated with immune checkpoint inhibitors (ICIs), but clinical outcomes associated with different ICI regimens remains uncertain. In this study, we investigate melanoma patients with and without LM and assess differential treatment outcomes associated with anti-PD-1 monotherapy and combination ipilimumab/nivolumab (I/N).

Methods We conducted a single-center, retrospective review of advanced stage melanoma patients with and without LM treated with anti-PD-1 monotherapy (nivolumab or pembrolizumab) or I/N between 2012 and 2018. Overall survival (OS) and progression free survival (PFS) were measured from the first dose of treatment to date of death and clinical or radiographic progression, respectively. Univariate and multivariate analysis were performed using Cox proportional hazard (CPH) models and logistic regression models. Inverse probability of treatment weighting using propensity scores in CPH models was used to account for the following baseline covariates: age, ECOG performance status, BRAF status, pre-treatment LDH level, prior therapy status, and number and sites of metastases.

Results 327 patients were identified, 87 with LM and 240 without LM. Patients with LM was associated with worse PFS [HR: 2.1, 95% CI, 1.5 – 3.1] (figure 1) and OS [HR: 3.4, 95% CI, 2.2 – 5.2] (figure 2). Respective 3-year PFS and OS estimates associated with anti-PD-1 monotherapy were 21.8% and 28.7% in patients with LM (figure 3, figure 4); and 36.5% and 57.6% without LM (figure 5, figure 6). Respective 3-year PFS and OS estimates associated with I/N were 46.7% and 56.7% in patients with LM; and 58.0% and 74.4% without LM.
Abstract 241 Figure 5  Kaplan-Meier curves comparing advanced stage melanoma patients without liver metastases treated with anti-PD-1 monotherapy (nivolumab or pembrolizumab) versus ipilimumab/nivolumab by progression free survival. n = 240

Conclusions In melanoma patients treated with PD-1 inhibitor-based regimens, the presence of LM leads to poorer survival outcomes. Our study suggests the poor prognosis associated with LM can be substantially mitigated by treatment with combination I/N over anti-PD-1 monotherapy. Further studies are warranted to investigate the anti-immunotherapy effect associated with LM.

Ethics Approval The study was approved by the University of Michigan institutional ethical guidelines and patients’ consents were waived following Institutional Review Board protocol review (HUM00156014).

REFERENCE


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Abstract 242 MONITORING MDSC – A HURDLE TO IMMUNE CHECKPOINTS INHIBITORS

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Background Certain cell subsets have been identified to have a negative impact on cancer immunotherapies by promoting angiogenesis and immunosuppression in the tumor microenvironment. One of these cell subsets is a heterogeneous population of immature myeloid cells that have been named Myeloid Derived Suppressor Cells (MDSC). MDSC are increased in states of cancer and their numbers have been shown to inversely correlate with a positive clinical outcome. These findings have prompted the measurement of MDSC in order to predict clinical outcome during treatment with immunotherapies. In this study, flow cytometry was used to measure M-MDSC in frozen PBMC samples and hence predict medical outcome in melanoma patients treated with an anti-CTLA-4 drug (ipilimumab).

Methods M-MDSC were measured in frozen PBMC from 20 healthy donors and 68 patients with melanoma treated with ipilimumab. M-MDSC were enumerated using a Lin-CD14+CD11b−HLA-DRlow/- phenotype. In order to prevent subjectivity during gating, caused by the lack of bi-modality with HLA-DR staining, a computational algorithm was used. As distinct HLA-DR spread can be observed in the different subjects, measuring the CV (a ratio between GMFI and SD) of this spread allows to calculate a standardized ad hoc quantitative measure of MDSC frequency in cancer patients. This measurement enables identification of M-MDSC in an objective manner and was used to determine whether the percentage of M-MDSC in patients could be linked with overall survival.

Results The relative frequency of M-MDSC was determined in 68 melanoma patients treated with two different doses of ipilimumab. By comparing the percentage of M-MDSC at baseline (pre-treatment) and after two doses of ipilimumab with the overall survival data and applying log-rank statistics, a cut-off was defined allowing the separation of ‘high’ and ‘low’ M-MDSC expressers. Patients with ‘low’ M-MDSC were associated with improved overall survival with a hazard ratio of 0.35.

Conclusions The reliable measurement of immune suppressive cells such as MDSC gives the ability to predict the clinical outcome of cancer treatments. In turn, these measurements will permit the design of patient-specific treatments as inhibitors to these cell subsets become available, making personalized medicine a reality in contemporaneous cancer treatment. The identification of specific phenotypes and activation markers for MDSC may improve the prediction ability of the test described in this study. These results highlight the importance of linking the frequency of immune suppressive cells with clinical outcome.

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Abstract 243 REAL WORLD EXPOSURE SURVIVAL RELATIONSHIP OF PEMBROLIZUMAB IN METASTATIC MELANOMA

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REFERENCE


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**Background** Despite the paradigm shift heralded by immune checkpoint blockade (ICB), only a small proportion of patients have a meaningful response. Dose selection of ICB agents was significantly based on in-silico modelling.\(^1\) Trial data has shown that clearance of these agents varies over time, with a reduction in clearance associated with improved best overall response (BOR).\(^2\),\(^3\) Real world data has shown patients with higher exposure to ICB, manifested as higher plasma trough concentrations, experience improved BOR and longer survival.\(^4\) This study aimed to determine the relationship between longitudinal ICB exposure and BOR, progression free survival (PFS) and overall survival (OS) in patients with metastatic melanoma receiving pembrolizumab monotherapy.

**Methods** 28 patients with metastatic melanoma receiving weight based pembrolizumab (2 mg/kg Q3w) had serial pharmacokinetic trough draws prior to their next scheduled dose, up to a maximum of 22 cycles. BRAF mutation positive patients were pre-treated with BRAFi/MEKi therapy, otherwise pembrolizumab was given first line. Plasma trough levels were determined using the Abcam\(^\circledR\) pembrolizumab ELISA kit. The cohort was split by best overall response (BOR), determined by iRECIST. No statistically significant differences were determined, using one-way ANOVA. The cohort was stratified into high versus low pembrolizumab trough concentrations, split by the median. Trough is an established surrogate for drug exposure.\(^5\) Kaplan-Meier survival analysis for progression-free and overall survival was performed based on pembrolizumab drug exposure groups.

**Results** Median follow up was 32.5 months. Complete responders (CR) (n=11) had 29.8% higher geometric mean pembrolizumab trough levels (90.8 mcg/mL) than partial responders (PR) (n=9) (63.7 mcg/mL, p=ns). CR patients had 16.1% higher trough levels than patients with progressive disease (PD) (n= 6) (76.2 mcg/mL, p=ns). 2 patients with stable disease had mean trough pembrolizumab levels of 106.4 mcg/mL. The high pembrolizumab exposure group experienced significantly longer median OS (not reached versus 48 months, p=0.021) (figure 1), than the low exposure group. No significant difference was found in mean PFS between the groups (49.2 versus 37.9 months, p=ns) (figure 2). The median PFS was not reached in either group.

**Conclusions** A positive exposure survival relationship for pembrolizumab in metastatic melanoma is described in a real world setting. Whether this relationship indicates a true causal effect of variation in drug exposure on clinical outcomes remains to be determined. Further pharmacokinetically driven dosing studies are required to identify whether therapeutic drug monitoring of pembrolizumab in the clinic is a necessity.

**Acknowledgements** This study was funded by a Hunter Medical Research Institute Project Grant (HMR983) - Hunter Cancer Biobank Serial Blood Collection Project.

**Ethics Approval** This study was approved by the Hunter New England Health Local Health District (14/12/10/4.02) and University of Newcastle Human Research Ethics Committee institutional review board (H-2018-0159).

**Consent** Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

**REFERENCES**


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**Abstract 243 Figure 1** OS Kaplan-Meier survival curves stratified for the group with the 50% highest trough concentrations (red) and 50% lowest trough concentrations (blue). The median OS for high pembrolizumab exposure group was not reached, which was significantly longer than the low pembrolizumab exposure median of 48 months (p=0.021)

**Abstract 243 Figure 2** PFS Kaplan-Meier survival curves stratified for the group with the 50% highest trough concentrations (red) and 50% lowest trough concentrations (blue). The mean PFS for the high pembrolizumab exposure group was 49.2 months, which was not significantly longer than low pembrolizumab exposure mean PFS of 37.9 months. The median PFS was not reached in either group.

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**244 NOVEL RESPIRABLE ANTIMUSE OLGONUCLEOTIDE (RASON) APPROACH TO PRIMARY AND METASTATIC HUMAN LUNG CANCER: PRELIMINARY RESULTS IN A MODEL SYSTEM EmployING SPONTANEOUS LUNG TUMORS IN DOGS**

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**Background** Antisense oligonucleotides function by targeting the messenger RNA coding for a target protein, rather than the protein itself. This laboratory previously introduced Respirable Antisense Oligonucleotides (RASONs) into human clinical
In this preliminary, proof-of-principal study, two dogs with lung tumors presenting to their veterinarians. sis, we applied a RASON protocol to dogs with spontaneous lung tumors, they would arrive in the tumor-associated microenvironment in a state impervious to checkpoint ligands. Such a state might be achieved by RASON treatment, administered by inhalation; the ability to deliver RASONs into the lung tissue is supported by the observation that the RASON approach might prove to be an effective addition to immune checkpoint inhibition. It possesses certain advantages over small molecule or antibody approaches to checkpoint inhibition. For example, rather than being delivered systemically, RASONs are delivered by inhalation directly to the target tissues– the bronchial epithelium and BAL. Furthermore, it may be possible to reduce the toxicity of systemic treatments targeting checkpoint proteins on tumor cells, by reducing or eliminating their ligands on immune effector cells. In as much as the RASON approach to the treatment of human asthma failed in clinical trials as a result of its induction of an influx of macrophages into the lung, the ability to render TAMs impervious to the presence of tumor-associated immune checkpoints suggests that the RASON approach may hold considerable promise for the treatment of lung tumors.

**Ethics Approval** All research reported here involved informed consent by owners of dogs with spontaneous lung neoplasms, for which no satisfactory alternative treatment was available, and was performed in strict compliance with both the Basle Declaration, to which the laboratory is a signatory member, as well as guidelines published by the International Council for Laboratory Animal Science (ICLAS).

**Consent** N/A

**REFERENCES**


**Abstract**

244 HUMAN TLR8 KNOCK-IN MICE POTENTIATE IMMUNOTHERAPY RESPONSES OF MC38 SYNGENEIC TUMORS

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**Background** Toll-like receptors (TLRs) serve critical roles in mediating innate immune responses against many pathogens. However, they may also bind to endogenous ligands and lead to the pathogenesis of autoimmunity. Although TLR8 belongs to the same TLR family as TLR7, its role in inflammation and tumor progression is not yet fully understood due to the lack of suitable animal models. In humans, both TLR7 and TLR8 recognize single-stranded self-RNA, viral RNA, and synthetic small molecule agonists. However, mouse Tlr8 is non-functional due to the absence of 5 amino acids necessary for RNA recognition. In order to create a mouse model with functional TLR8, we replaced exon 3 of mouse Tlr8 with human TLR8, therefore developing a hTLR8 knock-in (KI) trial for asthma. In that work we demonstrated that RASONs delivered by inhalation are absorbed into surfactant lining the surface of the lung; are distributed with high efficiency throughout the bronchial epithelium; and are taken up with therapeutic effect by both bronchial epithelial cells and immune effector cells resident throughout the bronchial epithelium, as well as in bronchial-associated lymphoid tissue (BALT). We have now re-engineered this technology to adapt it to the treatment of primary and metastatic lung tumors via immune checkpoint inhibition. While immune checkpoints expressed on lung tumors are not amenable to RASON inhibition, immune cells resident in the bronchial epithelium and BALT represent good targets for the RASON approach to checkpoint inhibition. E.g., SIRP-alpha is a receptor expressed by myeloid lineage cells such as dendritic cells (DCs), tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). When CD47, found on the surface of tumor cells, binds to SIRP-alpha on immune effector cells, the anti-tumor action of such immune effector cells becomes significantly diminished. We hypothesized that RASONs targeting mRNA of immune checkpoint proteins found on immune effector cells would eliminate checkpoint proteins from their surface, such that when they were signaled to home in on lung tumors, they would arrive in the tumor-associated microenvironment in a state impervious to checkpoint ligands expressed on the surface of tumor cells. To test this hypothesis, we applied a RASON protocol to dogs with spontaneous lung tumors presenting to their veterinarians.

**Methods** In this preliminary, proof-of-principal study, two dogs with histologically confirmed metastatic lung tumors were administered RASONs targeting PD1, CTLA-4 and SIRP-alpha, by inhalation, twice weekly for eight weeks.

**Results** X-ray analysis performed two weeks after the conclusion of RASON treatment showed dramatic results. One dog showed complete tumor dissolution (figure 1), and the second dog showed near total tumor dissolution, with faint shadows remaining (figure 2).

**Conclusions** While these are preliminary results, and need to be dramatically expanded, they provide an initial indication that the RASON approach might prove to be an effective addition to immune checkpoint inhibition. It possesses certain advantages over small molecule or antibody approaches to checkpoint inhibition. For example, rather than being delivered systemically, RASONs are delivered by inhalation directly to the target tissues– the bronchial epithelium and BALT. Furthermore, it may be possible to reduce the toxicity of systemic treatments targeting checkpoint proteins on tumor cells, by reducing or eliminating their ligands on immune effector cells. In as much as the RASON approach to the treatment of human asthma failed in clinical trials as a result of its induction of an influx of macrophages into the lung, the ability to render TAMs impervious to the presence of tumor-associated immune checkpoints suggests that the RASON approach may hold considerable promise for the treatment of lung tumors.
model. Both heterozygous and homozygous hTLR8 KI mice are viable with inflammatory phenotypes, i.e. enlarged spleens and livers, and significantly higher IL-12 p40 levels under TLR8 agonist treatment. In this study, we evaluated the potential use of hTLR8 mice for cancer immunotherapy studies.

Methods hTLR8 mice, together with naïve C57BL/6 mice, were inoculated with MC38 syngeneic tumor cells. Tumor bearing mice were grouped at a mean tumor volume of approximately 100 mm³ for treatment with PBS or 10 mg/kg anti-PD-1 (RMP1-14) antibody. At the efficacy endpoint, spleens and tumors were collected for flow cytometry profiling.

Results Anti-PD-1 treatment of MC38 tumors in naïve C57BL/6 led to moderate tumor growth inhibition (TGI = 54%). Interestingly, anti-PD-1 treatment showed improved efficacy in hTLR8 mice (TGI = 79%), including 2/10 tumors with complete tumor regression. In comparison, non-treated MC38 tumor growth rate was slower in hTLR8 mice than in naïve mice. Anti-PD-1 treated hTLR8 mice also had significantly increased IFN-γ and TNF-α positive CD4+ T cells in the spleen, along with higher numbers of differentiated effector T cells. In addition, hTLR8 mice have activated dendritic cells and macrophages, acting as critical steps in initiation of the inflammatory process, with higher levels of pro-inflammatory cytokines, such as IL-6, IFN-γ, TNF-α, and IL-1β, which may promote Th1 priming and differentiation of T cells into IFN-γ or TNF-α producing cells.

Conclusions hTLR8 mice offer a great tool to model cancer immunotherapy in an inflammatory/autoimmunity prone background. Moreover, hTLR8 mice can be effectively used to shift a ‘cold’ tumor phenotype to ‘hot’ tumors in a syngeneic setting.

Ethics Approval Animal experiments were conducted in accordance with animal welfare law, approved by local authorities, and in accordance with the ethical guidelines of Crown-Bio (Taicang).

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247 ASSESSMENT OF SENSITIVITY TO A PD-1 CHECKPOINT INHIBITOR AND CISPLATIN IN BLADDER CANCER PATIENT-DERIVED XENOGRAFTS WITH VARIOUS LEVELS OF PD-L1 EXPRESSION IN HUCD34NCG MICE

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Background Bladder cancer is the fifth most common cancer in the US, and the ninth most common cancer worldwide. Treatment of bladder cancer has evolved over time to encompass traditional modalities of chemotherapy and surgery, but has been particularly impacted by the recent use of immunotherapy. Modern immunotherapy has focused on checkpoint protein inhibitors that impede immune function. The inhibitors for several checkpoint targets (programmed death-ligand 1 [PD-L1], programmed cell death protein 1 [PD-1], and cytotoxic T-lymphocyte-associated protein 4 [CTLA4]) were either approved or in late-stage development. In this study we examined the effect of PD-1 inhibitor pembrolizumab and cisplatin in a panel of bladder patient-derived xenografts (PDX) with distinct patterns of PD-L1 expression in CD34+ stem cell humanized NCG (HuCD34NCG) mice.

Methods Three bladder PDX models PNX0428, PNX0434 and PNX1028 have been established under informed consent from the patients at the Fox Chase Cancer Center, Philadelphia. These models have been profiled for the levels of PD-L1 protein using immunohistochemical staining with SP263 antibody (Ventana) and used to establish the growth kinetics and sensitivity to the PD-1 check point inhibitor pembrolizumab and standard of care chemotherapeutic agent cisplatin in female HuCD34NCG and standard NCG mice from Charles River Laboratories.

Results We have established the ability of three bladder PDX models to grow in both the HuCD34NCG and standard NCG mice. The tumor growth kinetics of these models was slightly delayed in HuCD34NCG animals compared to NCG. We observed variable responses to cisplatin and pembrolizumab treatments among the PDX models that did not correlate with the level of PD-L1 expression in these tumors. Despite the presence of ~70% PD-L1 positive cells in the PNX0428 model, these tumors produced minor responses to pembrolizumab in HuCD34NCG mice that correspond to progressive disease in patients. Interestingly, pembrolizumab treatment in the PNX1028 model and even more significantly in the PNX0434 model in HuCD34NCG mice produced strong statistically significant tumor growth inhibition that correlates with stable disease in patients despite negative staining for PD-L1 protein in these tumors. The standard of care treatment cisplatin produced significant tumor growth inhibition in all three PDX models in both HuCD34NCG and standard NCG mice.

Conclusions Our data indicates that abundant expression of PD-L1 protein in tumors should not be used as the only biomarker for patient stratification for the treatment with PD-L1/PD-L1 check point inhibitors. The HuCD34NCG mouse model is an effective tool for supporting tumor growth and evaluating immunotherapies.

Ethics Approval Animal studies were approved by Nexus Pharma, IACUC number 08-22. Three bladder PDX models PNX0428, PNX0434 and PNX1028 have been established under informed consent from the patients at the Fox Chase Cancer Center, Philadelphia, IRB protocol 11-866.

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246 CLINICOPATHOLOGIC AND GENOMIC CORRELATES OF TUMOR MUTATIONAL BURDEN AND ITS IMPACT ON PD-(L)1 INHIBITION EFFICACY IN NON-SMALL CELL LUNG CANCER ACCORDING TO DIFFERENT PD-L1 EXPRESSION SUBGROUPS

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Background High tumor mutational burden (TMB) and PD-L1 expression are associated with improved clinical outcomes in patients (pts) with non-small cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICIs). However, how
TMB performs as a predictive biomarker to ICIs in different PD-L1 expression subgroups is not well characterized.

Methods

We collected clinicopathologic and genomic data from NSCLCs which underwent targeted NGS and TMB assessment at DFCI. An unbiased recursive partitioning (URP) algorithm was used to investigate an optimal TMB cut-off with respect to objective response rate (ORR) in the subset of pts treated with ICIs. This TMB cut-off was then validated in the prospective POPLAR/OAK cohort.

Results

Among 3560 NSCLCs identified, median TMB was significantly higher among current smokers compared to former (P<0.0001) and never smokers (P<0.0001), and there was a significant correlation between TMB and pack-years (figure 1A-B). Pts with BRAF or KRAS mutations had the highest median TMB (10.9 and 9.8 mutations/Megabase [mut/Mb], respectively), while tumors with RET and ALK alterations had the lowest median TMB of 5.3 mut/Mb (figure 2A-B). Tumors with PD-L1 expression of ≥50% had significantly higher median TMB compared to those with a PD-L1 expression of 1–49% (P=0.002) and <1% (P<0.0001). Among pts treated with ICIs (N=690), URP identified an optimal grouping TMB cut-off for ORR of 19.0 mut/Mb, which corresponded to the 90th percentile. Pts with a TMB of ≥19.0 mut/Mb had a significantly higher ORR (45.2% vs 20.1%, P<0.0001) and longer median PFS (11.0 vs. 2.9 months, HR:0.49, P<0.0001) and OS (20.8 vs. 11.2 months, HR:0.59, P=0.001) compared to those with a TMB of <19.0 mut/Mb (figure 3A-C). A TMB of ≥19.0 mut/Mb was an independent predictor of improved PFS and OS at multivariable analysis (table 1). A TMB within the top 10th percentile was confirmed to correlate with improved ORR and PFS in atezolizumab arm but not in the docetaxel arm of the POPLAR/OAK trials (figure 4A-B). When TMB and PD-L1 where integrated in the URP, we identified an optimal cut-off of 19 mut/Mb among cases with a PD-L1 expression of ≤25%, and of 8.4 mut/Mb among those with a PD-L1 expression of >25%, suggesting that TMB differentially impacts response to immunotherapy among PD-L1 high versus low NSCLCs (figure 5).
Conclusions The impact of TMB may vary across PD-L1 expression subgroups. Rational integration of TMB and PD-L1 expression may identify NSCLCs with the greatest likelihood of response or resistance to ICIs.

Ethics Approval Clinicopathologic data were collected from patients with advanced NSCLC who had consented to a correlative research study (DF/HCC protocol #02-180).

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Abstract 248 IMMUNOTHERAPY PERSISTER CELLS UNCOVERED BY DYNAMIC SINGLE-CELL RNA-SEQUENCING

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Background To understand fundamental mechanisms of immune escape, we leveraged our functional ex vivo platform of murine derived organotypic tumor spheroids (DOTS)1 to determine if drug-tolerant persister cells analogous to oncogene targeted therapies limit efficacy of programmed death (PD)-1 blockade, and to identify therapeutic vulnerabilities to overcome anti-PD-1 (αPD-1) resistance.

Methods Murine syngeneic cancer models with well-characterized response to αPD-1 therapy were chosen: MC38 (sensitive) and CT26 (partially resistant). Bulk and single-cell (sc) RNA-sequencing (RNA-seq) were performed on αPD-1 treated DOTS. In vitro culture studies were conducted with or without cytokines (100 ng/ml) or drugs (500 nM). In vivo studies in mice bearing MC38 or CT26 tumors evaluated the combinatorial strategy with PD-1 blockade. We further evaluated our findings in scRNA-seq of an αPD-1 refractory colorectal cancer (CRC) patient tumor.2

Results Bulk RNA-seq of αPD-1 treated DOTS revealed a mesenchymal resistant phenotype with upregulated TNF-α/ NFκB signaling (figure 1). scRNA-seq further identified a discrete sub-population of immunotherapy persister cells (IPCs). These cells expressed a stem-like phenotype including down-regulation of E2F targets indicative of quiescence, suppression of interferon-γ response genes, induction of hybrid epithelial-to-mesenchymal state, and active IL-6 signaling (figure 1). Ly6a/stem cell antigen-1 (Sca-1) and Snai1 were found to be differentially upregulated in IPCs resistant to PD-1 blockade (not shown). Sca-1 positivity was confirmed in pre-existing tumor populations in vitro (figure 2). When enriched via sorting, these cells remained more persistently Sca-1+ at 96 hours in culture of CT26 compared to MC38 cells, related to increased autocrine IL-6 production by CT26 Sca-1+ cells. Indeed, IL-6 supplementation was capable of expanding Sca-1+ cells in culture (figure 2). Sca-1+ cells expressing ovalbumin peptide were refractory to OT-1 T cell mediated killing and failed to upregulate MHC class-1 antigen presentation (H-2Kb) in response to IL-6, in contrast to interferon-γ (not shown). Analysis of RNA-seq data further identified Birc2/3 as potential targets limiting TNF-mediated apoptosis of these cells (not shown). Notably, Birc2/3 antagonism depleted Sca-1+ IPCs in vitro and significantly potentiated the impact of PD-1 blockade in vivo in MC38, and less robustly in CT26 (figure 3). Evaluation in a microsatellite-instability high CRC patient identified a pre-existent IPC sub-population within the αPD-1 refractory pre-treatment tumor, with high SNAI1 expression compared to CRC samples in TCGA (figure 4).

DOTS. In vitro culture studies were conducted with or without cytokines (100 ng/ml) or drugs (500 nM). In vivo studies in mice bearing MC38 or CT26 tumors evaluated the combinatorial strategy with PD-1 blockade. We further evaluated our findings in scRNA-seq of an αPD-1 refractory colorectal cancer (CRC) patient tumor.2

Abstract 248 Figure 1 Bulk and single-cell (sc) RNA-sequencing (RNA-seq) of DOTS identifies an anti-PD-1 (αPD-1) resistant subpopulation of persister cells. IgG: isotype control
Abstract 248 Figure 2  Pre-existent population of stem cell antigen-1 (Sca-1)+ cells expands in response to interleukin-6 (IL-6), as characterized by flow cytometry evaluation in murine syngeneic cancer models at baseline and after purification by fluorescence-activated cell sorting (FACS). H = hours.

Abstract 248 Figure 3  Combination of anti-PD-1 therapy with Birc2/3 antagonism increases tumor responses and improves survival. CR = complete response.

Abstract 248 Figure 4  Single-cell RNA-sequencing (scRNA-seq) of a pre-treatment microsatellite-instability (MSI-H) colorectal cancer (CRC) patient tumor, refractory to anti-PD-1 (αPD-1) therapy, reveals presence of SNAI1-high immunotherapy persister cells.

Conclusions High-resolution functional ex vivo profiling identified Sca-1+/Snai1high stem-like ‘immunotherapy persister cells’ and uncovered their anti-apoptotic dependencies targetable with Birc2/3 antagonism to augment αPD-1 efficacy.

Ethics Approval This study was approved by the Dana-Farber Animal Care and Use Committee and Novartis Institutional Animal Care and Use Committee. Informed written consent to participate in Dana-Farber/Harvard Cancer Center institutional review board (IRB)-approved research protocols was obtained from the human subject. A copy of the written consent is available for review by the Editor of this journal. The study was conducted per the WMA Declaration of Helsinki and IRB-approved protocols.

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249  TARGETING IFNβ-REGULATED SECRETORY PROFILES TO OVERCOME ACQUIRED ANTI-PD-L1 RESISTANCE

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Background Therapeutic targeting of programmed cell death protein ligand 1 (PD-L1) has led to durable benefits for many cancer patients; however, the development of acquired resistance is common. Dysregulated type II interferon (IFN) signaling on tumor cells can contribute to resistance via altered expression of IFN stimulated genes (ISGs), which include cytokines and growth factors capable of immune-suppression and tumor promotion. However, the role of type I IFNs, including IFNα and IFNβ, in acquired resistance remain understudied. Here we examined the impact of chronic PD-L1 blockade to evaluate the role of IFN-related secretory changes in preclinical models of resistance.

Methods Using a mouse breast EMT6 orthotopic tumor model, we selected PD-L1 drug resistant (PDR) cells from tumors initially responsive to PD-L1 blockade, but that later relapsed. Using transcriptomic and proteomic approaches, we evaluated secreted proteins associated with IFN signaling. To test for direct connections between PD-L1 and IFN signaling in secretory profile modulation, genetic and therapeutic disruption of PD-L1/IFNAR1 were conducted in vitro.

Results We identified a unique gene signature for secreted proteins following acquired resistance to PD-L1 blockade that associated with IFN signaling. This secretory signature was validated using publicly available datasets derived from preclinical tumors and clinical biopsies after anti-PD-L1 treatment failure. Interestingly, genetic and antibody inhibition of PD-L1 in vitro enhanced PDR secretory signatures following IFNβ stimulation suggesting PD-L1 tumor-intrinsic functions may regulate IFN responses following acquired resistance. To test whether secretory profiles impact tumor growth, inhibition of specific ISGs (IL-6) or ISG regulators (IFNAR1) were examined and found to inhibit PDR tumors in vivo, compared to parental controls.

Conclusions Together, these findings identify a secretory profile associated with acquired resistance to PD-L1 blockade that may be modulated, at least in part, by IFNβ. Selective targeting of secreted ISGs may provide a benefit for patients after anti-PD-L1 treatment failure.

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Background TIGIT is an immunoregulatory receptor expressed on activated and memory T cells, T regulatory cells (Tregs), and NK cells. TIGIT binding to CD155 and CD112 on tumor cells drives an inhibitory signal resulting in decreased T cell functionality. TIGIT targeting has been reported to release these inhibitory signals, drive Treg depletion, augment CD8 T cell function, and promote antitumor responses.

Methods To evaluate the impact of antibody backbone on anti-TIGIT action three distinct antibodies with differential backbone effector functions, wild type, Fc(gamma)R null (LALA), and Fc(gamma)R enhanced (nonfucosylated, SEA-TGT), were incorporated onto a human anti-TIGIT antibody and assessed. The nonfucosylated SEA-TGT backbone was distinct from the LALA and wild type backbone through increased binding to activating FcyRIIIa receptor while concomitantly decreasing binding to the inhibitory Fc(gamma)RIIB receptor.

Results Independent of backbone all TIGIT antibodies blocked ligand binding and restored CD226 signaling. The effector null backbone neither mediated Treg depletion nor naïve or memory CD8 T cell activation. However, the effector enhanced SEA-TGT significantly increased Treg depletion and activation of CD8 T cells over the comparator wild type anti-TIGIT antibody. The enhanced SEA-TGT also induced innate cell activation not seen with the other backbones. These in vitro results translated to curative in vivo antitumor activity in multiple syngeneic models as a single agent. Again, the effector null antibody was inactive in all models whereas the effector enhanced SEA-TGT drove curative responses beyond those seen with the standard wild type backbone. Increased activity correlated with a slight decrease in intra-tumoral Tregs and increases in CD8 memory T cells and innate cell activations. Anti-tumor response was associated with generation of long-term, antigen-specific immunity that resulted in complete tumor rejection upon tumor rechallenge.

Conclusions Collectively, these data indicate that modification of CD8 T cell functionality is not solely through alterations in the TIGIT/CD226 signaling axis and that our nonfucosylated FcR enhanced antibody uniquely activates both adaptive and innate arms of the immune system for maximal CD8 T cell responses. They also underscore the anti-tumor therapeutic potential of a nonfucosylated TIGIT targeting antibody (SEA-TGT) as a monotherapy agent and in combination with PD(L)1 agents. We have initiated a phase 1 trial testing the safety and activity of SEA-TGT in patients with advanced solid tumors and select lymphomas (NCT04254107).

Trial Registration NCT04254107

Ethics Approval Animals studies were approved by and conducted in accordance with Seattle Genetics Institutional Care and Use Committee protocol #SGE-029.

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IMMUNE-RELATED ADVERSE EVENTS ASSOCIATED WITH IMMUNE CHECKPOINT INHIBITOR THERAPY ARE ASSOCIATED WITH ENHANCED SURVIVAL AND DISEASE-SPECIFIC INCIDENCE

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Background Immune checkpoint inhibitors (ICIs) are now approved for several cancer types due to superior outcomes compared to chemotherapy. PD-1/PD-L1 and CTLA-4 inhibitors reactivate T-cell mediated anti-tumor immunity but may also lead to immune-related adverse events (irAEs). Growing evidence suggests the onset of irAEs could be correlated with the efficacy of ICIs.1 2 In this study, we investigated patterns and incidence of irAEs and their relationship to overall survival (OS) and progression-free survival (PFS) in multiple cancer types.

Methods The electronic medical record was queried at the University of Cincinnati Medical Center for the administration of ICIs for the identification of irAEs. Data on new irAEs diagnosed after administration of at least one dose of ICI was collected along with relevant demographic and clinicopathologic variables including treatment type, cancer type, staging information, and the administration of immune suppression following the identification of an IRAE either inpatient or outpatient. Univariate and multivariate analysis were conducted and survival analysis was determined according to log-rank testing.

Results Of our 210 initial patients, the median age was 64 (range 22–93), 37% were female, 72% had ECOG 0-1, and 79% were white. Cancer types included melanoma 24%, non-small cell lung cancer (NSCLC) 34%, small cell lung cancer 2%, renal cancer 12%, urothelial cancer 11%, head and neck cancers 12%, and 16% other primaries while 19% remained on ICI at the time of data entry. The most common ICIs were pembrolizumab, nivolumab, followed by ipilimumab-nivolumab, ipilimumab, and durvalumab. The overall incidence of irAEs was 22.6%. Overall survival and progression-free survival was improved for those who suffered an IRAE (median OS 8.3 vs. 3.5 years, HR 0.56, p=0.0092; median PFS 5.0 vs 2.5 years, HR 0.57, p=0.0052) (figure 1 and 2 respectively). ICI treatment in NSCLC was associated with decreased overall IRAE events by univariant analysis (Odds Ratio 0.39, 95% CI 0.17 - 0.86). Our multivariate analysis showed ICI treatment in hepatocellular carcinoma to be significantly associated with irAEs, however, this was likely due to low enrollment (n=4) and was not significant by unIVariant analysis.

Conclusions In our data set, irAEs were associated with increased OS and PFS regardless of disease site. ICI treatment of NSCLC was associated with significantly fewer irAEs compared to other malignancies. Further research is needed to...
determine irAE type-specific incidence, the incidence of multiplet irAEs in a single patient, and response to corticosteroid therapy.

Acknowledgements We would like to thank the Department of Hematology/Oncology of the University of Cincinnati, the University of Cincinnati Cancer Center, and the Department of Internal Medicine of the University of Cincinnati for their support.

Ethics Approval Considered and Approved by the University IRB Approval #2019-0610.

REFERENCES

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ANTI-TIGIT ANTIBODIES REQUIRE ENHANCED FCγR CO-ENGAGEMENT FOR OPTIMAL T AND NK CELL-DEPENDENT ANTI-TUMOR IMMUNITY

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Background T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is an important negative regulator of the immune response to cancer that contributes to resistance/relapse to anti-PD-1 therapy. In clinical trials, anti-human (h) TIGIT antibodies have shown promising activity in combination with anti-PD-1/PD-L1 antibodies for the treatment of various solid tumors. However, the optimal format for anti-TIGIT antibodies remains controversial. Here we describe a novel Fcγ receptor (FcγR)-dependent mechanism of action that is critical for enhancing T and NK cell anti-tumor immunity, and further informs on the optimal design of anti-TIGIT antibodies.

Methods We investigated a panel of Fc-silent, Fc-competent, and Fc-engineered anti-mouse (m) TIGIT antibody variants in syngeneic murine CT26 tumor-bearing or B16F10 pseudometastases models. To further elucidate the relative contribution of T and NK cells in controlling tumor growth, we assessed the activity of Fc-engineered anti-TIGIT antibodies in NK cell-depleted or T cell-deficient (Nu-Foxn1nu) CT26 tumor-bearing mice. Immune-related pharmacodynamic changes in the tumor microenvironment were assessed by flow cytometry. We further validated these findings in primary human T and NK cell activation assays using Fc-engineered anti-human TIGIT antibodies.

Results The Fc-engineered anti-mTIGIT antibody, which demonstrates enhanced binding to mouse FcyRI, was the only variant to deliver single agent anti-tumor activity. The Fc-enhanced variant outperformed the Fc-competent variant while the Fc-inert variant had no anti-tumor activity. Tumor control by anti-mTIGIT antibodies was not dependent on Treg depletion, but rather on increased frequency of CD8+ T cells and activated NK cells (Ki67, IFNγ, CD107a and TRAIL) in the tumor microenvironment. Concordant with observations in the mouse, Fc-engineered anti-hTIGIT antibodies with improved binding to FcγRIIIA demonstrate superior T and NK cell activation in PBMC-based assays compared to a standard hlgG1 variant. Notably, superior activity of the Fc-engineered anti-hTIGIT antibody was observed from PBMC donors that express either high or low affinity FcγRIIIA. Blockade of FcγRIIIA or depletion of CD14+ and CD56+ cells reduced the functional activity of the Fc-enhanced anti-TIGIT antibody, confirming the requirement for FcγR co-engagement to maximize T cell responses.

Conclusions Our data demonstrate the importance of FcγR co-engagement by anti-TIGIT antibodies to promote immune activation and tumor control. First generation anti-TIGIT antibodies are not optimally designed to co-engage all FcγRIIIA variants. However, Fc-enhanced anti-TIGIT antibodies unlock a novel FcγR-dependent mechanism of action to enhance T and NK cell-dependent anti-tumor immunity and further improve therapeutic outcomes.

REFERENCES

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CTLA-4 BLOCKADE PROMOTES TREG GLUCOSE METABOLISM AND REDUCES TREG FUNCTIONAL STABILITY IN GLYCOLYSIS-DEFECTIVE TUMORS

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Background Durable clinical responses to immune checkpoint blockade (ICB) occur in a limited fraction of patients. We thus hypothesized that the characteristic tumor metabolic switch towards aerobic glycolysis could contribute to ICB resistance. High glucose consumption and lactate production by tumor cells can indeed restrict nutrient availability for tumor-infiltrating T cells, which also rely on glycolysis to proliferate and function. Therefore, we investigated whether targeting tumor glucose metabolism potentiates ICB anti-tumor activity.

Methods We modeled tumor-selective glycolysis inhibition by knocking down the critical glycolytic enzyme lactate dehydrogenase A (LDHA-KD) in the murine metastatic breast carcinoma 4T1 and melanoma B16, which are known immunorefractory tumor models. Anti-CTLA-4 and anti-PD-1 were tested in immunocompetent mice orthotopically implanted with control vs. LDHA-KD tumor cells. Changes in glucose metabolism were assessed by Seahorse and fluorescent-glucose flow-cytometry staining. Changes in immune cells were measured by multiparameter flow cytometry. Glucose-dependent effects of anti-CTLA-4 in regulatory T cells (Tregs) were tested in standard suppression assays with increasing glucose concentration (0.5–10 mM). Pearson correlations between glycolysis and intra-tumor immune-cell infiltration by CIBERSORT immune-deconvolution method were analyzed in bulk RNA-
sequencing data sets from human and murine tumors treated with ICB.

Results
Comparison of ICB activity in LDHA-KD vs. control tumor-bearing mice revealed improved anti-tumor effects and overall survival in the setting of glycolysis-defective tumors specifically upon CTLA-4 blockade. Anti-tumor CD8+ T-cell responses correlated with Treg phenotypic and functional destabilization in anti-CTLA-4-treated LDHA-KD tumors. CTLA-4 blockade led to CTLA-4 and CD25 downregulation associated with increased IFN-gamma and TNF-alpha production in Tregs from glycolysis-defective vs. control tumors. We next mimicked high- vs. low-glycolysis tumor microenvironment (TME) in vitro using control vs. LDHA-KD tumor cocultures with Tregs, control vs. LDHA-KD tumors, or directly modulating glucose concentrations. In these assays, we observed that CTLA-4 blockade promotes IFN-gamma±TNF-alpha production and glucose uptake by Tregs and more efficiently counteracts Treg suppression and enhances CD28 co-stimulation at higher glucose concentrations. Lastly, by interrogating transcriptomic data from human melanoma and murine 4T1 tumors, we found that CTLA-4 blockade promotes immune-cell infiltration and metabolic fitness especially in glycolysis-defective tumors.

Conclusions
Our findings indicate that increasing glucose availability in the TME may improve anti-CTLA-4 therapeutic activity and reveal a new mechanism through which CTLA-4 blockade interferes with Treg immunosuppression in a glucose-dependent manner. These results suggest that CTLA-4 blockade can be more effective in tumors with low glycolysis and/or can be best exploited in combination with inhibitors of tumor glycolysis.

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EFFICACY OF SEQUENTIAL IMMUNE CHECKPOINT INHIBITION (ICI) IN PATIENTS WITH GENITOURINARY MALIGNANCIES

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1Emory University School of Medicine, Atlanta, GA, USA; 2Winship Cancer Institute of Emory University, Atlanta, GA, USA

Background
Immune checkpoint inhibitors (ICI) have become a standard of care for treatment of both metastatic renal cell carcinoma (mRCC) and metastatic urothelial carcinoma (mUC). Additional treatment with ICI following disease progression on first-line therapy has become increasingly common for patients with severe disease, but the clinical outcomes of sequential therapy have not been well studied. We report here the clinical outcomes in a cohort of patients with mRCC and mUC who received two regimens of ICI-based therapy.

Methods
We performed a retrospective review of 31 mRCC patients and 11 mUC patients with follow-up data available who received at least 1 dose of a 2nd ICI-based regimen at the Winship Cancer Institute of Emory University from 2015–2020. Radiographic responses were determined using response evaluation criteria in solid tumors version 1.1 (RECISTv1.1). An objective response (OR) was defined as a complete response (CR) or partial response (PR). Clinical benefit (CB) was defined as an objective response or stable disease (SD) > 6 months.

Results
Most patients were white (81%) and male (69%). 31 had mRCC (table 1) and 11 had mUC (table 2). Overall, most patients (58%) received anti-PD-1 (Programmed cell death protein 1) monotherapy as first line, with anti-PD-L1 (Programmed death-ligand 1) monotherapy (33%) and anti-PD-1/CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4) combination therapy (9%) being less prevalent. Patients spent an average of 27.1 weeks on first ICI therapy. Second ICI-based treatment was most commonly anti-PD-1/CTLA-4 (62%), followed by anti-PD-1 monotherapy (38%). A subset of patients (33%) had clinical benefit with combination anti-PD-1/CTLA-255 Table 1
Demographics and treatment data for patients with metastatic renal cell carcinoma

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<th>Variable</th>
<th>n (%)</th>
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<td>Male</td>
<td>32 (71)</td>
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<tr>
<td>Female</td>
<td>9 (29)</td>
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<tr>
<td>Race</td>
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<tr>
<td>White/Asian</td>
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<tr>
<td>Black</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Chemcell histology</td>
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</tr>
<tr>
<td>Yes</td>
<td>24 (77)</td>
</tr>
<tr>
<td>No</td>
<td>7 (23)</td>
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<tr>
<td>Prior Lines of Therapy</td>
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<tr>
<td>2</td>
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<tr>
<td>≥3</td>
<td>11 (37)</td>
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<tr>
<td>Number of distant metastatic sites</td>
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<td>1-2</td>
<td>9 (29)</td>
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<tr>
<td>≥3</td>
<td>10 (32)</td>
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<tr>
<td>First ICI Regimen</td>
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<tr>
<td>PD-1 monotherapy</td>
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<tr>
<td>PD-L1 monotherapy</td>
<td>4 (13)</td>
</tr>
<tr>
<td>PD-L1/CTLA-4 combination</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Reason for 2nd ICI Regimen</td>
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<tr>
<td>Progression</td>
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<tr>
<td>Toxicity</td>
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<tr>
<td>Completion</td>
<td>2 (6)</td>
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<tr>
<td>Second ICI Regimen</td>
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<td>PD-1 monotherapy</td>
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<tr>
<td>PD-L1/CTLA-4 combination</td>
<td>15 (48)</td>
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<tr>
<td>Best Radiographic Response to 2nd ICI Regimen</td>
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<tr>
<td>PD</td>
<td>10 (31)</td>
</tr>
<tr>
<td>SD</td>
<td>7 (23)</td>
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<tr>
<td>CR</td>
<td>4 (13)</td>
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<tr>
<td>CB Rate: 16.7%</td>
<td>11 (35)</td>
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255 Table 2
Demographics and treatment data for patients with urothelial cell carcinoma

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<td>Gender</td>
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</tr>
<tr>
<td>Male</td>
<td>7 (64)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (36)</td>
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<tr>
<td>Race</td>
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<tr>
<td>White/Asian</td>
<td>5 (45)</td>
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<tr>
<td>Black</td>
<td>2 (18)</td>
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<tr>
<td>Prior Lines of Therapy</td>
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</tr>
<tr>
<td>0-2</td>
<td>4 (36)</td>
</tr>
<tr>
<td>3-4</td>
<td>4 (36)</td>
</tr>
<tr>
<td>≥5</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Number of distant metastatic sites</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>6 (54)</td>
</tr>
<tr>
<td>≥3</td>
<td>2 (18)</td>
</tr>
<tr>
<td>First ICI Regimen</td>
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<tr>
<td>PD-1 monotherapy</td>
<td>1 (9)</td>
</tr>
<tr>
<td>PD-L1 monotherapy</td>
<td>10 (91)</td>
</tr>
<tr>
<td>Reason for 2nd ICI Regimen</td>
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</tr>
<tr>
<td>Progression</td>
<td>30 (93)</td>
</tr>
<tr>
<td>Toxicity</td>
<td>0</td>
</tr>
<tr>
<td>Completion</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Second ICI Regimen</td>
<td></td>
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<tr>
<td>PD-1 monotherapy</td>
<td>10 (91)</td>
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<tr>
<td>PD-L1/CTLA-4 combination</td>
<td>1 (9)</td>
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<tr>
<td>Best Radiographic Response to 2nd ICI Regimen</td>
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</tr>
<tr>
<td>PD</td>
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</tr>
<tr>
<td>SD</td>
<td>4 (40)</td>
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<td>CR</td>
<td>0</td>
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<tr>
<td>CB Rate: 40%</td>
<td>0</td>
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4-based second ICI therapy, with 4 (10%) having PR and one (2%) having CR of disease following second ICI-based treatment. Patients spent an average of 21.4 weeks on the second ICI regimen. The response rate for the entire cohort was 11.9% (16.7% for RCC and 0% for UC). The CR rate for the entire cohort was 40% (40% for RCC and 40% for UC). Immune-related adverse events were experienced in a subset of patients (28%).

Conclusions Although we observed a low OR rate to a second ICI-based regimen, a select subset of patients did have CB from a second ICI regimen. Current studies exploring the addition of CTLA4 inhibitors to anti-PD-1 therapy may provide insight into the greater efficacy of treatment within a subset of patients. Further analysis of a larger cohort receiving sequential immunotherapy is necessary to better identify patients who may be more likely to derive CB from sequential ICI.

Ethics Approval This retrospective study was approved by the Emory University Institutional Review Board.

Consent Not applicable.

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Trial Registration Not applicable.

REFERENCES
Not applicable

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Clinical trials completed

256 SINGLE-AGENT ZALIFRELIMAB (ANTI-CTLA-4) SHOWS CLINICAL BENEFIT IN RARE TUMORS – CASE REPORT FROM PHASE 2 STUDY (NCT03104699)

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Background Zalifrelimab is a fully human monoclonal antibody against cytotoxic T-lymphocyte -associated protein 4 (CTLA-4). Preliminary data demonstrated clinical benefit and tolerability, as monotherapy, in patients with recurrent solid tumors including rare tumor types. Previously presented Phase 1 data reported one durable complete response in recurrent cutaneous angiosarcoma (cAS).1 Here we report additional clinical responses from an ongoing Phase 2 study of zalifrelimab monotherapy including clinical benefit in rare solid tumors.

Methods In an ongoing, phase 2 study (NCT03104699), the safety and efficacy of zalifrelimab, as monotherapy, was evaluated in patients who progressed on prior anti-PD-1/L1 therapy. All patients were treated intravenously (IV) with zalifrelimab at 1 mg/kg every 3 weeks until disease progression or up to 2 years.

Results Overall, 44 patients were treated and 29 patients were response-evaluable at the time of report. In patients with refractory solid tumors treated with zalifrelimab, we report a disease control rate (CR, PR, and SD) of 51.7%, objective response rate (ORR) of 10.3% (3/29), disease stabilization of 41.3% (12/29). Clinical activity was observed in five solid tumors considered rare, including; cAS (N=1), glucagonoma (N=1), chondrosarcoma (N=1), spindle-cell sarcoma (N=1) and fibroblastic sarcoma (N=1). In these rare tumors, durable partial responses of 45 and 30 weeks were observed in cAS of the scalp with lymph node metastases and glucagonoma, respectively. Both patients remain on zalifrelimab with no evidence of disease progression. Additionally, durable disease stabilization was observed in a patient with spindle-cell sarcoma. Patients with chondrosarcoma and fibroblastic sarcoma progressed on therapy. Zalifrelimab was well tolerated with the most commonly reported treatment-related adverse events including fatigue, nausea, anemia, diarrhea and vomiting, consistent with the drug class. Most events were mild or moderate and resolved with standard treatments.

Conclusions Our data demonstrates the potential for Zalifrelimab to promote meaningful clinical benefit in difficult to treat tumors, including patients that progress on prior PD-1/PD-L1 therapy or chemotherapy. Notably, responses were observed in rare tumor types such as recurrent cutaneous angiosarcoma and glucagonoma. Treatment with zalifrelimab is safe and well tolerated in patients with advanced malignancies.

Trial Registration NCT03104699.

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0256

257 DEVELOPMENT OF A DIAGNOSTIC PLATFORM WHICH MATCHES THERAPIES TO THE TUMOR MICROENVIRONMENT DOMINANT BIOLOGY

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Background Tumor microenvironment (TME)-targeting agents such as anti-angiogenic therapies and check-point inhibitors (CPIs), have shown both promise and variability in effectiveness depending on the tumor type. For immune-targeting agents like CPIs, efforts to identify features or biomarkers that predispose responding patients include but are not limited to genomic stability, tumor mutation burden, and PD-L1 expression. Oncologie is developing a RNA-based platform that identifies subsets of patients based on multiple aspects of the biological processes (dominant biology) existing within the tumor microenvironment.

Methods RNA data from publicly available sources including microarray, RNASeq exome and whole RNA were analyzed with respect to gene signatures that describe four different microenvironmental phenotypes. Phenotypes were then evaluated for relationships to clinical efficacy endpoints. From these RNA signatures and driven by machine learning methodologies, drug-specific algorithms were developed and applied to retrospectively to clinical data. Comparative analyses were explored between gene signatures, commonly used biomarkers (eg. presence of microsatellite DNA, expression levels of PD-L1, etc) and within-patient metadata to better understand better how this approach can be utilized in prospective clinical studies.

Results Attributes in RNA expression identified using Oncologie’s platform have retrospectively characterized responders to CPIs or anti-angiogenic drugs, demonstrating a relationship between clinical response and biomarker positive and negative patient populations. Exploratory data summarizing the use of this platform demonstrates its utility for enriching
response to both immune- and angiogenesis-targeting drugs. Relative expression changes between archival and fresh biopsies demonstrate changes in the TME with time and/or following targeted therapy. Lastly, cross-tumor comparisons support a tumor-agnostic utility of this approach. Detailed comparisons of this biomarker approach relative to other available biomarkers will be presented for standard of care drugs and those in the Oncologie pipeline based on retrospective analyses.

Conclusions RNA based descriptors of biology may be a useful approach to enrich for response to targeted therapies whose mechanism of action is to modify the TME biology.

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SCIENTIFIC CORRELATES FROM LCCC 1525: A PHASE II STUDY OF A PRIMING DOSE OF CYCLOPHOSHAMIDE PRIOR TO PEMBROLIZUMAB TO TREAT METASTATIC TRIPLE NEGATIVE BREAST CANCER

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Background In metastatic triple negative breast cancer (mTNBC), median progression-free survival (PFS) with chemotherapy alone is approximately 2–4 months and improvements with single agent checkpoint inhibitors (CI) are limited by modest response rates. Murine breast cancer models have demonstrated a role for intratumoral regulatory T cells (Tregs) in modulating response to CIs. A phase II clinical trial was conducted to test the hypothesis that a single, priming dose of cyclophosphamide prior to pembrolizumab would improve...
Phases Ib/II open-label, randomized evaluation of pembrolizumab (I) versus those who did not, in pre-treatment PBMC samples. G-I: Similarities between patients who derived clinical benefit (E) or response (D) and patients who did not (B). E-F: Inter-group comparisons showed fewer TRB chain similarities between patients who derived clinical benefit (E) or response (F) versus those who did not, in pre-treatment PBMC samples. G-I: Univariate Cox proportional hazards models for PFS showing immune diversity measures derived from pre-treatment tumor RNA-Seq (G), PBMC-derived amplicon sequencing pre-pembrolizumab (H), and PBCM-derived amplicon sequencing pre-pembrolizumab (I).
Methods MORPHEUS-PDAC, MORPHEUS-TNBC and MORPHEUS-CRC enrolled 1L metastatic (m) PDAC, 2L locally advanced or mTNBC or 3L mCRC patients, respectively. Experimental arm patients received atezo (840 mg IV q2w) and seli (16 mg SC on D1 every 28-day cycle for C1-4 and every third cycle thereafter). Patients also received gem (1000 mg/m²) and nabP (1000 mg/m², 125 mg/m² respectively, IV on D1, 8, 15 every 28-day cycle) in PDAC or bev (10 mg/kg IV q2w) in TNBC and CRC. Control treatments were gem + nabP in PDAC, capectabine in TNBC, and regorafenib in CRC. Primary endpoints were safety and objective response rate (ORR; investigator-assessed RECIST 1.1). PD-L1 and CD8/panCK IHC were tested in all biopsies.

Results All treated patients were safety evaluable. MORPHEUS-PDAC (20-week interim analysis): 9 patients received atezo+seli+gem+nabP and 4 received control. Treatment-related adverse events (TRAEs) were seen in all. Treatment-related serious AEs (SAEs) occurred in 6 patients (67%) receiving atezo+seli+gem+nabP and 4 (50%) receiving control. TRAEs were seen in all patients receiving atezo+seli+bev and 1 (8%) receiving control. Treatment-related SAEs occurred in 3 patients (50%) receiving atezo+seli+bev and 1 (8%) receiving control. All 6 patients receiving atezo+seli+bev were PD-L1 negative (SP142 IHC assay) at baseline; the only patient with partial response (PR) showed upregulation of PD-L1 expression at week 3. MORPHEUS-TNBC (27-week interim analysis): 6 patients received atezo+seli+bev and 24 received control. TRAEs were seen in 5 patients (83%) receiving atezo+seli+bev and 18 (75%) receiving control. Treatment-related SAEs occurred in 1 patient in each arm (17% and 4%, respectively). Confirmed ORRs: 17% (95% CI:0.4–64) and 21% (95% CI:0.7–42), respectively. All 6 patients receiving atezo+seli+bev were PD-L1 negative (SP142 IHC assay) at baseline; the only patient with partial response (PR) showed upregulation of PD-L1 expression at week 3. MORPHEUS-CRC (18-week interim analysis): 6 patients received atezo+seli+bev and 13 received control. TRAEs were seen in all patients receiving atezo+seli+bev and 12 (92%) receiving control. Treatment-related SAEs occurred in 3 patients (50%) receiving atezo+seli+bev and 1 (8%) receiving control. No responses occurred in either study arm. Paired biopsies for 3 patients (60%) receiving atezo+seli+bev suggest on-treatment increases in CD8 T-cell infiltration into tumors.

Conclusions Toxicities related to the atezo+seli combinations were consistent with individual study treatments. Preliminary efficacy was observed for atezo+seli+gem+nabP in PDAC. Together with preliminary evidence of on-treatment pharmacodynamic effects in CRC and TNBC tumor samples, CD40 agonist strategies warrant further investigation.

Trial Registration MORPHEUS-PDAC: NCT03193190; MORPHEUS-TNBC: NCT03424005; MORPHEUS-CRC: NCT03555149.

Ethics Approval The trial was conducted according to the principles of the Declaration of Helsinki. All patients provided written informed consent. Protocol approval was obtained from independent review boards or ethics committees at each site.

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Abstract 260 Figure 1 Following neoadjuvant therapy, patients with resectable pancreatic cancer with a higher than median intratumoral TCR Vβ Diversity 50 (n=9, 4.624 HR; 95 CI [0.971, 21.83]) have greater overall survival compared to patients with a lower than median intratumoral TCR Vβ Diversity 50 (n=10, 0.2163 HR; 95 CI [0.458, 60 total CD3s). Patients with a higher than median TCR Vβ Diversity 50 Index (D50, proportion of uCD3s that make up 50% of the total CD3s) had significantly higher tumor CD4 (p = 0.003) and CD8 (p = 0.031) counts. Patients with a higher than median TRC Vβ D50 also had a reduced lymph node ratio (p = 0.039) and greater overall survival (p = 0.037, figure 1). Conversely, patients with a higher than median BCR IgH D50 had worse overall survival (p = 0.0241). Given the dichotomy of the TCR and BCR repertoire diversity and association with clinical outcome, we further analyzed the individual ratio of TRC Vβ:BCR IgH CDR3s and found that patients with a higher than median TRC Vβ:BCR IgH ratio had a greater Evans’s Grade histopathologic response (p = 0.069).

Conclusions PDAC TIL repertoire with high TCR Vβ diversity is associated with decreased positive lymph node ratio and greater overall survival following neoadjuvant therapy. The
Abstracts

261 ASSOCIATION OF T-CELL–INFILAMED GENE EXPRESSION PROFILE AND PD-L1 STATUS WITH EFFICACY OF PEMBROLIZUMAB IN PATIENTS WITH ESOPHAGEAL CANCER FROM KEYNOTE-180

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Background Key biomarkers under investigation for the ability to predict response to monotherapy PD-1 inhibitors such as pembrolizumab include PD-L1, TMB, MSI, and T-cell–inflamed gene expression profile (GEP). The KEYNOTE trial (NCT02559687) was a single-arm phase 2 study of pembrolizumab as third-line or greater therapy in advanced/metastatic esophageal/gastroesophageal junction adenocarcinoma or squamous cell carcinoma (SCC). ORR was 9.9% and median DOR was NR at the primary analysis. We investigated the relationship in KEYNOTE-180 between response to pembrolizumab and T-cell–inflamed GEP or PD-L1 expression by histology.

Methods Patients received pembrolizumab 200 mg Q3W for ≤2 years until disease progression, toxicity, or withdrawal. The end points for this analysis were ORR, DOR, and PFS per RECIST v1.1 by central review and OS in the SCC and adenocarcinoma populations by GEP (non-low [≥1.540]; high [<1.540]; cutoff prespecified) and PD-L1 (CPS ≥10 or <10). Tumor GEP was determined using the NanoString nCounter Analysis System. PD-L1 expression was characterized using PD-L1 IHC 22C3 pharmDx. Data cutoff date was July 30, 2018.

Results Of 121 total patients, 118 had an evaluable GEP score and 121 had an evaluable PD-L1 CPS. Fifty-one patients (42.1%) had GEPnon-low tumors, 58 (48.0%) had CPS ≥10 tumors, and 31 (25.6%) had GEPnon-low/CPS ≥10 tumors; 63 patients (52.1%) had SCC and 58 (47.9%) had adenocarcinoma. ORR was 15.4% with GEPnon-low and 13.5% with GEPlow among patients with SCC and 12% and 0% among patients with adenocarcinoma, respectively (table 1). ORR was 20% with CPS ≥10 and 7.1% with CPS <10 among patients with SCC and 4.3% and 5.7%, respectively, among patients with adenocarcinoma (table 2). Median OS was slightly higher among patients with SCC in the GEPnon-low subgroup and the CPS ≥10 subgroup versus GEPlow and CPS <10 subgroups, respectively (tables 1, 2); this trend was reversed among patients with adenocarcinoma (tables 1, 2). Median PFS ranged from 1.9 to 2.1 among histology/biomarker subgroups. Median DOR was NR regardless of GEP or CPS status (tables 1, 2).

Abstract 261 Table 1 Response by GEP status and histology

<table>
<thead>
<tr>
<th>GEP Status</th>
<th>SCC</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>SCC</td>
<td></td>
</tr>
<tr>
<td>GEPnon-low</td>
<td>n = 36</td>
<td>6 (16.7)</td>
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<tr>
<td>GEPlow</td>
<td>n = 37</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>GEPnon-low/CPS ≥10</td>
<td>n = 35</td>
<td>3 (8.6)</td>
</tr>
<tr>
<td>GEPlow/CPS &gt;0</td>
<td>n = 30</td>
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</table>

<table>
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<tr>
<th>ORR (%)</th>
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<th>(n = 5)</th>
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</thead>
<tbody>
<tr>
<td>Median DOR (months)</td>
<td>2.1 (1.9-4.1)</td>
<td>2.1 (1.9-3.8)</td>
</tr>
<tr>
<td>GEPnon-low</td>
<td>2 (1.0-1.2)</td>
<td>2 (1.0-2.1)</td>
</tr>
<tr>
<td>GEPlow</td>
<td>1.9 (1.6-2.0)</td>
<td>1.9 (1.6-2.0)</td>
</tr>
</tbody>
</table>

*Analysis by biomarker status was not possible because of the small sample size.

Abstract 261 Table 2 Response by PD-L1 status and histology

<table>
<thead>
<tr>
<th>GEP Status</th>
<th>SCC</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>SCC</td>
<td></td>
</tr>
<tr>
<td>GEPnon-low</td>
<td>n = 35</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td>GEPlow</td>
<td>n = 29</td>
<td>2 (7.0)</td>
</tr>
<tr>
<td>GEPnon-low/CPS ≥10</td>
<td>n = 23</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>GEPlow/CPS &gt;0</td>
<td>n = 10</td>
<td>2 (20.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ORR (%)</th>
<th>(n = 7)</th>
<th>(n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median DOR (months)</td>
<td>2.0 (1.7-2.0)</td>
<td>2.0 (1.7-2.0)</td>
</tr>
<tr>
<td>GEPnon-low</td>
<td>2.0 (1.0-2.1)</td>
<td>2.0 (1.0-2.1)</td>
</tr>
<tr>
<td>GEPlow</td>
<td>1.9 (1.7-2.0)</td>
<td>1.9 (1.7-2.0)</td>
</tr>
</tbody>
</table>

*Analysis by biomarker status was not possible because of the small sample size.

Conclusions In KEYNOTE-180, a data in small number of patients suggested that measures of inflammation, like PD-L1...
and GER may enrich for responses to pembrolizumab. In SCC, some trends toward enrichment were observed for both biomarkers, although the trend was stronger for PD-L1 CPS ≥10. In adenocarcinoma, a trend was observed for GEP but not for PD-L1; the small number of responders is limiting, and further studies are needed to understand molecular correlates in adenocarcinoma.

Acknowledgements Medical writing and/or editorial assistance was provided by Tim Peoples, MA, ELS, and Holly C. Cappelli, PhD, CMP, of the ApotheCom pembrolizumab team (Yardley, PA, USA). This assistance was funded by Merck Sharp & Dohme Corp, a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

Trial Registration ClinicalTrials.gov, NCT02559687

Ethics Approval The study and the protocol were approved by the institutional review board or ethics committee at each site. Consent All patients provided written informed consent to participate in the clinical trial.

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262 TUMORAL DKK1 EXPRESSION CORRELATES WITH BETTER CLINICAL OUTCOMES IN PATIENTS WITH ADVANCED ESOPHAGOASTRIC CANCER (EGC) TREATED WITH DKN-01

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Background Dickkopf-1 (DKK1) modulates Wnt signaling and contributes to an immune suppressive tumor microenvironment. DKN-01 (D), a neutralizing DKK1 antibody, has demonstrated safety and clinical activity in advanced EGC either as a monotherapy or in combination with paclitaxel (pac) or pembrolizumab (pem). We report response and survival outcomes in EGC patients (pts) by high/low tumoral DKK1 expression treated with D.

Methods We enrolled advanced EGC pts in a Phase 1b/2a study of D as monotherapy or in combination with pac or pem. Tumoral DKK1 mRNA expression was assessed by an in situ hybridization RNAscope assay. Objective response rate (ORR), disease control rate (DCR), progression free survival (PFS) and overall survival (OS) were compared between DKK1 high and low groups. Kaplan-Meier method and Cox PH model were used for survival analysis and logistic regression was used for clinical benefit/response outcome.

Results 69 EGC pts were enrolled to receive D alone or in combination with pac or pem and had tumoral DKK1 expression available. 60 pts (87%) were male, median age 65 (range 28, 81). 59 pts had adenocarcinoma [19 esophageal (28%), 40 GEJ/gastric (58%)] and 10 pts with ESCC. 65% had ≥2 prior therapies (range 1, 5). 23 pts with DKK1 high (H-score ≥ upper-tertile [≥39]) had an ORR of 22% (5 PR/23), DCR of 57% (13/23) while DKK1 low (H-score <39) had an ORR of 2% (1/46) and DCR of 26% (12/46). Median PFS was 12.1 weeks in DKK1 high vs. 6.0 weeks in DKK1 low; HR of 0.58 (95%CI: 0.34, 1.0). Median OS was 31.6 weeks in DKK1 high vs. 13.4 weeks in DKK1 low; HR of 0.70 (95%CI: 0.38, 1.3). Subgroup of pts (n=9) with immune checkpoint inhibitor (ICI) refractory disease treated with D + pem demonstrated longer PFS and OS for DKK1 high pts (H-score ≥39, n=4) vs DKK1 low (n=5); PFS 12.8 weeks vs 6.0 weeks; HR of 0.16 (95%CI: 0.02, 1.5) and OS 46 weeks vs. 16 weeks, respectively; HR of 0.22 (95%CI: 0.03, 2.0).

Conclusions High levels of tumoral DKK1 expression correlate with improved clinical outcomes in heterogeneous EGC pts treated with D monotherapy or in combination. Previously we have demonstrated greatest clinical benefit in ICI-naïve, DKK1 high G/GEJ adenocarcinoma treated with D + pem.1 DKK1 high ICI refractory pts treated with D + pem experienced longer PFS and OS compared with pts with low DKK1 expression. DKK1 as a predictive biomarker for DKN-01 is being evaluated in ongoing studies.

Trial Registration NCT02013154

Ethics Approval WIRB (Western Institutional Review Board) Institution’s Ethics Board, approval number 20140759

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0262

263 PLEIOTROPIC EFFECTS OF IL-7 IN PROSTATE CANCER PATIENTS RECEIVING SIPULEUCEL-T VACCINATION

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Background Sipuleucel-T (Provenge) is the first therapeutic vaccination approved by the FDA so far, indicated for advanced metastatic prostate cancer patients. Despite an improvement of the overall survival, the benefits of the therapy are still short-term so increasing the duration of the efficacy is necessary. Specifically, T-cell anergy is one of the challenges that we need to overcome to improve the overall efficacy. IL-7 is known to promote the naive T cell activation and to increase the proliferation and activation of the T cell memory subsets. Therefore, in this phase II clinical trial, we tested the therapeutic potential of a human recombinant glycosylated IL-7 after completion of the Provenge therapy on asymptomatic advanced prostate cancer patients.

Methods To get a comprehensive analysis of the immune landscape in these patients, we performed CyTOF analysis on PBMC samples obtained at week 1 (baseline) and week 6 after the beginning of the IL-7 therapy. After stimulation with PMA/Ionomycin, we proceeded to surface and intracellular cytokine staining before acquisition on the CyTOF. The data were then analyzed by expert gating on Cytobank.

Results At 6 weeks post therapy, our data showed an increase in the number of circulating T lymphocytes in the IL-7 cohort, especially CD8 T cells, in accordance with previous literature. Even though of the frequency of CD4 T cells did not increase, the cells showed greater functionalities, with increased expression of IL-2, TNFα and IL-6 upon stimulation by PMA-Ionomycine. Cytotoxic subsets were also positively affected, with increased expression of IFNγ in CD8 T cells, TNFα in NK cells and IL-2 in γδ T cells. Moreover, PD-1 expression was decreased on CD4, CD8 and γδ T cells while CD137 increased on CD4, CD8 and NK cells. In addition, despite a reduction in the pool of circulating monocytes, we observed higher TNFα expression in these cells.
Conclusions  Altogether, our data revealed multiple effects of IL-7 in these patients, highlighting a complex set of in vivo mechanisms. In the future, knowledge of these effects may help in choosing the best agents to use in combination with IL-7 and/or the best patients to benefit from IL-7 as part of their therapeutic approach.

Trial Registration  NCT01881867

Ethics Approval  The study was last approved by Fred Hutchinson Cancer Research Center Institutional Review Board, IR file 8037, on January 23, 2020.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0263

Abstract 270 Figure 1

a. Waterfall plot indicating maximum change in baseline tumor measurement following treatment. Crosshatch indicate patients failed therapy and do not have tumor measurements available due to early clinical progression or progression due to new lesions without RECIST measurable changes (N=2). b. Swimmer’s plot of PFS following therapy. Triangles idicated confirmed PR, targets indicate unconfirmed PR and squares indicate SD.

Conclusions  Combination therapy with ipilimumab plus nivolumab in thyroid cancer resulted in an ORR of 12% with two partial responses in seventeen treated patients.

Trial Registration  NCT02834013

Ethics Approval  The study was approved by the NCI Adult Central Institutional Review Board, approval number 02834013.

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Abstract 264

Correlation of Virus-Specific CD8+ T Cells to Clinical Response Following Treatment with PEXA-VEC and Cemiplimab in Patients with Advanced Renal Cell Carcinoma

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Background  To better understand the immune stimulatory mechanisms of Oncolytic virus (OV), we evaluated the circulating OV-specific T cell response in patients during the course of OV therapy. Patients with histologically confirmed advanced clear cell renal cell carcinoma, who were naïve or refractory to prior systemic treatment and who had no prior treatment with immune checkpoint inhibitors, were treated with 4 weekly intravenous infusions of Pexa-Vec at 1 × 10^9 plaque forming units starting at Day -7 plus Cemiplimab (350 mg every 3 weeks) from Day 1. Radiographic assessments per RECIST 1.1 were performed centrally every 9 weeks from baseline. Crosshatch indicate patients failed therapy and do not have tumor measurements available due to early clinical progression or progression due to new lesions without RECIST measurable changes (N=2).
Methods

We performed functional IFNγ ELISPOT analysis on longitudinal PBMC samples using a custom panel of OV epitopes and culture conditions designed to measure existing OV-specific memory T cell cytolytic activity [1]. PBMC samples were tested for IFNγ release following stimulation with OV peptides using two different assay conditions: 1) measurement following direct ex vivo stimulation with OV peptides alone, and 2) measurement following 10 days of T cell expansion in the presence of OV peptides, T cell supportive cytokines (GM-CSF, IL-4, IL-7, and IL-15), and autologous dendritic cells. The number of OV-specific IFNγ spots was correlated with the clinical response and tumor regression.

Results

In preliminary analyses, 8 of the 11 (72.7%) patients showed tumor burden reduction, 4 of whom had ≥30% confirmed reduction that qualify as RECIST1.1 PRs (figure 1 and 2). OV-specific IFNγ+ T cells were detected in only 3 out of 11 patients in the non-expanded ELISPOT culture conditions (figure 3A), but in 8 out of 11 patients when T cells were first expanded for 10 days in the presence of OV peptides prior to ELISPOT, which trended toward a correlation with the preliminary clinical response assessment (figure 3B). Prolonged stimulation with CMV, EBV, and Influenza peptides did not show any correlation (R2 = 0.005), suggesting that the treatment and culture expansion influenced relevant OV-specific memory T cell proliferation.

Conclusions

These results suggest that OV-specific T cell responses can be induced by OV therapy. In addition, 10-day expansion of low levels of OV-specific circulating T cells can amplify signals in ELISPOT analysis and might enable systemic tracking of patient responses in blood samples collected at early time points. The observed CD8+ T cell response to oncolytic vaccinia virus in patients supports the rationale for combination treatment with Pexa-Vec and immune checkpoint inhibitors.

Acknowledgements

Sun Young Rha, Yonsei Cancer Center, Severance Hospital, Yonsei University Health System, Seoul, Republic of Korea; Jamie Merchan, University of Miami Health System, Miami, FL, USA; Sung Yong Oh, Dong-A University Hospital, Busan, Republic of Korea; Chan Kim, Cha University Bundang Medical Center, Seongnam, Republic of Korea; Woo Kyun Bae, Chonnam National University Hwasun Hospital, Hwasun, Republic of Korea; Hyun Woo Lee, Ajou University Hospital, Suwon, Republic of Korea.

Trial Registration

NCT03294083

Ethics Approval

The study was approved by University of Miami Institutional Review Board, approval number 20180055.

REFERENCE


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0264
Background
Avelumab is an anti–PD-L1 monoclonal antibody approved for the treatment of advanced UC after disease progression during or following platinum-based chemotherapy and as maintenance treatment in patients whose disease has not progressed with first-line platinum-based chemotherapy.1–3 M9241 is an immunomodulatory cytokine composed of 2 heterodimers of IL-12 fused to the heavy chains of a human antibody targeting DNA released from necrotic tumor cells.4,5 During dose-escalation, avelumab + M9241 was well tolerated and showed promising antitumor activity in patients with advanced solid tumors, including 2 objective responses in patients with UC.5 We report on an interim analysis of efficacy and safety from the dose-expansion part of JAVELIN IL-12 (NCT02994953).

Methods
Eligible patients had locally advanced or metastatic UC that had progressed on first-line therapy, were aged ≥18 years, had an Eastern Cooperative Oncology Group performance status of 0/1, and were immune checkpoint inhibitor naive. Patients received the recommended phase 2 dose5 of avelumab 800 mg intravenously once weekly (QW) in combination with M9241 16.8 μg/kg subcutaneously Q4W for the first 12 weeks, then continued the combination with avelumab Q2W. The primary endpoints were confirmed best overall response status of 0/1, and were immune checkpoint inhibitor naive. Patients received the recommended phase 2 dose5 of avelumab 800 mg intravenously once weekly (QW) in combination with M9241 16.8 μg/kg subcutaneously Q4W for the first 12 weeks, then continued the combination with avelumab Q2W. The primary endpoints were confirmed best overall response (BOR) per investigator assessment (RECIST 1.1) and safety. The expansion cohort followed a 2-stage design. During stage 1 (single-arm part of the study), 16 patients were enrolled and treated. A futility analysis based on BOR was planned to determine if stage 2 (randomized controlled part of the study) would be initiated.

Results
At data cut-off (Jun 3, 2020), 16 patients had received avelumab + M9241 for a median duration of 8 weeks (range, 4.0–25.0 weeks). No complete or partial responses were observed; the study failed to meet the criterion (>2 responders) to initiate stage 2. Two patients (12.5%) had stable disease, 13 (81.3%) had progressive disease, and 1 (6.3%) was not evaluable. Any-grade treatment-related adverse events (TRAEs) occurred in 15 patients (93.8%); the most common (in ≥4 patients) were pyrexia (50.0%), nausea (37.5%), asthenia (31.3%), anemia (25.0%), and hyperthermia (25.0%); grade 4 gamma-glutamyltransferase increased occurred in 1 patient (6.3%). No TRAEs led to death. Pharmacodynamic effects on the peripheral immune system and results of pharmacokinetic and biomarker analyses will also be reported.

Conclusions
The predefined efficacy criterion to proceed to stage 2 was not met. The combination was well tolerated; no new safety signals emerged and the profile was consistent with the dose-escalation part of the study.5

Trial Registration
NCT02994953

Ethics Approval
The study was approved by each site’s independent ethics committee.

Consent
N/A

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0265
Conclusions Both bTMB and rTMB are potentially useful biomarkers for enriching responses to D+T in previously untreated, advanced UC. Neither bTMB nor rTMB was associated with better outcomes for D monotherapy. Cutoffs of 24 mut/Mb for bTMB and 10 mut/Mb for rTMB appear optimal for D monotherapy. bTMB and rTMB are potentially useful biomarkers for enriching responses to D+T in previously untreated, advanced UC.

**References**


**Ethics Approval**

The study protocol was approved by the Ethics Board at each investigator’s institution.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0266

**267 PSEUDOPROGRESSION PATTERNS: ANALYSIS FROM 2 INDEPENDENT PHASE-2 STUDIES WITH IMMUNOTHERAPY FOR RECURRENT CERVICAL CANCER**


**Background**

The phenomenon of pseudoprogression (PsP) may appear with cancer immunotherapy. The underlying etiology is not fully elucidated, tumor flare is the suspected mechanism of early pseudoprogression that may resolve gradually while continuing treatment. Further, immunotherapy-induced sarcoidosis may mimic PsP. Here we present examples of 3 observed patterns of PsP in cervical cancer (CC) patients treated with balstilimab (BAL; anti-PD-1), alone or in combination with zalifrelimab (ZAL; anti-CTLA-4).

**Methods**

The evaluated patients received either BAL 3 mg/kg every 2 weeks alone (NCT03104699) or in combination with ZAL dosed at 1 mg/kg every 6 weeks (NCT03495882). PsP was defined as radiologic disease progression per RECIST1.1 followed by a significant reduction of measurable baseline lesions, disappearance of the non-measurable lesions, or no further progression for at least two tumor assessments after initial progressive disease (PD) by Independent Evaluation Review Committee (IERC). PsP was divided into 3 categories – early (before or at week 12 of treatment), delayed (after week 12) and serial (at least 2 PsP occurrences).

**Results**

Overall, 313 patients with post-chemotherapy recurrent CC with baseline measurable disease were treated with either BAL (n=160) or in combination with ZAL (n=153). Early PsP was observed in 7 patients treated with BAL and 8 with BAL/ZAL while 5 patients experienced delayed PsP (BAL (n=1); ZAL(n=4)). Serial PsP was observed in 1 patient (BAL only) and another (n=1) BAL treated experienced showed PsP (new Mediastinal lesions) present in 2 consecutive CT scan evaluations before disappearance – hence were classified as PD even by iRECIST. Immune-related sarcoidosis was confirmed histologically in 2 patients following confirmation by mediastinal lymph node biopsy. PsPs were accompanied with clear clinical benefit of disease improvement and weight stabilization, improvement in performance status, and decreased pain.

**Conclusions**

This is the first report of PsP in CC population. PsP-confounded IERC evaluation of tumor response was seen in some CC patients treated with BAL or combination of BAL and ZAL. The differentiation of PD and PsP has important consequences for disease assessment in clinical trials and disease management and outcomes. Further efforts to elucidate the underlying mechanisms and clearly define the characteristics of PsP are crucial for better treatment management of affected patients. Standard response evaluation systems including iRECIST may need further refinement to recognize the importance of PsP.

**Trial Registration**

NCT03104699 and NCT03495882

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0267

**268 A PHASE 1 STUDY OF RETIFANLIMAB (INCMGA00012), A PD-1 INHIBITOR, IN PATIENTS WITH ADVANCED SOLID TUMORS: PRELIMINARY RESULTS IN RECURRENT MSI-HIGH OR DMMR ENDOMETRIAL CANCER (POD1UM-101)**

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**Background**

The evaluated patients received either BAL 3 mg/kg every 2 weeks alone (NCT03104699) or in combination with ZAL dosed at 1 mg/kg every 6 weeks (NCT03495882). PsP was defined as radiologic disease progression per RECIST1.1 followed by a significant reduction of measurable baseline lesions, disappearance of the non-measurable lesions, or no further progression for at least two tumor assessments after initial progressive disease (PD) by Independent Evaluation Review Committee (IERC). PsP was divided into 3 categories – early (before or at week 12 of treatment), delayed (after week 12) and serial (at least 2 PsP occurrences).

**Results**

Overall, 313 patients with post-chemotherapy recurrent CC with baseline measurable disease were treated with either BAL (n=160) or in combination with ZAL (n=153). Early PsP was observed in 7 patients treated with BAL and 8 with BAL/ZAL while 5 patients experienced delayed PsP (BAL (n=1); ZAL(n=4)). Serial PsP was observed in 1 patient (BAL only) and another (n=1) BAL treated experienced showed PsP (new Mediastinal lesions) present in 2 consecutive CT scan evaluations before disappearance – hence were classified as PD even by iRECIST. Immune-related sarcoidosis was confirmed histologically in 2 patients following confirmation by mediastinal lymph node biopsy. PsPs were accompanied with clear clinical benefit of disease improvement and weight stabilization, improvement in performance status, and decreased pain.

**Conclusions**

This is the first report of PsP in CC population. PsP-confounded IERC evaluation of tumor response was seen in some CC patients treated with BAL or combination of BAL and ZAL. The differentiation of PD and PsP has important consequences for disease assessment in clinical trials and disease management and outcomes. Further efforts to elucidate the underlying mechanisms and clearly define the characteristics of PsP are crucial for better treatment management of affected patients. Standard response evaluation systems including iRECIST may need further refinement to recognize the importance of PsP.

**Trial Registration**

NCT03104699 and NCT03495882

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0267
Tumor and Peripheral Landscape of Viral- versus Carcinogen-Driven Head and Neck Cancer

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Astrazeneca, Munich, Germany

Background: Head and neck squamous cell carcinoma (HNSCC) is composed of a heterogeneous group of tumors arising through environmental carcinogens or infection by human papillomavirus (HPV). Treatment interventions such as immunotherapy and targeted therapy have shown clinical benefit in HNSCC patients. Despite these encouraging results, resistance to treatment is still observed in the majority of patients. Additionally, clinical effectiveness of treatment options has also been shown to be associated with HPV status. Here we investigate the tumor and peripheral landscape of HPV(-) vs. HPV(+) head and neck cancers to identify features able to expand treatment options for patients with Viral- and Carcinogen-Driven Head and Neck Cancer.

Methods: Biopsies and serum samples derived from 502 primary and metastatic HNSCC patients were leveraged for genomic, proteomic and immunohistochemistry evaluations. Tumor biopsies from HNSCC patients commercially obtained (n=143) or derived from patients enrolled in CP1108 trial (n=19, NCT01693562) were profiled by gene expression. Primary tumor biopsies (N=198) from HNSCC have been assessed by Whole Exome Sequence (WES). Expression of immune markers including CD8, NKP 46 was evaluated by immunohistochemistry (IHC) on 186 and 214 tumors biopsies, respectively. The expression of 80 immune related soluble factors was evaluated in serum derived from n=285 patients of HNSCC enrolled in EAGLE (NCT02369874), a randomized, open-label, study assessing Durvalumab and Tremelimumab vs. Standard of Care (SoC). Statistical comparison between HPV (-) vs. HPV (+) samples were conducted using R software.

Results: Patients with HPV(-) vs. HPV(+) HNSCC were characterized by worse prognosis. Increased levels of immunosuppressive factors including VEGF (p=0.01), IL-8 (p=0.02), IL6 (p=0.07) and macrophages chemo attractive factor CCL4 (p=0.07) was observed in the serum of HPV(-) vs HPV(+) HNSCC patients. In the tumor microenvironment, higher mRNA expression of immune signatures associated with MDSc, Cancer Associated Fibroblast (CAF), and Metalloproteinase (MMP) was observed in HPV(-) vs. HPV (+) HNSCC patients. In contrast, HNSCC HPV(+) patients were characterized by increased mRNA expression of DC signatures and IFNγ related genes (i.e. CXCL9). No differential infiltration of T and NK cells (CD8+ and NKP46+) were found in HPV (-) vs. HPV(+) patients. Enrichments of mutations in EGFR, and DNA repair genes (PMS1, POLK, ATM) was observed in HPV(+) patients. On the contrary, enrichments of mutations in TP53 was observed in HPV(-) patients.

Conclusions: Deep evaluation of tumoral and peripheral landscape of viral- versus carcinogen-driven HNSCC might help understanding differential outcome of treatments regimens in HPV(+) vs HPV (-) HNSCC thus leading to novel therapeutic interventions.

Trial Registration: NCT01693562, NCT02369874

Ethics Approval: The study was approved by Astrazeneca.
Abstracts

Consent Patients provided written consent to perform evaluations here described.

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271 CONSISTENT HIGH-QUALITY DENDRITIC CELL VACCINES PRODUCED POST-CHEMOTHERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA FOR USE IN A PHASE III TRIAL

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Background A Phase III dendritic cell (DC) vaccine trial was completed in 20 patients with acute myeloid leukemia (AML) in complete remission or CRi after chemotherapy who were ineligible for hematopoietic stem cell transplantation (NCT02405338). The DC vaccines were designed to delay disease progression by mobilizing natural killer (NK) cells through secretion of IL-12(p70) and activating T cells by stimulation with WT-1 and PRAME, two prominent antigens in AML. DC vaccination was carried out in weeks 1, 2, 3, 4, 6 and monthly thereafter for 2 years. Two questions were prominent at the trial start. First, could mature DCs (mDCs) be efficiently prepared to accommodate the vaccine regimen, including use of separate DC-fractions for each antigen. Second, could quality DC vaccines be generated from patients with myeloid disease, since all had received intensive chemotherapy, impairing hematopoiesis, such that several patients showed extended times for monocyte recovery in peripheral blood before being able to undergo apheresis for production.

Methods Immune monitoring tools were used to assess DC vaccines: multi-color flow cytometry for surface and intracellular protein antigen expression, dual-color ELISpot for secretion of IL-10/IL-12, and chemokine-directed trans-well migration.

Results Adequate regeneration of monocytes occurred post-chemotherapy in all patients, allowing production of sufficient numbers of cryopreserved vaccine cells (2.5 or 5.0 × 10^6 mDCs/antigen/ampule) to be completed. In 15/20 patients one batch was sufficient to cover all vaccinations, while 5 patients with lower initial monocyte counts required an additional production. Phenotypic and functional parameters of patient DC vaccines were compared to cells of a healthy control (HC). Patient mDCs expressed CD83, CD40, CD80, CD86 and HLA-DR at frequencies/levels comparable to the HC. Both DC-fractions displayed intracellular protein antigen expression in most cells. Polarized secretion of IL-12(p70) without IL-10 was seen with few exceptions. Furthermore, mDCs displayed chemokine-directed migration. Detection of delayed type hypersensitivity responses post-vaccination at six weeks indicated the DC vaccines were active in vivo in all patients.

Conclusions DC vaccine production feasibility was clearly fulfilled and high quality mDCs were generated for every patient. Quantity and quality of DC vaccines did not differ in the patient groups that relapsed or remained in remission, nor in patients who succumbed to disease during the trial. DC vaccines were remarkably consistent, although originating from patients differing in age, AML subtype and receiving varied amounts of standard chemotherapy regimens.

Ethics Approval The study was approved by the responsible Norwegian ethics committee, approval number 2014/1677.

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272 USE OF LIOCYX-M, AUTOLOGOUS HEPATITIS B VIRUS (HBV)-SPECIFIC T CELL RECEPTOR (TCR) T-CELLS, IN ADVANCED HBV-RELATED HEPATOCELLULAR CARCINOMA (HCC)

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Background We have demonstrated the ability of Hepatitis-B-virus (HBV)-specific T cell receptor (TCR) bioengineered T cells to recognize and lyse Hepatocellular carcinoma (HCC) cells expressing HBV antigens derived from HBV-DNA integration in patients with liver transplant. LioCyx-M is an immunotherapeutic product composed of autologous T cells transiently modified with *in-vitro* transcribed mRNA encoding HBV-specific TCR. The transient TCR expression makes LioCyx-M amenable to a dose escalating posology.

Methods The primary endpoint of this phase 1 trial is to assess the safety and tolerability of LioCyx-M in patients with advanced HBV-HCC without curative treatment options. Eligible patients were diagnosed with Barcelona clinic liver cancer stage B or C HCC (Child-Pugh < 7 points), receiving >1 year antiviral treatment prior to enrollment. These patients had matching HLA class I genotypes which present HBV encoded antigen. Peripheral blood was collected from each patient prior to each dose for LioCyx-M manufacturing. Patients received 4 escalating doses of 1 × 10^4 cells/kg, 1 × 10^5 cells/kg, 1 × 10^6 cells/kg, 5 × 10^6 cells/kg bodyweight (BW) in the first treatment cycle, each intravenously administered weekly. Patients underwent 1-month safety assessment post the 4th infusion, according to Common Terminology NCI CTCAE Version 4.0.3. If there were no dose associated toxicities, patients were eligible to continue administration of LioCyx-M at dose of 5 × 10^6 cells/kg BW weekly. Tumor response per RECIST 1.1 criteria and survival time were assessed.

Results At data cutoff (30 April 2020), eight patients were enrolled, with a median age of 53 (range: 49 - 67). These patients received a median number of 6 (range: 4 - 12) infusions of LioCyx-M. 1 patient developed Grade 3 elevations in alanine aminotransferase (ALT), gamma-glutamyl transferase in the 4th, 5th and 6th infusions. No treatment-related adverse events (trAEs) such as cytokine release syndrome or neurotoxicity were observed. No fatal trAEs were observed. The median time to progression was 1.9 months (range: 0.2 - 9.5 months). The median overall survival was 34 months (range: 3 - 38.2 months).

Conclusions The encouraging clinical outcome and tolerable safety highlight the good benefit-risk profile of LioCyx-M. Therefore, further exploration of efficacy of LioCyx-M treatment for advanced HBV-HCC is warranted in a Phase 2 proof-of-concept clinical study.

Acknowledgements Funding: Lion TCR.

Trial Registration NCT03899415

Ethics Approval The study was approved by Fifth Medical Center of Chinese PLA General Hospital’s Ethics Board, approval number R2016185D1010.
Background LioCyx-M is an immunotherapeutic product based on autologous T cells transiently modified with in vitro transcribed mRNA encoding HBV-specific T-cell receptors (TCR). We have previously shown, in a compassionate setting, the ability of LioCyx-M cells to recognize and lyse hepatocellular carcinoma (HCC) expressing HBV antigens derived from HBV-DNA integration in patients with HCC recurrence post-liver transplant.1 Here, we report our phase I study aimed to determine the feasibility, safety and preliminary efficacy of LioCyx-M in recurrent HBV-related HCC post-liver transplantation.

Methods Eligible patients with HBsAg-positive recurrent HCC as well as HLA-matched to selected TCRs were enrolled in this study. All patients underwent leukapheresis prior to treatment and peripheral blood mononuclear cells (PBMC) were collected for LioCyx-M manufacturing. During the 1st treatment cycle, patients received 4 escalating doses of 1×10⁴ cells/kg BW intravenously every 7 days. Adverse events were collected for LioCyx-M manufacturing. During the 1st treatment cycle, patients received 4 escalating doses of 1×10⁴ cells/kg BW (n=3) respectively. No cytokine release events were evaluated by Common Terminology Criteria for Adverse Events Version 4.0. In the second treatment cycle, one infusion of LioCyx-M at dose of 1–5 × 10⁶ cells/kg BW was intravenously administrated every 7 days for 4 weeks. The anti-tumour efficacy of LioCyx-M was evaluated per RECIST 1.1 criteria and survival was followed-up during the study.

Results Six patients were enrolled, with a median age of 35.5 months (range: 28 - 47). These patients received a median number of 6.5 doses of LioCyx-M therapy (range: 4 - 12). Only fever was observed as treatment-related AEs. Grade 1 fever was observed at dose levels of 1 × 10⁶ cells/kg BW (n=1) and 1–5 × 10⁶ cells/kg BW (n=3) respectively. No cytokine release syndrome- and neurotoxicity-like AEs were observed. Out of 4 patients evaluable for tumor response, the median of time to progression was at 1.3 months (range: 1.2 - 1.6 months). The median overall survival was 14 months (range: 4 - 22 months). At data cutoff (30 April 2020), one patient was still alive and 5 were deceased.

Conclusions Our data showed that multiple infusions of LioCyx-M are well tolerated at all dose levels administrated in recurrent HCC post liver transplantation, with no adverse effect to the transplanted liver. This calls for further assessment in a Phase 2 study.

Acknowledgements Funding: Lion TCR.

Trial Registration NCT02719782

Ethics Approval The study was approved by Sun Yat-Sen Third Affiliated Hospital’s Ethics Board, approval number [2015]2-157.
TRANSLATIONAL ENDPOINTS ASSOCIATED WITH STK11 MUTATIONS IN PATIENTS WITH NON-SQUAMOUS NSCLC

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Background Emerging data suggest poor outcome to anti-PD(L)1 blocking agents in patients with STK11mut tumors. In the current study, we undertook in-depth translational evaluation of three Ph1/Ph2 independent studies of Durvalumab ± Tremelimumab to elucidate the biology associated with STK11 mutations leading to reduced clinical response in patients with non-squamous NSCLC.

Methods Mutational status was evaluated by ctDNA or Foundation One CDxs as previously described. RNA sequencing was conducted on baseline frozen biopsies (N=70). Selected proteins (N=66) were measured by Myriad RBM multiplexed immunoassays on baseline serum (n=91). Screening and longitudinal whole blood was assessed for circulating quantities of T, B or NK cells and activated or memory T cell subsets using bioanalytically-validated, flow cytometry-based immunophenotyping assays. Exploratory analyses of translational endpoints according to STK11 mutational status were conducted by Wilcoxon rank-sum test.

Results In a periphery, a reduced number (> 2-fold decreased in median quantities) of NK cells, CD4+ effector memory, CD4+ HLA-DR+, CD8+ effector memory and CD8 + HLA-DR+ T cells was observed at baseline and following treatments in patients with STK11mut vs. STK11wt tumors. At baseline, increased levels of IL6 (p=0.002) and the neutrophil-attracting cytokine IL8 (p=0.02) were found in serum of patients with STK11mut tumors. In the tumor microenvironment, significantly increased expression (p< 0.05; fold change > 2) of markers associated with neutrophils, (i.e. CXCL2, IL6, CSF3), Th17 contexture (i.e. IL17A) and immune checkpoints (i.e. KIRs, PD-L1) was found in STK11mut vs. STK11wt tumors.

Conclusions The poor outcomes to immunotherapy observed in NSCLC patients with STK11mut tumors might be determined by a compromised peripheral and intra-tumoral immune phenotype. These results might help the development of novel therapeutic interventions able to unleash response to immune checkpoints in NSCLC patients harboring STK11 mutations.

Trial Registration NCT01693562, NCT02087423, NCT02000947

REFERENCE

PROGNOSTIC VALUE OF TUMOR SIZE VARIES BY TREATMENT IN A META-ANALYSIS OF 15 RANDOMIZED CLINICAL TRIALS IN ADVANCED NON-SMALL CELL LUNG CANCER ACROSS IMMUNOTHERAPY, TKI, AND CHEMOTHERAPY REGIMENS

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Background RECIST is commonly used to characterize intermediate outcomes for clinical trials in the context of solid tumors, and it is largely based on a standardized measure of tumor size known as the sum of longest diameters (SLD). In recent years, the FDA has granted accelerated approvals for several new compounds based on improvements in RECIST-based surrogate outcomes like overall response rate and progression-free survival. However, there are concerns regarding the robustness of these surrogate endpoints relative to overall survival (OS). 4 and it is not known whether their prognostic value is similar across TKI, chemotherapy, and immunotherapy regimens.

Methods We have developed a Bayesian meta-analytic joint model for longitudinal SLD and OS in order to predict Phase III outcomes from early Phase II data. We validated this model in extensive simulation studies. The model utilizes a generalized Stein-Fojo equation to characterize SLD over time in terms of 3 parameters: f (proportion of tumor that is treatment-susceptible), ks (the decay rate among susceptible cells), and k (the growth rate among resistant cells). Two quantities [tumor shrinkage (f * ks) and tumor regrowth ((1-f) * kg)] are then associated with survival in the context of a proportional-hazards survival model. We estimated this model using Stan on a dataset of >6,000 subjects in 15 randomized clinical trials in advanced non-small cell lung cancer.

Results Both tumor shrinkage and tumor regrowth were found to be associated with OS (HR for tumor shrinkage: median 0.51, 90% CrI 0.42 - 0.61; HR for tumor regrowth: median 1.24, 90% CrI 1.18 - 1.32). There is a stronger association between tumor shrinkage and OS among patients randomized to a PD-1/PD-L1 inhibitor, either as a monotherapy or in combination with a CTLA-4 inhibitor, than among patients in other trial arms (figure 1). By contrast, there were negligible

Abstract 276 Figure 1
Hazard associated with SLD submodel parameters varies according to the class of treatment in a joint model for SLD and overall survival with varying association by assigned treatment regimen. The points represent posterior median values per treatment, with lines representing 90% posterior credible intervals (CrI). Two treatment classes demonstrated posterior probability greater than 90% of a non-zero treatment-specific effect for the response term: the combination PD-1/PD-L1 inhibitor + CTLA-4 inhibitor [interaction HR = 0.64 (90% CrI 0.39 - 1.00; posterior probability of HR<1: 95.2%)] and the PD-1/PD-L1 inhibitor alone [interaction HR = 0.62 (90% CrI 0.42 - 0.89; posterior probability of HR<1: 99.2%)].
differences across treatment classes in the association between tumor regrowth and OS.

**Conclusions** Our results suggest that not all reductions in tumor size are equal. A patient with a certain degree of tumor shrinkage on the PD-1/PD-L1 inhibitor will have lower mortality risk than a patient with a similar degree of shrinkage on the other regimens evaluated. More research is needed to determine whether the result is unique to this particular PD-1/PD-L1 inhibitor, to determine what mechanisms of action mediate these treatment-specific effects, and to develop improved surrogate measures of treatment efficacy.

**REFERENCES**


administered at Dose Level (DL) 1 = 0.1×10⁹, DL2 0.5–1.2×10⁹, and DL3/Expansion = 1.2–15×10⁹ transduced cells.

Results As of Jan 10, 2020, 11 pts (6 male/5 female) with NSCLC (3 squamous cell, 7 adenocarcinoma, 1 adenosquamous) were treated. Five, 3 and 3 pts received cells at DL1, DL2, and DL3/Expansion, respectively. The most frequently reported adverse events ≥ Grade 3 were lymphopenia (11), leukopenia (9), neutropenia (8), anemia (6), thrombocytopenia (5), and hyponatremia (5). Three pts reported CRS (Grades 1, 2, and 3, respectively). One pt received the highest dose of LD (Flu 30 mg/m² Day 1 4 and Cy 1800 mg/m² Day 1–2) prior to a second infusion and had a partial response (PR). This pt subsequently developed aplastic anemia and died. Responses included: 1 pt – PR, 3 pts - stable disease, 2 pts – progressive disease, 1 pt – too early to determine, 4 pts - off-study prior to tumor assessment. SPEAR T-cells were detectable in peripheral blood from pts at each dose level, and in tumor tissue from pts at DL1 and DL3.

Conclusions ADP-A2M10 SPEAR T-cells have shown acceptably safe and no evidence of toxicity related to off-target binding or alloreactivity. Given the minimal antitumor activity and the discovery that MAGE-A10 expression frequently overlaps with MAGE-A4 expression, the clinical program has closed. Several trials with SPEAR T-cells targeting MAGE-A4 are ongoing (https://bit.ly/35hrsZK).

Trial Registration NCT02592577

Ethics Approval The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and was approved by local authorities. An independent ethics committee or institutional review board approved the clinical protocol at each participating center. All the patients provided written informed consent before study entry.

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**DURVALUMAB AFTER CHEMORADIOThERAPY FOR PD-L1 EXPRESSING INOPERABLE STAGE III NSCLC IMPACTS LOCAL-REGIONAL CONTROL AND OVERALL SURVIVAL**

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**Background** Chemoradiotherapy (CRT) followed by maintenance treatment with the PD-L1 inhibitor durvalumab is a new standard of care for inoperable stage III NSCLC. The present study aims to evaluate the oncological outcome of patients treated with CRT alone to those treated with CRT and durvalumab (CRT-IO) in the real-world setting.

**Methods** Retro- and prospectively collected data of 133 consecutive inoperable stage III NSCLC patients treated between 2011–2019 were evaluated. Local-regional-recurrence-free-survival (LRPFS - defined as progression in the mediastinum, hilum and/or supraclavicular region at both sides and the involved lung), progression-free survival (PFS) and overall survival (OS) were evaluated from last day of thoracic radiotherapy (TRT).

**Results** Median age at diagnosis was 68.5 years; 44 (33%) were female; 58 (44%) were diagnosed with adenocarcinoma. All patients were irradiated to a total dose of at least 60 Gy (EQD2). Median PTV was 709.8 cc (range: 181–1958 cc). 113 (85%) patients were treated with CRT and 20 (15%) PD-L1 expressing patients with CRT-IO. 83% of patients received two cycles of concomitant platinum-based chemotherapy. Median time to initiation of durvalumab after CRT was 0.8 months (range: 0.4–2.1). Median follow-up for entire cohort was 33.3 months (range: 4.8–111.8) and median overall survival (OS) was 24.7 (95% CI: 18.9–30.4) months. In the CRT-IO cohort after a median follow-up of 15.5 (range: 5.1–20.2) months, no deaths were reported at the time of evaluation (August 2020). Improved LRPFS (p = 0.013), PFS (p = 0.033) and OS (p = 0.002) were correlated with CRT-IO compared to the historical cohort of conventional CRT patients. After propensity-scoring matching (PSM) analysis with age, gender, histology, tumor volume and treatment mode and exact matching for T-and N-stage, 18 CRT-IO patients were matched 1:2 to 36 CRT patients. 12-month LRPFS, PFS and OS rates in the CRT-IO vs CRT cohort were 80% vs 38.8% (p = 0.001), 50% vs 22% (p = 0.013) and 100% vs 75% (p = 0.002), respectively. Also regarding intracranial failure, 6-month brain metastases rates were 0% vs. 6% in the CRT-IO vs CRT cohort (p = 0.290).

**Conclusions** This real-world analysis demonstrates that durvalumab after CRT has led to significant improvement of local-regional control, PFS and OS in PD-L1 expressing inoperable stage III NSCLC patients compared to a historical cohort.

**Acknowledgements** The study was partly presented at 2020 Annual Meeting of the American Society of Clinical Oncology (ASCO).

**Trial Registration** N/A

**Ethics Approval** The study was approved by Ludwig-Maximilians-University (LMU), Munich, Germany: Institution’s Ethics Board, approval number 17-230.

**Consent** Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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**BOTH TUMOR INTRINSIC AND EXTRINSIC FACTORS CONTRIBUTE TO TIL RESISTANCE IN LUNG CANCER PATIENTS**

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**Background** Although cancer immunotherapies have achieved great success, many patients either do not respond or initially respond but later relapse. Several resistance mechanisms have been proposed from trials using immune checkpoint inhibitors or CAR-T therapy, but few studies have been conducted on resistance mechanisms to TIL therapy. In our trial, anti-PD1 refractory lung cancer patients were treated using TIL therapy. Several patients responded while others did not. We hypothesize that both tumor intrinsic and extrinsic factors may contribute to TIL resistance in lung cancer patients.

**Methods** We performed whole exome sequencing on resected baseline tumors and predicted neoantigens using the netMHCpan algorithm. Neoantigen-reactive TIL were screened using IFN-gamma ELISPOT assays in a T-DC-neoantigen co-culture system. We also did the same co-culture for TCRVβ sequencing to identify neoantigen-specific TCR clonotypes. Therefore, we have been able to track tumor antigen-specific T cells over...
Abstract 280 Figure 1  In vivo TIL persistence in patients with durable TIL benefit VS patients with no durable TIL benefit
time. Combined with single cell RNA sequencing & TCR sequencing, functional features of neoantigen-specific T cells in both baseline and progressive disease (PD) tumors were analyzed.

Results Our data show that the presence of neoantigen-specific TIL is associated with durable TIL benefit (p = 0.031). We also identified tumor antigen-specific TCR clonotypes for 3 TIL-treated patients and followed these cells longitudinally in PBMCs. We found that although neoantigen-specific T cells had a dramatic increase after TIL infusion, patients with durable TIL benefit had a longer TIL persistence (p = 0.048, figure 1). RNA sequencing on baseline tumors showed that in patients with no durable TIL benefit, genes contributing to extracellular matrix formation were highly expressed, preventing infused TILs from migrating into tumor sites.

In 2 TIL-treated patients, we found that neoantigens which were recognized by infused TILs were missing in PD tumors. In one patient, further investigation of TRM cells from both baseline and PD tumors showed that although T cells in the PD tumor can recognize PD tumor antigens, the T cells highly expressed PD-1, CTLA-4, Lag3 and TIGIT (figure 2), indicating an inability to control tumor progression. Enumeration of immunocyte compositions using CIBERSORT showed that higher M1/M2 ratios were found in patients with durable TIL benefit.

Conclusions In summary, higher expression of tumor antigens, longer TIL persistence and more M1 macrophages are associated with durable TIL benefit, while lack of tumor antigens, expression of immune checkpoint molecules, and upregulated formation of extracellular matrix may cause TIL resistance. Therefore, both tumor intrinsic factors and extrinsic factors contribute to TIL resistance in lung cancer patients.

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Trial Registration NCT03215810

Ethics Approval The study was approved by Chesapeake IRB, approval number Pro00021984.

REFERENCES

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Abstract 280 Figure 2  PD tumor neoantigen specific T cells (red circled) express immune checkpoint molecules

JAVELIN MEDLEY VEGF: PHASE 2 STUDY OF AVELUMAB + AXTINIB IN PATIENTS WITH PREVIOUSLY TREATED NON-SMALL CELL LUNG CANCER (NSCLC) OR TREATMENT NAIVE, CISPLATIN-INELIGIBLE UROTHelial CANCER (UC)

1Gabriella Galfy*, 2Iwona Lugowska, 3Elena Podubskaya, 4Byoung Chul Cho, 5Myung-Ju Ahn, 6Ji-Youn Han, 7Wu-Chou Su, 8Ralph Hauke, 9Stephen Dyer, 10Dae Ho Lee, 11Piotr Serwatowski, 12David Lorente Estelles, 13Viran Holden, 14Yu Jung Kim, 15Vladimir Vladimirov, 16Zsofia Horvath, 17Abhimanyu Ghose, 18Allison Goldman, 19Alessandra di Pietro, 20Mikhail Laskov, 21Taiwain, Province of China

Abstracts

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0280
for patients with locally advanced or metastatic UC who have not progressed with platinum-based chemotherapy and as maintenance treatment for those who have not progressed with platinum-based chemotherapy.

**Methods** Eligible patients with NSCLC had received ≥1 prior platinum-containing therapy and ≤2 prior lines of systemic therapy for locally advanced or metastatic disease; patients with UC were treatment naive in the locally advanced or metastatic setting and ineligible for cisplatin-containing chemotherapy. Patients were immune checkpoint inhibitor naive and received avelumab 800 mg intravenously every 2 weeks + axitinib 5 mg orally twice daily. The primary endpoint was confirmed objective response (OR) per investigator assessment (RECIST 1.1). Secondary endpoints included progression-free survival (PFS) and safety. PD-L1 expression was assessed in baseline tumor samples (Ventana SP263 assay). Data have not undergone standard quality checks and are subject to change due to COVID-19–related healthcare burden.

**Results** A total of 41 patients with NSCLC and 20 with UC received avelumab + axitinib. The confirmed OR rate was 31.7% (95% CI, 18.1–48.1) in the NSCLC cohort and 10% (95% CI, 1.2–31.7) in the UC cohort (all partial responses); 16 patients (39.0%) and 5 (25.0%) had stable disease, respectively. Responses were observed regardless of PD-L1 expression status. Median PFS was 5.3 months (95% CI, 2.5–7.0) in the NSCLC cohort and 2.3 months (95% CI, 1.8–5.6) in the UC cohort. Grade ≥3 treatment-related adverse events (TRAEs) occurred in 24 patients (58.5%) in the NSCLC cohort; the most common was hypertension (n=7 [17.1%]). Grade ≥3 TRAEs occurred in 9 patients (45.0%) in the UC cohort; the most common were amylase increased, asthenia, decreased appetite, and palmar-plantar erythrodysesthesia syndrome (n=2 [10%] each). One patient in each cohort experienced a TRAE that led to death (gastric perforation and urinary bladder hemorrhage).

**Conclusions** Avelumab + axitinib showed antitumor activity and a manageable safety profile in patients with advanced or metastatic NSCLC or UC consistent with findings from studies of each drug alone and in combination.

**Trial Registration** NCT03472560

**Ethics Approval** The study was approved by each site’s independent ethics committee.

**Consent N/A**

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**REFERENCES**


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**Abstract 282 Table 1** Response Rates and Sensitivity at Individual CPS Cutpoints for Pembrolizumab-Treated Patients

<table>
<thead>
<tr>
<th>Population</th>
<th>Prevalence, %</th>
<th>ORR, %</th>
<th>Sensitivity</th>
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<tr>
<td>Overall</td>
<td>100</td>
<td>17.6</td>
<td>1</td>
</tr>
<tr>
<td>CPS = 0</td>
<td>34.8</td>
<td>11.1</td>
<td>0.22</td>
</tr>
<tr>
<td>CPS &gt;1</td>
<td>65.2</td>
<td>21</td>
<td>0.78</td>
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<td>CPS &gt;10</td>
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<tr>
<td>CPS &gt;20</td>
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<td>0.33</td>
</tr>
<tr>
<td>CPS &gt;50</td>
<td>10</td>
<td>33.1</td>
<td>0.19</td>
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CPS, combined positive score; ORR, objective response rate.
SAFETY AND EFFICACY SIGNALS IN THE COMPLETE PHASE I STUDY OF LIVE BIOTherAPEUTIC MRX0518 IN COMBINATION WITH PEMBROLIZUMAB IN PATIENTS REFRACTORY TO IMMUNE CHECKPOINT INHIBITORS (ICIS)

Background MRx0518 is a novel, human gut microbiome-derived, single-strain, live biotherapeutic in clinical development for treatment of solid tumors. Preclinically, MRx0518 induced broad immunostimulatory activity and demonstrated anti-tumorigenic effects in a range of murine tumor models. MRx0518 increased CD4+ and CD8+ T cell and NK cell tumor infiltration and decreased Tregs. Activation of tumor TLR5 was observed and linked to the bacterial flagellin moiety, which was shown to strongly induce NFκB, cytokine responses and IFNγ+ CD4+ and CD8+ T cells.

Methods Heavily pre-treated patients refractory to ICIs were enrolled from March 2019 to March 2020. Patients had experienced at least SD from previous ICI (monotherapy or combination) but eventually progressed as confirmed by two radiological scans ≥4 weeks apart in the absence of rapid clinical progression and within 12 weeks of last dose of ICI. Patients were treated with 1 capsule of MRx0518 (1 × 10^{10} to 1 × 10^{11} CFU) BID and pembrolizumab (200 mg every 3 weeks) for up to 35 cycles or disease progression. Tumor response was assessed every 9 weeks per RECIST 1.1. The primary objective was to evaluate safety of the combination by monitoring toxicities in the first cycle of treatment. Secondary objectives were to evaluate efficacy via ORR, DOR, DCR and PFS.

Results In Part A, patients with mRCC (n=9) and mNSCLC (n=3) were recruited. At data cut-off (21 Aug 20), 5 patients remain on study treatment. 83% of patients were male and 17% were female. Median number of prior lines of therapy was 3. 10 patients received nivolumab previously (83%), one received avelumab (8%) and one received pembrolizumab and nivolumab (8%). 83% of patients had experienced SD as best response to prior ICI and 17% had PR as best response. Of 6 patients with available PD-L1 results, 5 had a positive CPS/TPS (>1) and 1 negative (<1). The combination shows a positive safety profile with no treatment-related SAEs or toxicity-related drug discontinuations. No increase in irAEs has been reported. On study treatment, 2 RCC patients and 1 NSCLC patient experienced a PR, with an additional 2 RCC patients experiencing durable SD (6 and 13 months), a protocol defined DCR of 42%. Median PFS is 2.14 months at data cut-off (table 1).

Conclusions This data represents first-in-class proof of concept for a live biotherapeutic in an oncology setting. The combination was tolerable and there were preliminary signals of efficacy. Part B (phase II) in NSCLC, RCC and bladder cancer is ongoing.

Trial Registration www.clinicaltrials.gov NCT03637803

Ethics Approval This study was approved by University of Texas MD Anderson’s Institutional Review Board; approval ref. 2018-0290

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0283
REAL WORLD DATA OF SEQUENCING IMMUNE CHECKPOINT INHIBITORS (ICI) AFTER INITIAL ICI

Krishna Guntryu, Muhammad Avidii, Rojer Ranjit, Brendan Connell, Rachel Cararasquillo, Brigitte Gil, Natalie Dalbo, Lewena Maher, Seanna Reilly, Stephanie McDonald, Philip Tseukas, Linda Voner. Lahey Hospital and Medical Center, Burlington, MA, USA

Background ICI revolutionized modern Oncology landscape and being utilized in metastatic to adjuvant and neo-adjuvant settings. As Oncologists, we are treating cancer patients with ICI every day, yet there is still a lot that is unknown about these drugs. We don’t have clear understanding of the efficacy and toxicity when sequencing one ICI for another. We conducted a retrospective review of real world data at Lahey Hospital and Medical Center to understand further and to pave path for prospective studies to understand this issue further to improve patient care.

Methods We retrospectively reviewed Oncology patient charts who received ICI between January 1, 2014 to December 18, 2018. Total 483 patients received ICI during this time frame and 22 of these patients received a second ICI either as monotherapy or in combination with other ICI or chemotherapy.

Results A total of 22 patients received subsequent ICI after the initial ICI as showed in table 1. 15 of the 22 (68%) patients were transitioned from one ICI to another monotherapy, 11 of these patients were transitioned secondary to disease progression (73%), three had immune related adverse events and one was switched per standard of care. One patient had ICI re-challenge. Three patients had a transition from ICI monotherapy to combination ICI therapy. One patient went onto chemo-immunotherapy and 2 patients transitioned from combination ICI to chemo-immunotherapy.

Conclusions ICI therapy is evolving and patients are being treated with multiple lines of ICI. In current practices, ICI is frequently being transitioned from cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) or PD-L1 to PD-1/L1 agonist or checkpoint inhibitors (ICI) after initial ICI.

Abstract 284 Table 1 Real world data of sequencing immune checkpoint inhibitors (ICI) after initial ICI

<table>
<thead>
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<th>Therapy</th>
<th>Drug</th>
<th>Drug interaction</th>
<th>Relapse</th>
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<td>A10</td>
<td>Monotherapy</td>
<td>Nonsignificant</td>
<td>Progression</td>
</tr>
<tr>
<td>A10</td>
<td>Combination</td>
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<td>Progression</td>
</tr>
<tr>
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<td>Monotherapy</td>
<td>Nonsignificant</td>
<td>Progression</td>
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<tr>
<td>A10</td>
<td>Combination</td>
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<td>Progression</td>
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<tr>
<td>A10</td>
<td>Monotherapy</td>
<td>Nonsignificant</td>
<td>Progression</td>
</tr>
<tr>
<td>A10</td>
<td>Combination</td>
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<td>Progression</td>
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<tr>
<td>A10</td>
<td>Monotherapy</td>
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<td>Progression</td>
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<tr>
<td>A10</td>
<td>Combination</td>
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</tr>
<tr>
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<td>Monotherapy</td>
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<tr>
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<td>Combination</td>
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</tr>
<tr>
<td>A10</td>
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<tr>
<td>A10</td>
<td>Combination</td>
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<tr>
<td>A10</td>
<td>Monotherapy</td>
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<td>Combination</td>
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<td>Combination</td>
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<tr>
<td>A10</td>
<td>Monotherapy</td>
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<tr>
<td>A10</td>
<td>Combination</td>
<td>Nonsignificant</td>
<td>Progression</td>
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</table>

Abstract 285 PHASE 1 CLINICAL TRIAL EVALUATING THE SAFETY OF ADP-A2M10 IN PATIENTS WITH MAGE-A10+ HEAD AND NECK, MELANOMA, OR UROTHELIAL TUMORS

1David Hong*, 2Marcus Butler, 3Russell Pachynski, 4Ivan Ryan, 5Parmod Kebriaei, 6Sarah Boross-Harmer, 7Matthew Frigault, 8Ecaterina Dumbrava, 9Amy Sauer, 10Francine Brophy, 11Jean-Marc Navenot, 12Svetlana Fayngerts, 13Jane Bai, 14Elliot Norry, 15Paula Fracasso, 16MD Anderson Cancer Center, Houston, TX, USA; 17Princess Margaret Cancer Centre, Toronto, Ontario, Canada; 18Washington University School of Medicine, St Louis, MO, USA; 19Harvard Medical School, Boston, MA, USA; 20Massachusetts General Hospital, Somerville, MA, USA; 21Adaptimmune, Philadelphia, PA, USA

Background ADP-A2M10 SPEAR T-cells are genetically engineered autologous T-cells that express a high affinity MAGE-A10-specific T-cell receptor targeting MAGE-A10+ tumors in the context of HLA A*02. This trial is now no longer enrolling (NCT02989064).

Methods This ADP-A2M10 dose escalation trial utilized a modified 3+3 design to evaluate safety and antitumor activity. Patients (pts) with advanced head and neck squamous cell carcinoma (HNSCC), melanoma, or urothelial cancer (UC) were enrolled. Pts were HLA A*02+ with tumors expressing MAGE A10. Pts underwent apheresis; T cells were isolated, transduced with a lentiviral vector containing the MAGE-A10 TCR, and expanded. Eligible pts underwent lymphodepletion with fludarabine and cyclophosphamide prior to receiving ADP-A2M10. ADP-A2M10 was administered at Dose Level (DL) 1 = 0.1 × 10⁹, DL2 = >1.2 – 6 × 10⁹, and Expansion = 1.2–15×10⁹ transfused cells.

Results As of January 10, 2020, 10 pts (8 male and 2 female) with HNSCC (4), melanoma (3), and UC (3) cancers were treated. Three pts each were treated at DL1 and DL2 and 4 pts were treated in Expansion. The most frequently reported adverse events ≥ Grade 3 were lymphopenia (10 pts), neutropenia (10), anemia (8), leukopenia (7), and thrombocytopenia (5). Two pts reported CRS (1 Grade 1, 1 Grade 3) with resolution. Responses included: 3 pts - stable disease, 5 pts not evaluable, and 1 pt too early to determine. ADP-A2M10 SPEAR T-cells were detectable in peripheral blood from pts at each dose level and in tumor tissues from several pts at Expansion.

Conclusions There was no evidence of on- or off-target toxicity. Given the minimal antitumor activity and the discovery that MAGE-A10 expression frequently overlaps with MAGE-A4 expression, the clinical program has closed. Several trials with SPEAR T-cells targeting MAGE-A4 are ongoing (https://bit.ly/35htsZK).

Trial Registration NCT02989064

Ethics Approval The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and was approved by local authorities. An independent ethics committee or institutional review board approved the clinical protocol at each participating center. All the patients provided written informed consent before study entry.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0284
Background RO7122290 (RO) is a potent next generation 4-1BB agonist simultaneously targeting fibroblast activation protein (FAP). FAP is highly expressed on cancer-associated fibroblasts in many solid tumors, while expression in healthy tissue is low. 4 1BB stimulation via FAP mediated cross linking augments the cytotoxicity, proliferation, and longevity of immune effector cells in FAP-positive tumors. RO was labelled with Zirconium-89 (89Zr) to visualize FAP-dependent tumor accumulation and assess the biodistribution in patients (pts) after successful proof of principle of 89Zr-based PET in preclinical pilot studies.

Methods Pts with advanced and/or metastatic solid tumors were eligible for this sub-study of an ongoing Phase 1/1b trial (EUDRACT 2017-003961-83). RO was administered intravenously once at a total dose of 5, 45, 200 or 500 mg consisting of RO (cold) + 5 mg 89Zr-RO (hot, 37.0 ± 3.4 MBq). Up to three 89Zr-RO PET scans were performed over a period of nine days followed by a single tumor biopsy before pts could switch to Part A (single agent) and subsequently to Part B (combination with atezolizumab) of the main study to continue treatment. Tracer uptake was calculated as peak standardized uptake value (SUVpeak) of the lesion with the highest uptake.

Results 14 pts were exposed to a total dose of 5, 45, 200 (all n=3) or 500 mg (n=5); median age was 60 years (range 26–74) with seven male and female pts each. Primary tumor sites included colon (n=4), lung (n=3), thymus (n=2), anus (n=1), breast (n=1), bile duct (n=1), pleura (n=1), and uvea (n=1). In healthy tissues, uptake of 89Zr-RO was predominantly observed in the liver and spleen across all doses and in non-malignant lymph nodes in 2/3 patients at 5 mg. Consistent with the target expression, tracer uptake was detected in FAP-positive scarring tissue and tissues with ongoing remodeling. Intra-tumor accumulation of 89Zr-RO was observed at all doses. 96 hours p.i., SUVpeak values were 12.1 ± 4.1, 10.0 ± 4.9, and 7.0 ± 3.7 for the 5, 45 and 200 mg cohorts, respectively, while SUVpeak for the 500 mg cohort was even lower at 4.9 ± 0.5.

Conclusions 89Zr-PET confirmed tumor-specific uptake and expected biodistribution pattern of 89Zr-RO. The decrease in SUV with increasing doses suggest that more cold antibodies saturated FAP binding sites in the tumor. 89Zr-PET results supported together with clinical PK, PD, safety and response data the selection of the recommended phase 2 dose and schedule of RO in combination with atezolizumab.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0286
Abstract 287 Table 1  Study 2455-001: Treatment-Emergent Adverse Events (>10% by Preferred Term)

<table>
<thead>
<tr>
<th>System Organ Class</th>
<th>Any Cyclea</th>
<th>N=56</th>
<th>N (%)</th>
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<tr>
<td>任何不良事件</td>
<td>31 (55.4)</td>
<td>34 (60.7)</td>
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<tr>
<td>任何免疫不良事件</td>
<td>12 (21.4)</td>
<td>13 (23.2)</td>
<td></td>
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<tr>
<td>KHK2455-related</td>
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<td>13 (23.2)</td>
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</tbody>
</table>

a. Includes both KHK2455 monotherapy and KHK2455 + mogamulizumab combination therapy cycles.

Abstract 287 Figure 1  Study 2455-001: Overall Survival

Conclusions KHK2455 in combination with mogamulizumab was well-tolerated and manageable at all doses tested, suppressed Kyn production in a dose-dependent and sustained manner, and demonstrated signals of antitumor activity. These data support the continued development of this combination.

Acknowledgements Medical writing assistance was provided by Susan E. Johnson, PhD, S.E. Johnson Consulting, LLC, New Hope, PA, USA.

Trial Registration NCT02867007 (www.clinicaltrials.gov)

Ethics Approval This study was approved by Ethics Committees at all participating study institutions.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0287

288  A PHASE 1 STUDY OF IMC-001, A PD-L1 BLOCKER, IN PATIENTS WITH METASTATIC OR LOCALLY ADVANCED SOLID TUMORS

Bhumuk Keam*, 1Bae Min Kim, 1Do-Youn Oh, 1Chan-Young Ock, 2Won Ki Kang, 2Yeon Hee Park, 2Jaeyaun Lee, 1Ji Hye Lee, 1Yun Jeong Song, 2Young Suk Park, 1Seoul National University Hospital, Seoul, Korea, Republic of; 2Samsung Medical Center, Seoul, Korea, Republic of; 3ImmuneOnCia Therapeutics Inc., Gyeonggi-do, Korea, Republic of

Background IMC-001 is a fully human IgG1 monoclonal antibody that binds to human PD-L1 and mediates the antibody-dependent cell-mediated cytotoxicity. The main objectives of this study were to evaluate the safety, pharmacokinetics, and pharmacodynamics of IMC-001 in patients with advanced solid tumors. Here, we report final result of the phase 1 study of IMC-001.

Methods This open-labeled phase 1 study used standard 3+3 dose-escalation design, dose ranging from 2 to 20 mg. IMC-001 was administered intravenously every two weeks until disease progression or unacceptable toxicity. Dose limiting toxicity (DLT) window was defined as 21 days from the first dose. Adverse events (AEs) were assessed using CTCAE v4.03, and tumor response was assessed by and the Response Evaluation Criteria In Solid Tumors (RECIST) version v1.1.

Results Fifteen subjects (8 Male, 7 Female; Median age : 58 [range 39–69]) were included in 5 dose escalation cohorts. No DLT was observed and the maximum tolerated dose was not reached. Most common AEs were general weakness, decreased appetite, fever, and cough. No Grade 4 or 5 treatment emergent AEs (TEAEs) were reported during the study and no TEAE or serious AE led to treatment discontinuation or death. There were no infusion-related reactions during this study. Grade 2 immune-induced thyroiditis and diabetes mellitus suspected to be related to IMC-001 were seen in one subject at 2 mg/kg cohort. Over the dose range of 2 to 20 mg/ kg IMC-001, AUC 0-14d, AUC 0 —¥, and Cmax generally appeared to increase in a dose proportional manner for each step of dose escalation. Of the 15 enrolled patients, one subject with colon cancer showed partial response, and disease control rate was 33.3%. There were total 3 biliary tract cancer patients (1 GB cancer, 2 Cholangiocarcinoma) who received ≥3 lines of systemic therapies prior to this trial. They all had stable disease during IMC-001 treatment, and one cholangiocarcinoma subject received the treatment for 434 days.

Conclusions IMC-001 demonstrated a favorable safety profile up to 20 mg/kg given IV every 2 weeks and showed encouraging preliminary efficacy in patients with advanced solid tumors. Based on PK and PD data, 20 mg/kg was selected as recommended Phase 2 dose (RP2D).

Ethics Approval This study was approved by Institutional Review Board; approval number SMC 2018-01-007-001 and H-1801-042-913.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0288
Background The efficacy of T cell directed immunotherapies relies on adequate priming of T cells to tumor-specific neoantigens, which some studies have augmented with synthetic neoantigen vaccines. This is the first report of a personalized genomic vaccine (PGV-001) in multiple histologies in the adjuvant setting.

Methods Tumor and germline RNA and DNA were sequenced, and neoantigen peptides were selected using our OpenVax custom computation pipeline that identifies and ranks mutant sequences by a combination of predicted MHC-I binding affinity and neoantigen abundance within tumor. Up to 10 peptides were synthesized per patient and were administered over the course of 27 weeks in combination with the poly-ICLC. Primary objectives were to determine 1) the safety and tolerability; 2) the feasibility of PGV-001 production and administration; and 3) the immunogenicity of PGV-001. Secondary objectives included immunophenotyping neoantigen-specific T cells in peripheral blood, and characterization of peripheral blood lymphoid, myeloid and humoral responses. We report here for the first time on the primary endpoints.

Results Vaccine was synthesized for 15 patients. A mean of 1619 somatic variants (range 521–5106) were detected. Our pipeline identified a mean of 67.1 neoantigens/patient (range 8–193) and 9.7 peptides/patient were synthesized (range 7–10). 13 patients received PGV-001 (11 patients received all 10 doses and 2 patients received at least 8 doses) while 2 had progressive disease before vaccine initiation. Transient grade 1 injection site reactions were seen in 31% of patients, and one patient experienced grade 1 fever. There were no other significant adverse events. Ex vivo ELISPot analysis of patient blood demonstrated significant induction of T cell responses following receipt of 10 vaccines that were not present after the 6th vaccine, supporting the need for a prolonged dosing schedule. Robust responses were seen in both CD4 and CD8 T cells by intracellular cytokine staining for TNF-a, IFN-a, and IL-2 following in vitro expansion in the presence of vaccine antigens. Additional studies are ongoing to define the most immunogenic neoantigens.

Conclusions A personalized neoantigen vaccine of synthetic mutant peptides and adjuvant poly-ICLC was successfully synthesized for 15 patients and administered successfully to 87% patients over the course of 27 weeks. The vaccine was well tolerated, and T cell expansion and reactivity to synthetic neoantigens confirms immunogenicity of neoantigens identified with OpenVax.

Trial Registration NCT02721043

Ethics Approval This study was approved by the IRB of The Mount Sinai Hospital in accordance with Federal law. HSM #15-00841.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0289

Background The development of oncolytic viruses for the treatment of cancer has been significantly hampered by their rapid clearance in circulation due to complement and antibody-mediated neutralization. In our recent first-in-human Phase I clinical trial, we evaluated the safety and feasibility of our approach to enhance virus delivery and improve tumor targeting by utilizing an autologous stromal vascular fraction (SVF) based cell delivery system. Patient sample analysis demonstrated that patients could be stratified based on the level of vaccinia virus amplification in vivo, as evidenced by analysis of persistent viral DNA in the blood.

Methods In the current study, we evaluated the immunomodulatory potential of vaccinia virus delivered by autologous stromal vascular fraction (SVF)-based cells and attempted to identify immunological correlates of successful vaccinia virus amplification in vivo. To this end, we performed an extensive time-course analysis of cytokines in patients’ plasma as well as various peripheral blood immune subpopulations using Luminex multi-analyte profiling and multiparameter flow cytometry, respectively. We also analyzed the impact of this therapeutic approach on the innate and adaptive immune subpopulations, including NK cells, myeloid cells, as well as effector, regulatory and memory T cells.

Results Therapy with SFV-delivered oncolytic vaccinia virus induced a coordinated activation of cytokine, T cell and NK cell responses in patients as early as 1 day after treatment, which peaked around 1-week and lasted for up to 1-month post treatment. The ability of the oncolytic virus to effectively amplify in cancer patients correlated with significant changes of multiple innate (NK) and adaptive (T cell) immunological parameters. Interestingly, patient stratification into groups with transient versus persistent viral DNA was linked to opposing and mutually exclusive patterns of robust activation of NK versus T cell responses, respectively. Our study also identified intriguing cytokine and immune subset frequency signatures present at baseline and associated with successful amplification and persistence of oncolytic vaccinia virus in vivo.

Conclusions Overall, this study establishes the timeline of treatment-related immunological changes and identifies biomarkers present at baseline and potential immunological correlates associated with the persistence of virus amplification in vivo. Therefore, our findings provide new insights into the role of interpatient immunological variability and will contribute to the proper evaluation of the therapeutic potency of oncolytic virotherapy in future clinical trials.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0290
Background Selicrelumab is a human IgG2 agonistic anti-CD40 monoclonal antibody. Binding of the antibody to CD40 expressed on antigen-presenting cells results in T-cell priming and T-cell dependent anti-tumor activity. In response to T-cell activation, tumor cells express programmed-death ligand 1 (PD-L1) that can suppress effector T-cells. Atezolizumab interrupts this feedback loop by blocking PD-L1, thereby supporting the combination with selicrelumab.

Methods This phase Ib open-label, multicenter, dose escalation (DE)/expansion clinical study (NCT02304393) investigated safety, pharmacokinetic (PK), pharmacodynamics (PD) and efficacy of selicrelumab in combination with atezolizumab in unselected patients with advanced/metastatic solid tumors, not amenable to standard therapy. In DE cohorts, a single dose of selicrelumab was given, either by intravenous (IV) infusion at a 16 mg fixed dose or subcutaneously (SC) at a range from 1 to 64 mg/dose. In dose-expansion cohorts (small bowel and colorectal cancer, head and neck squamous cell carcinoma [HNSCC] and non-small cell lung carcinoma), patients received multiple doses of selicrelumab SC at a dose of 16 mg. In all treatment cohorts, patients received atezolizumab at varying doses (16–64 mg/dose). In dose-expansion cohorts (small bowel and colorectal cancer, HNSCC, non-small cell lung carcinoma), patients received multiple doses of selicrelumab SC at a dose of 16 mg.

Results In this study, 140 patients were treated. This included 95 patients in DE cohorts (6 patients in the IV cohort, 89 patients in the SC cohorts) and 45 patients in dose-expansion cohorts. In the IV cohort, infusion-related reaction was the most frequent treatment-related adverse event (TRAE; 50%), while Grade ≥ 3 TRAE occurred in 1 patient (16.7%). In this cohort one dose-limiting toxicity (DLT) was reported (Grade 3 pancytopenia). In the SC cohorts, the most frequent TRAE was injection site reaction (ISR; 92%). Four DLTs were reported in four patients: three Grade 3 ISR and one Grade 3 TRAE occurred in 1 patient (16.7%). In this cohort one dose-limiting toxicity (DLT) was reported (Grade 3 pancytopenia). In the SC cohorts, the most frequent TRAE was injection site reaction (ISR; 92%). Four DLTs were reported in four patients: three Grade 3 ISR and one Grade 3 transaminase increase. Grade 3 TRAE were reported in 22 patients (16.4%). Anti-tumor activity was observed across cohorts receiving SC selicrelumab (dose range 1 to 36 mg). Eight of 80 evaluable patients in DE cohorts experienced objective responses (9%) ORR. In the dose-expansion HNSCC cohort, three of 16 evaluable patients responded (15.8%) ORR. There were no objective responses in the IV cohort. Treatment with selicrelumab resulted in significant peripheral B-cell depletion and activation and CD8+ T-cell proliferation.

Conclusions Treatment with selicrelumab in combination with atezolizumab was well tolerated in patients with advanced solid tumors. Signals of clinical and PD activity were observed. However, efficacy of the combination in this unselected population was limited, when compared to monotherapy efficacy of atezolizumab.
In steroid refractory cases, we propose early initiation of steroidal sparing immunosuppressive therapy after 3 days.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0292

RESULTS OF THE FIRST-IN-HUMAN CLINICAL TRIAL WITH PERSONALIZED MULTI-TARGET ADOPTIVE CELL THERAPY (ACTOLOG IMA101)

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Background ACTolog (IMA101) is a personalized multi-target adoptive cell therapy (ACT) approach in which autologous T-cell products are redirected against multiple novel defined peptide–HLA (pHLA) cancer targets identified by the target discovery platform XPRESIDENT®. The primary endpoint was to assess the safety of ACTolog. Secondary endpoints were to assess the in vivo persistence of transferred T-cells and antitumor activity (www.clinicaltrials.gov NCT02876510).

Methods HLA-A*02:01 positive patients with relapsed/refractory solid tumors whose tumor expressed ≥1 cancer target underwent leukapheresis and endogenous T-cells specific for up to 4 targets were primed and expanded in vitro. Patients received lymphodepletion (fludarabine 40 mg/m² and cyclophosphamide 500 mg/m² on days -6 to -3) followed by up to 4 targets were primed and expanded in vitro. Patients received lymphodepletion (fludarabine 40 mg/m² and cyclophosphamide 300 mg/m² on days -6 to -3) followed by up to 4 × 10^8 cells (day 0), and IL-2 (1 × 10^6 IU/m² SC twice daily, 14 days) (Cohort 1). In addition, cohort 2 received alezolizumab (1200 mg IV) every 21 days upon hematologic recovery. Infused T-cells were tracked in patients' blood via flow cytometry-based immunomonitoring as well as TCR sequencing. TCRs from target specific T-cells were identified from patients' T-cell products and characterized.

Results From 07/2017–03/2020, 214 patients were screened, and 14 heavily pre-treated patients with various tumor types were infused with 1–3 T-cell products each (table 1). The treatment was generally well tolerated. The most common adverse events observed to date were expected cytopenias, mostly attributed to the lymphodepleting regimen, and Grade 1–2 cytokine release syndrome. Prolonged disease stabilization was noted in three patients for 12 months, 12+ months, and 7 months. High frequencies of target-specific T-cells up to 78.7% of CD8+ cells were detected in the blood of treated patients, persisted for >1 year and were detected in post-treatment tumor biopsies. Characterization of individual TCRs contained in T-cell products showed a broad variation of TCR avidities with the majority of TCRs being of low avidity. High-avidity TCRs were identified from products of some patients, including a patient with 26% decrease in tumor measurements 6 weeks post-treatment. Tracking the respective T-cell clonotypes in patients' blood and tumor provides insights into the mechanism of tumor control. Six-month data will be presented at the conference.

Conclusions This is the first reported trial demonstrating the feasibility and tolerability of a personalized ACT approach using multiple defined T-cell products directed to multiple precisely defined pHLA cancer targets. These results support further exploration of a multi-target ACT approach, particularly in the context of T-cells expressing high-avidity TCRs to such defined pHLA targets.

Trial Registration https://clinicaltrials.gov/ct2/show/NCT02876510

Ethics Approval The study was approved by WCG WIRB, IRB tracking number 20162233. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. All the study participants provided written informed consent before enrollment.

Consent Patient consent for publication is not required. Patients consented to participate in the study.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0293

CD8 PET IMAGING OF TUMOR INFILTRATING T CELLS IN ADVANCED SOLID TUMORS: A PHASE I FIRST-IN-HUMAN STUDY OF 89Zr-IAB22M2C, A RADIOLABELED ANTI-CD8 MINIBODY

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Background Tumor infiltration by CD8 T cells is associated with favorable outcomes to immunotherapy (IOT). However, biopsies to assess T cell infiltration are invasive and prone to sampling error. CD8 PET imaging could provide an effective non-invasive method of visualizing T cell trafficking and tumor infiltration and predicting early response to IOT.

Methods A phase 1 first-in-human PET imaging study using an anti-CD8 radiolabeled Minibody, 89Zr-IAB22M2C (CD8-tracer), to detect whole body and tumor CD8 distribution in patients with metastatic solid tumors was completed. Patients received 3 mCi 89Zr-IAB22M2C followed by serial PET scans over a 5–7-day period. A two-stage design included protein dose escalation phase1 (n=6, 0.2 mg to 10 mg API) to
establish safety and optimal scanning parameters and a dose expansion phase focusing on two API doses (0.5 mg (n=4) or 1.5 mg (n=5) API). All patients were monitored for drug-related adverse events with blood chemistry, hematology, cytokine assay and anti-drug antibodies (ADA). Biodistribution, radiodosimetry and SUV PET uptake was performed in all patients.

**Results**

15 subjects (31–82 years, M/F = 9/6) with metastatic melanoma (n=8), NSCLC (n=6), and HCC (n=1) were enrolled. Treatment histories included naïve (n=2), discontinued prior IOT (n=3), active IOT (n=10). No drug-related AEs nor abnormal laboratory tests were noted except for a transient increase in ADA in 1 subject. The CD8-tracer accumulated in tumors and CD8 rich tissues (e.g. spleen, marrow, nodes) with maximum uptake at 24–48 hours post injection along with low background activity in non-T cell rich tissues (e.g. muscle, heart). More favorable dosimetry was seen at 1.5 mg versus 0.5 mg API (effective dose=0.64 mSv/MBq versus 0.67 mSv/MBq, respectively). Comparison of 1.5 mg and 0.5 mg API in expansion cohorts demonstrated similar uptake in nodes but with reduced uptake in marrow and spleen at the higher API. Tracer-uptake in tumors was noted in 10/15 (67%) subjects, favoring slightly higher tumor uptake in the 1.5 mg cohort. One patient with advanced melanoma on IOT had increased CD8-tracer uptake in several metastases on an early post treatment scan, which correlated with response (figure 1).

**Conclusions**

89Zr-IAB22M2C targets CD8+ rich tissues and visualizes whole-body biodistribution of CD8+ cells in tumors and reference tissues and may predict early response to IOT. A 1.5 mg protein dose provides similar distribution to 0.5 mg dose, with more favorable dosimetry and is used in the ongoing Phase 2 study.

**Acknowledgements**

None

**Trial Registration**

ClinicalTrials.gov Identifier: NCT03107663

**Ethics Approval**

The study was approved by Institutional Review Boards of MSKCC (IRB #16-1109), Honor Health (West IRB #1179278) and University of Pennsylvania (IRB #828992).

**Consent**

Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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**Reference**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0294
IMMUNE CORRELATES OF CLINICAL RESPONSE TO AVELUMAB IN PATIENTS WITH ADVANCED THYMIC EPITHELIAL TUMORS
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Background Thymic epithelial tumors (TET), consisting of thymomas and thymic carcinomas, are PD-L1-expressing tumors characterized by varying degrees of lymphocytic infiltration and a predisposition towards the development of paraneoplastic autoimmunity. As part of a phase I study (NCT01772004), the anti-tumor activity of patients with relapsed, advanced TET to avelumab (anti-PD-L1), was demonstrated and was accompanied by a high frequency of immune related adverse events (irAE). The current study aimed to identify immune related signatures that associate with clinical response and/or the development of irAE.

Methods Eight patients with recurrent TET were treated with avelumab at doses of 10 mg/kg to 20 mg/kg every 2 weeks until disease progression or development of intolerable side effects. Peripheral blood mononuclear cells (PBMC) were obtained before and during therapy, and interrogated by multicolor flow cytometry to evaluate 123 immune subsets, as well as by T-cell receptor (TCR) sequencing to evaluate TCR diversity.

Results Four of 8 TET patients had partial responses and 3 had stable disease. All responders developed irAEs that resolved with immunosuppressive therapy, compared to only 1 of 4 non responders. Analyses of PBMC subsets prior to therapy showed that responders had higher absolute lymphocyte counts, and lower frequencies of B cells, Tregs, conventional dendritic cells (cDCs), and NK cells, compared to non-responders. There was also a trend towards a higher level of TCR diversity in those patients who subsequently had a radiological response and developed irAE.

Conclusions Immune profiling identified specific immune measures prior to therapy that differed between responders and non-responders, that may serve as predictive biomarkers to identify patients with relapsed TET most likely to benefit from avelumab and/or to develop irAE.

Trial Registration NCT01772004

Ethics Approval All patients provided written informed consent for participation in a clinical trial that was approved by the Institutional Review Board at the National Cancer Institute (NCT01772004).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0296

MODELING THE EFFICACY OF NY-ESO-1 TCR T CELLS (LETETREGENE AUTOULECEL; GSK3377794) IN PATIENTS WITH SYNOVIAL SARCOMA: CORRELATIONS OF RESPONSE WITH TRANSDUCED CELL KINETICS AND BIOMARKERS

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Background NY-ESO-1–specific T cells (letetregene autoleucel [lete-cel]; GSK3377794) are autologous CD4+ and CD8+ T cells transduced to express a high-affinity T-cell receptor that recognizes NY-ESO-1 antigen in complex with HLA-A*02. NY-ESO-1 is a cancer testis antigen that is expressed in many cancers, including synovial sarcoma (SS). Study 208466 (NCT01343043) is a Phase I clinical trial that assessed the safety and efficacy of lete-cel in patients with advanced SS (presented in complementary abstract). This abstract presents correlations of transduced cell kinetics and biomarkers with response.

Methods Patients with unresectable, metastatic, or recurrent SS were enrolled to 4 cohorts based on NY-ESO-1 expression levels and received different lymphodepleting regimens (LDR) prior to lete-cel infusion (N=45) (table 1). Response was assessed per RECIST v1.1. Transduced cell kinetics (persistence) were measured by quantitative PCR of transgene vector copies in DNA extracted from peripheral blood mononuclear cells. Serum cytokines were measured by Mesoscale Discovery (MSD) immunoassay. Gene expression within tumor biopsies was evaluated by Nanostring. Post hoc analyses were evaluated in a hypothesis-driven manner using logistic and linear regression. Potential determinants of peak persistence and clinical response were tested using generalized linear models.

Results Higher peak persistence (Pmax) was associated (p=0.012) with response across cohorts. Higher weight-normalized cell dose (p=0.00421) and LDR (p=0.000910) were associated with Pmax according to the generalized linear model: Pmax ~ cell dose + LDR. These relationships allowed for accurate retrospective prediction of probability of response. Low LDR resulted in higher endogenous lymphocyte counts on the day of dosing, which trended with lack of response within and across cohorts. While the impact of fludarabine on IL-15 levels has been previously reported, data presented here show a novel, positive correlation between IL-15 levels pre-infusion and response (p=0.0332). Post lete-cel infusion, the concentrations of IFNy, IL-6, and IL-2RA within the first week were increased in responders vs non-responders. The peak expression of IL-2RA within the first week showed a linear correlation to Pmax. Analysis of tumor biopsies showed good correlation between NY-ESO-1 mRNA and protein expression.

Abstract 297 Table 1 NY-ESO-1 expression, lymphodepletion regimen, overall response rate, mean transduced cell dose, and mean peak persistence in Cohorts 1–4

| Table 1 | NY-ESO-1 expression, lymphodepletion regimen, overall response rate, mean transduced cell dose, and mean peak persistence in Cohorts 1–4 |
|---|---|---|---|---|
| | Cohort 1 | Cohort 2 | Cohort 3 | Cohort 4 |
| | mean (SD) | mean (SD) | mean (SD) | mean (SD) |
| NY-ESO-1 expression | mean (SD) | mean (SD) | mean (SD) | mean (SD) |
| High | 300 copies in DNA extracted from peripheral blood mononuclear cells | 300 copies in DNA extracted from peripheral blood mononuclear cells | 300 copies in DNA extracted from peripheral blood mononuclear cells | 300 copies in DNA extracted from peripheral blood mononuclear cells |
| Low | 300 copies in DNA extracted from peripheral blood mononuclear cells | 300 copies in DNA extracted from peripheral blood mononuclear cells | 300 copies in DNA extracted from peripheral blood mononuclear cells | 300 copies in DNA extracted from peripheral blood mononuclear cells |
| LDR (mg/kg IV daily) | 10 | 10 | 10 | 10 |
| Response rate (%) | 4/10 (40) | 2/10 (20) | 2/10 (20) | 2/10 (20) |
| Mean transduced cell dose in Billions (mean, SD) | 6.00 (5.00, 1.00) | 6.00 (5.00, 1.00) | 6.00 (5.00, 1.00) | 6.00 (5.00, 1.00) |
| Mean (SD) peak persistence (week regimens (WRR)) | 70.00 (5.00) | 70.00 (5.00) | 70.00 (5.00) | 70.00 (5.00) |

Conclusions Exposure–response analysis of study 208466 reveals that efficacy appears to be driven by weight-normalized...
cell dose and LDR via Pmax. Biomarker correlation analysis indicates that LDR impacts the level of IL-15 pre-infusion, which correlates with response directly. IFNγ, IL-6, and IL-2RA levels appear to be promising pharmacodynamic markers. Optimizing dose and LDR may offer opportunities to maximize antitumor efficacy.

Acknowledgements This study (208466) was funded by GlaxoSmithKline (GSK).

Trial Registration Clinicaltrials.gov NCT01343043

Ethics Approval This study was approved by the appropriate institutional review boards and independent ethics committees.

Background NY-ESO-1–specific T cells (letetresgene autoleucel [lete-cel]; GSK3377794) are autologous T cells transduced with a self-inactivating lentiviral vector to express an engineered NY-ESO-1–specific TCR that recognizes HA-A*02–presented peptides derived from NY-ESO-1, a cancer/testis antigen expressed in 70%–80% of SS. NCT01343043 was a Phase I, open-label trial assessing safety, efficacy, and pharmacokinetics of lete-cel in patients with SS; activity was evaluated after different lymphodepletion conditioning regimens and in patients with differing levels of NY-ESO-1 expression.

Methods Patients with unresectable, metastatic, or recurrent SS who were intolerant/nonresponsive to standard first-line chemotherapy enrolled in 4 cohorts based on NY-ESO-1 tumor expression and more intensive lymphodepletion regimen in Cohorts 1/2/3/4, respectively, median DoR was 31.0/8.6/24.3/9.9/19.9 months; Cohort 4 median OS was immature (table 1).

Overall, 50 patients enrolled; 45 received lete-cel infusion to compare cohorts. Primary endpoint was investigator-assessed overall response rate (ORR) per RECIST v1.1; secondary endpoints included duration of response (DoR), progression-free survival (PFS), overall survival (OS), and safety. Transduced cell persistence was measured by qPCR of transgene vector copies in DNA extracted from PBMCs. Study was not designed/powered to compare cohorts. Results Overall, 50 patients enrolled; 45 received lete-cel infusion (modified intent-to-treat population). Demographics were similar between cohorts. Median time in study was 480/278/605/643 days in Cohorts 1/2/3/4, respectively. At study completion, ORR ranged from 20%–50% between cohorts, with 1 complete (lasting 34 weeks) and 14 partial responses included (table 1). Primary endpoint was investigator-assessed overall response rate (ORR) per RECIST v1.1; secondary endpoints included duration of response (DoR), progression-free survival (PFS), overall survival (OS), and safety. Transduced cell persistence was measured by qPCR of transgene vector copies in DNA extracted from PBMCs. Study was not designed/powered to compare cohorts.

Conclusions In patients with advanced SS who need effective treatment, lete-cel had a manageable safety profile; responses occurred in all cohorts, but patients with high NY-ESO-1 expression and more intensive lymphodepletion regimen received greatest benefit.

Acknowledgements This study (208466) was funded by GlaxoSmithKline. Medical writing assistance was provided by Gemma Corr, DPhil, and Tiffany Brake, PhD, of Fishawack Indicia, UK, and funded by GlaxoSmithKline. We thank Ran Ji for contributions to statistical analysis.
IMMUNO-METABOLIC SIGNATURES OF DENDRITIC CELLS ASSOCIATE WITH T-CELL RESPONSES IN MELANOMA PATIENTS

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Background The therapeutic efficacy of Dendritic cells (DC) vaccines remains low and there is an unmet need for more effective vaccine design to achieve durable clinical outcomes. Our study analyzed the transcriptomic and energetic metabolism profile of an adenoviral-based DC vaccine targeted against three commonly shared melanoma antigens: Tyrosinase, MART-1 and MAGE-A6 from 35 subjects enrolled in a Phase I study of autologous DC vaccines in late-stage melanoma.1

To further investigate the immuno-metabolic features of mono-cyte-derived DC vaccines, we are employing a novel flow cytometry-based-method, called SCENITH™ to integrate functional metabolic states with multiparametric DC immune phenotypes.

Methods iDC were generated from HD and patient monocytes using GM-CSF+IL-4 for 5d. DC were matured (mDC) using IFN?+LPS for additional 24 hrs. Tolerogenic DC (Tol DC) were generated using vitamin-D3 and dexamethasone. Seahorse™ was used to measure DC metabolic profile. Cytek/Aurora spectral flow cytometry was used for multiparametric-phenotypic and metabolic analysis by SCENITH™.

Results Melanoma patient mDC used for autologous vaccine generation showed significantly altered metabolic gene signatures associated with enhanced oxidative phosphorylation (OXPHOS) and lipid metabolism pathways as compared to HD mDC. Furthermore, increased enrichment for mitochondrial respiration genes involved in the TCA cycle, electron transport chain and fatty acid oxidation (FAO) correlated with inferior tumor antigen-specific T cell responses and clinical outcome in patients. Seahorse analyses confirmed that HD and good outcome patient DC demonstrated the highest maturation-induced reduction in maximal oxygen consumption rate/ OXPHOS and exogenous FAO. Interestingly, while the glycolytic rate of non-responding patient DC was the lowest, overall, we observe only a moderate increase in glycolytic capacity during DC maturation. SCENITH analysis showed that unlike monocytes, which are primarily glycolytic, differentiated mono-derived iDC and mDC utilize both glycolysis and mitochondrial respiration. Interestingly, under tolerogenic (Tol) differentiation conditions Tol iDC shift from glucose dependence into FAO and/or glutaminolysis while Tol mDC strongly depend on OXPHOS. Consistent with dependence on mitochondrial respiration, Tol mDC exhibit reduced HIF1α levels together with enhanced p-AMPK:p-mTOR ratio. Additionally, we show that the altered metabolism of Tol mDC is linked to retention of CD14-monocyte antigen with reduced DC markers HLA-DR, CD86, CD206, CD11c, CD33, with increased PD-L1 and ILT3 expression. Furthermore, we show that unlike HD mDC, tolerogenic and melanoma patient-derived mDC populations exhibit similar metabolic and immune characteristics.

Conclusions We demonstrate that metabolic profile of DCs is tightly associated to the immuno-stimulatory potential of DC vaccines from cancer patients. Using SCENITH, we linked phenotypic and functional metabolic changes associated to immune signatures that correspond to heathy and immuno-suppressed DC differentiation.

Ethics Approval The clinical trial reported was fully approved by the Univ. Pittsburgh PRC and IRB (PRO12010416, #09–021) and had FDA IND #15044 and NCT01622933.
**Abstract 300 Figure 1** 36-month disease free survival for patients receiving TLPLDC vs placebo by PT analysis

**Abstract 300 Figure 2** 36-month disease free survival for subset of stage IV melanoma patients receiving TLPLDC vs placebo by PT analysis

**Results** Overall, 103 patients received TLPLDC and 41 placebo. In PT analysis, 65 patients received TLPLDC and 32 placebo. Total adverse events (AEs), grade 3+ AEs, and serious AEs (SAEs) were similar in placebo vs TLPLDC groups, with one related SAE per treatment arm. By ITT analysis, 36-month OS was 76.2% for TLPLDC vs 70.3% for placebo (HR 0.72, p=0.437) and 36-month DFS was 35.6% vs 27.1% (HR 0.95, p=0.841). By PT analysis, 36-month DFS was improved with TLPLDC (57.5% vs 35.0%; HR 0.50, p=0.025, figure 1). This effect was even more dramatic in resected stage IV patients (36-month DFS: 60.9% vs 0%; HR 0.12, p=0.001, figure 2).

**Conclusions** This phase IIb trial again demonstrates the safety of the TLPLDC vaccine, and an improved 36-month DFS in patients with resected stage III/IV melanoma who complete the primary vaccine series, particularly in the stage IV subgroup. Next, a phase III trial will evaluate the efficacy of TLPLDC vaccine as adjuvant treatment for resected stage IV melanoma, with patients randomized to receive standard of care PD-1 inhibitors + TLPLDC versus PD-1 inhibitors + placebo.

**Trial Registration** This is a phase IIb clinical trial registered under NCT02301611

**Ethics Approval** This study was approved by Western IRB, protocol 20141932.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0300

**Background** The phase 3 IMspire150 study (NCT02908672) demonstrated improved progression-free survival (PFS) with first-line atezolizumab (A) vs placebo (P) combined with vemurafenib (V) + cobimetinib (C) in patients with BRAF mutation–positive advanced melanoma (15.1 vs 10.6 months; hazard ratio [HR] 0.78; 95% confidence interval [CI] 0.63–0.97; P=0.0249). Objective response has been associated with increased survival with chemotherapy and targeted therapies, but it is unclear whether the association holds for immunotherapy. In this exploratory analysis, we evaluated the impact of response on survival outcomes in patients treated with A+V+C or P+V+C in the IMspire150 study.

**Methods** 514 patients were randomized 1:1 to A+V+C (n=256) or P+V+C (n=258). Patients received V+C in cycle 1; A or P was added on days 1+15 from cycle 2 onward. The primary endpoints for this exploratory analysis were PFS and overall survival (OS), estimated using the Kaplan-Meier method. Outcomes were analyzed by investigator-assessed best overall response (BOR) per RECIST v1.1 (complete response [CR] vs partial response [PR] vs stable disease [SD]).

**Results** Median follow-up was 18.9 mo. In the A+V+C arm, BOR was CR (n=41), PR (n=129), and SD (n=258); in the P+V+C arm, BOR was CR (n=46), PR (n=122), and SD (n=58). An imbalance in baseline prognostic factors (eg, lactate dehydrogenase, tumor burden measures) was noted across response categories in both treatment arms, with favorable factors more prevalent in patients with CR and unfavorable factors more prevalent in patients with PR/SD. Improvement in
PFS and OS was observed with A+V+C vs P+V+C in patients with PR, with 2-year PFS rates of 42.1% vs 24.6% and 2-year OS rates of 69.1% vs 56.1% with A+V+C vs P+V+C (table 1). In patients with CR, median PFS and OS were not yet reached in either arm, with 2-year PFS rates of 64.6% vs 59.8% and 2-year OS rates of 82.6% vs 82.8% with A+V+C vs P+V+C. PFS and OS outcomes were poor in both treatment arms in patients with SD, with 2-year PFS rates of 10.7% vs not estimable (NE) and 2-year OS rates of 36.6% vs 29.3% with A+V+C vs P+V+C.

Conclusions PFS and OS improvement was observed for A+V+C vs P+V+C for patients who achieved CR. PR is associated with improved PFS and OS with both A+V+C and P+V+C. Further follow-up is required to determine the impact of A+V+C vs P+V+C on survival outcomes.

**Trial Registration** ClinicalTrials.gov, NCT02908672

**http://dx.doi.org/10.1136/jitc-2020-SITC2020.0301**

**Abstract 301 Table 1** PFS and OS outcomes with A+V+C vs P+V+C by BOR per RECIST v1.1

<table>
<thead>
<tr>
<th></th>
<th>A+V+C</th>
<th>P+V+C</th>
<th>HR for A+V+C vs P+V+C (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CR</strong></td>
<td>NR (23.0-NE)</td>
<td>NR (23.0-NE)</td>
<td>0.83 (0.40-1.72)</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>18.4 (15.3-21.4)</td>
<td>12.3 (10.4-14.7)</td>
<td>0.64 (0.46-0.87)</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>4.6 (3.6-5.6)</td>
<td>5.5 (4.4-7.4)</td>
<td>0.91 (0.61-1.36)</td>
</tr>
<tr>
<td><strong>2-year PFS rate (% (95% CI)</strong></td>
<td>64.6 (48.6-80.6)</td>
<td>50.8 (43.5-76.0)</td>
<td>–</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>42.1 (32.5-51.6)</td>
<td>24.6 (16.5-32.8)</td>
<td>–</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>10.7 (1.4-20.3)</td>
<td>NE</td>
<td>–</td>
</tr>
<tr>
<td><strong>Median OS, months (95% CI)</strong></td>
<td>NR (NE-NE)</td>
<td>NR (NE-NE)</td>
<td>0.97 (0.35-2.07)</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>28.8 (27.4-NE)</td>
<td>25.0 (22.7-NE)</td>
<td>0.67 (0.44-1.02)</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>14.1 (10.6-NE)</td>
<td>14.6 (10.6-21.2)</td>
<td>0.93 (0.58-1.48)</td>
</tr>
<tr>
<td><strong>2-year OS rate, % (95% CI)</strong></td>
<td>82.0 (69.4-95.7)</td>
<td>62.8 (71.1-94.5)</td>
<td>–</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>69.1 (60.3-77.9)</td>
<td>56.1 (46.4-65.6)</td>
<td>–</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>36.6 (22.5-50.7)</td>
<td>20.3 (15.2-43.5)</td>
<td>–</td>
</tr>
</tbody>
</table>

**Abstract 302 Table 1** PVSRIPO anti-tumor response relative to ICI administration and post-study disease status

<table>
<thead>
<tr>
<th>Time to last anti-PD-1 relative to PVSRIPO</th>
<th>ORR per irRC</th>
<th>Proportion treated with ICI post-PVSRIPO</th>
<th>Progression-free post-PVSRIPO alone or PVSRIPO followed by ICI</th>
<th>Median duration of follow-up (11 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 30 days</td>
<td>60% (1 of 3)</td>
<td>70% (4 of 6)</td>
<td>60% (1 of 3)</td>
<td>6 months</td>
</tr>
<tr>
<td>&gt; 30 days</td>
<td>14% (1 of 7)</td>
<td>47% (2 of 7)</td>
<td>47% (2 of 7)</td>
<td>14 months</td>
</tr>
</tbody>
</table>

3 different lesions) injections of PVSRIPO monotherapy, 21 days apart.

**Results** PVSRIPO injections were well tolerated with no SAEs or DLTs reported; all TEAEs were grade (G) 1 or 2 (grade 1 pruritus most common at 38%), with all but 2 PVSRIPO-related TEAEs localized to the injected or adjacent lesions (n=1 G1 hot flash, n=1 G1 fatigue). Despite the limited number of PVSRIPO treatments relative to the overall lesion burden (67% patients >5 lesions), 4 of 12 patients (33%) achieved an objective response per irRC, including 4/6 (66%) who received 3 injections (maximum administered). Pathologic complete response (ie, no viable tumor detected in injected and non-injected lesions biopsied) was observed in 2 of 4 (50%) patients with in-transit disease. PVSRIPO response relative to time since prior anti-PD-1 exposure is summarized in table 1. Following study completion/PVSRIPO therapy, 10/12 patients (83%) again received immune checkpoint inhibitor (ICI)-based therapy and 6/12 patients (50%) remained progression free at the data cutoff.

**Conclusions** Intratumoral PVSRIPO was well tolerated. When taken together with preclinical data, the anti-tumor responses observed relative to prior or subsequent ICI therapy suggests that PVSRIPO, either alone or in combination with anti-PD-1, may be an effective treatment in anti-PD-1 refractory melanoma. An amendment exploring higher PVSRIPO dose levels is ongoing and a phase 2 study with and without anti-PD-1 in the refractory population is initiating.

**Ethics Approval** This study (NCT03712358) was approved by WIRB; ID 20181772.

**http://dx.doi.org/10.1136/jitc-2020-SITC2020.0302**

**Abstract 303** PHASE II TRIAL OF NEOADJUVANT NIVOLUMAB (NIVO) AND INTRA-TUMORAL (IT) CMP-001 IN HIGH-RISK RESECTABLE MELANOMA (NEO-C-NIVO): FINAL RESULTS

**Background** Neoadjuvant PD-1 blockade produces major pathological responses (MPR) in ~30% of patients (pts) with high-risk resectable melanoma (MEL) with durable relapse-free benefit, and increased circulating activated CD8+ T cells.2 1 CMP-001 is a type A CpG packaged within a virus-like particle that activates tumor-associated plasmacytoid dendritic cells (pDC) via TLR9 inducing type I interferons and anti-tumor immune responses (1D). Neoadjuvant PD-1 blockade produces major pathological responses (MPR) in ~30% of patients (pts) with high-risk resectable melanoma (MEL) with durable relapse-free benefit, and increased circulating activated CD8+ T cells.2 1 CMP-001 is a type A CpG packaged within a virus-like particle that activates tumor-associated plasmacytoid dendritic cells (pDC) via TLR9 inducing type I interferons and anti-tumor immune responses (1D). Neoadjuvant PD-1 blockade produces major pathological responses (MPR) in ~30% of patients (pts) with high-risk resectable melanoma (MEL) with durable relapse-free benefit, and increased circulating activated CD8+ T cells.2 1 CMP-001 is a type A CpG packaged within a virus-like particle that activates tumor-associated plasmacytoid dendritic cells (pDC) via TLR9 inducing type I interferons and anti-tumor immune responses (1D). Neoadjuvant PD-1 blockade produces major pathological responses (MPR) in ~30% of patients (pts) with high-risk resectable melanoma (MEL) with durable relapse-free benefit, and increased circulating activated CD8+ T cells.2 1 CMP-001 is a type A CpG packaged within a virus-like particle that activates tumor-associated plasmacytoid dendritic cells (pDC) via TLR9 inducing type I interferons and anti-tumor immune responses (1D). Neoadjuvant PD-1 blockade produces major pathological responses (MPR) in ~30% of patients (pts) with high-risk resectable melanoma (MEL) with durable relapse-free benefit, and increased circulating activated CD8+ T cells.2 1 CMP-001 is a type A CpG packaged within a virus-like particle that activates tumor-associated plasmacytoid dendritic cells (pDC) via TLR9 inducing type I interferons and anti-tumor immune responses (1D).
Methods 30 pts with stage III B/C/D MEL were enrolled. Preoperatively, CMP-001 was dosed at 5 mg subcutaneous (SC, 1st), then 10 mg IT (2nd-7th) weekly; Nivo was dosed 240 mg q2 weeks for 3 doses – both agents given for 7 weeks. Post-operatively, Nivo was dosed 480 mg q4 weeks with CMP-001 5 mg q4 weeks SC for 48 weeks. Primary endpoints included major pathologic response rate (MPR), and incidence of dose-limiting toxicities (DLT). Secondary endpoints were radiographic response, relapse-free survival (RFS) and overall survival (OS). Pathological response was scored blinded by pathologists based on residual volume of tumor (RVT) using prior specified cutoffs: 46.0% (complete response, pCR); 0%<rvt<rvt50% (non-response, pNR). Radiographic response was assessed using RECIST v1.1. Sequential blood draws and tumor biopsies were collected and analyzed for CD8+ T cell infiltrate (TIL), multiparameter flow cytometry (MFC) and multiplex immunofluorescence (mIF).

Results 30 pts with regionally advanced MEL were enrolled, of stages IIIB (57%), IIIC (37%), IIBD (7%). 29/30 (97%) of pts completed 7 weeks of neoadjuvant Nivo/CMP; while 1 pt had a delay in surgery related to a pre-operative infection unrelated to therapy. No DLTs were reported; grade 3/4 irAE were reported in 3 pts (11%) leading to CMP-001 discontinuation in 2 pts (7%). Radiographic responses were seen in 13 pts (43%), while 9 pts (30%) had stable disease and 8 pts (27%) had progressive disease. Pathological responses (RVT <50%) were seen in 70% of pts: pCR 15 (50%), pMR 3 (10%), 3 pPR (10%); only 9 (30%) had pNR. Pathological responders (pCR/ pMR) had increased CD8+ TIL and CD303+ pDC intratumorally by mIF; and peripherally activated PD1+Ki67+ CD8+ T cells by MFC.

Conclusions Neoadjuvant CMP/Nivo has acceptable toxicity and promising efficacy. MPR is 60% in 30 pts. I-year RFS was 82% (all pts) and 89% (among those with pCR/pMR); median RFS is 9 months (among pNR/pPR) and not reached (among pCR/pMR). Response is associated with evidence of immune activation intra-tumorally and peripherally. IT CMP001 increases clinical efficacy of PD-1 blockade with minimal additional toxicity in pts with regionally advanced MEL. Further study of this combination in high-risk resectable MEL is planned.

Acknowledgements We thank Dr. Jagjit Singh and the pathology grossing room staff for their assistance and Checkmate Pharmaceuticals for funding and CIMP-001.

Trial Registration Clinical trial information: NCT03618641

Ethics Approval The study was approved by University of Pittsburgh’s Institutional Review Board, approval number MOD19040237-002.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Background Therapeutic options are limited for patients with advanced melanoma that is refractory to PD-1 blockade. This study was performed in this patient population to assess the safety and antitumor activity of CMP-001, a CpG-A TLR9 agonist packaged within a virus-like particle.

Methods Patients were eligible for this 2-part, open-label, multicenter, phase 1b study if they had metastatic/unresectable melanoma and stable disease after =12 weeks or progressive disease (PD) on/after anti-PD-1 therapy. Part 1 evaluated CIMP-001 plus pembrolizumab dose-escalation and dose-expansion. Part 2 evaluated CIMP-001 monotherapy. Accessible lesion(s) were injected intratumorally with CIMP-001, at a polysorbate 20 (PS20) concentration of either 0.01% or 0.00167%. The Part 1 primary objective was to identify the recommended phase 2 dose (RP2D) and schedule of CIMP-001 plus pembrolizumab, while the Part 2 primary objective was to assess the safety of CIMP-001 monotherapy. Secondary objectives for both parts were a preliminary assessment of antitumor activity of CIMP-001 plus pembrolizumab and CIMP-001 monotherapy, and the overall safety profile and pharmacodynamics of the combination.

Results In Part 1 (N=159) and Part 2 (N=40), 93.1% and 80.0% of patients had PD as their last response to prior anti-PD-1 therapy, respectively. The most common treatment-related adverse events (TRAEs; >25%) were flu-like symptoms (Parts 1 and 2) and injection-site reactions (Part 1). Grade 3/4 TRAEs were reported in 36.5% (Part 1) and 22.5% (Part 2) of patients, the most common being hypotension (Part 1:...
6.9%; Part 2: 5.0%). No Grade 5 TRAEs were observed. In Part 1, the best objective response rate (ORR; RECIST v1.1) in patients treated with pembrolizumab and CMP-001 (PS20 0.01%) was 23.5% (23/98), while CMP-001 PS20 (0.00167%) resulted in a lower ORR of 11.5% (7/61). Seven additional patients had a delayed response after initial PD (table 1). The median duration of response was >1 year. In the 37 RECIST v1.1 and post-progression responders, the mean regression in injected and noninjected target lesions was 54.7% and 52.7%, respectively. In Part 2, the best ORR with CMP-001 monotherapy was 17.5% (7/40 patients); the response duration was shorter than in Part 1. Intratumoral CMP-001 PS20 0.01% 10 mg was selected as the RP2D.

Conclusions Intratumoral CMP-001 was well-tolerated and provided both local and distant responses in patients with advanced melanoma with disease progression on prior PD-1 blockade. CMP-001 monotherapy induced systemic tumor regression in some patients, but duration of response was substantially increased by the addition of pembrolizumab.

Acknowledgements This work was supported by Checkmate Pharmaceuticals. Medical writing assistance was provided by Cindy Rigby, PhD, of ApotheCom (San Francisco, CA) and was funded by Checkmate Pharmaceuticals.

Trial Registration NCT02680184

Ethics Approval This study was approved by the WCG-WIRB, WIRB approval tracking number 20152597.

Consent N/A

REFERENCES


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306 PREDICTORS OF IMMUNOTHERAPY BENEFIT IN MERKEL CELL CARCINOMA

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Background Merkel cell carcinoma is a rare cancer for which the standard-of-care is immune checkpoint blockade in the recurrent/metastatic setting. However, immunotherapy is not effective in all patients. A greater understanding of molecular mechanisms and potential predictive biomarkers are unmet needs for clinicians and researchers.

Methods We undertook a retrospective analysis of 45 patients treated at our institution from 2013 to 2020 to understand the clinical and genomic correlates of clinical benefit from immunotherapy. We gathered data from the electronic health record, including provider notes and results from our institutional next-generation sequencing panel of actionable genomic alterations.

Results Our cohort predominantly included individuals with stage III disease at diagnosis and stage IV disease at the time of diagnosis of recurrent/metastatic disease. Most patients received immunotherapy in the first line. 43% of patients experienced an objective response to immunotherapy (median duration of response 24.2 months, 95% confidence interval 15.5 months (95% confidence interval 9.0–28.7) (median follow-up 25.2 months). Lower stage at diagnosis of primary disease and shorter disease-free interval between completion of initial treatment and recurrence were each associated with greater odds of response (odds ratio 0.06, p=0.04 for stage; odds ratio 0.75, p=0.05 for disease-free interval). The most common single-nucleotide variants among the sequenced cohort were those in TP53 (59%) and RB1 (51%). Single-nucleotide variants in the ARID2 and NTRK1 genes were associated with response without Bonferroni correction (p=0.05), while none of Merkel cell polyomavirus status, total mutational burden, ultraviolet mutational signatures, and copy-number alterations predicted outcomes (figure 1).

Conclusions Patients with shorter disease-free interval after definitive treatment may be particularly suitable candidates for immunotherapy. Our molecular findings point to ARID2 and NTRK1 as potential predictive markers and/or therapeutic targets (e.g., with Trk inhibitors), although this association needs to be confirmed in a larger sample.

Acknowledgements AJK receives research funding from the American Society of Hematology and from the Pritzker School of Medicine.

Ethics Approval The study was approved by the Dana-Farber institutional review board, protocol numbers 11–104 and 17–000.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0306

A307 ATEZOLIZUMAB PLUS VEMURAFENIB AND COBIMETINIB PROVIDES FAVORABLE SURVIVAL OUTCOMES IN PATIENTS WITH HIGH TUMOR MUTATION BURDEN AND PROINFLAMMATORY GENE SIGNATURE IN THE PHASE 3 IMSPIRE150 STUDY

Karl Lewis*, 1Paolo Ascierto, 2Caroline Robert, 3Rodrigo Munhoz, 2Gabriella Lisciay, 5Luis De La Cruz Marino, 7Judit Olah, 6Paola Queirolo, 3Jack Mackiewicz, 5Kalpit Shah, 1Harper Forbes, 11Christian Hertig, 10Yibing Yan, 12Ralf Gutzmer, 11Grant McArthur.

1University of Colorado Comp. Cancer Ctr, Aurora, CO, USA; 2Istituto Nazionale Tumori IRCCS, Napoli, Italy; 3Gustave Roussy and Université Paris-Saclé, Villejuif-Paris, France; 4Instituto do Câncer do Estado, São Paulo, Brazil; 5Orságos Onkológiai Intézet, Budapest, Hungary; 6Hospital Universitario Virgen Macarena, Seville, Spain; 7University of Szeged Szent-Györgyi, Szeged, Hungary; 8IRCCS Istituto Europeo di Oncologia, Milan, Italy; 9Greater Poland Cancer Centre, Poznan, Poland; 10Genentech, Inc., South San Francisco, CA, USA; 11Haut-Tumour-Zentrum Hannover (HTZH), Hannover, Germany; 12Peter MacCallum Cancer Centre, Melbourne, Australia

Background The phase 3 IMspire150 study (NCT02908672) showed that first-line atezolizumab (A) combined with vemurafenib (V) + cobimetinib (C) improved progression-free survival (PFS) vs placebo (P) + V + C in patients with BRAFV600 mutation–positive advanced melanoma (15.1 vs 10.6 months; hazard ratio [HR] 0.78; 95% CI 0.63–0.97; P=0.0249). Insights into the clinical benefit of the A+V+C triple combination in prognostic molecular subgroups of patients can inform treatment selection and future clinical research.

Methods 514 patients were randomized 1:1 to A+V+C (n=256) or P+V+C (n=258). The efficacy endpoints analyzed included PFS and duration of response (DOR) estimated using the Kaplan-Meier method. Outcomes were based on investigator-assessed best overall response per Response Evaluation Criteria in Solid Tumors v1.1. Patients were primarily categorized into binary subgroups defined by tumor mutation burden (TMB; low or high: <10 or ≥10 mutations/Mb, respectively) or by the < or ≥ median values

Abstract 306 Figure 1 Mutation landscape by immune checkpoint inhibitor response. Mutational plot showing the most frequently mutated genes (top-to-bottom, ≥15%) ordered by response and by total number of SNVs, with gene frequency listed at left (%), and Fisher exact test p values (response versus no response) at right. Asterisks denote values less than 0.05 (significant before Bonferroni correction, for which cutoff for significance is 0.0001 for our panel of 447 genes). The bar graph at top shows the total number of panel single nucleotide variants detected per sample by mutation signature. Blank MCPyV and TMB denote unknown values.
of interferon (IFN)-gamma or CD8+ tumor cells. In addition, these subgroups were further broken down based on the proportion of programmed death-ligand 1 (PD-L1)-expressing tumor-infiltrating cells as PD-L1+ (≥1%) or PD-L1− (<1%).

Results Patients treated with P+V+C with high and low TMB had similar PFS outcomes. However, the magnitude of the PFS benefit with A+V+C vs P+V+C was markedly higher in patients with high TMB (≥10 mutations/Mb) compared with patients with low TMB (<10 mutations/Mb) in whom the benefit between treatment arms was comparable (figure 1A). The magnitude of the PFS benefit with A+V+C was further enhanced in patients with high TMB and PD-L1− compared with patients with high TMB and PD-L1+. Overall, patients with potential for increased antitumor immunity (IFN-gamma ≥ median or CD8+ ≥ median) who received A+V+C had more favorable outcomes compared with their counterparts with IFN-gamma < median or CD8+ < median. In general, the PFS benefit with A+V+C vs P+V+C was more readily apparent in PD-L1− subgroups. Similar trends were seen with DOR (figure 1B).

Conclusions There was a trend of larger magnitude of PFS benefit with A+V+C vs P+V+C in PD-L1− patient subgroups, who benefit less with single-agent immunotherapy. The PFS and DOR benefits were more evident in patients with high IFN-gamma or TMB >10 mutations/Mb. Additional multivariate analyses are ongoing to delineate the PFS trends observed.

Trial Registration ClinicalTrials.gov, identifier NCT02908672
http://dx.doi.org/10.1136/jitc-2020-SITC2020.0307

Abstract 307 Figure 1  Forest plot of PFS (A) and DOR (B). mo, months; NE, not evaluable; Neg, negative; NE, not estimable; Pos, positive.

INDIRECT TREATMENT COMPARISON OF NIVOLUMAB VERSUS PLACEBO AS ADJUVANT TREATMENT FOR MELANOMA

Background We have previously performed indirect treatment comparisons (ITCs) to demonstrate improvements in recurrence-free survival (RFS) and distant metastasis-free survival with nivolumab versus placebo as adjuvant treatment for resected melanoma; however, overall survival (OS) data were not available at the time. Recently, results of the phase 3 CheckMate 238 trial in patients with resected stage IIIIB–IIIC/ IV melanoma (American Joint Committee on Cancer [AJCC],...
The role of tumor-draining lymph nodes in the tuning of systemic T cell immunity by CTLA-4 blockade is revealed by local delivery of tremelimumab in early-stage melanoma: data from a phase I trial

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Background The sentinel lymph node (SLN) is the first node to receive lymphatic drainage from the primary tumor and the site where naïve T cells are first primed. As such it is of great importance in initiating an effective anti-tumor immune response and an attractive target for immunomodulatory agents. Pre-clinical studies have reported that i.t. administration of anti-CTLA-4 is as effective in inducing tumor eradication as systemic delivery, without the risk of treatment related side effects. However, it remains unclear whether this is due primarily to modulation of the tumor microenvironment or of tumor-draining lymph nodes (TDLN). Here, we have evaluated the safety, tolerability and immunomodulatory effects in the SLN and peripheral blood mononuclear cells (PBMC) of anti-CTLA-4/tremelimumab, delivered locally at the tumor excision site in patients with early-stage melanoma. This unique setting (post tumor excision but prior to SLN biopsy) allowed us to clinically assess the role of TDLN in the biological efficacy of CTLA-4 blockade.

Methods In this phase 1 dose-escalation trial, patients with clinical stage I-II melanoma received one intradermal injection of tremelimumab at four dose levels (2, 5, 10 [n=3 each] or 20 mg [n=4]) around the primary excision site of the tumor, seven days prior to re-excision and SLN biopsy. Flow cytometry was performed to study viable cells from melanoma SLN and PBMC (prior to tremelimumab administration [day 0], and at 7 days, 3 weeks and 3 months after tremelimumab injection). Systemic melanoma antigen (MART-1/NY-ESO-1)-specific T cells responses were assessed by IFN-γ ELISPOT assay.

Results Intradermal delivery of tremelimumab was safe and well tolerated. In terms of biological efficacy it selectively induced profound and durable decreases in Treg frequencies in both SLN and PBMC, decreased systemic MDSC rates, activated migratory dendritic cell subsets in the SLN, and induced T cell activation (by HLA-DR and ICOS up-regulation), both in SLN and PBMC. Moreover, systemic anti-melanoma T cell responses were induced (n=5) or boosted (n=2), in association with T cell activation and central-memory T cell differentiation. Of note, tumor recurrences so far were only observed in two patients who did not develop a systemic anti-tumor T cell response.

Conclusions These findings indicate that i.d. administration of anti-CTLA-4 may offer a safe and promising adjuvant treatment strategy for patients with early-stage melanoma. Moreover, they demonstrate a central role for TDLN in the biological efficacy of CTLA-4 blockade and warrant the development of TDLN-targeted delivery methods for anti-CTLA-4.

Acknowledgements This study received funding from the Harry J. Lloyd Charitable Trust; tremelimumab was provided by Pfizer Inc.

Trial Registration NCT04274816

Ethics Approval The study was approved by the Medical Ethics Committee of the VU University Medical Center and Spaarne Gasthuis.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0309
Background We have completed a prospective, randomized, multi-center, double-blind, placebo-controlled phase IIb trial of the tumor lysate, particle loaded, dendritic cell (TLPLDC) vaccine given to prevent recurrences in patients with resected stage III/IV melanoma. During the trial, granulocyte colony stimulating factor (G-CSF) was administered to some patients to mobilize dendritic cells (DCs) precursors prior to harvest, allowing for similar DC yield with reduced blood draws. This study examines the impact of DC collection methods on vaccine effectiveness.

Methods TLPLDC is produced by loading tumor lysate into pre-prepared yeast cell wall particles (YCWPs) and exposing them to autologous DCs. DC precursors were isolated either by collection of 50–70 mL of blood following pre-administration of 300 μg of G-CSF 24–48 hrs prior, or collection of 120 mL of peripheral blood without G-CSF pretreatment based on patient and provider preference. Patients were randomized 2:1 to receive TLPLDC or placebo (DCs exposed to empty YCWPs). 1–1.5 × 10⁶ cells/dose were injected dermally at 0, 1, 2, 6, 12, and 18 months. Differences in disease-free survival (DFS) and overall survival (OS) were analyzed by log rank.

Results Of 144 patients randomized, 103 received TLPLDC and 41 received placebo. Within the TLPLDC group, 57 received pretreatment with G-CSF (TLPLDC+G-CSF) and 46 did not (TLPLDC–G-CSF). There were no significant clinicopathologic or treatment differences between the three treatment arms. 36-month DFS was significantly improved in TLPLDC–G-CSF vs. TLPLDC+G-CSF or placebo (51.8% vs. 23.4% and 27.1% respectively, p=0.027) (figure 1).

Conclusions TLPLDC–G-CSF had correspondingly improved OS (92.9% vs. 62.8% and 72.3% respectively, p=0.022) (figure 2). Subgroup analysis revealed TLPLDC–G-CSF had increased DFS over TLPLDC+G-CSF or placebo in stage IV (68.6% vs. 18.8% and 0.0% respectively, p=0.058). Similarly, the DFS survival benefit of TLPLDC–G-CSF was enhanced in patients who received prior immunotherapy (IO) (61.9% vs. 11.5% and 35.7% respectively, p=0.007) or checkpoint inhibitors (CPI) (48.5% vs. 10.6% and 37.5% respectively, p=0.039).

Trial Registration ClinicalTrials.gov Identifier: NCT02301611
Ethics Approval This study was reviewed and approved by the IRB or Independent Ethics Committee (IEC) of each participating center prior to study initiation.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0310
after transfer. Dendritic cells (DC) are professional antigen presenting cells and have the ability to optimally activate T lymphocytes. We hypothesized that the combination of autologous TIL containing a population of HLA-A*0201 restricted MART-1 reactive CD8+ TIL with autologous MART-1 antigen-pulsed DCs will result in enhanced proliferation and prolonged survival of the transferred antigen-specific T cells in vivo, thus leading to improved clinical responses.

Methods This is a randomized phase II trial of lymphodepleting chemotherapy followed by autologous TILs ± DC vaccine and high dose Interleukin-2 (IL-2) for patients with metastatic melanoma. Patients were randomly assigned to receive TIL alone or TIL + DCs pulsed with MART-1 peptide. The primary objective was to determine whether patients receiving TIL + DCs have sustained persistence of infused T cells compared to patients treated with TIL alone. Secondary endpoints included evaluation of tumor response and survival.

Results A total of 18 patients with stage IV melanoma were treated; 89% with stage M1c, including 56% with brain metastasis; 17% had high LDH level. All but one patient were checkpoint naïve prior to TIL. Ten patients received TIL alone and eight received TIL + DC. Treatments were well tolerated with no grade 5 adverse events. There were no toxicities conferred by the DC vaccination. The ORR was 63% (5/8) in TIL + DC arm (1 CR, 4 PR) and 40% (4/10) in TIL arm alone (1 CR, 3 PR) (P=0.64). There was no statistically significant difference in survival between the arms. The median progression-free survival (PFS) was 3.6 months in the TIL arm and 7.2 months in the TIL+DC arm, while the median overall survival (OS) was 4.1 years in the TIL arm and 7.2 months in the TIL+DC arm. Tracking of the infused MART-1 reactive CD8+ T cells in the blood over time by flow cytometry showed no difference in persistence between the two arms.

Conclusions ACT with TILs has robust response in checkpoint naïve advanced melanoma patients. Despite numerically higher response rate in the TIL+DC arm, due to small patient number there was no statistically significant difference between the arms. Further testing of this approach in a prospective trial post-ICI is warranted.

Trial Registration All metastatic melanoma TIL lines were derived from tumor tissue obtained from patients enrolled on the TIL ACT clinical trial [institutional review board (IRB)-approved protocol# 2004-0069, NCT00338377] at The University of Texas MD Anderson Cancer Center.

Ethics Approval The United States Food and Drug Administration and the Institutional Review Board at MD Anderson Cancer Center approved the study. This study was conducted according to the principles from the Declaration of Helsinki.

Consent All study participants granted a written informed consent prior to treatment initiation.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0311

312 FEMALE SEX INDEPENDENTLY PREDICTS ADJUVANT IMMUNOTHERAPEUTIC BENEFIT FROM CTLA4 IMMUNE CHECKPOINT INHIBITION

1Ahmad Taheen’s, 2Ni Kang, 3Sandra Lee, 4Stephen Hodi, 5Gary Cohen, 6Omid Hamid, 7Laura Hutchins, 8Jeffrey Sosman, 9Kariette Kluge, 10Zeynep Eroglu, 11Henry Sondak, 12Donald Lawrence, 13Kari Kendra, 14David Minor, 15Carrie Lee, 16Mark Albertini, 17Lawrence Flaherty, 18Teresa Petrella, 19Howard Stiecher, 20Yemon Sondak, 21John Kirkwood, 22J. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA; 23Dana Farber Cancer Institute – ECOG-ACRI, Boston, MA, USA; 24Dana Farber Cancer Institute ECOG-ACRI, Boston, MA, USA; 25Dana Farber Cancer Institute, Boston, MA, USA; 26Greater Baltimore Medical Center, Baltimore, MD, USA; 27Aileses Clinic and Research Institute, Los Angeles, CA, USA; 28University of Arkansas, Little Rock, AR, USA; 29Northwestern University, Chicago, IL, USA; 30Yale University, New Haven, CT, USA; 31H. Lee Moffitt Cancer Center, Tampa, FL, USA; 32Bristol Myers Squibb, Cleveland, OH, USA; 33Massachusetts General Hospital, Boston, MA, USA; 34Ohio State University, Columbus, OH, USA; 35Sutter-California Pacific Medical Center, San Francisco, CA, USA; 36University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 37University of Wisconsin, Madison, WI, USA; 38Wayne State University/Karmanos Cancer I, Detroit, MI, USA; 39Odette Cancer Center, Toronto, ON, Canada; 40National Cancer Institute, Rockville, MD, USA; 41University of Pittsburgh, Pittsburgh, PA, USA.

Background Sex differences in tumor immunity and response to immunotherapy were shown in murine models and descriptive analyses from recent clinical trials. Female sex hormones have been implicated in melanoma development and response to systemic therapy. We hypothesized a gender difference in response to adjuvant immunotherapy with ipilimumab (3 or 10 mg/kg; ipi3 or ipi10) versus high dose IFNα (HDI) as tested in the E1609 trial.

Methods E1609 demonstrated significant overall survival (OS) benefit with iipi3 versus HDI1. We investigated treatment efficacy between iipi and HDI in the subgroups by sex (female, male), age (<55 or ≥55), stage at study entry (IIIB, IIIC, M1a/b), ECOG performance status (PS 0, 1), ulceration (yes, no), primary tumor (known, unknown), number of lymph nodes involved (0, 1, 2–3, 4+). Forest plots were created to compare OS and RFS with iipi3 vs. HDI and iipi10 vs. HDI using the concurrently randomized IIT populations. For the estimated HRs, 95% confidence intervals were created for all subgroups.

Results The subgroups of female, stage IIIC, PS=1, ulcerated, in-transit without lymph node involvement demonstrated significant improvement in overall survival (OS) and/or relapse free survival (RFS) with iipi3 versus HDI as summarized in table 1. Female sex was significant for both OS and RFS and was further explored. In investigating RFS with iipi3 versus HDI, a multivariate Cox regression model including sex, treatment and interaction term of sex×treatment, indicated a significant interaction between sex and treatment (P = 0.026). Including sex, PS (0 vs. 1), age (<55 vs. 55+), ulceration (yes vs. no), stage (IIIB, IIIC, M1a, M1b), treatment and interaction term of sex×treatment, indicated a significant interaction between sex and treatment (P = 0.024). While similar trends were seen, no significant interactions between sex and treatment effect were found in the OS multivariate analysis or in the comparison of iipi10 versus HDI. When exploring age, in the univariate analyses in the iipi3 versus HDI comparison older women appeared to drive most of the difference (age ≥55: OS, P=0.02 and RFS, P=0.08; differences non-significant for women <55). Table 1.

Conclusions Female sex was independently associated with RFS adjuvant immunotherapeutic benefit from iipi3, supporting a potentially important role for female related factors in the immune response against melanoma, and these warrant further investigation.
Methods This study characterizes safety, PK/PD, and preliminary antitumor activity of tebotelimab plus margetuximab in patients with advanced HER2+ malignancies. A one-step 3+3 dose escalation phase of tebotelimab (300 and 600 mg) combined with margetuximab 15 mg/kg, both every 3 weeks, was followed by cohort expansion of patients with breast, gastric or gastroesophageal, and other HER2+ tumors. Results At data-cutoff, 31 patients (2.0 median lines of prior therapy; 64.5% with prior HER2-directed therapy) were treated. Median duration of treatment is 10.3 weeks with 17 patients remaining on treatment. No maximum tolerated dose was defined. Treatment-related adverse events (TRAEs) occurred in 23/31 (74.2%) patients, most commonly diarrhea (n=6), nausea, ALT increased (n=5, each), AST increased, and myalgia (n=4, each). The rate of Grade 3 TRAEs was 19.4%, with no Grade 4–5 TRAEs observed. Immune-related AEs were consistent with events observed with anti-PD-1 antibodies and were manageable with supportive treatment. Among 20 response-evaluable patients (i.e., received on-treatment scan), 8 objective responses (6 confirmed) per RECIST v1.1 have been observed, including a confirmed complete response (cholangiocarcinoma) and 7 partial responses (breast [2], microsatellite stable colorectal cancer [2], esophageal adenocarcinoma [1], ovarian cancer [1], and microsatellite stable gastroesophageal junction carcinoma). Immunohistochemistry (IHC) of available baseline tumor specimens (n=17) demonstrated low PD-L1 expression with combined positive scores of either 0 (n=16) or 1 (n=1, colorectal cancer). Investigations into other potential correlative biomarkers, including LAG-3 and PD-1 by IHC and gene expression profiling by NanoString, remain ongoing.

Conclusions Tebotelimab in combination with margetuximab has demonstrated an acceptable safety profile and encouraging early evidence of anti-tumor activity, with a preliminary overall response rate (ORR) of 40% (8/20) [including unconfirmed responses] among late-line patients with various advanced HER2+ malignancies.

Trial Registration NCT03219268

Ethics Approval This study was approved by each Institution’s Ethics Board prior to enrollment of subjects.

REFERENCE


Clinical trials in progress

### Abstract 312 Table 1 Treatment efficacy between ipi3 and HDI by subgroup

<table>
<thead>
<tr>
<th>Group</th>
<th>HR, 95% CI</th>
<th>OS</th>
<th>RFS</th>
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</thead>
<tbody>
<tr>
<td>Female sex</td>
<td></td>
<td>0.60 (0.40, 0.92)</td>
<td>0.66 (0.49, 0.89)</td>
</tr>
<tr>
<td>In-transit, LN-v</td>
<td>0.55 (0.29, 1.02)</td>
<td>0.58 (0.38, 0.88)</td>
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</tr>
<tr>
<td>Ulceration</td>
<td>0.70 (0.50, 0.98)</td>
<td>0.83 (0.65, 1.07)</td>
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<tr>
<td>Stage III</td>
<td>0.67 (0.48, 0.95)</td>
<td>0.78 (0.61, 1.01)</td>
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</tr>
<tr>
<td>PS = 1</td>
<td>0.55 (0.32, 0.95)</td>
<td>0.74 (0.49, 1.12)</td>
<td></td>
</tr>
</tbody>
</table>

### Background

Tebotelimab, also known as MGD013, is an investigational, Fc bearing bispecific tetravalent DART molecule designed to bind PD-1 and LAG-3 and sustain/restore the function of exhausted T cells. Margetuximab, an investigational Fc-engineered anti-HER2 monoclonal antibody, has similar HER2 binding and antiproliferative properties to trastuzumab, but with enhanced Fc-mediated effector function. In vitro studies have demonstrated upregulation of LAG-3/PD-L1 expression on immune cells after margetuximab exposure, along with enhanced lytic activity of immune cells primed by margetuximab in the presence of tebotelimab.

### Methods

This study characterizes safety, PK/PD, and preliminary antitumor activity of tebotelimab plus margetuximab in patients with advanced HER2+ malignancies. A one-step 3+3 dose escalation phase of tebotelimab (300 and 600 mg) combined with margetuximab 15 mg/kg, both every 3 weeks, was followed by cohort expansion of patients with breast, gastric or gastroesophageal, and other HER2+ tumors.

### Results

At data-cutoff, 31 patients (2.0 median lines of prior therapy; 64.5% with prior HER2-directed therapy) were treated. Median duration of treatment is 10.3 weeks with 17 patients remaining on treatment. No maximum tolerated dose was defined. Treatment-related adverse events (TRAEs) occurred in 23/31 (74.2%) patients, most commonly diarrhea (n=6), nausea, ALT increased (n=5, each), AST increased, and myalgia (n=4, each). The rate of Grade 3 TRAEs was 19.4%, with no Grade 4–5 TRAEs observed. Immune-related AEs were consistent with events observed with anti-PD-1 antibodies and were manageable with supportive treatment. Among 20 response-evaluable patients (i.e., received on-treatment scan), 8 objective responses (6 confirmed) per RECIST v1.1 have been observed, including a confirmed complete response (cholangiocarcinoma) and 7 partial responses (breast [2], microsatellite stable colorectal cancer [2], esophageal adenocarcinoma [1], ovarian cancer [1], and microsatellite stable gastroesophageal junction carcinoma).

Immunohistochemistry (IHC) of available baseline tumor specimens (n=17) demonstrated low PD-L1 expression with combined positive scores of either 0 (n=16) or 1 (n=1, colorectal cancer). Investigations into other potential correlative biomarkers, including LAG-3 and PD-1 by IHC and gene expression profiling by NanoString, remain ongoing.

### Conclusions

Tebotelimab in combination with margetuximab has demonstrated an acceptable safety profile and encouraging early evidence of anti-tumor activity, with a preliminary overall response rate (ORR) of 40% (8/20) [including unconfirmed responses] among late-line patients with various advanced HER2+ malignancies.

### Trial Registration

NCT01274338

**Ethics Approval** The study protocol was approved by the institutional review board (IRB) of each participating institution and conducted in accordance with Good Clinical Practice guidelines as defined by the International Conference on Harmonisation. This study was monitored by the ECOG-ACRIN DataSafety Monitoring Committee and the NCI.

**Consent** All patients provided IRB-approved written informed consent.

**REFERENCE**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0312

**Clinical trials in progress**

**A559 A193**

**A1609.**

**REFERENCE**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0313

**A1808.**

**REFERENCE**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0313

**A315.**

**REFERENCE**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0313

**A313.**

**REFERENCE**

This confirms that VISTA may play a key role as a mechanism of resistance to the currently used immunotherapies. VISTA/PSGL1 pH-selective biochemical interaction has been recently demonstrated. VISTA and PSGL1 expression pattern, their correlation and their relationship to myeloid infiltrates have been evaluated in samples from patients with solid tumors. K01401-020 (W0180) is a novel anti-VISTA antibody that has the potential to activate T cells when given as a monotherapy, and thus to generate added activity when combined with anti-PD-1/L1 antibodies in cancer patients.

**Methods**

This phase I/ib for W0180 consists of 2 parts: an initial dose escalation phase I followed by an expansion cohorts phase Ib. In the dose escalation phase, 2 cohorts of patients will be assessed in parallel: the first cohort will be given W0180 as a single agent and the second cohort will receive W0180 in combination with pembrolizumab. The first dose and the schedule of administration of W0180 in combination with pembrolizumab will be determined using safety and pharmacokinetic data generated in monotherapy. The phase I will allow to determine the Maximum Tolerated Dose and Schedule (MTDS), to characterize Dose-Limiting Toxicities (DLTs) and explore pharmacodynamic activity of W0180 in monotherapy and combination with pembrolizumab. The dose-toxicity relationships will support the dose escalation process and will be used to assess the MTDS and recommended doses for expansion. Following completion of the dose escalation phase, the expansion phase will enroll cohorts of patients with homogeneous tumors to validate the dose/schedule, assess preliminary activity and to explore the potential relationship with VISTA and PSGL1 expression.

**Results**

N/A

**Conclusions**

N/A

**Trial Registration**

N/A

**Ethics Approval**

The study was approved by National French Ethic committee (CPP Ile de France V) and National Spanish Ethic committee (Comité Ético de Investigación Clínica de Navarra) and was registered in the European database (EudraCT: 2019-002299-15).

**Consent**

N/A

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VISTA antibody monotherapy increases specific CD8 T cell response in non-human primates. AACR Annual meeting 2020.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0315

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**Abstracts**

**316 EVICTION STUDY: PRELIMINARY RESULTS IN SOLID TUMOR PATIENTS WITH ICT01, A FIRST-IN-CLASS, GAMMA9 DELTA2 T CELL ACTIVATING ANTIBODY TARGETING BUTYROPHILIN-3A**

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**Background**

Gamma9 Delta2 (γ9Δ2) T cells are an important component of the innate anti-tumor immune response whose infiltration into solid tumors has been associated with a positive prognosis, making γ9Δ2 T cells an attractive target for the next generation of cancer immunotherapy. Butyrophilin (BTNs) are a family of immune checkpoint molecules that regulate γ9Δ2 T cell activity, including BTN3A that is a potent endogenous activator of γ9Δ2 T cells following phosphoantigen (pAg) binding to the intracellular domain of BTN3A1. This observation led to the design and development of ICT01, a humanized, monoclonal antibody that binds all 3 isoforms of BTN3A1/A2/A3 and induces pAg-independent γ9Δ2 T cell activation, for the treatment of patients with solid or hematologic tumors.

**Methods**

EVICTION (www.clinicaltrials.gov NCT04243499; EudraCT Number: 2019-003847-31) is a first-in-human, two-part, open-label, clinical study to assess the safety, tolerability and activity of intravenous doses of ICT01 as monotherapy and in combination with pembrolizumab, in patients with advanced-stage, relapsed/refractory cancer. Following Competent Authority and Ethics Committee approvals, the study is being conducted at cancer centers in France, Belgium, Spain, Germany, and the UK. Patients provide signed informed consent prior to screening. Eligible patients receive ICT01 (Range: 20 µg to 200 µg) every 3 weeks with blood samples collected at multiple timepoints for immunophenotyping and cytokine analysis (INFγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-13, TNFα).

**Tumor biopsies are collected at baseline and Day 28 and stained by immunohistochemistry for BTN3A, γ9Δ2 T cells and other markers of anti-tumor immunity.**

**Results**

Cohort 1 comprising 6 patients with solid tumors (3 Colorectal, 1 Pancreatic, 1 Ovarian, 1 Melanoma) has been enrolled and treated with ICT01 doses ranging from 20 to 700 µg. No dose-limiting toxicities or related SAEs have been reported. Target occupancy on T cells at 4 hours post first dose was 10% at 70 µg (n=1), 31% at 200 µg (n=2) and 34% at 700 µg (n=2), which was reflected at 24 hours post dose by a 73%, 91% and 97% decrease from baseline in the number of circulating γ9Δ2 T cells, respectively. On Day 7, γ9Δ2 T cells remained decreased by 37%, 75% and 76%, respectively. There were no effects on CD4 or CD8 T cells, NK cells, or B cells. Transient increases in IFNγ, secreted by activated γ9Δ2 T cells, were observed in 4/6 patients. No cytotoxicity release syndrome was observed. Data from the paired tumor biopsy specimens are still being generated and will be presented.

**Conclusions**

The preliminary results demonstrate that ICT01 has the potential to safely activate the innate anti-tumor potential of γ9Δ2 T cells through BTN3A.

**Acknowledgements**

Trial Registration www.clinicaltrials.gov NCT04243499; EudraCT Number: 2019-003847-31
A PHASE 1/1B STUDY OF SBT6050, A HER2-DIRECTED MONOCONAL ANTIBODY CONJUGATED TO A TOLL-LIKE RECEPTOR 8 AGONIST, IN SUBJECTS WITH ADVANCED HER2-EXPRESSING SOLID TUMORS

1Leisha Emens*, 2Muralidhar Beeram, 3Erika Hamilton, 4Sarina Pha-Pau, 5Valerie Odegard, 6Massachusetts General Hospital, Boston, MA, USA

Background New strategies are needed to improve outcomes in human epidermal growth factor receptor 2 (HER2)-expressing cancers. SBT6050 is a novel therapeutic comprising a specific small molecule toll-like receptor (TLR) 8 agonist conjugated to a HER2-directed monoclonal antibody. TLR8 is highly expressed in myeloid cells that are prevalent in human tumors, including dendritic cells (DCs) and macrophages, and modulates their pro-inflammatory activity. SBT6050 is designed to activate human myeloid cells only in the presence of moderate-to-high HER2 expression (immunohistochemistry [IHC] 2+ or 3+) and binds to the same epitope as pertuzumab. In preclinical studies, SBT6050 potently induces a broad spectrum of antitumor immune mechanisms, including proinflammatory cytokine and chemokine production, inflammasome activation, and indirect activation of T and natural killer (NK) cells. TLR8 agonism has emerged as a promising approach to overcome resistance to immune checkpoint inhibitors in tumors lacking T-cell infiltrates, as these cancers are often replete with myeloid cells. Using an SBT6050 mouse surrogate in vivo, curative single-agent efficacy was observed in multiple murine tumor models, including a model deficient in T, B, and NK cells. In preclinical toxicity studies in nonhuman primates, SBT6050 was well tolerated, supporting a first-in-human starting dose that is predicted to be pharmacologically active, with a short escalation to projected clinically active doses. Preclinical studies also support combinations with checkpoint inhibitors and with trastuzumab to further enhance antitumor activity.

Methods SBT6050-101 is an ongoing phase 1/1b, first-in-human, open-label, multicenter study. Eligible subjects are adults with histologically confirmed, HER2-expressing (IHC 2+ or 3+), locally advanced (unresectable) and/or metastatic cancer. Subjects must have measurable disease per the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and have previously received all therapies known to confer clinical benefit. SBT6050 is given subcutaneously every 2 weeks and treatment may continue for up to 2 years or until disease progression, unacceptable toxicity, or other reason for discontinuation. The trial objectives are to evaluate the safety and tolerability of SBT6050 and to identify the maximum tolerated dose and recommended phase 2 dose (RP2D). The study has 2 parts: Part 1, consisting of a dose escalation using a standard 3+3 design, and Part 2, consisting of 5 parallel expansion cohorts based on tumor type and HER2 expression level and treated with SBT6050 at the RP2D. Pharmacokinetics, immunogenicity, and antitumor activity will be evaluated and pharmacodynamic markers of myeloid cell activation will be assessed in peripheral blood and on-treatment tumor biopsies.

Results N/A

Conclusions N/A

OLAPARIB PLUS PEMBROLIZUMAB IN PATIENTS WITH PREVIOUSLY TREATED ADVANCED SOLID TUMORS WITH HOMOLOGOUS RECOMBINATION REPAIR DEFICIENCY: KEYLYNK-007

1Timothy A Yap*, 2Malika Dhawan, 3Andrew E Hendifar, 4Michele Maio, 5Tadeeek K. Owonikoko, 6Miguel Quintela-Fandino, 7Ronnie Shapira-Frommer, 8Sanatan Saraf, 9Ping Qiu, 10Fan Jin, 11Alexander Gomzan, 12Douglas A Levine. 1The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 2Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA, USA; 3Samuel Oschin Cancer Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA; 4Center for Immunology-Oncology, University Hospital of Siena, Siena, Italy; 5Winship Cancer Institute, Emory University, Atlanta, GA, USA; 6Centro Nacional de Investigaciones Oncológicas, Madrid, Spain; 7Chaim Sheba Medical Center, Tel HaShomer, Israel; 8Merk and Co., Inc., Kenilworth, NJ, USA; 9Laura and Isaac Perlmutter Cancer Center, New York, NY, USA.

Background Treatment with the anti–PD-1 antibody pembrolizumab has improved clinical outcomes in multiple previously treated advanced solid tumors. The poly (ADP-ribose) polymerase (PARP) inhibitor olaparib has shown antitumor activity as monotherapy in patients with previously treated advanced ovarian, breast, pancreatic, and prostate cancers with BRCA1/BRCA2 mutations (BRCAm). Activity was also seen in patients with previously treated advanced solid tumors with other homologous recombination repair mutation (HRRm) and in those with ovarian cancer with homologous recombination repair deficiency (HRD) phenotype. PARP inhibitors have been found to increase interferon signaling and tumor infiltrating lymphocytes, enhancing tumor susceptibility to immune checkpoint blockade. Antitumor activity of PD-1/L1 plus PARP inhibition was found to be higher than expected with either agent alone in patients with recurrent ovarian cancer regardless of BRCAm or HRD status and in patients with BRCAm breast cancer. KEYLYNK-007 (NCT04123366) evaluates the antitumor activity and safety of olaparib in combination with pembrolizumab in patients with previously treated advanced solid tumors with HRRm and/or HRD.

Methods This phase 2, nonrandomized, multicenter, open-label study will enroll approximately 300 patients aged ≥18 years.
with histologically/cytologically confirmed, previously treated, advanced solid tumors with HRRm and/or HRD per Lynparza HRR-HRD assay (Foundation Medicine, Inc., Cambridge, MA, USA), with an ECOG PS of 0-1. Patients will be grouped by biomarker status: subgroup 1: BRCAm; subgroup 2: HRRm without BRCAm; and subgroup 3: HRD positive without HRRm (loss of heterozygosity score ≥ 16 per Lynparza HRR-HRD assay). Patients will receive olaparib 300 mg twice daily + pembrolizumab 200 mg intravenously Q3W (35 cycles) until PD, unacceptable AEs, intercurrent illness, investigator decision, withdrawal of consent, or pregnancy. Tumor imaging assessment by blinded independent central review (BICR) per RECIST v1.1 or Prostate Cancer Working Group (PCWG)–modified RECIST v1.1 for prostate cancer will occur Q9W for 12 months, then Q12W until PD, start of new anticanter treatment, withdrawal of consent, pregnancy, or death. AEs will be monitored throughout the study and for 30 days after final dose (90 days for serious AEs). The primary endpoint is ORR (RECIST v1.1 or PCWG–modified RECIST version 1.1 by BICR). Secondary endpoints include duration of response (DOR) and PFS (RECIST v1.1 or PCWG–modified RECIST v1.1 by BICR), OS, and safety. Point estimate and exact Clopper-Pearson CI for ORR, and Kaplan-Meier estimates for DOR, PFS, and OS will be calculated. A total of 89 sites are currently enrolling in 20 countries.

Results N/A

Conclusions N/A

Trial Registration ClinicalTrials.gov identifier, NCT04123366

Ethics Approval An independent institutional review board or ethics committee approved the protocol at each study site, and the trial is being conducted in compliance with Good Clinical Practice guidelines and the Declaration of Helsinki.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0318

319 PHASE II TRIAL OF IMMUNOTHERAPY IN PRIMARY GliOBLASTOMA: ANTIGENS FROM SELF-RENEWING AUTOLOGOUS TUMOR CELLS PRESENTED BY AUTOLOGOUS DENDRITIC CELL VACCINE

1Daniela Bota*, 2David Piccioni, 3Christopher Duma, 4Renato LaRocca, 5Santosh Kesari, 6Jose Carillo, 7Robert O’Donnell, 8Robert Aiken, 9Frank Hsu, 10Xiao-Tang Kong, 2Thomas Taylor, 3Gabriel Nistor, 4Robert Dillman. 1University of California, Irvine, Orange, USA; 2UCI, Orange, USA; 3UCSD, San Diego, CA, USA; 4Hoag Hospital, Newport Beach, CA, USA; 5Norton Cancer Center, Louisville, KY, USA; 6John Wayne Cancer Institute, Santa Monica, California, USA; 7UCDavis, Davis, USA; 8Rutgers, New Brunswick, NJ, USA; 9Avita Biomedical, Irvine, CA, USA; 10Avita Biomedical, Irvine, California, USA

Background Primary glioblastoma (GBM) is associated with poor survival. Adjunctive vaccines may improve survival by inducing or enhancing anti-GBM immune responses.

Methods A multi-institutional phase II clinical trial was conducted with a primary objective of 75% survival 15 months after intent-to-treat enrollment. Key eligibility criteria were: (1) primary GBM diagnosis, (2) age < 70 years at time of tumor resection, (3) successful GBM cell culture, (4) successful monocyte collection by leukapheresis, (5) Karnofsky Performance Status (KPS) > 70 after surgical recovery. Dendritic cells (DC) were differentiated from autologous monocytes, then incubated with autologous tumor antigens (ATA) from the GBM cell line-lyse to produce each patient-specific DC-ATA vaccine. Doses were suspended in 500 mcg granulocyte-macrophage colony-stimulating factor (GM-CSF) at the time of subcutaneous injections at weeks 1, 2, 3, 8, 12, 16, 20 and 24. Patients were enrolled just prior to starting standard concurrent temozolomide (TMZ) and radiation therapy (RT) for the intent-to-treat after recovery from RT/TMZ.

Results Tumors were collected August 2018-January 2020. Cell line success rate was 71/73 (97%); monocyte collection success rate was 63/65 (97%), but 10 patients required a second leukapheresis. Patients were enrolled for in-to-treat October 2018-February 2020. The 60 patients included 42 men and 18 women with median age of 59 years (range of 27–70). Racial makeup was 43 White, 10 Hispanic, 2 Black, 1 Asian and 3 Other. KPS was 100 in 4, 90 in 25, 80 in 17 and 70 in 14 (mean 83.2). MGMT methylation was present in 13, absent in 31, and unknown in 16; IDH1 mutation was present in 7, absent in 50, and unknown in 3. 37 patients had received 380 doses with 9 still under treatment at time of abstract submission. 32 had completed all 8 doses; 16 had received fewer than 8 doses when they discontinued treatment. No patient discontinued treatment because of toxicity, but 28 have been hospitalized for 53 treatment-emergent central nervous system-related serious adverse events including seizures (15 episodes), falls and/or increased focal weakness (13 episodes), or severe headaches or visual changes (3 episodes).

Conclusions This patient-specific DC-ATA approach is feasible and may be increasing intratumor inflammation that is associated with on-target efficacy and/or toxicity. An interim survival analysis will be conducted in October 2020, 15 months after the median patient was enrolled; results will be available November 2020 as will immunologic data for 55 patients who received at least two injections.

Trial Registration ClinicalTrials.gov NCT03400917.

Ethics Approval The study was approved by UCI IRB, approval number 2018-4148.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0319

320 PHASE IIA STUDY OF ALPHA-DC1 VACCINE AGAINST HER2/HER3, CHEMOKINE MODULATION REGIMEN AND PEMBROLIZUMAB IN PATIENTS WITH ASYMPTOMATIC BRAIN METASTASIS FROM TRIPLE NEGATIVE OR HER2+ BREAST CANCER

1Shipra Gandhi*, 2Peter Forsyth, 3Mateusz Opuchal, 4Kamran Ahmed, 5Hung Khong, 1Kristopher Attwood, 1Ellis Levine, 1Tracey O’Connor, 1Amy Early, 1Robert Fenstermaker, 1Dheerendra Prasad, 1Kazuaki Takabe, 2Brian Czerniecki, 1Pawel Kalinski, 5Norton Cancer Center, Louisville, KY, USA; 6John Wayne Cancer Institute, Santa Monica, California, USA; 7UCDavis, Davis, USA; 8Rutgers, New Brunswick, NJ, USA; 9Aivita Biomedical, Irvine, CA, USA; 10Avita Biomedical, Irvine, California, USA

Background Brain metastases develop in up to 50% patients with metastatic triple negative breast cancer (TNBC) and HER2+ BC and are an increasing source of morbidity and mortality. HER3, overexpressed in triple negative and HER2 + brain metastatic breast cancer (BMBC), is a resistance factor to HER2-targeted therapies and a driver of CNS metastasis. Disease progression is associated with loss of anti-HER2/3 immunity. We have demonstrated that alphaDC1 loaded with glioma-specific peptides induce intratumoral production of chemokines (CXCL9, CXCL10, CXCL11, CCL5) which attract CXCR3- and CCR5- expressing cytotoxic T-lymphocytes (CTLs) and T-helper 1 (Th1) cells to brain tumors, inducing clinical responses and long-term disease stabilization.
in patients with aggressive recurrent primary brain tumors. Our preclinical data show that Chemokine modulating (CKM) regimen [rintatolimod, interferon (IFN)-α2b and COX-2 inhibitor] also selectively attracts effector CTLs and Th1 cells (but not suppressive regulatory T-cells or myeloid-derived suppressor cells) into tumors. Importantly, CKM preferentially promotes CTL migration into tumor rather than healthy tissues, providing rationale for its systemic use. We hypothesize that anti-HER2/3 type 1 polarized DC1s in combination with CKM and anti-PD1 will result in improved Th1/CTL response against HER2/3 epitopes, reduce brain recurrence and systemic progression.

Methods This is a phase II single-arm, non-randomized multicenter study (NCT04348747). Eligibility includes patients with triple negative and HER2+ BMBC ≥ 18 years, ECOG PS ≤ 1, normal marrow and organ function with asymptomatic untreated brain metastases who receive αDC1 q2 weeks x 3, with CKM [200 mg IV rintatolimod, IFN-α 20 million units/m2 IV, celecoxib 200 mg oral BID] on days 1-3 with second and third dose of αDC1, followed by pembrolizumab 200 mg IV. Thereafter, pembrolizumab is given every 3 weeks, along with αDC1 and CKM every 3 months as booster dose until disease progression, intolerable side effects or withdrawal from study, or up to 24 months. Baseline and 3-week post-CKM treatment peripheral (non-CNS) biopsies are required for six patients. Primary objective is CNS response rate (RR) using RANO-BM criteria. If no CNS response is observed after 12 patients, study will be terminated. If ≥ 3 CR observed, the proposed therapy will be considered promising for further study. Secondary objectives include non-CNS RR per RECIST v1.1, median CNS, non-CNS and overall progression-free survival, overall survival and safety. Analysis of change in intratumoral biomarkers is an exploratory objective.

Results N/A

Conclusions N/A

Trial Registration NCT04348747

Ethics Approval The study was approved by Roswell Park Comprehensive Cancer Center Institution’s Ethics Board, approval number I-19-04120.

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321 PHASE I CLINICAL TRIAL ASSESSING THE COMBINATION OF SYSTEMIC CHEMOKINE MODULATORY REGIMEN TARGETING TLR3 WITH NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER

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Background Neoadjuvant chemotherapy (NAC) with taxanes is the standard of care in triple negative breast cancer (TNBC). Intratumoral prevalence of CD8+ cytotoxic T-lymphocytes (CTLs) is associated with an improvement in relapse-free survival (RFS) and overall survival (OS), while regulatory T-cells (Treg) and myeloid derived suppressor cells (MDSC) are associated with poor survival. Higher ratio of CTL/Treg is associated with higher probability of obtaining pathological complete response (pCR), a surrogate marker for RFS. Intratumoral production of CCL5, CXCL9, CXCL10 and CXCL11 is critical for local infiltration with CTLs, while CCL22 is responsible for Treg attraction. Previous studies have shown that CXCL9 expression in the pre-treatment breast tissue is associated with a three-fold higher rate of achieving pCR. Our preclinical data show that Chemokine modulating (CKM) regimen, combining rintatolimod (TLR3 agonist), interferon (IFN)-α2b, and celecoxib (COX-2 inhibitor) increases CTL-attracting, and decreases MDSC, Treg favoring chemokines, increasing CTL/Treg ratio in tumor microenvironment, with preferential tumor tissue activation than adjacent healthy tissues. We hypothesize that the combination of CKM with paclitaxel will result in infiltration of TNBC with CTLs, and along with doxorubicin/cyclophosphamide (AC), result in higher pCR, translating into improved RFS and OS.

Methods In this phase I study NCT04081389, eligibility includes age ≥18 years, confirmed resectable TNBC, radio-graphically measurable disease ≥ 1 cm, ECOG PS ≤ 2, adequate organ and marrow function. Patients with autoimmune disease, serious mood disorders, invasive carcinoma within 3 years, history of peptic ulcers or hypersensitivity to NSAIDs will be excluded. We plan to treat three patients with early stage TNBC with paclitaxel 80 mg/m2 IV weekly for 12 weeks, rintatolimod 200 mg IV, celecoxib 200 mg oral twice daily, and accelerated titration of IFN-α2b at doses 0, 5, or 10 million units (MU)/m2 [Dose Levels (DL) 1, 2 and 3 respectively] on days 1–3 (no intra-patient dose escalation) in weeks 1–3. Dose-limiting toxicity (DLT) is defined as grade 3 or higher toxicities within the first 3 weeks. Any DLT will mandate recruitment per the 3+3 model. If no DLT, three patients will be enrolled at DL 4 at 20 MU/m2 IFN-α2b. This will be followed by standard dose-dense AC, and then surgery. The primary endpoint is safety and tolerability of combination and to identify the appropriate DL of CKM and paclitaxel for extended efficacy study. The secondary endpoints include investigation of efficacy (pCR and breast MRI response), along with RFS and OS. Intratumoral biomarkers will be analyzed in an exploratory manner.

Results N/A

Conclusions N/A

Trial Registration NCT04081389

Ethics Approval The study was approved by Roswell Park Comprehensive Cancer Center Institution’s Ethics Board, approval number I-73718.

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322 EFFICACY AND SAFETY OF GX-I7 PLUS PEMBROLIZUMAB FOR HEAVILY PRETREATED PATIENTS WITH METASTATIC TRIPLE NEGATIVE BREAST CANCER: THE PHASE 1B/2 KEYNOTE-899 STUDY

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Background Pembrolizumab monotherapy showed 9.6% ORR and did not significantly improve OS as 2L or 3L treatment for mTNBC compared to standard chemotherapy in phase 3 study (KEYNOTE-119) leading to high unmet needs of a new drug that could enhance the activity of pembrolizumab
when it is combined with. Higher lymphocyte count is an independent factor which correlates with better response to checkpoint blockade in cancer patients. Based on the MoA of GX-17, which induces increase of T cells in both the tumor microenvironment and peripheral blood, combining GX-17 with pembrolizumab can potentially enhance the anti-tumor effect.

**Methods** This is an open-label, phase 1b/2 study in patients with refractory or recurrent TNBC who failed standard chemotheraphy from 1st to 3rd line treatment in metastasis setting. Patients pretreated with cyclophosphamide received GX-17 from 360 µg/kg up to 1,440 µg/kg every 12 weeks and pembrolizumab 200 mg every 3 weeks (n=21). Patients without cyclophosphamide pretreatment received GX-17 from 720 µg/kg up to 1,440 µg/kg every 9 weeks or 12 weeks and pembrolizumab 200 mg every 3 weeks (n=24). The objectives were dose limiting toxicities (DLTs), safety, pharmacodynamic markers including lymphocyte increase and ORR.

**Results** GX-17 and pembrolizumab were given to 45 patients (pts) (median age 50 years [29–75], ECOG PS 1 [42.2%]). 1 DLT (skin rash, Gr 3) was reported in the 1,440 µg/kg cohort. Treatment-related AEs occurred in 97.8% of pts with Gr 1–2, 15.6% with Gr 3 and 2.2% with Gr 4. Common TEAEs were injection site reaction (75.6%), rash (40.0%), pyrexia (40.0%) which were manageable. GX-17 treatment induced up to 7-fold increase in absolute lymphocyte counts in all dose levels ranging from 360 µg/kg to 1,440 µg/kg with or without cyclophosphamide. A total of 33 evaluable mTNBC pts showed ORR of 0/3 in 360 µg/kg, 1/9 in 720 µg/kg, 2/9 in 960 µg/kg and, 4/12 in 1,200 µg/kg. Interestingly, 4 out of 6 pts received 1,200 µg/kg of GX-17 with cyclophosphamide achieved SD and, thus, 1,200 µg/kg of GX-17 regimens have been selected as candidates for RP2D. The tumor assessment for 1,440 µg/kg with or without cyclophosphamide is ongoing.

**Conclusions** GX-17 in combination with pembrolizumab with or without cyclophosphamide was safe and well tolerated in most study participants. GX-17 significantly suggested to increased T cell numbers in combination with pembrolizumab at doses from 360 µg/kg to 1,440 µg/kg. These results suggested GX-17 in combination with pembrolizumab as a potential treatment option for patients with metastatic TNBC.

**Trial Registration** ClinicalTrials.gov Identifier: NCT03752723

**Ethics Approval** The study was approved by the Samsung Medical Center, Gachon University Gil Medical Center, National Cancer Center, Korea University Anam Hospital, Korea University Guro Hospital, Severance Hospital, Ajou University Hospital, Seoul National University Bundang Hospital, Ewha Womans University Mokdong Hospital, Asan Medical center, Catholic Medical Center and Gangnam Severance Hospital Institutional Review Board, protocol number GX-17-CA-006 (KEYNOTE-899).

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**SYSTEMIC ADMINISTRATION OF LADIRATUZUMAB VEDOTIN ALONE OR IN COMBINATION WITH PEMBROLIZUMAB RESULTS IN SIGNIFICANT IMMUNE ACTIVATION IN THE TUMOR MICROENVIRONMENT IN METASTATIC BREAST CANCER PATIENTS**

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**Background** Ladiratuzumab vedotin (LV) is an investigational antibody-drug conjugate (ADC) composed of a humanized anti-LIV-1 IgG1 conjugated with monomethyl auristatin E (MMAE), a microtubule-disrupting agent. LV targets LIV-1, a protein expressed by various cancers. Along with a cytotoxic effect, LV has been shown to induce immunogenic cell death (ICD) in preclinical studies. LV is currently being investigated as a monotherapy and in combination with pembrolizumab in patients with metastatic breast cancer and other solid tumors. This correlative biomarker study aims to assess the ability of LV to modulate the tumor microenvironment (TME) in breast cancer patients.

**Methods** In the SGNLVA-001 trial, metastatic breast cancer patients, predominantly of the triple negative subtype (TNBC), received LV monotherapy (2.0 or 2.5 mg/kg, every 3 weeks [q3w]). In the SGNLVA-002 trial, patients with metastatic TNBC received LV (2.0 or 2.5 mg/kg, q3w) plus pembrolizumab (200 mg, q3w). To investigate the potential effect of LV or LV plus pembrolizumab on the TME, paired pre-treatment and on-treatment tumor biopsies (Cycle [C] 1 Day [D] 5 or C1D15) were collected and analyzed by RNAseq and immunohistochemistry (IHC) staining.

**Results** Gene expression analysis of paired biopsy TNBC samples (n=59; baseline and C1D5) showed that LV monotherapy treatment significantly induces immune response-related gene expression, MHC, co-stimulatory molecules, and PD-L1. Gene set enrichment analysis (GSEA) demonstrated enrichment of macrophage and tumor inflammation signature genes, supporting the induction of ICD and enhancement of innate immune response. Paired tumor samples from subjects treated with LV plus pembrolizumab (n=16; baseline and C1D15) showed a broader range of gene expression changes on RNAseq compared to LV monotherapy. GSEA evidenced enrichment of genes associated with cytotoxic CD8 T cells, CD4 T helper cells, dendritic cells, and macrophages, further demonstrating the induction of ICD and activation of an innate immune response. Importantly, the combination had a unique adaptive immune response induction signature. IHC analysis confirmed the increased infiltration of macrophages after LV monotherapy. The combination of LV plus pembrolizumab resulted in a further increase in macrophages and a prominent influx of CD8 T cells.

**Conclusions** Systemic administration of LV monotherapy resulted in immune activation in the TME and macrophage infiltration. The combination of LV plus pembrolizumab resulted in a more potent immune activation in the TME and a prominent influx of CD8 T cells in addition to macrophages. Together these results provide a rationale for the continued clinical investigation of LV alone or in combination with pembrolizumab.

**Trial Registration** NCT01969643 and NCT03310957
BDB001, a Toll-like receptor 7 and 8 (TLR7/8) agonist, can be safely administered intravenously and shows clinical responses in advanced solid tumors

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**Background** TLR agonists mediate antitumor activity through dendritic cell (DC) activation. Most TLR agonists in development are administered intratumorally allowing for less than 30% of advanced solid tumor to be treated. BDB001 is an intravenously administered novel TLR7/8 agonist that activates plasmacytoid and myeloid DCs and has shown to have activity in preclinical studies. Here we report on BDB001 administration in patients with advanced solid tumors.

**Methods** BDB001-101 is a Phase 1, open label, dose escalation/expansion trial of BDB001 administered intravenously weekly in patients with advanced solid tumors. The primary endpoint was safety and tolerability. Secondary endpoints included efficacy, pharmacokinetics and pharmacodynamic profiling of immune activation.

**Results** Thirty-six subjects with 16 different tumor types were enrolled across 5 dose levels. Sixty seven percent were female, median age was 66 years (range, 38–88), median number of prior therapies was 4 (range, 0–12), and 61% of tumors had progressed on prior anti-PD-(L)1 therapy. BDB001 was well tolerated and a maximum tolerated dose was not reached. Eleven (30.5%) subjects had no treatment related adverse events (AEs) and the majority of AEs were Grade 1 or 2. Three (8.3%) subjects had Grade 3 AEs, including 2 with cytokine release syndrome, both of whom were clinically stable and had symptoms fully resolved within 2 to 5 days. There were no Grade 4 or 5 AEs. The most common AEs included chills/rigor (19.4%), fever (19.4%), fatigue (11.1%), nausea (11.1%) and pruritus (11.1%). Of 32 subjects evaluable for efficacy, best overall response rate was: 6% durable partial response, 56% stable disease, 38% progressive disease, for a disease control rate of 62%. Durable responses were seen in renal cell carcinoma and non-small cell lung cancer. Interestingly, clinical activity favored subjects with tumors that had progressed on prior anti-PD-(L)1 therapy, compared to prior DNA-damaging chemotherapy, within 6 months of BDB001 initiation. Median time on treatment was 12.1 weeks (range, 3.1 – 68.0). Transcriptional profiling showed up-regulation of interferon inducible genes, activation of dendritic cells and macrophages. BDB001 also significantly increased serum levels of interferon gamma and interferon inducible protein-10 (IP-10).

**Conclusions** Intravenously administered BDB001 monotherapy was well tolerated. Clinical responses were achieved, supported by BDB001-induced immune activation. Preliminary findings suggest that BDB001 is a promising therapeutic option for patients with tumors that progress on anti-PD-(L)1 therapy. BDB001 is also being evaluated in combination with pembrolizumab (anti-PD-1, NCT03486301) and with atezolizumab (anti-PD-L1, NCT04196530).

**Trial Registration** NCT03486301

**Ethics Approval** This study was approved by the institutional review boards at the four participating institutions. All subjects signed informed consent before enrolling in the clinical trial.

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IMMUNOTHERAPY WITH B CELL ACTIVATING ANTIBODY CPI-006 IN PATIENTS (PTS) WITH MILD TO MODERATE COVID-19 STIMULATES ANTI-SARS-COV-2 ANTIBODY RESPONSE, MEMORY B CELLS AND MEMORY T EFFECTOR CELLS

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**Background** CD73 is present on subsets of B and T cells and is involved in lymphocyte activation. CPI-006 is a humanized IgG1, Fcγ receptor deficient anti-CD73 that has agonistic properties. In vitro studies and ongoing cancer clinical trials show that CPI-006 binds to B cells leading to expression of CD69, trafficking to lymph nodes, immunoglobulin class switching, transformation to plasmablasts and generation of memory B cells.1 Recently, a patient in the cancer trial with asymptomatic COVID-19 developed high titers of neutralizing anti-SARS-CoV-2 antibodies following administration of CPI-006. A phase 1 trial in COVID-19 was initiated to evaluate the use of CPI-006 to enhance anti-viral immune response (NCT04464395).

**Methods** Single intravenous dose escalation with N=5 per cohort of 0.3, 1.0, 3.0 and 5.0 mg/kg. Pt eligibility included PCR positive nasal swab for COVID-19; hospitalized with O2 saturation of ≥92% on <5 l/min of O2. Pts received standard care for COVID-19. Pts were monitored for safety, COVID-19 symptoms, inflammatory markers and anti-SARS-CoV-2 antibodies by ELISA. Immunophenotyping of blood by flow cytometry was performed.

**Results** 10 pts have been treated in the first 2 cohorts; median age 64 (range 28–76) and all had comorbidities: diabetes (4), hypertension (2), obesity (7) and/or cancer (2). Median duration of symptoms prior to CPI-006 was 8 days (range 1–21 days). No treatment-related adverse events were reported. There was no correlation between duration of symptoms and baseline anti-viral titers. Kinetics of anti-SARS-CoV-2 response to spike protein are shown for 7 pts with follow-up ≥ 7 days post CPI-006 (figure 1). One pt with lymphopenia (600/mm3) had delayed response to CPI-006; all other pts generated antibody response by Day 7 post-CPI-006 to both spike and RBD. Increasing titers of IgG and IgM antibodies were observed out to 28 days post treatment. In one pt examined, memory B cells increased from 1.81% to 4.83% of B cells 28 days after treatment with serum IgG titers to spike and to RBD of >1:50,000. 2 of 2 pts had increase in both CD4 and CD8 T effector memory cells at day 28. All pts were discharged (median 4 days) with clinical improvement.

**Conclusions** CPI-006 is well tolerated in COVID-19 pts. Low baseline titers of antibodies to virus were increased following CPI-006 in all treated pts. Immunomodulation with CPI-006...
represents a novel therapy for COVID-19 aimed at stimulating more robust and prolonged anti-SARS-CoV-2 immunity potentially after infection or with vaccination.

Trial Registration NCT04464395

Ethics Approval The study was approved by Temple University Hospital’s Ethics Board, Western IRB, approval number 1-1317457-1.

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0325

326 SARS-COV-2 SPECIFIC T-CELLS IN TIL FROM PATIENTS WITH EPITHELIAL CANCER

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Background SARS-CoV-2 primarily infects the upper and lower airway system, yet also endothelial cells and multiple tissues/organ systems. Anti-SARS-CoV-2-directed cellular immune responses may be deleterious or may confer immune protection – more research is needed in order to link epitope-specific T-cell responses with clinically relevant endpoints.

Analysis of epitope reactivity in blood from healthy individuals showed pre-existing (CD4+) reactivity most likely due to previous exposure to the common old coronavirus species HCoV-OC43, HCoV-229E, -NL63 or HKU1, or – not mutually exclusive - cross-reactive T-cell responses that would recognize SARS-CoV-2, yet also other non-SARS-CoV-2 targets. Detailed single cell analysis in PBMCs from patients with COVID-19 showed strong T-cell activation and expansion of TCR gamma - delta T-cells in patients with fast recovery or mild clinical symptoms. Previous studies examining antigen-specific T-cell responses in tumor-infiltrating T-cells (TIL) showed that EBV or CMV-specific cellular immune responses in TIL from patients with melanoma or pancreatic cancer. Such virus-specific T-cells may represent ‘bystander’ T-cell activation, yet they may also impact on the quality and quantity of anti-tumor directed immune responses. We tested therefore TIL expanded from 5 patients with gastrointestinal cancer, who underwent elective tumor surgery during the COVID-19 pandemic for recognition of a comprehensive panel of SARS-CoV-2 T-cell epitopes and compared the reactivity, defined by IFN-gamma production to TIL reactivity in TIL harvested from patients in 2018, prior to the pandemic.

Methods A set of 187 individual T-cell epitopes were tested for TIL recognition using 100IU IL-2 and 100 IU IL-15. Different peptide epitopes were selected: i) all epitopes were not shared with the 4 common old coronavirus species, ii) some peptides were unique for SARS-CoV-2, and iii) others were shared with SARS-CoV-1. Antigen targets were either 15 mers or 9mers for MHC class II or class I epitopes, respectively, derived from the nucleocapsid, membrane, spike protein, ORF8 or the ORF3a. The amount of IFN-gamma production was reported as pg/10⁴ cells/epitope/5 days. Controls included CMV and EBV peptides.

Results We detected strong IFN-gamma production directed against antigenic ‘hotspots’ including the ORF3a, epitopes from the SARS-CoV-2 nucleocapsid and spike protein with a range of 12 up to 30 targets being recognized/TIL.

Conclusions SARS-CoV-2 epitope recognition, defined by IFN production, can be readily detected in TIL from patients who underwent surgery during the pandemic, which is not the case for TIL harvested prior to the circulating SARS-CoV-2. This suggests a broader exposure of individuals to SARS-CoV-2 and shows that SARS-CoV-2 responses may shape the quality and quantity of anti-cancer directed cellular immune responses in patients with solid epithelial malignancies.

Acknowledgements We thank the Surgery, Pathology and Varium Units of Champalimaud Clinical Center (N. Figueiredo, A. Brandl, A. Beltran, M. Castillo, C. Silva ).

Ethics Approval This study was approved by the Champalimaud Foundation Ethics Committee.

Consent All donors provided written consent and the study was approved by the local ethics committee. The study is in compliance with the Declaration of Helsinki.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0326

327 STUDY OF ANTI-PD-1 ANTIBODY MULTIMODAL COMBINATION AS FIRST-LINE TREATMENT ON TIME WINDOW OF ADVANCED SOLID TUMOR

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Background Immune checkpoint inhibitors (ICIs) targeting the programmed cell death-1 (PD-1) has dramatically shifted the therapeutic paradigm of advanced tumor. However, a large proportion of patients do not achieve durable responses with anti-PD-1 monotherapy. Strategically combining immunotherapies with other systemic therapies to harness potential synergies is critical for maximizing their clinical activity and realizing the greatest benefits for patients with cancer. Chemotherapy drugs induce a form of tumor cell death that is immunologically active, thereby inducing an adaptive immune response specific for the tumor. Apatinib (VEGFR2 inhibitor) in combination with an anti-PD-1 has demonstrated synergistic antitumor effects. In our previous research, steady-state of apatinib (250 mg qd) plasma drug concentration was achieved by day 3. Camrelizumab and sintilimab are humanized anti-PD-1 antibody. We aim to assess time window, efficacy and safety of patients who receive anti-PD-1 antibody multimodal combination as first-line treatment of advanced solid tumor.

Methods This multicentre, open-label, exploratory cohort study. Eligible patients were aged 18–70 years, and had historically or cytologically confirmed advanced solid tumors, an Eastern Cooperative Oncology Group performance status of 0 or 1, and received no previous anti-tumor treatment for advanced disease. 180 patients were assigned to three group: Camrelizumab/sintilimab (200 mg iv,d4,q3w,24 months) plus standard chemotherapy (d1-3), Camrelizumab/sintilimab (200 mg,iv,d4,q3w,24 months) plus apatinib (250 mg, po, d1,qd), Camrelizumab/sintilimab (200 mg,iv,d7,q3w,24 months) plus standard chemotherapy(d1-3) and apatinib (230 mg,po, d1,qd). Tumor tissue and matched blood of all patients will be collected for NGS-based 727 genes panel assay, and the blood samples will be collected until disease progression. Meanwhile, plasma drug concentrations were detected by daily measurement of trough and peak concentrations (d0, 1, 2, 3, 4, 6, 9, 12 weeks thereafter. Study treatments will continue until disease progression. Response specific for the tumor. Apatinib (VEGFR2 inhibitor) in combination with an anti-PD-1 has demonstrated synergistic antitumor effects. In our previous research, steady-state of apatinib (250 mg qd) plasma drug concentration was achieved by day 3. Camrelizumab and sintilimab are humanized anti-PD-1 antibody. We aim to assess time window, efficacy and safety of patients who receive anti-PD-1 antibody multimodal combination as first-line treatment of advanced solid tumor.

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Background SPICE is a phase 1 study of the oncolytic adenovirus enadenotucirev in combination with nivolumab in patients with advanced epithelial tumors (NCT02636036). Preliminary data has indicated a survival advantage in patients with mCRC resulting in a median OS of 14 months.1 To further understand this OS signal, a comparison to historical patient-level data from the placebo arm of the CORRECT study (NCT01103323) was performed using data obtained from Project Data Sphere.

Methods Individual patients from SPICE were matched with patients in the placebo arm of the CORRECT study in terms of covariates known to be associated with OS (ECOG, presence of liver mets, haemoglobin, albumin, LDH and platelet count). The OS outcomes were then compared between the matched SPICE and CORRECT patients to minimise any bias due to patient selection. The distribution of the covariates was broadly similar between studies with minor differences favouring the SPICE study.

Results The mOS in confirmed microsatellite stable mCRC patients (n=25) in the SPICE study was 15.4 months (95% CI; 11.8 m, 21.0 m) as compared to 5.0 months for patients in the placebo arm of the CORRECT study (n=231). Two different statistical analyses were performed to compare the outcomes between studies: (1) A comparison of OS matching each SPICE patient to a maximum of 10 (average of 5.5) placebo patients from CORRECT using M:1 variable nearest neighbour propensity score matching; (2) Multivariate analysis of SPICE vs CORRECT adjusting for all covariates in a Cox regression model. The Hazard Ratio (SPICE:CORRECT) from the regression model was 0.28 with an upper 2-sided 95% confidence limit of 0.48, which was consistent with results using propensity score matching. The upper 95% CL for the HR for method (2) was 0.61.

Conclusions The results appear promising, particularly in a population that has historically shown little response to PD-1 intervention and warrant further exploration in a randomised study. However, these analyses cannot be regarded as definitive, due to the possible presence of unmeasured confounders between a small phase 1 cohort and a large phase 3 control group.

Ethics Approval The study was approved by the Western Institutional Review Board, study approval number 1160755.

REFERENCE


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0329
Abstract 331 Table 1

| Dose level | Imunotherapy (Y90)-Radioembolization | Administration* | Number of Administration of Drug
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<td>Pre-Y90 dose</td>
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Once the recommended phase-2 dose is determined through an acceleration titration design, a total of 18 patients are being planned to be treated on this study at the University of Iowa Holden Comprehensive Cancer Center. The study has strong correlational components from a tumor microenvironment (pre- and post-biopsies) as well as ‘liquid biopsies’ - circulating tumor DNA (ctDNA) testing already integrated into the protocol. This would provide an opportunity to understand better the changes to the tumor microenvironment from such an approach in addition to understanding mechanisms of immune evasion/resistance.

Results N/A

Conclusions N/A

Trial Registration NCT04108481

REFERENCES


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0330
who achieved objective response assessed by RECIST v1.1/ iRECIST or progression free survival more than 24 weeks. Vactosertib responsive gene signature (VRGS) that showed significantly different expression among previously identified TGF-β responsive gene signature and IFN-γ signature in responders than in non-responders was identified and VRGS score was calculated by a mean value of VRGS filtered-in gene expressions divided by 6 housekeeping gene expressions.

Results As of July 1, 2020, of the total evaluable 24 patients, 71% were CMS4 subtype and 33% were with high TMB (>10 mut/Mb). Clinical benefit rate was 33.3% including 3 PR and 1 IPR patients. No significant associations in response rate were observed with CMS subtypes or TMB status. VRGS score was significantly enriched in responders than in non-responders (P value = 0.006; AUC = 0.836). A preliminary cut-off value of 2.179 resulted in 94% specificity and 75% sensitivity with 85.7% patients correctly classifying as a responder. After treatment of vactosertib plus pembrolizumab, TGF-β related VRGS was significantly decreased and the extent of decrease was greater in responders, compared to non-responders.

Ethics Approval The study was approved by Ethics Board of Asan Medical Center, Yonsei University College of Medicine, Samsung Medical Center, and Seoul National University Bundang Hospital with approval number 2018-1215, 4-2018-0728, SMC 2018-07-146-006, and B-1808/487-003, respectively.

Conclusions Development of VRGS as a predictive biomarker for this combination treatment with vactosertib and pembrolizumab is ongoing and its potential clinical utility for patient selection will be explored.

Trial Registration NCT03724851

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### Abstracts

#### 333 TARGETING THE APICAL INTRACELLULAR CHECKPOINT CISH UNLEASHES T CELL NEOANTIGEN REACTIVITY AND EFFECTOR PROGRAM

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Background Neoantigen-specific T cells isolated from tumors have shown promise clinically but fail to consistently elicit durable tumor regression. Expression of the intracellular checkpoint CISH is elevated in human tumor infiltrating lymphocytes (TIL) and has been shown to inhibit neoantigen reactivity in murine TIL.

Methods To explore CISH function in human T cells we developed a CRISPR/Cas9-based strategy to knock out (KO) CISH in human T cells with high-efficiency (>90%) and without detectable off-target editing.

Results CISH KO in peripheral blood T cells enhanced proliferation, cytokine polyfunctionality, and cytotoxicity in vitro. To determine if CISH KO similarly enhances TIL function, we developed a clinical-scale, GMP-compliant manufacturing process for CISH disruption in primary human TIL. In process validation runs we achieved CISH KO efficiencies >90% without detectable off-target editing while maintaining high viability and expansion. Compared to WT controls, CISH KO in patient-derived TIL demonstrated increased proliferation, T cell receptor (TCR) avidity, neoantigen recognition, and unmasked reactivity to common p53 mutations. Hyperactivation in CISH KO TIL did not increase differentiation, suggesting that CISH KO may uncouple activation and differentiation pathways. Single cell profiling identifies a pattern of CISH expression inverse to key regulators of activation, suggesting CISH KO may unmask reactivity to common p53 mutations. Hyperactivation in CISH KO TIL did not increase differentiation, suggesting CISH KO in human TIL increases PD1 expression. Adoptive transfer of CISH KO T cells synergistically combines with PD1 inhibition resulting in durable tumor regression in mice, highlighting orthogonal dual cell surface and intracellular checkpoint inhibition as a novel combinatorial approach for T cell immunotherapy.

Conclusions These pre-clinical data offer new insight into neoantigen recognition and serve as the basis for a recently initiated human clinical trial at the University of Minnesota (NCT04426669) evaluating inhibition of the novel intracellular immune checkpoint CISH in a CRISPR-engineered, neoantigen-specific T cell therapy for solid tumors. Updates from the clinical trial will be highlighted.

Trial Registration NCT04426669

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#### PHASE II STUDY EVALUATING A CHEMOKINE-MODULATORY (CKM) REGIMEN IN PATIENTS WITH COLORECTAL CANCER (CRC) METASTATIC TO THE LIVER

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Background CRC remains the 2nd most common cause of cancer-related death in the US. Hepatic metastases develop in 20–50% of CRC patients. Median overall survival (OS) of patients with metastatic CRC is poor, even with the advent of biologics. A high density of CRC-infiltrating effector cytotoxic T lymphocytes (Teff; CTL) is known to predict long-term outcomes and the responsiveness of tumors to immune checkpoint inhibitors (ICIs). In our ex vivo tumor explant models and CRC-bearing experimental animals, the combination of toll-like receptor-3 (TLR3) ligands with interferon (IFN)α and cyclooxygenase (COX)-2 inhibitors resulted in increased production of Teff attracting chemokines CXCL10 and CCL5, along with suppression of regulatory T cells (Treg) attracting chemokine, CCL22 in the tumor microenvironment. A combination of all three factors was needed to uniformly elevate the desirable chemokines and counteract CCL22 induction. Based on these studies and on prior clinical safety data, we developed this phase IIa study combining IFNα2b, celecoxib (COX-2 inhibitor) and rintalotimod (selective TLR3 agonist) as a chemokine-modulating (CKM) regimen for CRC patients with unresectable liver-metastatic disease. We aim to study the immunological impact, potential clinical efficacy and safety
of this CKM regimen in a non-randomized, single-arm prospective phase II trial.

Methods Eligible patients have recurrent/metastatic unresectable CRC with hepatic metastases that are amenable to biopsy. Enrolled patients have prior treatment with or contra-indication to a fluoropyrimidine, irinotecan, oxaliplatin, anti-VEGF treatment, and an anti-EGFR targeted therapy (if RAS wt), as well as a PD-1 or PD-L1 targeted drug if MSI-H/dMMR. Patients receive celecoxib (200 mg orally PO BID), IFNα2b IV (20 million units/m2 IV QD), and rintalimod (200 mg IV QD) on days 1, 2, 3, 8, 9, 10, 15, 16 and 17 in the absence of disease progression or unacceptable toxicity. Response assessment via liver biopsies (pre-treatment and on D20) and CT imaging (RECIST v1.1) on D46. If stable disease/response is demonstrated during repeat CT imaging, patients will continue to follow-up with CT imaging q8 weeks until progression, clinical deterioration, or withdrawal from the study. Primary endpoint assessment compares the change in CD8+ T-cells before treatment, with that seen post-treatment (measured by quantitative RT-PCR and expressed as a ratio of CD8α to a housekeeping gene). Secondary endpoints include objective response rate and safety profile. Subjects are monitored continuously for safety, based on Bayesian analysis. Exploratory endpoints include progression-free survival and overall survival. With a sample size of n=12 evaluable pts, the study design has a 90% power to detect a 0.77 standard deviation increase (pre- to post-treatment) at a significance level of 0.1.

Results N/A

Conclusions N/A

Trial Registration ClinicalTrials. gov Identifier: NCT03403634.

Ethics Approval The study was approved by Roswell Park Comprehensive Cancer Center’s Institutional Review Board, approval number: MOD000067221/L-52917.

Consent N/A

REFERENCES


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NOVEL COUPLED CAR T CELL TECHNOLOGY FOR TREATING COLORECTAL CANCER

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Background Chimeric antigen receptor (CAR) T cell therapy has made significant progress in the treatment of blood cancers such as leukemia, lymphoma, and myeloma. However, the therapy faces many challenges in treating solid tumors. These challenges include physical barriers, tumor microenvironment immunosuppression, tumor heterogeneity, target specificity, and limited reactive cell expansion in vivo. Conventional CAR T cell therapy has thus far shown weak cell expansion in solid tumor patients and achieved little or no therapeutic responses. Here, we developed CAR T cells based on a novel CoupledCAR® technology to treat solid tumors. In contrast to conventional CAR T cells, CoupledCAR T cells significantly improved the expansion of the CAR T cells in vivo and enhanced the CAR T cells’ migration ability and resistance to immunosuppression by the tumor microenvironment. The enhanced migration ability and resistance allow the CAR T cells to infiltrate to tumor tissue sites and increase anti-tumor activities.

Methods We designed a ‘CoupledCAR’ lentivirus vector containing a single-chain variable fragment (scFv) targeting human TSHR. The lentivirus was produced by transfecting HEK-293T cells with ‘CoupledCAR’ lentiviral vectors and viral packaging plasmids. Patient’s CD3 T cells were cultured in X-VIVO medium containing 125U/mL interferon-γ (IL-2), and transduced with ‘CoupledCAR’ lentivirus at certain MOI. Transduction efficiency and viral quality controls for fungi, bacteria, mycoplasma, chlamydia, and endotoxin were performed. After infliximab, serial peripheral blood samples were collected, and the expansion and the cytokine release of CART cells were detected by FACS and QPCR. The evaluation of response level for patients were performed at month 1, month 3, and month 6 by PET/CT.

Results Specifically, we engineered CoupledCAR T cells with lentiviral vectors encoding an anti-GCC (guanylate cyclase 2C) CAR molecule. Furthermore, anti-GCC CAR T cells showed anti-tumor activities in vitro and in vivo experiments. To verify the safety and efficacy of CoupledCAR T cells for treating solid tumors, we conducted several clinical trials for different solid tumors, including seven patients with colorectal cancer. These seven patients failed multiple rounds of chemotherapy and radiotherapy. In the clinical trial, the patients were infused with autologous anti-GCC CoupledCAR T cells. Specifically, we observed that CoupledCAR T cells expanded significantly in the patients and infiltrated tumor tissue sites, demonstrating enhanced anti-tumor activities. PET/CT showed significant tumor shrinkage and SUV max declined, and the ongoing responses were monitored. Patient 3 achieved complete response and the best overall response rate (ORR, include complete remission, complete metabolic response, partial response, and partial metabolic response,) was 71.4% (5/7), complete remission (CR) was 14.3% (1/7).

Conclusions The clinical data demonstrated that CoupledCAR T cells effectively expanded, infiltrated tumor tissue sites, and kill tumor cells in patients with colorectal cancer. We used immunotherapy to achieve complete remission in patients with advanced colorectal cancer for the first time. We are recruiting more colorectal cancer patients to further test the safety and efficacy of anti-GCC CoupledCAR T cells. Since our CoupledCAR® technology is a platform technology, we are expanding it to treat other solid tumors using different target tumor markers.

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Abstracts

337 PEMBROLIZUMAB IN COMBINATION WITH XELOX BEVACIZUMAB IN PATIENTS WITH MICROSATELLITE STABLE (MSS) METASTATIC COLORECTAL CANCER AND A HIGH IMMUNE INFILTRATE: A PROOF OF CONCEPT STUDY. FFCD 1703 POCHI

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Background Immune checkpoint inhibitors (ICI) are very effective in deficient DNA mismatch-repair system (dMMR)/microsatellite instable (MSI) metastatic colorectal cancer (mCRC). About 15% of MSS/pMMR CRCs are highly infiltrated by tumor infiltrating lymphocyte (TIL) with a good prognosis. Some immune scores based on CD3+ and/or CD8+ T-cells infiltration are validated and reproducible, especially TuLIS® and Immunoscore®. No data are available concerning efficacy of ICI in this subpopulation of mCRC. Pembrolizumab, an anti-PD1 (programmed death-1) monoclonal antibody has been recently reported very effective in patients with MSI/dMMR mCRC. Immunogenic cell death induced by chemotherapy, such as oxaliplatin, could increase the efficacy of ICI. We formulated the hypothesis that patients with a pMMR mCRC with a high immune infiltrate can be sensitive to ICI plus oxaliplatin-based chemotherapy.

Methods POCHI is a multicenter, open-label, single-arm phase II trial to evaluate efficacy of pembrolizumab in combination with chemotherapy as first-line treatment of pMMR mCRC with a high immune infiltrate. Primary objective is PFS at 10 months, i.e. number of patients alive and without radiological progression or unacceptable toxicity. The clinical hypotheses are to increase PFS at 10 months from 50% to 70%. With a secondary resection rate, depth of response and early tumour shrinkage. Main inclusion criteria are pMMR mCRC untreated for metastatic disease and with at least one measurable metastatic target according to RECIST v1.1 criteria. Patients must have resected primary tumor to evaluate two different immune scores (Immunoscore® and TuLIS®) and patients are eligible if one score is ‘high’. Patients will receive combination of pembrolizumab (200 mg), bevacizumab (7.5 mg/kg), oxaliplatin (130 mg/m²) and capecitabine (2000 mg/m²/day, on day 1 to 14). Treatment will be repeated every 3 weeks until disease progression or unacceptable toxicity. The clinical hypotheses are to increase PFS at 10 months from 50% to 70%. With a one-sided type error of 5%, power of 85%, 10% rate of patients lost to follow-up or not evaluable, 55 patients have to be included. If 32 patients or more are alive and without progression at 10 months, the treatment will be considered as effective. Thus, with 15% ‘high’ immune score, about 400 patients must be tested in order to include 55 patients in POCHI trial. The ancillary study will consist to identify predictive biomarkers of response and included expression of PD-L1, circulating lymphocytes circulating tumour DNA, mutational load and gut microbiota. Inclusions will start in September 2020 and theoretical end of recruitment is 2023.

Results N/A

Conclusions N/A

Acknowledgements We thank all the cooperative groups (FFCD – UNICANCER GI– GERCOR) for their contribution and participation to the present trial. We thank MSD and HalioDX for their support.

Trial Registration NCT04262687

Ethics Approval This study was approved by ‘Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM)’ on 24/03/2020; approval number MEDIACNAT-2020-01-00038_2019-002407-18.

Consent N/A

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338 EMERGING INSIGHTS ON THE ASSOCIATION OF TUMOR MOLECULAR PHENOTYPE WITH CLINICAL BENEFIT IN METASTATIC COLORECTAL CANCER (MCRC) SUBJECTS TREATED WITH AB928 + MODIFIED FOLFOX-6 (MFOLFOX-6)

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Background The release of ATP from dying cancer cells in response to platinum-based chemotherapy increases extracellular adenosine, which binds to and activates A2aR and A2bR receptors to generate a n immunosuppressive microenvironment. This is mediated by the activation of A2aR on intra-tumoral T and NK cells, A2aR and CD73, an adenosine-producing enzyme, on cancer cells is upregulated by oncogenic drivers such as KRAS. Consistent with this, tumors from CRC subjects express high levels of A2bR. Adenosine receptor blockade may therefore enhance the therapeutic efficacy of certain chemotherapeutic agents. AB928 is the first clinical-stage small-molecule dual adenosine receptor antagonist, targeting both A2aR and A2bR to generate a n immunosuppressive microenvironment.

Methods A total of 35 subjects enrolled in this study: 12 (1L); 4 (2L); and 19 (3L+). Baseline and on-treatment biopsy samples were subjected to immunofluorescent staining as well as WES and RNAseq analysis.

Results Analysis of the primary CRC dataset in TCGA highlights this tumor type as having high levels of CD73, coupled with a paucity of Tissue Nonspecific Alkaline Phosphatase (TNAP), another enzyme that can produce adenosine. In our mCRC study samples, TNAP was often present, being expressed on either stroma or tumor and in a non-overlapping manner with CD73. Analysis of the expression levels of these
Background HER2 potently inhibits innate immunity through cGAS–STING signalling,1 meanwhile HER2 antibody induced ADCP will also lead to macrophage mediated immune suppression. Preclinical and clinical studies suggested a coordination of engagement of innate and adaptive immunity with the combination of an anti-HER2 antibody and an immune checkpoint blockade. KN026 is a novel bispecific antibody that simultaneously binds to two distinct HER2 epitopes. KN046 is a novel bispecific antibody that blocks both PD-L1 interaction with PD-1 and CTLA-4 interaction with CD80/CD86. Here we reported the interim results from an ongoing phase Ib dose escalation and expansion study assessing the safety, tolerability and preliminary efficacy for KN026 in combination with KN046.

Methods This study enrolled pts with solid tumors who failed available standard of care, HER2 aberration status confirmed locally (HER2 mutation, HER2 amplification and/or HER2 overexpression). Eligible pts received combination of KN026 and KN046 at two dose levels until disease progression, unacceptable toxicity or withdrawal of informed consent (DL1: KN026 20 mg/kg Q2W + KN046 3 mg/kg Q2W; DL2: KN026 20 mg/kg Q2W with loading on Days 1, 8 of Cycle 1 + KN046 5 mg/kg Q3W). Tumor response was evaluated Q8W per RECIST 1.1. Primary endpoint was DLT and key secondary endpoints were efficacy parameters (ORR, DOR, PFS).

Results As of the Jul. 13, 2020, 21 pts were enrolled into DL1 (n = 18, 3 for dose escalation) and DL2 (n = 3) (mGC/GEJ 12 pts; mCRC 7 pts; other solid tumors 2 pts). 11 pts remained on the study treatment and 10 pts discontinued treatment due to disease progression (n=5), death (n=2) and other reasons (n=3). 15 pts had HER2-positive status (11 of 15 failed previous trastuzumab therapy), 1 pt had HER2 mutation and 5 pts had HER2 low expression (without FISH amplification). No DLTs were observed. No pts experienced LVEF decreased or other clinically meaningful cardiac AEs. Treatment-related TEAEs occurred in 13 pts, of which 1 pt experienced grade 3 or above treatment-related TEAEs. 7 pts experienced irAEs, all of which were grade 1 or 2. The most common (≥10%) KN026 or KN046 related TEAEs were anaemia (n=5, 23.8%), AST increased (n=4, 19.0%), rash (n=4, 19.0%), diarrhea (n=4, 19.0%), blood bilirubin increased (n=3, 14.3%) and infusion related reaction (n=3, 14.3%). The objective response rate in pts with HER2-positive tumors (n = 7 efficacy evaluable pts) was 4/7 (57.1%, 95% CI 18.4–90.1%) and disease control rate 6/7 (85.7%, 95% CI 42.1–99.6%). 3 pts with HER2 mutation or low expression achieved SD including one patient with SD for more than 24 weeks. 2 death cases only received one cycle of KN026 plus KN046 due to COVID-19 restriction before died from clinical deterioration from underlying tumors.

Conclusions KN026 combined with KN046 is well tolerated and has demonstrated profound anti-tumor activity in HER2-positive solid tumors.

Trial Registration NCT04040699

Ethics Approval The study was approved by Beijing Cancer Hospital Institution’s Ethics Board, approval number SSU00070639

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

REFERENCE

Abstracts

PSMA on cancer cells and CD3 on T cells, leading to T-cell activation, tumor-cell killing, and T-cell expansion. As the BiTE mode of action leads to an upregulation of immune checkpoints, combining AMG 160 with a PD-1 inhibitor may lead to sustained T-cell-dependent killing of tumor cells. Cytokine release syndrome (CRS) is a first-dose effect induced by BiTE molecule-mediated T-cell activation. An approach to mitigate CRS is prophylaxis with an anti-inflammatory agent.

Methods The phase 1 study (NCT03792841) has four parts: AMG 160 monotherapy; AMG 160 in combination with pembrolizumab; AMG 160 monotherapy with etanercept prophylaxis; and AMG 160 monotherapy administered in outpatient centers with 24-hour monitoring. Included in the study are men with histologically/cytologically confirmed mCRPC who are refractory to novel androgen receptor signaling inhibitors: abiraterone, enzalutamide, darolutamide, and/or apalutamide and have failed, refused, or are unsuitable for taxanes; and who have ongoing castration with evidence of progressive disease. Patients who received prior PSMA radionuclide therapy are eligible. Patients with CNS metastases, leptomeningeal disease, spinal cord compression, or active autoimmune disease are excluded. Primary objectives are to evaluate safety and tolerability and determine the MTD or RP2D of AMG 160 monotherapy or in combination with pembrolizumab. Secondary objectives are to characterize pharmacokinetics and preliminary antitumor activity. Evaluation of preliminary antitumor activity will be based on RECIST 1.1 with Prostate Cancer Working Group 3 modifications, PSA response, CTC response, progression-free survival (radiographic and PSA), and overall survival. PSMA PET/CT and FDG PET/CT imaging will be used for evaluation of exploratory objectives (figure 1). The study opened in February 2019 and is currently recruiting patients.

Results N/A

Conclusions N/A

Trial Registration NCT03792841

Ethics Approval The study was approved by all institutional ethics boards.

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PHASE 1B/2 STUDY OF BXCL701, AN ORAL ACTIVATOR OF THE SYSTEMIC INNATE IMMUNITY PATHWAY, COMBINED WITH PEMBROLIZUMAB (PEMBO), IN MEN WITH METASTATIC CASTRATION-RESISTANT PROSTATE CANCER (MCRPC)

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Background BXCL701 (talabostat) is an oral small molecule inhibitor of dipeptidyl peptidases (DPP) primarily DPP8 and DPP9, which triggers inflammasome mediated pyroptosis in macrophages leading to induction of IL-18 and IL-1beta, bridging between innate and adaptive immunity. PD-L1 expression correlates with amplification of DPP8 and DPP9. In syngeneic animal models, significant tumor growth inhibition was observed with BXCL701 plus checkpoint inhibition. In a prior clinical study, single-agent BXCL701 resulted in objective responses in patients (pts) with Stage IV melanoma (unpublished).

Methods In Phase 1b portion of this multicenter study, eligible pts had progressing mCRPC (PCWG3), at least 1 prior systemic therapy, ≤ 2 lines of cytotoxic chemotherapy for mCRPC, no prior anti-PD-1/PD-L1 or other T-cell directed anticancer therapy. Using a 3+3 design, pts received fixed-dose pembro (200 mg IV q21-days) with escalating doses of BXCL701 on days 1–14. The primary endpoint was determination of the recommended Phase 2 dose (RP2D). Response (RECIST 1.1, PSA, CTC), plasma drug concentration and change in relevant immune effector cytokines were also evaluated.

Results 13 pts were treated in 3 cohorts of BXCL701: 0.4 mg qd (n = 3); 0.6 mg qd (n = 3) and 0.6 mg split dose (n=7). 7 pts had adenocarcinoma, 6 had small cell/neuroendocrine prostate cancer features. Prior treatment included ADT (n = 10), 2nd-generation androgen signaling inhibitors (n = 9), chemotherapy (n = 11), RT (n = 11). On-target AEs consistent with cytokine activation were seen at the highest dose levels. In the 0.6 mg qd cohort, all pts had events consistent with cytokine release: 3/3 had hypotension (including 1 grade 3 syncope (DLT)) and 2pts each had dizziness and LE edema. Splitting the 0.6 mg dose improved the tolerability while maintaining the TDD previously associated with objective response; 3/7 pts had fatigue, and 1pt each had low grade hypotension, dyspnea, chills, myalgia. Preliminary anti-tumor activity was seen with 1 pt achieving a PSA response and 3 pts with RECIST1.1 stable disease. BXCL701 was quantifiable in plasma. Consistent dose and time dependent increases in serum IL-18 levels were observed with 0.6 mg split dose.

Conclusions BXCL701 0.3 mg BID (0.6 mg TDD) administered on days 1–14 was identified as the RP2D when administered with pembro 200 mg every 21 days. Splitting the TDD was associated with improved tolerability as evidenced by no reported DLTs and lower rates of other adverse events of interest such as hypotension and peripheral edema. The Phase 2 portion of the study is enrolling.

Acknowledgements All patients, their families, and caregivers who make this study possible; the participating investigators

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**Trial Registration** NCT03910660EUDRAC'T 2018-003734-32

**Ethics Approval** This study was approved by Institution Review Boards or Ethics Committees affiliated with participating institutions.

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**Abstracts**

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A PHASE 3 STUDY (COSMIC-313) OF CABOZANTINIB IN COMBINATION WITH NIVOLUMAB AND IPIлимУМAB IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED RENAL CELL CARCINOMA OF INTERMEDIATE OR POOR RISK

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**Background** Cabozantinib (C) inhibits tyrosine kinases involved in tumor growth, angiogenesis, and immune regulation, including MET, VEGFR, and TAM kinases (Tyro3, AXL, MER), and may promote an immune-permissive tumor environment, resulting in enhanced response to immune checkpoint inhibitors. C has shown preliminary clinical activity and tolerability in combination with the PD-1 inhibitor nivolumab (N) and as part of a triplet combination with N and the CTLA-4 inhibitor ipilimumab (I) in patients (pts) with advanced renal cell carcinoma (aRCC) (Nadal et al. ASCO 2018). C is approved for pts with aRCC, and N+I is approved as a combination therapy in pts with previously untreated aRCC of intermediate or poor risk. We present the study design of a phase 3 trial of C+N+I vs N+I in previously untreated pts with aRCC of IMDC intermediate or poor risk (NCT03937219).

**Methods** This randomized, double-blind, controlled phase 3 study evaluates the efficacy and safety of C+N+I vs N+I in previously untreated pts with IMDC intermediate or poor risk aRCC. Eligible pts are randomized 1:1 to receive C+N+I or N+I in combination with placebo, stratified by IMDC prognostic score and geographic region. Pts receive C (40 mg oral QD) + N (3 mg/kg IV Q3W) x 4 doses + I (1 mg/kg IV Q3W) x 4 doses, followed by C (40 mg oral QD) + N (480 mg IV flat dose Q4W). Control pts receive C-matched placebo and the same treatment regimen for N+I as the experimental arm. N will be administered for a maximum of 2 years. Eligibility criteria include histologically confirmed aRCC with a clear cell component, intermediate or poor risk RCC per IMDC criteria, measurable disease per RECIST 1.1, KPS ≥70%, adequate organ and marrow function and age ≥18 years. Exclusion criteria include prior systemic therapy for aRCC and uncontrolled significant illnesses. The primary endpoint is PFS per RECIST 1.1 by BICR; the secondary endpoint is OS. Additional endpoints include ORR, safety, correlation of biomarkers with outcomes, and pharmacokinetics of C in combination with N+I. The first patient was enrolled in June 2019 and enrollment is ongoing.

**Results** N/A

**Conclusions** N/A

**Trial Registration** ClinicalTrials.gov: NCT03937219

**Ethics Approval** This study is being conducted in compliance with Good Clinical Practice (GCP), including International Conference on Harmonisation (ICH) Guidelines, the most recent accepted version of the Declaration of Helsinki, and all applicable local laws and regulatory requirements. The appropriate Institutional Review Boards (IRBs) or Ethics Committees (ECs) of participating centers have approved the study protocol. All patients have provided written informed consent.

**Consent** All patients have provided written informed consent.

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**PHASE 3 STUDY OF PEMBROLIZUMAB + DOCETAXEL AND PREDNISONE/PREDNISOLONE FOR METASTATIC CASTRATION-RESISTANT PROSTATE CANCER (mCRPC) PRETREATED WITH NEXT-GENERATION HORMONAL AGENTS (NHAS) (KEYNOTE-921)

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**Background** Cohort B of the phase 1b/2 KEYNOTE-365 study (NCT02861573) found that docetaxel + pembrolizumab + prednisone demonstrated activity in patients previously treated with abiraterone acetate or enzalutamide for mCRPC. The prostate-specific antigen (PSA) response rate was 28%; objective response rate (ORR) was 18% (7 partial responses); duration of response (DOR) was 6.7 months; progression-free survival (PFS) was 8.3 months; overall survival (OS) was 20.4 months; and the 12-month PFS and OS rates were 24.0% and 75.8%, respectively. The safety and tolerability profile of this combination was consistent with the profiles of each individual agent. The KEYNOTE-921 (NCT03834506) phase 3 trial will evaluate efficacy and safety of pembrolizumab + docetaxel + prednisone/prednisolone in patients with mCRPC after prior treatment with NHA.

**Methods** Eligible patients are adults with histologically or cytologically confirmed mCRPC who experience disease progression or intolerance to NHA in the metastatic hormone-sensitive prostate cancer setting or CRPC setting, no prior treatment with chemotherapy for mCRPC, and tissue available for biomarker analysis. Treatment stratification factors are prior treatment with abiraterone acetate (yes or no) and metastases location (bone only, liver, other). Approximately 1000 patients will be randomly assigned to receive docetaxel 75 mg/m2 IV Q3W + prednisone/prednisolone 5 mg orally BID and pembrolizumab 200 mg IV Q3W or docetaxel 75 mg/m2 IV Q3W + prednisone/prednisolone 5 mg PO BID + placebo IV Q3W (1:1 ratio). Response and progression will be determined using imaging (CT/MRI/bone) according to PCWG3-modified RECIST v1.1 by blinded independent central review (BICR) Q9W during the first year and Q12W thereafter. Treatment maximums are 10 cycles for docetaxel + prednisone/prednisolone and 35 cycles for pembrolizumab or placebo. Treatment discontinuation regardless of therapy received is mandated for disease progression, unacceptable toxicity, or consent withdrawal. The dual primary end points are radiographic PFS per PCWG3-modified
REIST v1.1, as assessed by bICR and OS, and the key secondary end point is time to initiation of subsequent anticancer therapy or death. Other secondary end points include PSA response rate, time to PSA progression, ORR, DOR, time to radiographic soft tissue progression, time to radiographic bone progression, and safety. KEYNOTE-921 is ongoing or planned in 22 countries across Asia, Australia, Europe, and North and South America.

Results N/A

Conclusions N/A

Ethics Approval The study and the protocol were approved by the Institutional Review Board or ethics committee at each site.

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344 PHASE 3 TRIAL OF PEMBROLIZUMAB AND ENZALUTAMIDE VERSUS ENZALUTAMIDE IN PATIENTS WITH METASTATIC CASTRATION-RESISTANT PROSTATE CANCER (mCRPC) (KEYNOTE-641)

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Background Antitumor activity with pembrolizumab + enzalutamide was observed in cohort C of the phase 1b/2 KEYNOTE-365 (NCT02861573) study of abiraterone acetate–pretreated patients with mCRPC and in a phase 2 study (NCT02312557) of patients with mCRPC who experienced progression with enzalutamide alone. In KEYNOTE-365 cohort C, prostate-specific antigen (PSA) response rate was 22%, objective response rate (ORR) was 20%, and 12-month PFS and OS rates were 24.6% and 72.8%, respectively. Safety and tolerability of the combination was consistent with individual profiles of each agent. In the phase 2 study of enzalutamide–pretreated patients, 5 of 28 patients (18%) had a PSA decline of ≥50%, and 3 of 12 patients (25%) with measurable disease achieved objective response. KEYNOTE-641 (NCT03834493) is a randomized, phase 3 trial to assess efficacy and safety of pembrolizumab + enzalutamide versus placebo + enzalutamide in patients with mCRPC.

Methods Enrolled patients have biochemical or radiographic progression with androgen deprivation therapy/bilateral orchectomy within 6 months of screening, ECOG PS 0/1, ongoing androgen deprivation with serum testosterone <50 ng/dL, and tumor tissue availability for biomarker analysis. The study continues to enroll those who previously had abiraterone acetate therapy; the abiraterone-naive cohort is filled. Exclusion criteria are prior chemotherapy for mCRPC, checkpoint inhibition, or any treatment with a second-generation androgen receptor inhibitor. Treatment stratification factors are prior abiraterone acetate treatment (yes or no), metastases location (bone only or liver or other), and prior docetaxel treatment for metastatic hormone-sensitive prostate cancer (yes or no). Response and progression will be determined by imaging (CT/MRI/bone) per PCWG3-modified RECIST v1.1 on visits Q9W during the first year and Q12W thereafter. Approximately 1200 adults will be randomly assigned 1:1 in a double-blind fashion to receive enzalutamide 160 mg orally once daily + pembrolizumab 200 mg IV Q3W or enzalutamide 160 mg orally once daily + placebo for a maximum of 35 cycles or until disease progression, unacceptable toxicity, or consent withdrawal. Coprimary end points are radiographic PFS per PCWG3-modified RECIST v1.1, as assessed by blinded independent central review, and OS. The key secondary end point is time to subsequent anticancer therapy or death. Other secondary end points are ORR, DOR, PSA response rate, PSA undetectable rate, time to PSA progression, time to pain progression, time to symptomatic skeletal-related event, time to soft tissue progression, and safety. KEYNOTE-641 is ongoing or planned in 21 countries across Asia, Australia, Europe, and North and South America.

Results N/A

Conclusions N/A

Trial Registration ClinicalTrials.gov, NCT03834493

Ethics Approval The study and the protocol were approved by the Institutional Review Board or ethics committee at each site.

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345 PHASE 3 STUDY OF COMBINATION PEMBROLIZUMAB + OLAPARIB THERAPY VERSUS ENZALUTAMIDE/ABIRATERONE IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER (mCRPC) AFTER PROGRESSION ON CHEMOTHERAPY (KEYLYNK-010)

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Background Cohort A of the phase 1b/2 KEYNOTE-365 study (NCT02861573) demonstrated promising antitumor activity with pembrolizumab + olaparib in patients with mCRPC unselected for homologous recombination deficiency. The prostate-specific antigen (PSA) and objective response rates (ORR) were both 9%, progression-free survival (PFS) was 4.3 months, overall survival (OS) was 14.4 months, and 12-month PFS and OS rates were 23.3% and 58.2%, respectively. The safety profile of the combination therapy was also aligned with the individual profiles of each agent. KEYLYNK-010 (NCT03834519) is a phase 3 trial to evaluate efficacy and safety of pembrolizumab + olaparib in molecularly unselected enzalutamide- or abiraterone-pretreated patients with mCRPC who progressed with docetaxel chemotherapy.

Methods Eligibility criteria include histologically confirmed mCRPC unselected for homologous recombination repair (HRR) gene mutation, progression on docetaxel chemotherapy, progression on androgen deprivation therapy within 6 months before screening, received either abiraterone for metastatic castration-sensitive prostate cancer/mCRPC or enzalutamide for metastatic hormone-sensitive prostate cancer/mCRPC or enzalutamide after bilateral orchiectomy within 6 months of screening, ECOG PS 0 or 1, patients will also be required to provide tumor tissue for biomarker analysis. Approximately 780 adults will be randomized in a 2:1 ratio to pembrolizumab + olaparib 300 mg PO QD or abiraterone 1000 mg PO QD + prednisone 7.5 mg PO QD + prednisolone 5 mg PO QD (enzalutamide-pretreated patients) or enzalutamide 160 mg PO QD (abiraterone-pretreated patients). Randomization will be stratified by prior treatment (abiraterone or enzalutamide) and measurable disease (yes/no). Treatment for all patients will continue until disease progression, unacceptable toxicity, or withdrawal. Response will be assessed by imaging (CT/MRI/bone) per PCWG3-modified RECIST v1.1, as assessed by blinded independent central review, and OS. The key secondary end point is time to subsequent anticancer therapy or death. Other secondary end points are ORR, DOR, PSA response rate, PSA undetectable rate, time to PSA progression, time to pain progression, time to symptomatic skeletal-related event, time to soft tissue progression, and safety. KEYNOTE-641 is ongoing or planned in 21 countries across Asia, Australia, Europe, and North and South America.

Results N/A

Conclusions N/A
Working Group 3 (PCWG3)-modified RECIST v1.1 by blinded independent central review (BICR) Q9W during the first year and then Q12W thereafter. The dual primary end points are radiographic PFS per PCWG3-modified RECIST v1.1, as assessed by BICR and OS. The key secondary end point is time to initiation of subsequent anticancer therapy or death. Other secondary end points include ORR, duration of response, time to PSA progression, time to first symptomatic skeletal-related event, and safety and tolerability. Patient-reported outcomes and identification of molecular biomarkers for treatment response are exploratory end points. KEYLYNK-010 is ongoing or planned in 19 countries across Asia, Australia, Europe, and North and South America.

Results N/A

Conclusions N/A

Trial Registration ClinicalTrials.gov, NCT03834519

Ethics Approval The study and the protocol were approved by the Institutional Review Board or ethics committee at each site.

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346 KEYNOTE-991: PHASE 3 STUDY OF PEMBROLIZUMAB PLUS ENZALUTAMIDE AND ANDROGEN DEPRIVATION THERAPY (ADT) FOR PATIENTS WITH METASTATIC HORMONE-SENSITIVE PROSTATE CANCER (mHSPC)

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Background Combination of pembrolizumab, an anti-PD-1 antibody, added to enzalutamide, a nonsteroidal antiandrogen agent, has shown antitumor activity in abiraterone-resistant mCRPC (KEYNOTE-365, NCT02861573) and in patients with mCRPC for whom enzalutamide was ineffective (KEYNOTE-199, NCT02787005). These data indicate that the combination of pembrolizumab + enzalutamide with ADT warrants phase 3 evaluation. Also, efficacy in enzalutamide may be proimmunogenic, suggesting that it may be additive or synergistic in antitumor activity when combined with pembrolizumab.

Methods The KEYNOTE-991 (NCT04191096) phase 3 trial will evaluate the efficacy and safety of enzalutamide + ADT (LHRH agonist/antagonist during study treatment or bilateral orchiectomy) + pembrolizumab or placebo in patients with mHSPC. Eligibility criteria include age ≥ 18 years, mHSPC, ≥ 2 bone lesions or visceral disease, no prior treatment with next-generation hormone agents, adequate organ function, and ECOG PS 0 or 1. Patients must provide tissue for biomarker analysis. Approximately 1232 patients will be randomized in a 1:1 ratio to receive enzalutamide 160 mg orally once daily + ADT + pembrolizumab 200 mg IV every 3 weeks (Q3W) or enzalutamide 160 mg orally once daily + ADT + placebo IV Q3W. Treatment will continue with pembrolizumab up to 35 cycles and treatment with enzalutamide will proceed continuously from day 1 of cycle 1 until disease progression, unacceptable toxicity, or withdrawal of consent. The stratification factors are prior docetaxel therapy (yes or no) and presence of high-volume disease (yes or no). CT or MRI and radionuclide bone imaging will be used to assess response according to Prostate Cancer Working Group 3 (PCWG3)-modified RECIST v1.1 by blinded independent central review (BICR) Q12W from the date of randomization. Imaging will continue until the end of treatment and will resume Q12W during the posttreatment period. The co-primary end points are BICR-assessed radiographic PFS (according to PCWG3-modified RECIST v1.1) and OS. Key secondary end points are time to first subsequent anticancer therapy and time to symptomatic skeletal-related event. Other end points are PSA2 progression (measurement of prior chemotherapy or death), PSA response rate, time to PSA progression, PSA undetectable rate, ORR, duration of response, time to soft tissue and radiographic bone progression per PCWG3-modified RECIST v1.1, safety, and patient-reported outcomes (eg, time to pain progression). Safety and tolerability will be evaluated using a tiered approach. KEYNOTE-991 is enrolling at 40 sites in Australia, Chile, Colombia, Israel, Japan, Poland, South Korea, Spain, Switzerland, Taiwan, and the United States.

Results N/A

Conclusions N/A

Trial Registration ClinicalTrials.gov: NCT04191096

Ethics Approval The study and the protocol were approved by the Institutional Review Board or ethics committee at each site.

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347 CLINICAL OUTCOMES OF OVARIAN CANCER PATIENTS TREATED WITH ALKS 4230, A NOVEL ENGINEERED CYTOKINE, IN COMBINATION WITH PEMBROLIZUMAB: ARTISTRY-1 TRIAL

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Background ALKS 4230 is a novel engineered cytokine that selectively targets the intermediate-affinity interleukin-2 receptor complex to activate CD8+ T cells and natural killer cells. The ARTISTRY-1 trial (NCT02799095) has shown encouraging efficacy and acceptable tolerability of ALKS 4230 among patients with advanced solid tumors. We report a detailed analysis of ovarian cancer (OC) patients who received combination therapy in ARTISTRY-1.

Methods ARTISTRY-1 is an ongoing multicohort phase 1/2 trial exploring intravenous ALKS 4230 as monotherapy and combined with pembrolizumab. OC patients were enrolled into a cohort with mixed anti PD 1/L1 unapproved tumor types who had progressed on prior chemotherapy. OC patients received ALKS 4230 (3 μg/kg) on days 1–5 and pembrolizumab (200 mg) on day 1 of a 21 day cycle. Outcomes
Abstract 347 Table 1 Summary of response observations among patients with ovarian cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Prior Therapies</th>
<th>Max. Reduction of Target Lesions (%)</th>
<th>CR*</th>
<th>CA125 (U/mL) Response From Baseline</th>
<th>Time on ALKS 4230 (Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>CBB/PCB/BEV, CMB/PCB/BEV, PCB/PCB/BEV</td>
<td>70.6</td>
<td>CBB Normalized from 283 to 245.3 at cycle 4</td>
<td>CA125 U/mL: 81.0</td>
<td>81*</td>
</tr>
<tr>
<td>2</td>
<td>83</td>
<td>CBB/PCB/BEV, PCB/BEV</td>
<td>76.3</td>
<td>Normalized from 125 to 10 at cycle 4</td>
<td>CA125 U/mL: 23*</td>
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</tr>
<tr>
<td>3</td>
<td>60</td>
<td>CBB/PCB/BEV, PCB/BEV</td>
<td>44.7</td>
<td>Reduced from 1400 to 200 at cycle 1</td>
<td>CA125 U/mL: 34</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>CBB/PCB/BEV, PCB/BEV</td>
<td>21.8</td>
<td>Reduced from 493 to 345 at cycle 3</td>
<td>On-treatment at cycle 3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>83</td>
<td>CBB/PCB/BEV, PCB/BEV</td>
<td>18.3</td>
<td>Normal at baseline at 19.0</td>
<td>21*</td>
<td></td>
</tr>
</tbody>
</table>

*As assessed by the investigator.
*CR due to radiographic shrinkage to <10 mm non-measurable, which is considered normal.

Abstract 347 Figure 1 Increased markers of lymphocyte tumor infiltration

An increase in CD3+CD8+ T cells (A, red = CD3; blue = CD8; purple = CD3+CD8+; teel = tumor marker), GranzymeB (B, red = CD8; green = granzymeB+; yellow = granzymeB+CD8+; teal = tumor marker), and PD-L1 (C, red = PD-L1; blue = tumor marker) in the tumor microenvironment of a single patient was observed after the patient received monotherapy ALKS 4230.

The data presented include antitumor activity (RECIST v1.1) and safety as of 7/24/2020. To evaluate changes in tumor microenvironment (TME), baseline and on-treatment biopsies were collected.

Results Fourteen heavily pretreated patients with OC were enrolled. Patients received a median of 5 (range, 2–11) prior regimens and all were previously treated with platinum based therapy. Among 13 evaluable patients with ≥1 assessment, 9 experienced disease control and 4 experienced disease progression; median treatment duration was approximately 7 weeks. Three patients experienced an objective response, including 1 complete response, 1 partial response (PR), and 1 unconfirmed PR; all were platinum resistant and negative for BRCA mutations. Five patients experienced treatment burden reductions (table 1). Treatment-related adverse events at the doses tested have generally been transient and manageable, with the majority being grade 1 and 2 in severity. Overall, based on preliminary data, the combination with ALKS 4230 did not demonstrate any additive toxicity to that already established with pembrolizumab alone. Additional safety and efficacy data are being collected in ongoing cohorts. In the monotherapy dose escalation portion of the study, ALKS 4230 alone increased markers of lymphocyte infiltration in 1 paired melanoma biopsy (1 of 1; on treatment at cycle 2); CD8+ T cell density and PD-L1 tumor proportion score increased 5.2- and 11 fold, respectively, supporting evidence that ALKS 4230 has immunostimulatory impact on the TME and providing rationale for combining ALKS 4230 with pembrolizumab (figure 1).

Conclusions The combination of ALKS 4230, an investigational agent, and pembrolizumab demonstrates an acceptable safety profile and provides some evidence of tumor shrinkage and disease stabilization in some patients with heavily pretreated OC. This regimen could represent a new therapeutic option for these patients.

Acknowledgements The authors would like to thank all of the patients who are participating in this trial and their families. The trial is sponsored by Alkermes, Inc. Medical writing and editorial support was provided by Parexel and funded by Alkermes, Inc.

Trial Registration ClinicalTrials.gov NCT02799095

Ethics Approval This trial was approved by Ethics and Institutional Review Boards (IRBs) at all trial sites; IRB reference numbers 16–229 (Dana-Farber Cancer Institute), MOD00003422/PH285316 (Roswell Park Comprehensive Cancer Center), 20160175 (Western IRB), i15-01394_MOD23 (New York University School of Medicine), TRIAL201909090 (Cleveland Clinic), and 0000097 (ADVARRA).

REFERENCES
2. Vaidhampayan UN, Muzaffar J, Velcheti V, Winer I, Hoimes CJ, Rosen SD, et al. ALKS 4230 monotherapy and in combination with pembrolizumab (pembro) in patients (pts) with refractory solid tumors (ARTISTRY-1). Oral presentation at: European Society for Medical Oncology Annual Meeting; September 2020; virtual.

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antibody against programmed cell death 1 (PD 1). In POD1UM-101, retifanlimab monotherapy demonstrated acceptable tolerability and durable clinical benefit in multiple advanced tumor types, including pretreated endometrial cancer. POD1UM-204 is designed to further investigate efficacy and safety of retifanlimab alone or in combination with other immunotherapy or targeted agents in patients with advanced/metastatic endometrial cancer.

Methods POD1UM-204 is a phase 2, multicenter, nonrandomized, open-label, umbrella study in women ≥18 years of age, with histologically confirmed diagnosis of advanced/metastatic endometrial cancer that has progressed on or after platinum-based chemotherapy. Patients must have an ECOG performance status =1, at least 1 measurable tumor lesion by Response Evaluation Criteria in Solid Tumors v1.1, and provide tumor tissue at baseline. Approximately 220 patients will be enrolled into 4 treatment groups: Group A—patients with dMMR (deficient DNA mismatch repair) or POLE (DNA polymerase epsilon) mutations, or alterations outside of the kinase domain and regardless of prior CPI treatment (up to 40 patients) receiving retifanlimab monotherapy; Group B—patients with dMMR (deficient DNA mismatch repair) or POLE (DNA polymerase epsilon) mutations, or alterations outside of the kinase domain and regardless of prior CPI treatment (up to 40 patients) receiving retifanlimab plus epacadostat (indoleamine 2,3-dioxygenase inhibitor); and Group D—patients with endometrial cancer and activating fibroblast growth factor receptor (FGFR1, 2 or 3) mutations or alterations outside of the kinase domain and regardless of prior CPI treatment (up to 40 patients) receiving retifanlimab plus pembrolizumab (FGFR1, 2, 3 inhibitor) (figure 1). Patients can receive up to 26 treatment cycles if they continue to derive benefit and have not met criteria for withdrawal. The primary study objective is evaluating retifanlimab monotherapy antitumor activity (objective response rate [ORR] determined by independent central review [ICR]) in Group A. Secondary study objectives include assessing additional efficacy measures (duration of response, disease control rate and progression-free survival by ICR, and overall survival) in Group A; determining clinical activity (ORR by the investigator) in Groups B, C and D; and evaluating safety and tolerability of retifanlimab.

Abstract 348 Figure 1 POD1UM-204 study design

Results N/A

Conclusions N/A

Acknowledgements This study is sponsored by Incyte Corporation (Wilmington, DE).

Trial Registration ClinicalTrials.gov Identifier: NCT04463771; EudraCT 2020-000496-20

Ethics Approval The study was approved by institutional review boards or independent ethics committees of participating institutions.

Consent N/A

References


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0348

Background Efficacy of anti-PD-1 therapy is attributed to the presence of infiltrating antigen-specific CD8+ T-cells. Despite the success of anti-PD-1 therapy, many patients with SCCHN present with immune desert or immune excluded tumors and only 13–18% of patients achieve tumor reductions. Given this low response rate, it is imperative to combine agents that generate or expand anti-tumor T cells, such as vaccines, with anti-PD-1 therapies. SNS-301 is a first-in-class, bacteriophage-based immune activating agent targeting human aspartate β-hydroxylase (ASPH), a tumor associated antigen overexpressed in multiple tumor types. SNS-301 is a self-adjuvanting vaccine consisting of λ-bacteriophage engineered to express an immunogenic fragment of ASPH fused to the phage gpD coat protein, previously shown to be well tolerated and generate an immune response (Phase 1, NCT03120832). The objectives of this trial are to evaluate safety, immunogenicity and preliminary efficacy of SNS-301 in combination with pembrolizumab in patients that did not achieve tumor reductions on anti-PD-1/anti-PD-L1 therapy alone.

Methods The study consists of an initial safety-run-in followed by a two-stage design. SNS-301 is delivered intradermally in addition to pembrolizumab in up to 30 patients with locally advanced unresectable or metastatic/recurrent SCCHN. Patients must have actively received anti-PD-1 therapy for ≥12 weeks, with a best response of stable disease (SD) or unconfirmed progressive disease (PD) per iRECIST. Patients provide pre, on-treatment and biopsies at PD (optional) to characterize the tumor microenvironment using Nanostring™, multiplex immunohistochemistry, and correlate with clinical outcomes. Blood
samples are collected to evaluate T cell responses using flow cytometry, ELISA, ELISPOT.

Results As of July 23, 2020, 9 patients were enrolled. Median duration of ongoing anti-PD therapy was 37 weeks (range 20–101). The combination was well-tolerated with no DLTs and mostly Grade 1–2 unrelated adverse events. Two Grade 3 events were reported: hypertension (not related) and dehydration (related), the later reported as serious adverse event. Of seven patients eligible for efficacy analysis, one patient with PD-L1 negative disease had a partial response with a reduction of 29% at week 6 with deepening of the response to 43% at week 12 and one patient with progressive disease at study entry had stabilization of disease at week 6 and 12. Another two patients had stable disease for 30+ weeks and three patients had PD. Additional efficacy and immunological analyses are ongoing.

Conclusions Early data show that the combination of SNS-301 and pembrolizumab has manageable toxicity and capacity to achieve long-term disease stability and objective tumor responses.

Trial Registration NCT04034225
Ethics Approval This study has been approved by WIRB (20190628) as well as several institutional IRBs.

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351 PEMBROLIZUMAB PLUS LENVATINIB VS CHEMOTHERAPY AND LENVATINIB MONOTHERAPY FOR RECURRENT/METASTATIC HEAD AND NECK SQUAMOUS CELL CARCINOMA THAT PROGRESS ON PLATINUM THERAPY AND IMMUNOTHERAPY: LEAP-009

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Background Pembrolizumab alone and in combination with platinum-based chemotherapy have become standard first-line treatment options for recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC), and there is a growing unmet need for safe and efficacious treatment options for R/M HNSCC that has progressed on or after platinum-based chemotherapy and immunotherapy. Data from Study 111/KEYNOTE-146 showed promising antitumor activity and acceptable safety for the PD-1 inhibitor pembrolizumab given in combination with the multikinase inhibitor lenvatinib in patients with metastatic HNSCC.1 LEAP-009 (NCT04428151), a global, randomized, open-label, phase 2 trial, will assess the efficacy and safety of pembrolizumab in combination with lenvatinib versus SOC chemotherapy, as well as the efficacy and safety of lenvatinib monotherapy, in patients with R/M HNSCC that has progressed after platinum-based chemotherapy and a PD-(L)1 inhibitor.

Methods Eligible patients are adults with histologically confirmed, locally incurable R/M HNSCC of the oral cavity, oropharynx, hypopharynx, or larynx, disease progression at any time during or after platinum-containing chemotherapy (with or without cetuximab), disease progression within 12 weeks from the last dose of treatment with ≥2 doses of a PD-(L)1 inhibitor, measurable disease based on RECIST v1.1 as confirmed by BICR, ECOG performance status of 0 or 1, and no major blood vessel invasion/infiltration. Patients will be randomized 3:3:2 to pembrolizumab (200 mg IV Q3W for up to 35 cycles) plus lenvatinib (20 mg orally once daily), investigator’s choice of SOC chemotherapy (docetaxel, paclitaxel, cetuximab, or capecitabine), or lenvatinib monotherapy (24 mg orally once daily). Randomization will be stratified by PD-L1 tumor proportion score (<50% versus ≥50%) and ECOG performance status (0 versus 1). Treatment will continue until centrally verified disease progression, unacceptable toxicity, or decision to withdraw. Patients in the chemotherapy and lenvatinib monotherapy arms may be eligible to receive pembrolizumab plus lenvatinib upon disease progression. The primary endpoint is ORR according to modified RECIST v1.1 as assessed by BICR. Secondary endpoints include PFS, OS, DOR, and safety. Interim futility analysis will be conducted for the lenvatinib monotherapy arm. Tumor imaging by CT or MRI will be performed 6 weeks after randomization, every 6 weeks through year 1, and every 9 weeks thereafter. Safety will be monitored throughout the study and for 30 days after treatment end (90 days for serious AEs if no new anticancer treatment is initiated, and at any time if the AE is considered treatment-related). Recruitment is ongoing; Planned enrollment is ~400 patients.

Results N/A

Conclusions N/A

Trial Registration ClinicalTrials.gov Identifier, NCT04428151
Ethics Approval The study and protocol were approved by the Institutional Review Board or ethics committee at each site.

Consent All patients provided written informed consent to participate in the clinical trial.

REFERENCE

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352 UPDATED CLINICAL DATA FROM THE SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK (SCCHN) EXPANSION COHORT OF AN ONGOING PH1/1B STUDY OF EGANELISIB (FORMERLY IPI-549) IN COMBINATION WITH NIVOLUMAB

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Background Eganelisib is a first-in-class, oral, selective PI3K-γ inhibitor. Preclinically, eganelisib reprograms macrophages/myeloid derived suppressor cells (MDSCs) from an immune-suppressive to an immune-activating phenotype and enhances efficacy of checkpoint inhibitors. Efficacy of eganelisib + nivolumab in patients with SCCHN resistant to immediate prior anti-PD-(L)1 therapy is presented.
Methods IPI-549-01 (NCT02637531) evaluates eganelisib in advanced solid tumors, as monotherapy and in combination with nivolumab. The combination expansion dose was eganelisib 40 mg QD PO + nivolumab 240 mg Q2W IV. Combination expansion cohorts include SCCHN patients resistant to immediate prior anti-PD(L)1 therapy. Safety, preliminary clinical activity, PK, and correlative study of blood and tumor biopsy samples were mandated.

Results As of June 1, 2020, 180 patients were treated with eganelisib + nivolumab including 21 with SCCHN. The most common (>20% of patients) treatment-emergent adverse events in patients treated with eganelisib + nivolumab (N = 180) were fatigue (34.4%), increased AST (30.0%), increased ALT (26.7%), nausea (25.0%), pyrexia (25.0%), anemia (22.8%), decreased appetite (20.6%), and cough (20.6%). 85 (47.2%) patients experienced at least 1 treatment-emergent serious adverse event (SAE) and 19 (10.6%) had a treatment-related SAE. There were no treatment-related grade 5 adverse events as assessed by investigators. Preliminary data from the SCCHN cohort show that in the efficacy-evaluable population which includes all patients (n=20) who had at least 1 post-baseline response assessment or discontinued treatment due to disease progression, the overall response rate (ORR, ie. CR [complete response] or PR [partial response] per RECIST v1.1) is 10.0%, the disease control rate (DCR, ie. CR, PR, or SD [stable disease]) is 45.0%, and the clinical benefit rate (CBR, ie. CR, PR, or SD of at least 24 weeks from first treatment) is 25.0%, per RECIST v1.1. For patients that received ≤2 lines of prior systemic therapy (n=11), the ORR is 20.0%, the DCR is 40.0%, and the CBR is 30.0%. In total, there are 2 patients with PR (duration of response 1.6–9.3 months) and 3 with SD for greater than 6 months’ treatment duration. Translation data including T cell proliferation in peripheral blood as well as markers of inflammation in baseline biopsy of PR patient will be presented.

Conclusions Eganelisib + nivolumab demonstrates an acceptable safety profile and preliminary clinical activity in patients with SCCHN who were resistant to immediate prior anti-PD(L)1 therapy. Updated clinical and translational data will be presented.

Ethics Approval The study was approved by WIRB, Study Number 1188591 and IRB Tracking Number: 20180297.

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353 SAFETY AND EFFICACY OF TUMOR INFILTRATING LYMPHOCYTES (TIL, LN-145) IN COMBINATION WITH PEMBROLIZUMAB FOR ADVANCED, RECURRENT OR METASTATIC HNSCC

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Background Single-agent checkpoint inhibitors (CPI) are an approved first or second-line therapy in head and neck squamous cell carcinoma (HNSCC), but their efficacy is limited. Adoptive cell therapy with tumor infiltrating lymphocytes (TIL, LN-145) has demonstrated efficacy in multiple malignancies alone or in combination with CPI. To improve HNSCC therapy, a combination of pembrolizumab and LN-145 was explored.

Methods IOV-COM-202 is an ongoing Phase 2 multicenter, multi-cohort, open-label study evaluating LN-145 in multiple settings and indications, and here we report cohort 2A which enrolled CPI naïve HNSCC patients who received the combination of LN-145 and pembrolizumab. Key eligibility criteria include up to 3 lines of prior therapy, ECOG <1, at least one resectable metastasis for LN-145 production, and at least another measurable lesion after tumor resection. Primary endpoints are ORR per RECIST v1.1 by investigator and safety as measured by the incidence of grade ≥3 treatment-emergent adverse events (TEAEs), LN-145 production method uses central GMP manufacturing in a 22-day process yielding a cryopreserved TIL product (figure 1). Preconditioning chemotherapy consists of cyclophosphamide/fludarabine, followed by LN-145, and then <6 doses of IL-2 over <3 days. Pembrolizumab is initiated post-tumor harvest but prior to LN-145 and continues after LN-145 infusion Q3W until toxicity or progression (figure 2).

Results Nine (N=9) HNSCC patients have received LN-145 plus pembrolizumab, with a median duration of follow up of 6.9 months. Nine and 8 patients were evaluable for safety and efficacy, respectively. Mean number of prior therapies was 1.1 with 89% of the patients having received prior chemotherapy. Four were HPV+, 2 HPV-, 3 unknown. The Treatment Emergent Adverse Event (TEAE) profile was consistent with the underlying advanced disease and the known AE profiles of pembrolizumab, the lymphodepletion and IL-2 regimens. The most common TEAE were chills, hypotension, anemia, thrombocytopenia, pyrexia, fatigue and tachycardia. Four patients had a confirmed, objective response with an ORR of 44% (1 CR, 3 PR, 4 SD, 1 NE) per RECIST 1.1. The disease control rate at data cutoff was 89% in 9 patients, and 7 of the 8 evaluable patients (87.5%) had a reduction in target lesions. Median DOR was not reached.
Early signals of expansion of HPV-16 E711-20-specific CD8+ T cells. Stable disease (SD), as determined by RECIST 1.1, was observed in several participants in these early dose cohorts, with one subject maintaining SD up to 19 weeks. The maximum tolerated dose (MTD) has not yet been reached. As of May 14, 2020 (the development safety update report (DSUR) data-lock date), no dose limiting toxicities and the following adverse events were observed in the first 12 patients treated with CUE-101: fatigue (n=3), decreased appetite (n=1), arthralgia (n=1), muscular weakness (n=1), parasthesia (n=1), bullous pemphigoid (n=1), and infusion-related reactions (n=1).

Conclusions CUE-101 is a novel agent that is demonstrating acceptable tolerability, favorable PK, and preliminary PD signals that support selective activation of tumor-specific T cells. Neither the MTD nor the monotherapy RP2D have been established. PD and PK analyses are ongoing as dose escalation continues.

Acknowledgements The authors would like to thank all the patients who are participating in this study. The study is sponsored by Cue Biopharma.

A PHASE 1 TRIAL OF CUE-101 A NOVEL HPV16 E7-PHLA-IL2-FC FUSION PROTEIN IN PATIENTS WITH RECURRENT/METASTATIC HPV16+ HEAD AND NECK CANCER

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Background Immuno-STATSTM are novel, modular fusion proteins designed to selectively activate tumor-antigen-specific CD8+ T cells. Human papillomavirus (HPV) associated cancers serve as a model system to assess the safety and efficacy of the Immuno-STAT platform. CUE-101 is comprised of human leukocyte antigen (HLA) complex, HLA A*0201, a peptide epitope derived from the HPV type 16 E7 protein, and 4 molecules of a reduced affinity human interleukin-2 (IL2) designed to bind and activate HPV-specific T cells for eradication of HPV16-driven cancers. In preclinical studies CUE-101 demonstrated selective binding, activation, and expansion of HPV16 E7-specific CD8+ T cells, which translated into anti-tumor activity.¹

Methods CUE-101-01 is a first-in-human (FIH) phase 1 study in patients diagnosed with HPV16+ recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC) refractory to one or more lines of therapy. Trial eligibility includes MHC class I type HLA-A*0201 and a diagnosis of an HPV16 + HNSCC, as assessed by p16 IHC and confirmed by HPV16 mRNA ISH. CUE-101 is administered intravenously over 60 minutes every 21 days. Objectives include determination of safety, pharmacodynamics (PD), pharmacokinetics (PK), recommended phase 2 dose (RP2D), and preliminary anti-tumor activity. The safety results from treated participants will be presented.

Results 19 participants have received CUE-101 monotherapy as of August 7, 2020. Doses ranging from 0.06 to 1 mg/kg were determined to be safe and well-tolerated, enabling dose escalation to 2 mg/kg. Preliminary PK data demonstrate dose-dependent increases in drug exposure which are sustained upon repeat dosing, and low inter-subject variability. Preliminary data from systemic blood analyses show
Background NKTR-255, an investigational IL-15Rα-dependent polymer-conjugated recombinant human IL-15 (rhIL-15) agonist, maintains the full spectrum of IL-15 biology and provides sustained pharmacodynamic (PD) responses without the need for daily dosing. NKTR-255 engages IL-15Rα and IL-2/IL-15Rβγ leading to natural killer(NK) and CD8+ T-cell expansion, proliferation and activation. In preclinical studies, NKTR-255 enhanced antibody-dependent cellular cytoxicity(ADCC) of each of daratumumab, rituximab, trastuzumab and cetuximab, resulting in synergistic anticancer activity. This ongoing phase 1 trial (NCT04136756) evaluates NKTR-255 in patients with hematologic malignancies.

Methods Heavily pretreated patients with relapsed/refractory multiple myeloma(MM) or non-Hodgkin lymphoma(NHL) received escalating doses of NKTR-255 intravenously q3w. Patients were observed for 3 weeks following the first NKTR-255 dose for dose-limiting toxicity(DLT). Preliminary safety, PK and PD were evaluated in all patients and bone marrow biopsy was evaluated in one patient. NKTR-255-mediated activation of the immune system was assessed by flow cytometry and plasma cytokine analysis.

Results As of June 25, 2020, 4 patients were dosed(1.5µg/ kg:3 patients; 3µg/kg:1 patient). NKTR-255 was well tolerated. Most common treatment-related adverse events(AEs): flu-like symptoms, muscle stiffness, and myalgia. One Grade 3 event(pyrexia) was reported, resolving <24 hours with no accumulation following repeat dosing. NKTR-255 was well tolerated treatments are required. DPX-Survivac is a unique T cell activation therapy that targets survivin-expressing tumor cells and has shown anti-tumor activity in clinical trials. This trial is evaluating a novel immunotherapy combination with DPX-Survivac, intermittent low dose CPA and pembrolizumab.

Methods ‘SPIReL’ is a Phase 2 non-randomized, open label, efficacy and safety study of a novel immunotherapy combination with DPX-Survivac (a unique T cell activation therapy that targets survivin-expressing tumor cells), intermittent low dose CPA and pembrolizumab, treatment regimen as described in figure 1. Subjects with r/r incurable DLBCL and survivin expression are eligible for participation. This study was approved by the Ontario Cancer Research Ethics Board, approval number 0981.ORR is assessed by modified Cheson criteria. For translational analyses, baseline and on-treatment PBMCs, along with tumor biopsy samples are collected from each subject. Survivin-specific systemic T cell responses are assessed using IFNy-ELISPOT assay and tumour immune-infiltrate profile by multiplex-IHC.

Results Twenty-two subjects have been enrolled to date, 19 are included in the intent to treat (ITT) population and 11 subjects are evaluable in the per protocol (PP) population. In the PP, the ORR is 63.6% including 3 CRs (27.3%), 4 PRs (36.4%) and the DCR is 81.8% (9/11). In the ITT, the ORR is 35% (7/19), and DCR is 52.0% (10/19). Preliminary results

Abstract 356 Table 1 Data summary of results

<table>
<thead>
<tr>
<th># Participants</th>
<th>Clinical Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td>All/ITT patients (n=19)</td>
<td>3</td>
</tr>
<tr>
<td>Immune infiltrates N=13 (%)</td>
<td></td>
</tr>
<tr>
<td>PD1+</td>
<td>1 (31)</td>
</tr>
<tr>
<td>CD4+</td>
<td>1 (33)</td>
</tr>
<tr>
<td>CD8+</td>
<td>2 (69)</td>
</tr>
<tr>
<td>Positive ELISPOT response</td>
<td>1 (33)</td>
</tr>
</tbody>
</table>

Abstract 356 Figure 1 SPIReL treatment regimen
show that non-GCB subjects had a higher proportion of clinical response (4/8, 50%), compared to 3/10 (30%) in GCB subjects. DPX-Survivac-induced T cell responses were observed in 8/19 subjects (42.1%) including 6 subjects with clinical response (PR, CR), one SD and one PD. Multiplex-IHC analyses demonstrated baseline tumor PD-L1 expression in 6/7 subjects with a clinical response (85.7%, p<0.05). Similarly, subjects with higher baseline CD4+ and CD8+ T cell infiltration demonstrated a trend towards clinical response (table 1).

Conclusions DPX-Survivac, intermittent low-dose CPA and pembrolizumab is generally well tolerated and can induce clinical responses in subjects with r/r DLBCL (7/11, 63.6% of evaluable subjects), including subjects with both non-GCB and GCB subtypes. Pre-treatment biopsies of clinical responders were characterized by higher baseline tumor PD-L1 expression and CD4 and CD8 infiltration. Extending this exploratory data in a larger cohort may define a r/r DLBCL patient population with a higher likelihood to respond to this novel combination immunotherapy.

Trial Registration NCT03349450
Ethics Approval This study was approved by the Ontario Cancer Research Ethics Board, approval number 0981.

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Abstract 357 Figure 1 Proposed Mechanism of Action of TAK-573

Abstract 357

TAK-573, AN ANTI-CD38–ATTENUATED INTERFERON ALPHA (IFNα) FUSION PROTEIN (ATTENUKINE™), HAS DEMONSTRATED IFNα RECEPTOR (IFNAR) PATHWAY MODULATION IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

Sabrina Collins*, Adarsh Joshi, Lei Shen, Subhasree Das, Kaveri Suryanarayan, Dean Bottino, Cheryl Li, Michael Curley, Dannie Wang, Michael Abadier, Ryan Larson, Xavier Parot. Millennium Pharmaceuticals, Inc., Cambridge, MA, USA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, MA, USA

Background TAK-573, a humanized, anti-CD38, IgG4, monoclonal antibody genetically fused to two attenuated IFNα/IFNβ molecules, was designed for targeted delivery of attenuated IFNα/β to CD38 expressing (CD38+)-cells, utilizing a unique epitope of CD38 that does not compete with current anti-CD38 therapies. Preclinical evaluation of TAK-573 confirmed activation of type I IFN signaling in CD38+ cells inducing direct anti-proliferative effects on multiple myeloma (MM) cells and direct and indirect immune cell activation. Here we provide the preliminary analyses of the pharmacodynamic data currently available from the ongoing Ph I/II TAK-573-1501 clinical study in patients with relapsed/refractory MM (NCT03215030).

Methods Peripheral blood (PB) and bone marrow (BM) aspirates were collected from patients at pre- and post-dose time points for exploratory biomarker analyses. CD38 receptor occupancy (RO) and receptor density (RD) were determined using a 9-color flow cytometry assay. Whole transcriptome sequencing of bulk RNA was performed and analyzed to assess the type I IFN gene signature. Serum samples were analyzed using Olink’s Proximity Extension Assay Immuno-Oncology panel to measure changes in cytokine levels. Mass cytometry-based immunophenotyping was utilized to characterize changes in immune cell prevalence and activation status of cryopreserved cells.

Results Administration of TAK-573 resulted in a dose dependent increase in CD38 RO of PB-derived immune cells with saturation detected 4 hours after the end of infusion (EOI) at doses ≥ 0.2 mg/kg. The duration of saturation was dose dependent with doses ≥ 0.75 mg/kg saturating CD38 RO through 24 hours. All dose levels tested resulted in increases in the type I IFN gene signature at 24 hours. Consistent with CD38 being an IFN stimulated gene, TAK-573 treatment resulted in CD38 RD increases most notably on NK cells, but also on other CD38+ cells including MM cells. Circulating levels of IFN-associated cytokines were also elevated, with maximal induction 4 hours after the EOI. CD8+ T-cells in BM showed increased CD69 expression in 7 of 9 patients analyzed, 3 of whom also showed increases in both IFNα and granzyme B positivity suggesting TAK-573 treatment results in increased BM cytolytic CD8+ T-cells, in a subset of patients.

Conclusions These preliminary biomass data indicate that TAK-573 is a pharmacologically active molecule that mediates its effect through IFNAR pathway modulation. Additional data are being collected to further refine the mechanism of action (Image 1), which will inform the recommended phase 2 dose and optimal schedule of administration for the development of TAK-573.

Trial Registration ClinicalTrials.gov: NCT03215030
Ethics Approval The TAK-573-1501 study is approved by WIRB-Copernicus Group, University of Nebraska Medical Center, Dana Farber Cancer Institute and Advarra IRBs.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0357

Abstract 358 TRIAL IN PROGRESS: A PILOT STUDY OF COMBINED IMMUNE CHECKPOINT INHIBITION IN COMBINATION WITH ABLATIVE THERAPIES IN SUBJECTS WITH HEPATOCELLULAR CARCINOMA (HCC)

1Hailey Carroll*, 2Umair Aleem, 3Pooja Varghese, 4Marie Galligan, 5Michèle Bourke, 6Katherine Hoey, 7Ronan Ryan, 8Peter Donan, 9Stephen Stewart, 10Cliona O’Farrell, 11Tim Greten, 12Diarmaid Houlihan, 13Raymond McDermott, 14Austin Duffy, 15Mater Hospital, Dublin, Ireland; 16St. Vincent’s University Hospital, Dublin, Ireland; 17University College Dublin, Dublin, Ireland; 18Trinity College, Dublin, Ireland; 19NIH, Bethesda, MD, USA

Background Locoregional therapies for hepato cellular carcinoma, such as trans catheter arterial chemo embolization (TACE) or ablation, can induce a peripheral anti-tumor immune response. This may be amplified by immune checkpoint inhibitors (ICI). Early and higher anti-CTLA4 dosing could potentially lead to better priming and a stronger immune response. Recent data has suggested that early (Day 1 only), increased doses of anti-CTLA4 therapy, was associated with encouraging clinical activity and a tolerable safety profile. This study will evaluate dual immune checkpoint, CTLA4 (tremelimumab, day 1-only dosing) and PD-L1 (durvalumab) blockade in combination with TACE in patients with advanced
HCC. Intensive peripheral immune-monitoring and longitudinal on-treatment tumor biopsies will focus on the role of the innate immune system, particularly Natural Killer cells, in anti-tumor responses.

Methods Patients with HCC (Childs Pugh A/B7; Barcelona Clinic Liver Cancer Stage B/C; ECOG 0/1; sorafenib-naïve or experienced) are being enrolled in a pilot study (Study Number UCDCRC/19/01) of tremelimumab at 2 dose levels (DL1 and DL2) in combination with durvalumab and TACE until disease progression (per irRECIST). DL1: tremelimumab (75 mg q28 days for 4 doses) and durvalumab (1500 mg q28 days). DL2: tremelimumab (300 mg in a single dose on day 1) and durvalumab (1500 mg q28 days). Subtotal TACE will be performed during study week 6 with the dose-limiting toxicity (DLT) evaluation period encompassing the first 8 weeks of the study. Primary endpoint is 6-month progression-free survival with secondary efficacy endpoints being safety, tolerability, and overall survival. Exploratory objectives will evaluate changes in immune parameters in the tumor and peripheral blood of patients undergoing anti-CTLA4 therapy pre- and post-RFA or TACE. A major focus will be on the role of the innate immune system, particularly Natural Killer cells, in anti-tumor responses. Patients will be enrolled and treated at St Vincent’s University Hospital in Dublin, Ireland. This study is currently open and actively recruiting.

Results N/A

Conclusions N/A

Trial Registration EudraCT Number 2019-002767-98

Ethics Approval St Vincent’s University Hospital Research Ethics Committee Study Number UCDCRC/19/01.

REFERENCES


Abstract 359 Figure 1  Tumor shrinkage over time in response to AMG 757
A PHASE 1 STUDY OF AN OFF-THE-SHELF, MULTI-NEOANTIGEN VECTOR (ADXS-503) ALONE AND IN COMBINATION WITH PEMBROLIZUMAB IN SUBJECTS WITH METASTATIC NON-SMALL CELL LUNG CANCER (NSCLC)

Jonathan Goldman*, Thomas Stinchcombe, Gregory Geistner, Misak Haigentz, Surya Vangala, Megan Pans, Victor Kabala, Dinesh Sinha, Andres Gutierrez, Suresh Ramalingam, University of California Los Angeles, CA, Los Angeles, CA, USA; Duke University, Durham, NC, USA; Illinois Cancer Care, Peoria, IL, USA; Morristown Medical Center, Morristown, NJ, USA; Advaxis Inc, Princeton, NJ, USA; Emory University, Atlanta, GA, USA

Background ADXS-503 (A503) is an off-the-shelf, attenuated Listeria monocytogenes (Lm)-based immunotherapy bioengineered to elicit potent T cell responses against 22 tumor antigens commonly found in NSCLC (i.e., 11 hotspot mutations and 11 tumor-associated antigens, TAAs). Pembrolizumab (Pembro) is a programmed death receptor-1 (PD-1)-blocking antibody approved for the treatment of advanced lung cancer. A503 and Pembro have complementary mechanisms of immune activation and reversal of immune tolerance.

Methods We conducted a phase 1 study of A503 ± Pembro in patients (pts) with metastatic squamous or non-squamous NSCLC. In Part A, A503 alone has been tested at two dose levels (i.e., 1 × 108 and 5 × 108 CFU) in pts refractory or intolerant to prior systemic therapy. In dose escalation Part B, A503 has been evaluated at the lower dose level (DL) in combination with Pembro within 6 weeks of presenting with disease progression per RECIST criteria v1.1. Part C dose expansion cohort with A503 + Pembro has started for frontline treatment in the metastatic setting. A503 ± Pembro (200 mg) are infused by IV every 3 weeks until disease progression or limiting toxicity. Main endpoints include safety, tolerability and immune-correlative data.

Results Twelve patients have been treated: 7 in Part A, 4 in Part B-DL1 and 1 in Part C. No pts in Part A experienced dose-limiting toxicities at the 2 DLs tested. A503 + Pembro has also been well tolerated in 4 pts treated in Part B-DL1 and in one in Part C. No immune related AEs have been reported in Part B or Part C. Three evaluable pts in Part A achieved stable disease (SD). Of the three evaluable pts in Part B-DL1 one has achieved SD for 8 months and the second one a partial response for over 6 months; both of these patients had been on Pembro therapy for 2 years before enrollment. The 3rd pt showed progressive disease, ADXS-503 induced transient release of pro-inflammatory cytokines, activation of cytotoxic- and memory-CD8+ T cells against antigens in the context and antigen spreading in peripheral blood across all cohorts. Preliminary data on on-therapy biopsies showed increased PD-L1 expression and decreased Treg cell counts. Part B-DL1 cohort has thus been expanded to further explore the potential reversal of Pembro resistance with ADXS-503 in these pts.

Conclusions ADXS-503 alone and in combination with Pembro has demonstrated a manageable safety profile and induction of antigen specific T cell responses. The potential effect of A503 to reverse resistance to Pembro is now being studied in an expansion cohort and this combination approach is also being evaluated in the first line treatment setting (Part C).

Acknowledgements Dr. S Miglani and Dr. M Chopra (AWINSA group) for PV review, Precision for Medicine for immune-correlative work and Abhay Sheeri for data analyses.

Trial Registration NCT03847519

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A RANDOMISED OPEN-LABEL PHASE II STUDY ADDING ONCOS-102 TO PEMETREXED/CISPLATIN IN PATIENTS WITH UNRESECTABLE MALIGNANT PLEURAL MESOTHELIOMA – 12 MONTH ANALYSIS OF BIOMARKERS AND CLINICAL OUTCOMES

Magnus Jaderberg, Susana Cedres, Luis Paz-Ares*, Xavier Serr, Charles Ricordel, Nicolas Isambert, Santiago Ponce Aix, Victor Levitsky, Lukasz Kunyk, Annette Geller, Sylvia Vethuis, Research and Development, Oslo, Norway; Medical Oncology, Sevilla, Spain; Radiotherapy, Barcelona, Spain; Pulmonology, Reims, France

Background Malignant pleural mesothelioma (MPM) is a rare, aggressive malignancy without curative treatment. Majority of patients receive pemetrexed/cisplatin as standard of care (SoC). Median overall survival in unresectable disease is 12 months. ONCOS-102 is a granulocyte-macrophage colony stimulating factor (GM-CSF) expressing oncolytic adenovirus (Ad5/3-D24-GMCSF) with a unique ability to both prime and boost immune responses. The aim of the study was to assess
immune and clinical responses as well as safety in patients with 1st and 2nd line unresectable MPM.

Methods Eligible patients (experimental arm, n=20) received ONCOS-102 given intratumorally under CT or US guidance at a dose of 3 × 10 × 11 on Day 1, 4, 8, 36, 78 and 120 plus six cycles of SoC starting on Day 22. The control group (n=11) received only SoC. Imaging was done at baseline, Day 43–64 and 127–148. Patients were monitored regularly for immunological assessment including lesional biopsies (baseline and Day 36). Primary objective was safety and tolerability. Secondary objectives were immunological activation, ORR, PFS and OS as well as correlation between immunological activation and clinical outcome.

Results There were no safety concerns nor DLTs. In 1st line patients ORR/DCR was 30%/90% in the experimental group and 33%/83% in the control group. 2nd line patients had ORR/DCR of 11%/67% in the experimental group and 60%/80% in the control group. 12-month survival rate for the 1st line pts was 64% in the experimental group and 50% in the control group. PFS and OS are still to be reported. The treatment with ONCOS-102 induced strong upregulation of multiple genes associated with immune activation in tumor lesions. Profound innate and adaptive immune activation was observed in the experimental vs control group that was associated with better clinical outcome. In addition to an increase in intratumoral cytotoxic T-cells (10/15 pts), the treatment with ONCOS-102 resulted in polarization from M2 to M1 macrophages. An upregulation of PD-L1 was reported in 9/15 pts in the experimental group vs 2/5 pts in the control arm, highlighting the potential of ONCOS-102 as an immunosensitizing agent for combinatorial therapies with checkpoint inhibitors.

Conclusions ONCOS-102 treated patients benefited from superior immune activation compared to patients receiving SoC with preliminary signals of clinical efficacy. Upregulation of adaptive immunity and cytokotoxicity related gene expression, PD-L1 level and M2 to M1 macrophage polarization indicate that ONCOS-102 can induce a favourable TME modulation thus providing a scientific rationale for combination with check point inhibition.

Trial Registration ClinicalTrials.gov nCT02879669

Ethics Approval This study was approved by the IRBs of all the sites in Madrid, Barcelona, Rennes and Poitiers.

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A PHII STUDY OF BEMCENTINIB, A FIRST-IN-CLASS SELECTIVE AXL KINASE INHIBITOR, IN COMBINATION WITH PEBROLIZUMAB IN PTS WITH PREVIOUSLY-TREATED ADVANCED NSCLC: UPDATED CLINICAL & TRANSCRIPTOMICAL ANALYSIS

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Background AXL is implicated in resistance to immunotherapy. Bemcentinib (BGB324), a first-in-class, oral, selective and potent AXL kinase inhibitor, enhances checkpoint inhibitor (CPI) efficacy in pre-clinical models through tumor-immune mechanisms.

Methods BGBC008 is a PhII single-arm, 2-stage study with bemcentinib (200 mg/d) and pembrolizumab (200 mg q3wk) for previously-treated stage IV lung adenocarcinoma comprising 3 cohorts: chemotherapy-pretreated IO-naïve patients (Cohort-A), patients progressing on prior IO therapy (Cohort-B) or chemotherapy/pembrolizumab combination (Cohort-C).

Primary endpoint was ORR according to RECIST1.1 with pre-defined criteria to proceed from the first to second stage in each cohort. Secondary endpoints included DCR, PFS, OS and safety. Exploratory endpoints include biomarker analysis and correlation with clinical endpoints, including composite (tumor and immune cell) cAXL score, PD-L1 TPS, and genom-wide mutational and transcriptome analyses.

Results As of July 2020, enrollment in Cohort-A and B (stage 1) is completed; a total of 66 NSCLC patients were dosed. Cohort-A (n=50) results were previously presented. All Cohort-B1 (n=16) patients received at least one prior line of therapy, the most recent including CPI; 4 patients had 1 and 12 had 2+ prior treatments. Of the Cohort-B1 patients, cAXL status was available for 13 patients: 8 cAXL-positive, 5 cAXL-negative. PD-L1 TPS was available for 13 patients: 5 TPS >50%, 5 TPS 1–49%, and 3 TPS <1%. Of patients who had previously undergone 1 line of CPI therapy (n=4), 75% were cAXL-positive and 25% were not evaluable for cAXL (median TPS of 20%). Patients who had previously undergone 2+ lines of therapy (n=12), 33% were cAXL-positive, 50% cAXL-negative, and 17% not evaluable for cAXL (median TPS of 50%). Of the treated pts, most common TEAEs (>25% of patients) were increased ALT (29%; 10% G3+), AST (29%; 5% G3+), and diarrhoea (29%; 1% G3+). All cases of treatment-related transaminase increase were reversible and managed with concomitant administration of steroids and treatment interruption. Of the 15 radiologically-evaluable patients in Cohort-B1, 1 PR was observed; 6/7 (86%) cAXL-positive patients (1 PR, 5 SD) achieved clinical benefit while none was observed in cAXL negative patients. mPFS was 4.7mo in cAXL-positive and 1.9mo in cAXL-negative patients. Ongoing transcriptional analysis of pre-treatment biopsies revealed a distinct gene profile correlating with clinical benefit from bencentinib + pembrolizumab combination treatment.

Conclusions Overall, bemcentinib in combination with pembrolizumab was well-tolerated and shows promising clinical activity in AXL-positive immunotherapy refractory disease. Updated survival and translation/biomarker data will be presented.

Acknowledgements The authors would like to thank all patients and their caretakers for participating in this trial.

Trial Registration NCT03184571

Ethics Approval This study was approved by all relevant institutions, including London Bridge Research Ethics Committee (UK), REC-South East (Norway), Drug Research Ethics Committee of the University Hospital Clinic of Barcelona (Spain), MCW/FH Institutional Review Board #4, Medical College of Wisconsin (USA).

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363 VACTOSERTIB AND DURVALUMAB AS SECOND OR LATER LINE TREATMENT FOR PD-L1 POSITIVE NON-SMALL CELL LUNG CANCER: INTERIM RESULT

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Background Targeting transforming growth factor-β (TGF-β) is reported to augment the efficacy of immune checkpoint inhibitors (ICIs) through either enhanced anti-tumor immunity or the correction of tumor microenvironment (TME). Therefore, the combination of vactosertib, a highly selective TGF-β RI kinase inhibitor, and durvalumab is anticipated to improve anti-tumor activity of the ICI. A phase 1b/2a study was conducted to evaluate the combination of vactosertib and durvalumab in patients with advanced NSCLC who progressed after platinum-based chemotherapy.

Methods Patients were treated with vactosertib at a dose of 200 mg twice daily (five days on and two days off) and durvalumab at a dose of 1500 mg every four weeks. Eligible patients were ≥19 years old with good performance status (ECOG 0–1) and have no prior exposure to immune checkpoint inhibitors or other TGF-β RI kinase inhibitors. The objectives of this analysis were to evaluate the safety, antitumor activity including objective response rate (ORR), duration of response (DOR), and time to response (TTR) as well as circulating pharmacodynamic biomarkers related to TGF-β signaling. Response was assessed per RECIST (v1.1).

Results By August 4 2020, twenty-six PD-L1 positive (SP263 assay) patients were analyzed. Median age was 61.5 years (range 48–83), 69.2% were male, median number of previous lines of chemotherapy was 1 (range 1–4), and all patients were PD-L1 positive (15 patients with PD-L1 ≥25% and 11 patients with PD-L1 1–24%). The most frequently reported treatment-related adverse events (TRAE) were itching (38.5%) and skin rash (34.6%), but no Gr≥3 itching and rash were observed. Each case of the following was reported as Grade 3 TRAEs: adrenal insufficiency, anemia, and pneumonitis; Grade 4 TRAE, CPK increase, was observed in one patient. Objective response rate was 30.8% and 40.0% in patients with PD-L1 ≥1% and ≥25% respectively. Circulating PAI-1 and CTGF evaluated in 15 patients decreased significantly on Cycle 1 day 5. Ongoing biomarker results will be presented.

Conclusions The combination of vactosertib and durvalumab has demonstrated a manageable safety profile and encouraging anti-tumor activity as a potential therapeutic strategy in patients with advanced NSCLC. The efficacy outcomes of this combination in a larger number of patients with advanced NSCLC will be followed.

Trial Registration NCT03732274

Ethics Approval The study was approved by Ethics Board of Severance Hospital (4-2018-0892), National Cancer Center (NCC2019-0057), St. Vincent’s Hospital (VC19MDDF0205), and Chungbuk National University Hospital (2019-08-015).

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364 A PERSONAL NEOANTIGEN VACCINE NEO-PV-01 IN COMBINATION WITH CHEMOTHERAPY AND PEMBROLIZUMAB INDUCES BROAD DE NOVO IMMUNE RESPONSES IN FIRST LINE, NON-SQUAMOUS NSCLC

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Background Neoantigens arising from mutations in cancer cell DNA are important targets for T cell mediated anti-tumor immunity. NEO-PV-01 is a personal neoantigen vaccine of up to 20 peptides (14–35 amino acids) based on a patient’s HLA profile and bioinformatic analysis of tumor neoantigens. We report here clinical and immune data for NT-002, a Phase 1b study of NEO-PV-01 with pemetrexed, carboplatin, and pembrolizumab as first-line therapy for advanced non-squamous NSCLC.

Methods Patients received 12 weeks of pembrolizumab (Q3W) plus carboplatin and pemetrexed. NEO-PV-01 was then given subcutaneously in a prime-boost format spanning 12 weeks, followed by pembrolizumab for up to 2 years. The primary objective was safety; secondary objectives included overall response rate (ORR), clinical benefit rate (CBR), progression-free survival (PFS), and overall survival (OS). Comprehensive immune assessments were performed with peripheral blood mononuclear cells and biopsies collected at weeks 0, 12, and 24.

Results A total of 38 patients initiated study treatment (ITT population); 21 patients received at least 1 dose NEO-PV-01 (vaccinated group, VAX). The demographics included 61% women and 82% with a smoking history. The regimen was well tolerated consistent with the pembrolizumab plus pemetrexed/carboplatin safety profile, with transient low-grade injection site reactions present in VAX (29%). Treatment-related study discontinuations were rare (2/38). The ORR/CBR for the ITT and VAX were 37%/69% and 57%/95%, respectively. Median PFS was 7.2 months (95% CI: 5.6,16.8) for both the ITT and VAX, and median OS 16.8 months (95% CI: 11.6, NR) for both groups. Interim immune analysis on 8 patients revealed neoantigen-specific CD4+ and CD8+ T cell responses against 48% of vaccine peptides. T cell responses were durable at 52 weeks and exhibited a memory phenotype with cytolytic potential. Epitope spread was observed in 3 of 5 patients analyzed thus far. Further, assessments of immune and molecular correlates of clinical response identified both tumor mutation burden and baseline levels of T cell infiltration in tumor as highly predictive of durable PFS (p = 0.005 and p= 7.2e-07 (for CD8)), respectively. Additional correlates of clinical outcomes with molecular and immunologic responses will be presented.

Conclusions NEO-PV-01 in combination with pembrolizumab and carboplatin/pemetrexed is feasible, has a good safety profile, and induces de novo immune responses in first line non-squamous NSCLC. The association of baseline disease characteristics to prolonged PFS suggests future patient enrichment strategies for evaluation of this novel regimen in a phase 2 trial.

Trial Registration NCT03380871
A RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED PHASE III STUDY EVALUATING PERIOPERATIVE TORIPALIMAB COMBINED WITH PLATINUM-BASED DOUBLET CHEMOTHERAPY IN RESECTABLE STAGE III NSCLC

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Background Surgery remains the mainstream of treatment for resectable stage III non-small cell lung cancer (NSCLC). The preliminary results from some pilot trials have shown that neoadjuvant immunotherapy in NSCLC is safe and tolerable.1 Hypothesizing that neoadjuvant toripalimab (a humanized anti-PD-1 antibody) plus chemotherapy can improve the outcome in resectable NSCLC, we are conducting a randomized, double-blind, placebo-controlled, phase III study to evaluate the efficacy and safety of toripalimab plus platinum-based doublet chemotherapy as neoadjuvant/adjuvant therapy for patients with resectable stage III NSCLC.

Methods This ongoing study enrolls patients aged 18–70 years with treatment-naive, histopathologically confirmed resectable stage III NSCLC without EGFR mutation or ALK translocation, ECOG PS 0–1, and adequate organ function. Eligible subjects are randomized (1:1) into experimental or control group, to receive perioperative toripalimab 240 mg or placebo combined with chemotherapy for 4 cycle in total (Docetaxel 60–75 mg/m² or Paclitaxel 175 mg/m² with platinum [squamous histology] or Pemtrexed 500 mg/m² with platinum [non-squamous histology]) every 3 weeks for three cycles followed by surgery, and one more cycle after surgery, then monotherapy of toripalimab 240 mg or placebo every 3 weeks up to 13 cycles is delivered. Adjuvant radiotherapy is allowed. Randomization is stratified by tumor stage(I/IIA vs IIIB), pathological type (squamous vs non-squamous), PD-L1 expression (PD-L1>1% vs <1% or not evaluable) and planned surgical procedure (pneumonectomy vs lobectomy). Radiographic response is assessed within 4–6 weeks after last dose of neo-adjuvant therapy, at 30 days after surgery and every 12 weeks thereafter. Primary endpoints are major pathologic response (MPR) rate evaluated by blind independent central pathology review (BIPR-MPR) and event-free survival evaluated by investigator (INV-EFS). Secondary endpoints include pathologic complete response (pCR) rate evaluated by BIPR and investigators (BIPR-pCR and INV-pCR), disease-free survival (DFS), 2–3 years OS rate, OS, safety, and feasibility of surgery.
Exploratory endpoints are potential correlations between biomarkers and efficacy. A stratified Cochran Mantel Haenszel method will be used to assess binary endpoints. A Kaplan-Meier method, a stratified log-rank test and a stratified Cox proportional hazards model will be used to assess survival endpoints. Planned enrollment is 406 patients. The study is actively enrolling at 52 Chinese sites.

**Results**

N/A

**Conclusions**

N/A

**Acknowledgements**

N/A

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**REFERENCES**


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**368 REVEAL: PHASE 1 DOSE-ESCALATION STUDY OF NKTR-262, A NOVEL TLR7/8 AGONIST, PLUS BEMPEGALDESLEUKIN: LOCAL INNATE IMMUNE ACTIVATION AND SYSTEMIC ADAPTIVE IMMUNE EXPANSION FOR TREATING SOLID TUMORS**

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Background NKTR-262 is a small-molecule agonist of toll-like receptors (TLR) 7/8. Given by intratumoral (IT) injection, NKTR-262 is retained within the tumor microenvironment (TME) and promotes an immunostimulatory milieu and tumor antigen release. Bempegaldesleukin (BEMPEG) is a CD122-preferential IL-2 pathway agonist, which increases proliferation and tumor infiltration of CD8+ T cells and natural killer (NK) cells. Preclinically, NKTR-262 plus BEMPEG combined innate immune signaling and enhanced antigen presentation, with sustained T-cell activation, resulting in tumor growth inhibition with treated and abscopal lesions.

Methods This phase 1 dose-escalation study enrolled patients with relapsed/refractory, advanced/metastatic solid tumors (REVEAL; NCT03435640). Patients received escalating doses of NKTR-262 (0.03 mg to 3.84 mg IT) followed 3 weeks later by BEMPEG (0.006 mg/kg IV) q3wk utilizing a 3+3 design. The primary endpoint was safety and tolerability, including definition of the recommended phase 2 dose (RP2D). Other endpoints included antitumor activity, pharmacodynamics, and pharmacokinetics.

Results As of June 15, 2020, 36 patients were enrolled. One dose-limiting toxicity, transient transaminase elevation, was observed at the highest NKTR-262 dose (3.84 mg). The most frequent treatment-related adverse events were flu-like symptoms, fatigue, nausea, and pruritus, consistent with the known profile of BEMPEG. Early evidence of clinical activity was observed in patients with metastatic melanoma, with a disease control rate (partial response [PR] + stable disease) of 41.2% (7/17 patients), including two patients with PRs after progression on two prior immunotherapy regimens. Preliminary analyses showed dose-dependent induction of CXCL10 and type 1 interferon genes, consistent with TLR7/8 engagement. CD11c+ target cells were significantly more abundant in baseline melanoma biopsies than other tumor types (p<0.001). Induction...
Background ATOR-1015 is a human CTLA-4 xOX40 bispecific antibody developed as a first in class tumor-localizing CTLA-4 antibody for improved efficacy and reduced toxicity.

Methods The study (NCT03782467) is a first-in-human dose escalation study followed by an expansion part. In the dose escalation patients with refractory solid malignancies are enrolled and the expansion part will enroll patients with cutaneous or mucosal malignant melanoma. Patients receive ATOR-1015 intravenously Q2W as a single agent until confirmed progressive disease, unacceptable toxicity or withdrawal of consent. Intra-patient dose escalation is allowed. The primary objective is to assess the safety and tolerability of ATOR-1015. Secondary objectives include pharmacokinetics, immunogenicity, pharmacodynamics, and clinical efficacy as assessed by iRECIST. Pharmacodynamic analyses include serum cytokines, immunophenotyping of peripheral blood mononuclear cells. Tumor biopsies before and after ATOR-1015 will be analyzed.

Results As of June 26, 2020, 23 patients have been exposed to ATOR-1015. The median age of the patients is 54 years (range 40–72). Patients have received a median of 5 prior lines of therapy (range 1–16). Most common cancer type is colorectal cancer. Dose levels from 0.043 mg to 600 mg have been evaluated and declared safe. Dose escalation is ongoing, and currently two patients have been enrolled at 750 mg dose level. The median time on study was 8.4 weeks (range 0.1–34.3). Five patients are on study and 18 patients have discontinued. Reasons for discontinuation included clinical deterioration (n=10), disease progression (n=5), death due to disease progression (n=2), and investigator’s decision (n=1). Twelve of the 23 patients experienced a drug-related adverse event (AE). Two patients experienced a grade 3 drug-related AE, for all other patients AEs were grade 1 or 2. Infusion-related reactions (IRR) were reported in nine patients. Predominant symptoms of the IRR were chills, rash and pain. Potentially immune-related AEs grade 1 were reported in three patients: one patient had rash, one vitiligo, and one exanthema and eczema. No dose-limiting toxicities have occurred. Best response is stable disease. Pharmacokinetic data show dose proportional kinetics up to 600 mg. Preliminary biomarker analysis shows pharmacodynamic activity of ATOR-1015.

Conclusions ATOR-1015 has been safe and well-tolerated up to 600 mg. Currently 750 mg is under evaluation. Best response is stable disease. Following the dose escalation phase, an expansion cohort for patients with advanced malignant melanoma will be initiated.

Ethics Approval The study is approved by the Ethic Boards in Sweden and Denmark.

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Background RO7122290 (RO) is a bispecific antibody-like fusion protein that simultaneously targets FAP, abundantly expressed by cancer-associated fibroblasts in most solid tumors, and 4-1BB, transiently expressed on activated T cells. Pre-clinical experiments revealed strong intra-tumoral CD8+ T cell infiltration in FAP-positive tumors, cytokines induction and significant anti-tumor activity mediated by RO (signal 2 of T cell activation), upon TCR/CD3 engagement (signal 1) or in combination with atezolizumab (ATZ). In this first-in-human study, the pharmacodynamic (PD) effects of RO were assessed, both as single agent (SA) (Part A) and in combination with ATZ (Part B).

Methods Pts with advanced and/or metastatic solid tumors were eligible for this ongoing Phase 1/1b trial (EUDRACT 2017-003961-83). RO was administered intravenously, weekly (QW) at escalating dose levels (DLs). In Part A, 62 pts were treated at 13 DLs of RO, dose range 5–2000 mg. In Part B, 39 pts were treated at 8 DLs of RO, dose range 45–2000 mg, with ATZ 1200 mg Q3W. Secondary biomarker objective was characterization of PD effects in tumor tissue and blood. The endpoints were change from baseline in intra-tumoral density (cell/mm²) and proliferation (Ki67) of CD8+ T cells measured by immunohistochemistry (IHC), and change in

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activation (4-1BB) and proliferation (Ki67) of peripheral CD8 + T cells measured by flow cytometry. Exploratory objectives were characterization of PD effects in plasma/serum and measurement of intra-tumoral immune activation. The endpoints were change in peripheral cytokines (TNF-alfa, IFN-gamma, IL-6) and soluble(s) factors (sCD25, s-4-1BB) concentration measured by ELISA, and intra-tumoral changes in gene expression measured by RNAseq.

**Results** In the periphery, we observed transient expression of 4-1BB on CD4+ and CD8+ T cells, along with secretion of s4-1BB and inflammatory cytokines, suggesting 4-1BB targeting and potent T cell activation. The concomitant induction of proliferating T cells indicated the potential association to priming and formation of tumor-reactive T cells. In the tumor, we detected increased CD8+ T cells infiltration and proliferation, in both Parts. Proliferating CD8+ T cells increased in both tumor nests and surrounding stroma, with a preferential accumulation in the latter. RNAseq analysis revealed induction of 4-1BB, PD-1 and IFN-gamma, indicating intra-tumoral T cells activation in Part B.

**Conclusions** PD activity was consistent with the postulated MoA, confirming RO to be a potent tumor-targeted 4-1BB agonist in the clinical setting. Our observations suggest that RO can synergize with endogenous T cell receptor stimulation, both as SA and in combination with ATZ.

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**THE ASSOCIATION OF THE GUT MICROBIOTA AND CLINICAL RESPONSE TO IMMUNE CHECKPOINT INHIBITORS IN PATIENTS WITH ADVANCED CANCER**

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**Background** The gut microbiome is associated with the immune function of the host. No consensus has been reached regarding to the association between microbiota and the treatment response to immune checkpoint inhibitor (ICI). This study is designed to explore the relationship between gut microbiome composition and clinical outcomes in patients with advanced cancer treated with ICI.

**Methods** Fifty patients were enrolled in this study. Fecal samples were collected at the baseline, 3 months after treatment and when disease progression was noted. To explore the gut microbiota as a potential predictive biomarker for immunotherapy, 165 ribosome RNA gene sequencing was used to analyze the gut microbiota profiles. Peripheral immunity parameters were determined by multicolor flow cytometry and cytokine array. Alpha-diversity of healthy individuals was used as a cut-off.

**Results** When subgrouping patients into benifter and non-benifter according to the clinical response assessed, non-benefiter patients harbored lower alpha-diversity of gut microbiome at the baseline. Patients with low microbiome diversity had poor progression-free survival (HR=0.569, p=0.219) when compared to those with high diversity. Compositional difference was observed between the two groups as well with the enrichment of g_Fusibactinibacter in benefiter whereas f_Veillonellaceae enriched in non-benefiter. Analysis of immune responses using multicolor flow cytometry revealed that patients with a high diversity of gut microbiota had decreased CD4+/CD25+ / FoxP3+ regulatory T cells in response to ICI. After ICI treatment the CD4/CD8 ratio of PBMCs was decreased in clinical benefiter. The serum MIF and CXCL12 levels were decreased in clinical benefiter.

**Conclusions** Low alpha diversity of the gut microbiota is associated with poor response to immune checkpoint inhibitors in patients with advanced cancer. Further confirmation in the clinical trials is warranted.

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**Ethics Approval** The study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, approval number 201801261B0.

**Consent** Written informed consent was obtained from the patients for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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treatment-related AEs were fever/pyrexia (Grade 1: n=3; Grade 2: n=8) and injection site reactions (Grade 1: n=1; Grade 2: n=9). Preliminary estimate of median plasma half-life for AMV564 after SC injection was >48 hours, with dose-related increases in peak plasma concentration (Cmax). Tumor responses were evaluable in 9 patients; 1 patient had not reached their first assessment and 1 patient was not efficac y evaluable due to a non-treatment-related AE resulting in study discontinuation. Single-agent activity has been observed including a complete response by RECISTv1.1 criteria in 1 patient with ovarian cancer refractory to all standard thera pie and anti-PD-1 therapy, and stable disease in 4 additional patients.

Conclusions AMV564 has been well tolerated across multiple dose levels, with good plasma exposure and evidence of antitumor activity when administered subcutaneously. Single-agent anti-tumor activity was observed in an ovarian cancer patient.

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Trial Registration NCT04128423

Ethics Approval The study was approved by the Institutional Review Board at each center where the study is being conducted.

REFERENCES

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A227
Background VT1021, a cyclic pentapeptide, reprograms myeloid-derived suppressor cells (MDSCs) and induces the production of thrombospondin-1 (Tsp-1) in the tumor microenvironment (TME). Tsp-1, via binding to CD36 and CD47, induces apoptosis in tumor and endothelial cells, blocks the ‘do-not-eat-me’ signal, increases the M1:M2 macrophage ratio and activates cytotoxic T lymphocytes (CTLs). Preclinical studies showed robust antitumor activities of VT1021 in multiple animal models.

Methods This is a first-in-human, Ph 1/2, open-label, multicenter dose escalation and expansion study in advanced solid tumors. The primary objectives are to determine the recommended Phase 2 dose (RP2D) and characterize the safety and tolerability of VT1021. Secondary objectives are to characterize the adverse event (AE) profile, evaluate pharmacokinetics (PK), and describe preliminary efficacy. Exploratory objectives include evaluation of pharmacodynamic effects of VT1021 in tumor, TME, and peripheral blood. The expansion phase focuses on ovarian, pancreatic, triple negative breast cancer, glioblastoma, and a basket cohort with high CD36-expressing tumors.

Results In the escalation phase, 46 subjects received between 0.5–15.6 mg/kg of VT1021 by IV infusion twice weekly. VT1021 has been well tolerated through all doses tested. One patient dosed at 1.0 mg/kg developed a grade 3 infusion reaction and 3 patients dosed at 1.0, 6.6, and 8.8 mg/kg respectively developed grade 2 infusion reactions. Other drug related AEs included grade 1–2 fatigue (n=7), nausea (n=4), constipation (n=2), increased aspartate aminotransferase (n=2) and blood bilirubin (n=2), hypomagnesaemia (n=2), and dizziness (n=2). Dose proportionality was observed in PK analysis. Among 28 evaluable subjects, one partial response (thymoma, n=1), one stable disease (ovarian cancer, n=1), and 3 patients dosed at 1.0 mg/kg developed a grade 3 infusion reaction. Other drug related AEs included grade 1–2 fatigue (n=7), nausea (n=4), constipation (n=2), increased aspartate aminotransferase (n=2) and blood bilirubin (n=2), hypomagnesaemia (n=2), and dizziness (n=2). Dose proportionality was observed in PK analysis. Among 28 evaluable subjects, one partial response (thymoma, n=1), one stable disease (ovarian cancer, n=1), and 3 patients dosed at 1.0 mg/kg developed a grade 3 infusion reaction. Other drug related AEs included grade 1–2 fatigue (n=7), nausea (n=4), constipation (n=2), increased aspartate aminotransferase (n=2) and blood bilirubin (n=2), hypomagnesaemia (n=2), and dizziness (n=2). Dose proportionality was observed in PK analysis. Among 28 evaluable subjects, one partial response (thymoma, n=1), one stable disease (ovarian cancer, n=1), and 3 patients dosed at 1.0 mg/kg developed a grade 3 infusion reaction.

Conclusions Through all doses tested, VT1021 was safe and well tolerated, with dose proportional PK properties. In addition, VT1021 has demonstrated activities in reprogramming the TME which resulted in a high disease control rate in subjects with tumors expressing both high CD36 and high CD47.

Trial Registration NCT03364400

Ethics Approval The study was approved by Northwestern University Medical School institutional review board (IRB), approval number 00000418, Horizon Oncology Center IRB, approval number 00001313, South Texas Accelerated Research Therapeutic IRB, approval number 00003657, University of Oklahoma Health Sciences Center IRB, approval number 00006075, Cleveland Clinic IRB, approval number 00000536, Florida Cancer Specialists IRB, approval number 00006075, Case Western IRB, approval number 00000536, Beth Israel Deaconess Hospital and Dana Farber Cancer Institute IRB, approval number 0000753 and MD Anderson IRB, approval number 00006023.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0374
Conclusions An early increase in HPV-16 specific T cells (after a single administration of bintrafusp alfa, prior to restaging) was associated with clinical activity in patients with HPV-related cancers undergoing bintrafusp alfa therapy. This evidence, and the pre-clinical finding of enhanced antitumor activity observed when combining bintrafusp alfa with an HPV-16 targeted vaccine and an immunostimulatory cytokine, have provided the rationale for an ongoing study evaluating this combination in patients with advanced HPV-associated malignancies (NCT04287868).

Ethics Approval All patients provided written informed consent for participation in a clinical trial that was approved by the Institutional Review Board at the National Cancer Institute (NCT02517398, NCT03427411).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0376

Background MRx0518 is a novel, human gut microbiome-derived, single-strain, oral live biotherapeutic. It is a bacterium of the Enterococcus genus that was selected for development in the treatment of solid tumours in its strong for in vitro and in vivo immunostimulatory activity. In vivo studies have shown that MRx0518 can inhibit tumour growth in different syngeneic cancer models as monotherapy and in combination with checkpoint inhibitors. MRx0518 has been shown to reduce Treg and increase Th1 and Tc1 lymphocyte differentiation in vitro, and increase intratumoral CD4+ and CD8+ T cells and NK cells in vivo. This phase I/II clinical study is evaluating the combination of MRx0518 and pembrolizumab in a cohort of heavily pre-treated patients refractory to immune checkpoint inhibitors (ICIs) to assess whether it is safe and can provide a clinical benefit.

Methods The study is being conducted in two parts. Part A is complete and evaluated safety of the combination therapy in a cohort of 12 mRCC and mNSCLC patients. This data was assessed by the Safety Review Committee and it was determined appropriate to proceed to Part B. Part B is now recruiting up to 30 additional patients per indication (RCC, NSCLC or bladder cancer) at several US sites. Patients in both parts must be refractory to checkpoint inhibition. This is defined as having had an initial benefit from PD-1 pathway targeting immune checkpoint inhibition (ICI) but developing disease progression confirmed by two radiological scans ≥4 weeks apart in the absence of rapid clinical progression and within 12 weeks of last dose of ICI. Patients are treated with 1 capsule of MRx0518 (1 × 10^10 to 1 × 10^{11} CFU) twice daily and pembrolizumab (200 mg every 3 weeks) for up to 35 cycles or until disease progression. Tumour response is assessed every 9 weeks per RECIST. Blood, stool and urine samples are collected throughout the study to evaluate immune markers and microbiome. Patients may choose to consent to tissue biopsies. The primary objective of the study is to evaluate safety of the combination by monitoring toxicities in the first cycle of treatment. Secondary objectives are to evaluate efficacy via ORR, DOR, DCR (CR, PR or SD ≥ 6 months) and PFS. Exploratory objectives are to evaluate biomarkers of treatment effect, impact on microbiota and OS and correlation of clinical outcome with PD-L1 CPS/TPS.

Results N/A

Conclusions N/A

Trial Registration NCT03637803

Ethics Approval This study was approved by University of Texas MD Anderson’s Institutional Review Board; approval ref. 2018-0290

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0376

Background CD137 (4-1BB) represents a costimulatory pathway that promotes T, NK, and dendritic cell effector functions favorable for antitumor immunity. The extracellular domain of CD137, comprised of four cysteine-rich domains (CRD-I, CRD-II, CRD-III, CRD-IV), trimerizes upon binding to CD137 ligand (CD137L) to induce cell stimulatory transcriptional and epigenetic changes. The investigation of CD137-targeting agonist antibody, urelumab (CD137L-binding, IgG4), in human subjects showed immunologic and pharmacodynamic effects, but poor efficacy due to dose-limiting liver toxicity. Preclinical studies using a murine surrogate antibody, clone H3 (CRD-I-binding, rIgG2a), also demonstrated hepatotoxicity. Agenus Inc., Lexington, MA, USA; Mary Crowley Cancer Research Center, Dallas, TX, USA; Columbia University Medical Center, New York, NY, USA; AgenTus Therapeutics, Inc., Cambridge, UK; Gilead Sciences, San Carlos, CA, USA; Next Oncology, San Antonio, TX, USA

Methods We investigated the molecular and cellular effects of AGEN2373 (CD137L-binding, IgG1), a conditionally active CD137-targeting agonist antibody designed to bind and induce CD137 signaling upon FcR cross-linking while permitting ligand binding to CD137. The role of epitope and FcR binding as critical factors for anti-CD137 therapeutic activity were elucidated in primary cell-based assays and syngeneic tumour-bearing mouse models using anti-mouse antibody clones S3B1 (CD137-binding) and 3H3 (CD137L-binding, rlgG2a), also demonstrated hepatotoxicity that correlated with activation of CD137-expressing myeloid cells and memory CD8+ T cells. In contrast, urelumab (CRD-II/III-binding, IgG2) showed acceptable tolerability, but limited clinical efficacy. These and more recent findings implicate epitope and Fc gamma receptor (FcγR)-dependent antibody cross-linking as critical factors for CD137 therapeutic antibody design.

Results AGEN2373 bound with high-affinity to CD137 CRD- IV and promoted potent agonist activity of CD137 that was
conditioned on Fc-dependent antibody cross-linking. AGEN2373 surrogate, S3B1, showed comparable binding and cross-link dependent agonist activity. In CT26 tumor-bearing mice, S3B1 and 3H3 demonstrated complete tumor control that was not reproducible with a Fc-silent S3B1 antibody. The Fc-dependent activity of S3B1 correlated with induced immunologic changes in the TME including CD8 T cell expansion, NK cell activation, and Treg depletion. Patients with advanced solid cancers, treated with AGEN2373 up to 1 mg/kg every 4 weeks, demonstrated clinical activity with no evidence of hepatotoxicity.

Conclusions: Conditional and potent agonist activity of AGEN2373 is dependent on binding to CD137 CRD-IV and FcγR. Preclinically, our data demonstrate that AGEN2373-like murine surrogate antibodies promote potent immune activation and anti-tumor immunity. Phase 1 clinical trials investigating the safety and efficacy of AGEN2373, alone or combination with baslitimab (anti-PD-1), are underway.

Trial Registration NCT04121676

REFERENCES


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0377

378 A FIRST IN-HUMAN, MULTICENTER, OPEN-LABEL, DOSE-FINDING PHASE 1 STUDY OF THE IMMUNE STIMULATOR ANTIBODY CONJUGATE NJH395 IN PATIENTS WITH NONBREAST HER2+ ADVANCED MALIGNANCIES

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Background: NJH395 is a first-in-class immune stimulator antibody conjugate (ISAC) consisting of a toll-like receptor 7 (TLR7) agonist conjugated to an anti-HER2 antibody. Antibody-mediated delivery of TLR7 may limit systemic toxicities previously seen with TLR agonists, while enhancing long-lasting antitumor immune response. In preclinical studies, NJH395 showed promising activity in HER2 expressing xenograft mouse models, and demonstrated immunogenicity and cytokine release in mice and non-human primates.

Methods: This phase 1, first-in-human, open-label, multicenter study (NCT03696771) is evaluating the safety, tolerability, pharmacokinetics, and preliminary efficacy of NJH395 in patients with nonbreast HER2+ advanced malignancies. The study design includes two parts: single-ascending dose (SAD), followed by multiple-ascending dose. Primary endpoint is safety; key secondary endpoints include assessment of pharmacokinetics, immunogenicity, and overall response rate. Tumor response was evaluated 3 weeks after treatment in SAD. Evaluation of pharmacodynamic markers including tumor-infiltrating lymphocytes is the key exploratory objective.

Results: Here, we report the results of the SAD part of this phase 1 study. As of July 01, 2020, 18 patients (10 males, 8 females; median age, 52.5 years [range, 42–74 years]) were enrolled in 5 dose cohorts (0.1–1.6 mg/kg). The tumor types included HER2+ colorectal cancer (N=11), gastroesophageal adenocarcinoma (N=2), non–small cell lung cancer (N=1), nasopharynx adenocarcinoma (N=1), pancreatic adenocarcinoma (N=1), bladder cancer (N=1), and small intestine adenocarcinoma (N=1). Seventeen patients reported 124 treatment-related adverse events. The most common (occurring in ≥ 20%) adverse events of any grade (G), regardless of study drug relationship were cytokine release syndrome (55.6%, G ≤ 2), pyrexia (44.4%), nausea (44.4%), vomiting (33.3%), headache (33.3%), increased aspartate aminotransferase (AST, 33.3%), increased alanine aminotransferase (ALT, 27.8%), and lymphopenia/lymphocyte count decrease (27.8%). The most common ≥ G3 AEs (occurring in ≥ 10%) were lymphopenia/lymphocyte count decrease (27.8%) and increased AST (11.1%). Five dose-limiting toxicities, all G3, were reported in 3 patients: 2 cases of AST increase (1 at 0.2 mg/kg; 1 at 1.6 mg/kg), 1 ALT increase (1.6 mg/kg), 1 septic meningitis (1.6 mg/kg), and 1 meningism (1.6 mg/kg). No complete/partial response was seen; 9 patients had stable disease by RECIST v1.1 at 3 weeks post treatment. An increase in CD8-positive T-cells was detected in on-treatment tumor biopsies in 5 patients. Pharmacokinetics showed a greater than dose proportional exposure of NJH395; anti-drug antibodies were detected in all tested patients (14/14).

Conclusions: Single dosing of NJH395 showed significant but manageable toxicities in patients with nonbreast HER2+ advanced malignancies. Biomarker analysis is ongoing.

Acknowledgements: The authors thank all patients who participated in the study. The authors acknowledge Kavita Garg, PhD of Novartis Healthcare Pvt Ltd for providing medical editorial assistance with this abstract.

Trial Registration ClinicalTrials.gov Identifier: NCT03696771

Ethics Approval: The study was performed in accordance with ethical principles of the declaration of Helsinki and good clinical practice guidelines. The protocol and its amendments were approved by institutional review boards of each participating site.

Consent: Written informed consent was obtained from each patient prior to enrolment in the study.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0378
INITIAL SAFETY, EFFICACY, AND PRODUCT ATTRIBUTES FROM THE SURPASS TRIAL WITH ADP-A2M4CD8, A SPEAR T-CELL THERAPY INCORPORATING AN AFFINITY OPTIMIZED TCR TARGETING MAGE-A4 AND A CD8α CO-RECEPTOR

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Background The ongoing SURPASS trial (NCT04044859) evaluates safety and efficacy of next-generation ADP-A2M4CD8 SPEAR T-cells co-expressing the CD8α co-receptor with the engineered MAGE-A4 TCR (TCR). Methods First-in-human trial in HLA-A*02 positive patients (pts) with advanced cancers expressing MAGE-A4 antigen by immunohistochemistry. Eligible pts undergo apheresis, T-cells are isolated, transduced with a Lentiviral vector containing the MAGE-A4 TCR and CD8α co-receptor, and expanded. Expansion, transduction level, cellular composition and function of the manufactured product (MP) are assessed in vitro. Prior to infusion, pts receive lymphodepletion with fludarabine 30 mg/m²/day for 4 days and cyclophosphamide 600 mg/m²/ day for 3 days. Results As of 16 July 2020, 5 pts (1 with MRCLS, 2 with esophagegoastic junction [EGJ] cancers, 1 with ovarian cancer, and 1 with head and neck cancer) were treated with ADP-A2M4 CD8 (range ~1 to 5.7 billion transduced cells). No DLTs or SAEs have been reported. To date, 1 pt with EGJ cancer had a partial response (PR per RECIST) and has had progression-free survival >6 months. One pt with head and neck cancer also had a PR. All other pts have had best overall response of stable disease, MP expanded by a average of 15.3 fold during manufacturing (range 5.9 to 25.6-fold). On average, 43% of T-cells in the MP expressed the TCR (range 23 to 63%). The fraction of CD4+ cells in the final MP varied (range 45 to 84%). Co-expression of the MAGE-A4 TCR and CD8α co-receptor in the patient MP enabled CD4+ T-cells to kill tumor target cells directly in vitro. MAGE-A4 expression in tumor biopsies varied (H-score range 55 to 300). Transduced T-cells were detected in peripheral blood of all pts. IFN-gamma increased transiently in the serum of 1 pt who responded. Conclusions ADP-A2M4CD8 SPEAR T-cells have shown an acceptable safety profile and pts with EGJ cancer and head and neck cancer have demonstrated evidence of antitumor activity. Translational data and early clinical results indicate that co-expression of the CD8α co-receptor on CD4+ SPEAR T-cells may increase the potency of the product by conferring additional killing activity to the helper T-cell subset. This dose escalation trial is ongoing and updated clinical and translational data will be presented. Trial Registration NCT04044859

Ethics Approval The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and was approved by local authorities. An independent ethics committee or institutional review board approved the clinical protocol at each participating center. All the patients provided written informed consent before study entry.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0379

PRELIMINARY RESULTS OF AN ONGOING PHASE I TRIAL OF FT500, A FIRST-IN-CLASS, OFF-THE-SHELF, INDUCED PLURIPOTENT STEM CELL (IPSC) DERIVED NATURAL KILLER (NK) CELL THERAPY IN ADVANCED SOLID TUMORS

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Background FT500 is an investigational, off-the-shelf NK cell cancer immunotherapy derived from a human clonal master iPSC line, a renewable cell source from which innate effector cells can be mass produced and made available off-the-shelf for broad patient access and multiple dose administration. FT500 has potent innate cellular cytotoxicity as compared to NK cells sourced from healthy donors and has been shown to synergize with T cells and anti-PD-1 blockade in preclinical studies. Methods FT500 is being investigated in a Phase I clinical trial as monotherapy and in combination with immune checkpoint inhibitors (ICIs) in patients with advanced solid tumors and lymphomas (ClinicalTrials.gov: NCT03841110). Treatment consists of 2 days lympho conditioning (fludarabine 25 mg/m² and cyclophosphamide 300 mg/m²) followed by 2 cycles of 3 once weekly doses of FT500 as monotherapy or combined with 1 of 3 approved ICIs (nivolumab, pembrolizumab, or atezolizumab) in patients who have failed prior ICI therapy. Key clinical and translational readouts include FT500 safety and tolerability, including immune mediated toxicities and anti-product immunogenicity. Results 15 patients with relapsed/refractory disease following a median of 4 prior therapies were treated in dose escalation, including 9 with FT500 monotherapy (3 with 1×108 cells, 6 with 3×108 cells) and 6 with FT500 (3 each with 1×108 and 3×108 cells) combined with ICI. No dose limiting toxicities, Grade ³3 related adverse events (AEs), Grade ³3 related serious AEs, or related AEs leading to treatment discontinuation were reported. No graft-versus-host disease (GVHD), cytokine release syndrome (CRS), or neurotoxicity (NT) was observed. The most common treatment-emergent AEs in >3 patients were nausea (9), fatigue (7), constipation, decreased appetite, decreased lymphocyte count, decreased white blood cell count (5 each), anemia, and decreased neutrophil count (4 each). Nine of 13 efficacy-evaluable solid tumor patients had best response of stable disease by iRECIST. One patient with classical Hodgkin lymphoma (cHL) refractory to prior experimental anti-PD-1 therapy had a 58% reduction in target lesions size following FT500 plus ICI. No evidence of robust B- or T-cell mediated anti product responses was observed despite endogenous immune cell recovery following lympho-conditioning. Conclusions Administration of 6 doses of up to 3×108 FT500 cells is safe and tolerable without evidence of GVHD, CRS, NT, or host immune rejection. Enrollment of advanced
non-small cell lung cancer and cHL patients at 3×108 FT500 cells per dose combined with ICI is ongoing.

Ethics Approval This study is being conducted in accordance with the Declaration of Helsinki and was approved by all Institutional Review Boards from each clinical site participating in the study. Specific approval numbers can be provided upon request.

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0380

381 ROLE OF CT SCANS OF ABDOMEN AND PELVIS IN MANAGEMENT OF PATIENTS WITH IMMUNOTHERAPY-INDUCED COLITIS
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Background Colitis is one of the most common immune-related adverse event in patients who receive immune checkpoint inhibitors targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death-1 (PD-1). Although radiographic changes are reported on computed tomography such as mild diffuse bowel thickening or segmental colitis, the utility of CT in diagnosis of patients with suspected immune-related colitis is not well studied.

Methods CT scans of the abdomen and pelvis of 34 patients on immunotherapy with a clinical diagnosis of immunotherapy-induced colitis and 19 patients receiving immunotherapy without clinical symptoms of colitis (control) were enrolled in this retrospective study. Segments of the colon (rectum, sigmoid, descending, transverse, ascending and cecum) were assessed independently by two fellowship trained abdominal imaging specialists with 7 and 13 years' experience who were blinded to the clinical diagnosis. Each segment was assessed for mucosal enhancement, wall thickening, distension, peri-serosal fat stranding. Any disagreements were resolved in consensus. The degree of distension and the spurious assignment of wall thickening were the most common causes for disagreement. The presence of any of the signs was considered as radiographic evidence of colitis.

Results CT evidence of colitis was seen in 16 of 34 patients with symptoms of colitis. 7 of 19 patients who did not have symptoms of colitis showed signs of colitis on CT. The sensitivity, specificity, Positive Predictive Value and Negative Predictive Value for colitis on CT is 47%, 63.2%, 69.5% and 40%, respectively.

Conclusions CT has a low sensitivity, specificity and negative predictive value for the diagnosis of immunotherapy-induced colitis. CT has no role in the diagnosis of patients suspected of having uncomplicated immune-related colitis and should not be used routinely for management.

Trial Registration This protocol is not registered on clinicaltrials.gov.

Ethics Approval This protocol was IRB approved on: 11/16/2015 - IRB 4 Chair Designee FWA #: 0000363 OHRP IRB Registration Number: IRB 4 IRB00005015

Consent This protocol utilizes an IRB approved waiver of consent.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0381
Background Intratumorally injected Clostridium novyi-NT (non-toxic), an attenuated strain of C. novyi that lacks production of the lethal alpha toxin, replicates within hypoxic tumor regions and elicits tumor-confined cell lysis. Early clinical and translational data suggest that intratumoral injection of C. novyi-NT is feasible, demonstrates early signals of anti-tumor activity and induction of the host immune response, which supports additional studies in combination with immune checkpoint inhibitors.

Methods This first-in-human study (NCT03435952) enrolls patients with injectable, treatment-refractory solid tumors to receive a single intratumoral injection of C. novyi-NT across 4 dose cohorts (3 × 104 to 100 × 104 spores, 3+3 dose-escalation design) in combination with intravenous pembrolizumab 200 mg every 3 weeks for up to 24 months to determine dose-limiting toxicities (DLTs), and the maximum tolerated dose (MTD).

Results As of August 24, 2020, 9 patients (breast cancer, n=2; colorectal cancer, n=1; fibrous histiocytoma, n=1; anal cancer, n=1; chondrosarcoma, n=1; appendiceal cancer, n=1; tongue squamous cell cancer, n=1; nasopharyngeal cancer, n=1) were treated. There were no DLTs to date. Signs and symptoms of C. novyi-NT germination (infection) including fever, injection site pain, erythema, swelling, tenderness, and in some cases, ulceration, spontaneous drainage, tissue sloughing, bleeding, and malodor were observed in 5 patients. Partial responses were noted in 2 of 9 patients (tongue squamous cell cancer, nasopharyngeal cancer).

Conclusions Single intratumoral injection of C. novyi-NT in combination with pembrolizumab has been demonstrating manageable toxicity profile and encouraging signals of anti-cancer activity. The enrollment continues.

Trial Registration NCT03435952

Ethics Approval The study was approved by MD Anderson IRB.
cancer treatment strategy. However, targeting CD47 leads to various hematological toxicities, particularly anemia and thrombocytopenia. Lemzoparlimab (also known as TJ011133 or TJC4) is a fully human, anti-CD47 IgG4 antibody that is endowed with a red blood cell (RBC) sparing property and unique binding epitope, potentially differentiating itself from other CD47 axis targeting therapies.

Methods This phase 1 study (NCT03934814) is comprised of 2 parts. Part 1 consists of lemzoparlimab monotherapy dose escalation and 2 separate dose schedules of combination therapy with pembrolizumab or rituximab. The study is a standard 3+3 design. Part 2 is a dose expansion study. During monotherapy dose escalation, patients with relapsed/refractory solid tumors were administered an intravenous weekly dose (1 to 30 mg/kg) of lemzoparlimab to determine tolerability, safety, pharmacokinetics (PK), pharmacodynamics (PD) and antitumor activity based on Response Evaluation Criteria in Solids Tumors (RECIST v1.1) and iRECIST. Preliminary data from fully enrolled monotherapy cohorts in Part 1 are reported as of 17 July 2020.

Results Twenty patients with relapsed/refractory solid tumors were enrolled to monotherapy dose escalation cohorts (1, 3, 10, 20 and 30 mg/kg). Lemzoparlimab toxicity was manageable up to 30 mg/kg without a dose-limiting toxicity (DLT) observed. The most common treatment-related adverse events (TRAEs) were anemia (30.0%, n=6), fatigue (25.0%, n=5), infusion-related reactions (20.0%, n=4), and diarrhea (15.0%, n=3). All TRAEs were Grade 1 or 2. A transient, non-dose-dependent average reduction of 1.5 mg/dL (range: 0.4–2.6 mg/dL) in hemoglobin during the first cycle was observed across all cohorts consistent with the results of pre-clinical good laboratory practice toxicity studies. Laboratory or clinical evidence of hemolysis was not observed in any cohort. Preliminary results indicate the PK of lemzoparlimab appears to be linear at mid- to high dose levels following a single dose. CD47 receptor occupancy shows complete saturation on peripheral T cells at peak concentrations of 20 mg/kg and above.

Conclusions Lemzoparlimab appears safe up to 30 mg/kg with favorable PK and PD characteristics in patients with relapsed/refractory solid tumors to date. No TRAEs greater than Grade 2 have been observed. Results will be updated at presentation including available tumor response data.

Trial Registration NCT03934814

Ethics Approval The study was approved by IRB, approval number 20190733.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0385

Background HEK-NIZ985 (NIZ985) is a recombinant heterodimer of IL-15/IL-15Rα that expands effector lymphocytes and antitumor activity in animal models and a human clinical trial. We report interim data from the first-in-human study of NIZ985.

Methods CNIZ985X2102J is an open-label Phase I/II dose-escalation/expansion trial evaluating the safety of subcutaneous NIZ985 three-times-weekly (TIW; 2-weeks-on/2-weeks-off) or once-weekly (QW; 3-weeks-on/1-week-off) as a single agent (SA) or in combination (CM) dosing with 400 mg of the PD-1 inhibitor spartalizumab every 4 weeks, in adults with metastatic/unresectable solid tumors. SA dosing was 0.25–4 μg/kg TIW or 2–10 μg/kg QW; CM dosing was 1 μg/kg TIW or 2–4 μg/kg QW. The primary objective was to characterize the safety and tolerability of NIZ985 ± spartalizumab. Data are presented for dose escalation, and CM-TIW expansion.

Results Overall, 83 patients entered dose escalation (n=47) or CM-TIW expansion (n=36), of whom 63.8% (30/47) and 69.4% (25/36), respectively, had received ≥3 prior lines of antineoplastic treatment. At data cut-off (March 2, 2020), 91.6% (76/83) had discontinued study treatment. Adverse events (AEs) are summarized below (table 1). There were no dose-limiting toxicities during the first 28-day cycle in any cohort. Systemic skin AEs (Cycle 2) occurred in three SA-TIW patients receiving 2 or 4 μg/kg (bullous pemphigoid, purpura, vasculitis), limiting TIW escalation and initiating QW dose exploration; these were not observed at 1 μg/kg TIW (± spartalizumab) or for QW doses up to 10 μg/kg. CM-TIW dose expansion was therefore at 1 μg/kg; the recommended QW expansion dose is currently undetermined. For SA NIZ985, best overall response (RECIST 1.1) was stable disease (SD; 8/27 patients [29.6%]). Objective responses for NIZ985 plus spartalizumab (3/56 partial response [PR; 5.3%], 15/56 SD [26.8%]) occurred in both immuno-oncology treatment (IO)-naive and IO-experienced patients, including 5/8 IO-experienced melanomas (cutaneous: 3 SD, 1 PR; uveal: 1 SD). Systemic NIZ985 exposure was approximately dose-proportional after first dose for ≥1 μg/kg TIW and <10 μg/kg QW, with time-dependent clearance without accumulation. Proliferation of peripheral CD8+ and NK lymphocytes, and increased inflammatory cytokines, were observed for both dosing schedules.

Abstract 386 Table 1 Adverse event summary

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic anemia</td>
<td>1/14 (7.1%)</td>
<td>0/13 (0.0%)</td>
<td>0/13 (0.0%)</td>
<td>0/19 (0.0%)</td>
</tr>
<tr>
<td>Myelosuppression</td>
<td>12/14 (85.7%)</td>
<td>10/13 (76.9%)</td>
<td>10/13 (76.9%)</td>
<td>10/19 (52.6%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>11/14 (78.6%)</td>
<td>10/13 (76.9%)</td>
<td>10/13 (76.9%)</td>
<td>10/19 (52.6%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>9/14 (63.6%)</td>
<td>8/13 (61.5%)</td>
<td>8/13 (61.5%)</td>
<td>14/19 (73.7%)</td>
</tr>
<tr>
<td>Platelet reduction</td>
<td>12/14 (85.7%)</td>
<td>10/13 (76.9%)</td>
<td>10/13 (76.9%)</td>
<td>10/19 (52.6%)</td>
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<tr>
<td>Diarrhea</td>
<td>6/14 (42.9%)</td>
<td>6/13 (46.2%)</td>
<td>6/13 (46.2%)</td>
<td>4/19 (21.1%)</td>
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<tr>
<td>Influenza-like illnesses</td>
<td>3/14 (21.4%)</td>
<td>2/13 (15.4%)</td>
<td>2/13 (15.4%)</td>
<td>2/19 (10.5%)</td>
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<tr>
<td>Gastrointestinal toxicity</td>
<td>9/14 (63.6%)</td>
<td>8/13 (61.5%)</td>
<td>8/13 (61.5%)</td>
<td>14/19 (73.7%)</td>
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Common AEs (all-causes >20% in all patients)

- Fatigue (63.6%, n=9)
- Platelet reduction (85.7%, n=12)
- Diarrhea (42.9%, n=6)
- Influenza-like illnesses (21.4%, n=3)
- Gastrointestinal toxicity (63.6%, n=9)

AE, adverse event; CM, combination treatment (NIZ985 + spartalizumab); QW, once-weekly; SA, NIZ985 single agent; TIW, twice-weekly; CM-TIW, triple therapy weekly.
A PHASE II, MULTICENTER STUDY OF THE SAFETY AND EFFICACY OF LAG525 IN COMBINATION WITH SPARTALIZUMAB IN PATIENTS WITH ADVANCED MALIGNANCIES

Viola Mihaljević, Chi-Chi Lin, Elena Garralda, Patrick Schönfisch, David Hong, Lillian Sui, Miguel Martin, Michela Maur, Rina Hui, Ross Soo, Joanne Chiui, Tian Zhang, Chirsann Kyi, Daniel Tan, Philippe Cassier, John Sarantopoulos, Andrew Weichhardt, Rich Carvajal, Jennifer Spratlin, Taito Esaki, Frederic Rolland, Wallace Akerley, Barbara Deschlen-Baire, Catherine Sabatos-Freyton, Niadri De Braud.

Background Expression of LAG-3, an inhibitory immunoreceptor, has been linked to reduced T-cell proliferation and cytokine production. LAG525 is a humanized IgG4 anti-LAG-3 antibody which inhibits LAG-3 binding to MHC class II. Spartalizumab is a humanized IgG4 anti-PD-1 mAb which inhibits PD-1 binding with its ligands PD-L1 and PD-L2. Preclinical data have shown promising antitumor activity when blocking PD-1/-L1 with PD-1/PD-L1 inhibitors, suggesting that this combination may salvage prior PD-1/L1 resistance. The combination was well tolerated, and no new safety signals were observed. Biomarker analysis is ongoing.

Conclusions LAG525 + spartalizumab exhibited antitumor activity across different indications, including patients with melanoma, RCC, and mesothelioma who had been pretreated with PD-1/L1 inhibitors, suggesting that this combination may salvage prior PD-1/L1 resistance. The combination was well tolerated, and no new safety signals were observed. Biomarker analysis is ongoing.

Trial Registration NCT02460224

Ethics Approval This study was approved by an independent ethics committee or institutional review board at each site.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0387
Background GB1275 is a first-in-class CD11b modulator that reduced myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), repolarized M2 immunosuppressive TAMs to M1 phenotype, and increased tumor infiltration of activated CD8+ T cells in preclinical models. Preclinical anti-tumor activity was observed with single-agent therapy and in combination with chemotherapy or immunooncology therapies. We report results from the dose escalation portion of an ongoing, first-in-human study of GB1275 monotherapy and combined with pembrolizumab in patients with specific advanced solid tumors. (NCT04060342)

Methods This study comprises phase 1 dose escalation followed by phase 2 expansion in specific tumor types. In phase 1, cohorts of 3 to 6 patients with histologically confirmed, locally advanced/metastatic pancreatic, esophageal, gastric, MSS colorectal, metastatic castrate-resistant prostate cancer, or triple negative breast cancer are sequentially assigned to one of the ascending dose levels of GB1275 orally twice daily (BID) in 1 of 3 regimens: A (GB1275 monotherapy); B (GB1275 + pembrolizumab) commenced after completion of 2 cohorts of A; and C (GB1275 + nab-paclitaxel + gemcitabine) will be initiated after A. Patients in Regimens A and B had previously exhausted standard of care treatment options. Dose escalation was based on safety, including dose-limiting toxicity (DLT). Serial blood samples were collected for pharmacokinetic (PK) and biomarker analyses; tumor tissue was also collected for biomarker analyses.

Results As of July 28, 2020, 36 patients were treated, 23 in Regimen A (GB1275 100 mg to 1200 mg BID) and 13 in Regimen B (GB1275 100 mg to 800 mg BID + pembrolizumab). No DLTs or adverse events requiring steroid treatment were reported. GB1275-related adverse events were reported in 19 (52.8%) patients; most were Grade 1 and most frequent events (≥10%) were dysesthesia (13.9%) and photosensitivity reaction (11.1%). Stable disease was reported in 4 (17%) patients in Regimen A and 6 (46%) in Regimen B with a median (range) exposure of 84 days (35–172). A dose-dependent increase in GB1275 exposure was observed. An increase in tumor infiltrating lymphocyte (TIL) counts was noted in both Regimens A and B. Other biomarker analyses in serial blood and tumor tissue are ongoing.

Conclusions Dose escalation of GB1275, up to 1200 mg and 800 mg BID in Regimens A and B, respectively, demonstrated tolerability as monotherapy and combined with pembrolizumab. The maximum tolerated dose has not been reached. Preliminary observation of an increase in TILs after treatment is encouraging.

Ethics Approval This ongoing study is being conducted in accordance with the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines. The study was approved by the Ethics Boards of the University of Colorado Hospital, Washington University School of Medicine - Siteman Cancer Center, Memorial Sloan Kettering Cancer Center, The Sarah Cannon Research Institute/Tennessee Oncology, South Texas Accelerated Research Therapeutics, and The Royal Marsden NHS Foundation Trust.

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0388
Emerging Safety and Activity Data from GEN-009: A First-in-Human Study of Intratumoral Neoantigen Vaccine in Combination with PD-1 Check-Point Inhibitors (CPI) in Advanced Solid Tumors

Background: GEN-009 is an adjuvanted personalized cancer vaccine containing up to 20 neoantigens selected by ATLAS™, an ex vivo bioassay screening autologous T cells to identify both neoantigens as well as Inhibigen™s empirically and without in silico predictions. Inhibigen-specific T cells suppress immunity and have been shown to accelerate tumor progression in mice. Inhibigenes are avoided in GEN-009. Previous data from patients treated with GEN-009 monotherapy showed 99% of selected peptides generated immune responses including ex vivo CD4+ and CD8+ fluorospot responses specific for 51% and 41% of immunized peptides respectively. Methods: GEN-009 is being evaluated in patients (pts) with advanced cancer who received standard-of-care (SOC) PD-1 inhibitor as monotherapy or in combination therapy during vaccine manufacturing; they subsequently received 5 vaccine doses over 24 weeks in combination with the PD-1 inhibitor. Patients who progressed prior to vaccination could receive alternate therapy followed by GEN-009 combined with an appropriate salvage regimen. Peripheral T cell responses were evaluated pre-and post-vaccination by dual-analyte fluorospot assays measured both directly ex vivo and after in vitro stimulation. Results: As of August 18, 2020, 15 pts received GEN-009 in combination with a PD-1 inhibitor. Their median TMB was treated with GB1275. Analyses of TIL count revealed an increase in lymphocyte trafficking into the tumor after treatment with GB1275 alone or in combination with pembrolizumab. CD8 expression and transcriptomic analysis are underway and will be presented. Conclusions: GB1275 alone or in combination with pembrolizumab demonstrates biological activity, which may be dose dependent. The observed increase in TILs after treatment is supportive of the mechanism of action of GB1275. Further biomarker analyses in blood and tissues are ongoing and will be correlated with clinical activity in a larger number of patients.

Ethics Approval: This ongoing study is being conducted in accordance with the Declaration of Helsinki and International Ethical Guidelines. The study was approved by the Ethics Boards of University of Colorado Hospital, Washington University School of Medicine - Siteman Cancer Center, Memorial Sloan Kettering Cancer Center, The Sarah Cannon Research Institute/Tennessee Oncology, South Texas Accelerated Research Therapeutics, and The Royal Marsden NHS Foundation Trust.

ClinicalTrials.gov NCT03633110

Ethics Approval: The study was approved by Western Institutional Review Board, approval number 1-1078861-1.

A First-in-Human Study of Intratumoral SAR441000, an MRNA Mixture Encoding IL-12SC, Interferon Alpha2B, GM-CSF and IL-15SUSHI as Monotherapy and in Combination with Cemiplimab in Advanced Solid Tumors

Background: mRNA-based-drugs can be applied for cancer immunotherapy. SAR441000 is a novel saline-formulated mixture of four MRNAs encoding interleukin-12 single chain, 1.37Mut/m (range 0.31-6.55), with a median of 24 (6-99) neoantigens and 16 (1-86) Inhibigenes. The number of neoantigens in each manufactured vaccine ranged from 4-18 (median 13). GEN-009-related adverse events were limited to Grade 1 injection site reactions. Ex vivo T cell responses peaked after the third vaccination for IFNγ and some patients showed evidence of epitope spread. The initial 5 patients are evaluable for antitumor activity with at least 3 months follow up after first vaccination. Three patients experienced early tumor responses followed by stabilization on PD-1 inhibitor SOC and demonstrated a further reduction in tumor volume after GEN-009 vaccination (figure 1). One patient experienced a complete response prior to vaccination and the 5th patient had progression on SOC, but had a Partial Response to salvage and remains stable after vaccination.

Conclusions: Vaccination with GEN-009 in combination with PD-1 CPI is feasible for patients with advanced solid tumors with little additive toxicity. Preliminary data demonstrate induction of robust, neoantigen-specific immune responses and a potential expansion of stimulatory targets with epitope spreading in the presence of PD-1 inhibitor. Possible additive antitumor activity in combination with PD-1 inhibitors is suggested by tumor shrinkage following GEN-009 dosing. More mature response and immunogenicity data on 10 additional patients is anticipated for November.

Trial Registration: ClinicalTrials.gov NCT03633110

Ethics Approval: The study was approved by Western Institutional Review Board, approval number 1-1078861-1.
Abstract 391 Table 1 Frequency of patients with a TEAE related to SAR44100* by dose group and grade

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Abstract 391 Table 2 Frequency of patients with a TEAE related to study treatment (SAR441000+cemiplimab) * by dose group and grade

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 interferon alpha-2b, granulocytre-macrophage colony-stimulating factor, and interleukin-15 sushii that we have identified as mediators of tumor regression across different murine tumor models. Local intratumoral administration of SAR441000 in immunocompetent mice, mediates successful antitumor immunity leading to tumor eradication. Effective antitumor activity of these cytokines involved multiple immune cell populations and was accompanied by intratumoral interferon gamma induction, systemic antigen-specific T-cell expansion, increased granzyme B+ T-cell infiltration, and formation of immune memory. Antitumor activity extended beyond the treated lesions and inhibited growth of non-injected distant tumors. Combining the mRNAs with checkpoint inhibitors enhanced antitumor responses in both injected and non-injected tumors, improving survival and tumor regression in mice. Based on these preclinical observations a clinical study was initiated. Methods In a phase 1 dose escalation study, patients with advanced solid tumors were treated with weekly intratumoral administration of SAR441000 monotherapy and in combination with fixed dose of cemiplimab 350 mg. Plasma samples for cytokine analysis and tumor biopsies were collected at baseline and throughout the study to characterize the PK/PD profile of SAR441000, immune cell tumor infiltration by immunohistochemistry and the presence of corresponding tumor proinflammatory signatures by RNA sequencing. Results As of July 2020, 17 patients received SAR441000 monotherapy (melanoma 7, breast 4, sarcoma 2, Cutaneous Squamous Cell 2, Basal Cell 1, and Merkel Cell 1) at dose levels 1 through 7. Six patients received SAR441000 in combination therapy (melanoma 3, breast 3) at dose levels 4 and 5. No patient experienced a Dose Limiting Toxicity. No grade 3, 4 or 5 adverse events related to study treatment were reported. Adverse events related to study treatment in two or more subjects in both treatment groups combined were nonserious grade 1 or 2 fatigue (43%;10/23), vomiting (17%; 4/23), nausea (13%;3/23); local injection site reaction (11.7%, 2/23); and chills, diarrhea, and rash were reported as 9% (2/23), respectively (table 1 and 2). In some patients, increases in plasma IP10 and IFN gamma and CD8+ T cell infiltration in tumor biopsies were observed. Conclusions SAR441000 administered as monotherapy and in combination with cemiplimab was generally well tolerated. An immunomodulatory effect is suggested by downstream effector cytokines and T cell infiltration. These data support further clinical evaluation of SAR441000. Ethics Approval The study was approved by each participating Institution’s Ethics or Institutional Review Board(s).

REFERENCE


392 PHASE 1 STUDY OF CI-8993 ANTI-VISTA ANTIBODY IN PATIENTS WITH ADVANCED SOLID TUMOR MALIGNANCIES

Elizabeth Martinez, Jason Fairs*, Reinhard Von Roemeling, Steven Angellides, Melissa Johnson, Curis Inc, Durham, NC, USA; Dartmouth-Hitchcock, Norris Cotton Cancer, Lebanon, NH, USA; Curis, Ridgefield, CT, USA; Tenessee Oncology, Nashville, TN, USA

Background VISTA (V-domain Ig suppressor of T cell activation) is a key negative immune checkpoint regulator, locking T cells in a quiescent state, unlike PD1 and CTLA4, which are expressed on activated T cells. Preclinically, VISTA monoclonal antibody treatment increased the number of tumor-specific T cells in the periphery, and enhanced the infiltration, proliferation and effector function of tumor-reactive T cells within the tumor microenvironment (TME). VISTA blockade alters the suppressive feature of the TME by decreasing the presence of monocyto myeloid-derived suppressor cells and increasing the presence of activated dendritic cells (DCs) within the TME leading to enhanced T cell mediated immunity. VISTA monoclonal antibody administration as a monotherapy has been shown to suppress the growth of both transplantable and inducible melanoma in preclinical models. CI-8993 is a first-in-class, fully human immunoglobulin (IgG) G1k monoclonal antibody (mAb) against the VISTA ligand. Prior human clinical evaluation of CI-8993 demonstrated target-related clinical findings and pharmacodynamic activity at 0.15 mg/kg.

Methods This phase 1 study is being conducted in the USA (NCT04475523) and is designed as a 3+3 dose escalation study beginning at 0.15 mg/kg. Patients with solid tumor malignancy (non-lymphoma) that is metastatic or unresectable and considered relapsed and/or refractory to prior therapy will be included, excluding prior CAR-T therapy or allogenic transplant. Patients will be treated with an initial step-dose of CI-8993 by IV infusion, followed by every 2 weeks of a full dose, until disease progression or toxicity. Efficacy, pharmacokinetics, pharmacodynamic and safety endpoints will be monitored and reported.
Background CTLA-4 pathway blockade with ipilimumab (IPI) ± nivolumab (NIVO) ± nivolumab is an effective treatment for several cancers. A nonfucosylated version of IPI, BMS-986218, was developed to increase the effects of CTLA-4 blockade and enhance intratumoral regulatory T-cell depletion via its increased affinity for Fcγ receptors (FcγR, CD16) on natural killer T cells and macrophages, resulting in enhancement of antibody-dependent cellular cytotoxicity. Preclinical data supported the mechanism of action of BMS-986218 and demonstrated greater antigen tumor activity in an MC38 tumor model vs IPI.1 Here, we present initial results from the first-in-human phase 1/2a trial of BMS-986218 ± NIVO in previously treated patients with advanced cancer (NCT03110107).

Methods Patients with ≥1 prior therapy received BMS-986218 2–70 mg intravenously Q4W. Safety, tolerability, pharmacokinetics, and pharmacodynamics were evaluated.

Results As of December 12, 2019, 65 patients (median age, 61 years [range, 24–85 years]) received BMS-986218 monotherapy. TRAEs occurred in 52% of patients; most were grade 1–2. The most common (≥10%) TRAEs (any grade; grade 3) were pruritus (12%; 0%) and diarrhea (11%; 3%). Eight patients (12%) had grade 3 TRAEs; most resolved with protocol-defined management. No grade 4 TRAEs were reported; 1 grade 5 TRAE (pneumonitis; 2 mg) occurred. Seven patients (11%) discontinued treatment due to TRAEs; 4 dose-limiting toxicities occurred. The maximum tolerated dose has not been reached. BMS-986218 exposure increased dose proportionally, and the half-life was ~2 weeks. Increased levels of serum chemokine ligands 9 and 10 and interferon-γ indicate that pharmacodynamic changes occurred at the lowest dose tested (2 mg [=0.03 mg/kg]), similar to previous findings with IPI 3 mg/kg, and at higher doses (40–70 mg [=0.06–1 mg/kg]), consistent with findings with IPI 10 mg/kg. In a subset of patients with paired biopsies, BMS-986218 was associated with an increased gene signature linked to CD8+ T-cell infiltration and inflammation. In a highly heterogeneous population, as part of dose escalation, BMS-986218 monotherapy treatment was associated with clinical activity in some patients. Updated data based on a September 2020 data cutoff will be presented.

Conclusions BMS-986218 monotherapy demonstrated an acceptable safety profile and signs of clinical benefit in this heterogeneous patient population with select advanced cancers. Preliminary pharmacodynamic activity was consistent with enhanced effects of CTLA-4 blockade. Data from continuing dose escalation of BMS-986218 ± NIVO along with preclinical results provide support for ongoing monotherapy expansions and for BMS-986218 + NIVO expansions in patients with advanced cancer.

Acknowledgements The authors acknowledge Dr Charles Drake while at Columbia University Medical Center, New York, NY, USA, for his contributions to the study.

Trial Registration NCT03110107

Ethics Approval This study was approved by the WCG Independent Review Board, approval number 20170464.
Results As of March 20, 2020, 120 patients (median age, 63 years [range, 35–87 years]) received BMS-986253 + NIVO; 97% of patients received prior anti–PD-(L)1 therapy, and 25% received prior anti–CTLA-4 therapy. BMS-986253 + NIVO was well tolerated with no dose-limiting toxicities observed. Most TRAEs were grade 1–2. The most common (>5% of patients) TRAEs (any grade; grade 3–4) were fatigue (9%; 1%), nausea (7%; 0%), rash/sha rash maculopapular (6%; 0%), pruritus (5%; 0%), and decreased appetite (5%; 0%). Grade 3–4 serious TRAEs were reported in 2 patients (inflammation-related reaction, BMS-986253 2400 mg Q2W + NIVO; AST/ ALT increased, BMS-986253 1200 mg Q4W + NIVO). BMS-986253 exposure increased dose proportionally and was not altered with NIVO. BMS-986253 resulted in dose-dependent reductions in free sIL-8 levels, with tumor IL-8 suppression detected in most patients evaluated; additional pharmacodynamic endpoints will be presented. Partial responses were observed in multiple tumor types, including 5 of 28 patients with melanoma who had progressed on/after prior anti–PD-(L)1 1; 4 of the 5 patients were also previously treated with anti–CTLA-4.

Conclusions BMS-986253 + NIVO demonstrated a tolerable safety profile with dose-proportional pharmacokinetics and robust sIL-8 suppression. Preliminary antitumor activity was observed across a range of doses/regimens in this biomarker-enriched, anti–PD-(L)1–experienced, heterogeneous patient population with advanced cancers. These findings support further evaluation of BMS-986253 in select advanced tumors.

Acknowledgements The authors acknowledge Dr Charles Drake while at Columbia University Medical Center, New York, NY, USA, for his contributions to the study.

Trial Registration NCT03440332

Ethics Approval This study was approved by the WCG Independent Review Board, approval number 20172711.

REFERENCES

Background Upregulation of immune checkpoints, such as LAG-3, plays an important role in promoting resistance to anti-PD-(L)1 therapy. Targeting PD-L1 and LAG-3 using a bispecific antibody may overcome resistance to PD-(L)1 blockade.1 We report initial data from a first-in-human study evaluating FS118 in patients with advanced cancer and resistance to PD-(L)1 therapy.

Methods The ongoing Phase I FIH study (NCT03440437) is being conducted to evaluate safety, tolerability, immunogenicity, PK/PD and clinical activity of FS118 administered IV weekly to heavily pre-treated patients who had previously received anti–PD-(L)1 therapy for a minimum of 12 weeks. Adverse events were assessed using CTCAE v4.03 and tumor responses assessed using RECISTv1.1 and iRECIST. Single subject dose escalation cohorts were followed by a 3+3 ascending dose design. Three cohorts (3, 10, 20 mg/kg) were expanded to evaluate PK, PD and clinical activity. Pharmacodynamic studies examined soluble LAG-3 production and peripheral T-cell expansion.

Results Forty-three patients (median 6 lines of prior therapy, including ICB) with solid tumors received FS118 at doses from 0.8 mg up to 20 mg/kg across 8 dose levels. Weekly administration of FS118 was well tolerated and did not result in dose- or treatment-limiting toxicities. An MTD was not reached. No safety signals unexpected for the drug class of immune-checkpoint inhibitors were identified in the early study population. The majority (95%) of treatment-emergent adverse events (TEAE) considered by the Safety Review Committee (SRC) to be treatment-related were Grade 1 and 2. Grade 3 TEAEs toxicities (elevated liver enzymes) were observed in 2 patients (5%). No SAEs or deaths were attributed to FS118 treatment. Anti-drug antibodies, observed in half of patients, were typically transient in nature. The pharmacokinetic profile confirmed preclinical predictions and PD parameters included a dose-dependent increase in serum soluble LAG-3 and expansion of peripheral T cells. Long-lasting disease stabilisation (>6 months) was observed in a subset of patients with acquired resistance (defined as a CR, PR or SD ≥3 months on previous PD-(L)1 treatment), but not in patients with primary resistance. Two patients remain on FS118 treatment as of 2 Jul 2020 (duration 10 and 16 months). Retrospective IHC analysis of PD-L1 and LAG-3 co-expression in the tumor was assessed as a potential biomarker associated with clinical outcome.

Conclusions Weekly treatment with FS118 was well tolerated up to 20 mg/kg and was associated with pharmacodynamic markers of FS118 activity. Encouraging signs of clinical activity were observed in highly pre-treated patients who had acquired resistance to prior PD-(L)1 therapy.

Trial Registration Registered at www.clinicaltrials.gov, NCT03440437

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0394
OVERCOMING RESISTANCE TO ANTI-PD-1 WITH TUMOR AGNOSTIC NBTXR3: FROM BENCH TO BED SIDE

1James Welsh*, 1, 2Cécile Shen, 3Jessica Frakes, 1, 2I lain Nku, 2Jared Weiss, 3Jimmy Caudell, 1, 2Hu Yun, 2Hamptonsoum Barsounian, 1, 5Juliette Thariat, 1, 5Sylve Bonvalot, 1, 5Zsuzsanna Papai, 5Maria Angelica Cortez, 1, 5P ing Zhang, 1, 6Katherine Jameson, 1, 5Patricia Said, 1, 5Sébastien Paris, 1, 5James Welsh*, 2Colette Shen, 3Jessica Frakes, 4Jiaxin Niu, 2Jared Weiss, 3Jimmy Caudell, 1, 2Hu Yun, 2Hamptonsoum Barsounian, 1, 5Juliette Thariat, 1, 5Sylve Bonvalot, 1, 5Zsuzsanna Papai, 5Maria Angelica Cortez, 1, 5P ing Zhang, 1, 6Katherine Jameson, 1, 5Patricia Said, 1, 5Sébastien Paris, 1, 5James Welsh*, 2Colette Shen, 3Jessica Frakes, 4Jiaxin Niu, 2Jared Weiss, 3

Background Despite recent advances, resistance to immune checkpoint inhibitors (ICI), observed in over 80% of treated patients, is currently the main challenge immuno-oncology is facing. Intense efforts are being made to identify combination therapies that could improve ICI response rates. Administered intratumorally, NBTXR3 enhances the energy dose deposited by ionizing radiation within tumor cells, increasing the anti-tumor efficacy of radiation therapy (XRT) without adding toxicity to surrounding tissues. Here we present evidence that NBTXR3 activated by XRT primes the immune system, producing an anti-tumor response, including activation of the cGAS-STING pathway, that overcomes anti-PD-1 resistance both in mice models and patients.

Methods Abscopal assays were conducted in immunocompetent mice. Tumor cell lines, sensitive or resistant to anti-PD-1, were injected in both flanks of mice. Intratumoral injection of NBTXR3 (or vehicle) followed by XRT was performed in right flank (primary) tumors only. Some mice also received anti-PD-1 injections. Tumor growth was monitored, and tumor immune cell infiltrates were analyzed by immunohistochemistry (IHC). Separately, in the phase II/III randomized trial Act.in. (NCT02379845) patients with locally advanced soft tissue sarcoma (STS) received either NBTXR3+XRT or XRT alone followed by wide tumor resection. Pre- and post-treatment tumor samples from patients in both groups were analyzed by IHC and Digital Pathology for immune biomarkers. The safety and efficacy (RECIST 1.1/RECIST) of NBTXR3 plus stereotactic ablative radiotherapy (SABR) in combination with anti-PD-1 is being evaluated in three cohorts of patients with advanced cancers [NCT03589339].

Results Pre-clinical studies demonstrated that NBTXR3+XRT induces an immune response a not observed with XRT alone and enhances systemic control. IHC showed significant increase of CD8+ T-cell infiltrates in both NBTXR3+XRT treated and untreated tumors compared to XRT alone. Similarly, increased CD8+ T-cell density (pre- vs post-treatment) was observed in tumor tissues from STS patients treated with NBTXR3+XRT. Tumor samples from the NBTXR3+XRT group also displayed increased PD-1+ cell density. Furthermore, in combination with anti-PD-1, NBTXR3+XRT improved local and systemic control in mice bearing anti-PD-1 resistant lung tumors, as well as resulted in reduced number of spontaneous lung metastases.Preliminary efficacy data from the first in human trial of NBTXR3+XRT in combination with anti-PD-1 showed tumor response in patients who progressed on prior anti-PD-1.

Conclusions The clinical efficacy of NBTXR3+XRT has been demonstrated as a single agent. We now demonstrate that it potentiates anti-PD-1 treatment to overcome resistance mechanisms. These results highlight the potential of NBTXR3+XRT to positively impact the immuno-oncology field.
immunotherapeutic agents with concomitant biopsy procedures to date are associated with a high technical success rate & favorable safety profile.

**Acknowledgements** Joshua Hein, Mara Castaneda, Jyotsna Pera, Yunfang Jiang, Shuang Liu, Holly Liu and Anna Lui

**Trial Registration** N/A

**Ethics Approval** The study was approved by Institution’s Ethics Board, approval number 2020-0536: A retrospective study to determine the safety, feasibility and technical challenges of real-time image guidance for intra-tumor injection and biopsy across multiple solid tumors.

**Consent** 2020-0536 Waiver of Informed Consent

**REFERENCE**


**http://dx.doi.org/10.1136/jitc-2020-SITC2020.0397**

**RESULTS**

Preclinically, AGEN1181 demonstrated superior T cell activation than a standard IgG1 anti-CTLA-4 analogue in donors expressing either the low or high affinity FcγRIIIA. In poorly immunogenic tumor-bearing mouse models, AGEN1181-like surrogate demonstrated robust tumor control in combination with anti-PD-1 and focal radiation or chemotherapy. As of August 25th, 2020, we observed a clinical benefit rate of 63–53% at 6 and 12 weeks respectively among evaluable treated patients. We observed two durable responses in patients with endometrial cancer that were BRCA-, microsatellite stable and PD-L1 negative. These patients progressed on prior PD-1 therapy or chemoradiation respectively. Notably, responders expressed either the low or high affinity FcγRIIIA. AGEN1181 showed potent dose-dependent increases in peripheral CD4+Ki67+, CD4+ICOS+ and CD4+HLA-DR+ T-cells. Treatment was well tolerated through the highest dose tested. Grade 3 or greater immune-related adverse events occurred in 28.5% patients and were consistent with CTLA-4 therapies.

**Conclusions** AGEN1181 is designed to expand the benefit of anti-CTLA-4 therapy to a broader patient population. AGEN1181, alone or in combination with balstilimab, demonstrates clinical activity in heavily pretreated patients.

**Trial Registration** NCT03860272

**http://dx.doi.org/10.1136/jitc-2020-SITC2020.0398**

**Abstracts**

**AGEN1181, AN FC ENGINEERED ANTI-CTLA-4 ANTIBODY, DEMONSTRATES CLINICAL ACTIVITY, ALONE OR IN COMBINATION WITH BALSTILIMAB (ANTI-PD-1), AND BROADENS THE THERAPEUTIC POTENTIAL OF CTLA-4 THERAPY**

**Background** Immune checkpoint therapies targeting CTLA-4, alone, or in combination with anti-PD-1 have shown durable responses in cancer patients. However, responses are limited to a small subset of patients in the most common immunogenic cancers. Here we describe, a novel anti-CTLA-4 antibody, AGEN1181, with enhanced FcγR-dependent functionality that harnessed a novel mechanism of action to promote superior T cell activation and anti-cancer immunity. Concordant with preclinical findings, we report preliminary safety, pharmacodynamic and efficacy data from a phase 1 study of AGEN1181 (NCT03860272), alone or in combination with balstilimab (anti-PD-1 antibody) in a range of immunogenic and non-immunogenic tumors.

**Methods** The functional activity of AGEN1181 or AGEN1181-like mouse surrogate were assessed in primary cell-based assays or in PD-1 refractory syngeneic tumor-bearing mouse models (B16F10 or KPC pancreatic tumor). Efficacy was evaluated as monotherapy, or in combination with anti-PD-1, focal radiation or chemotherapy. In an ongoing phase 1 study, AGEN1181 is administered intravenously once every 3- or 6-weeks as monotherapy (0.1–4 mg/kg), or every 6-weeks (1–4 mg/kg) in combination with balstilimab (3 mg/kg) dosed every 2 weeks. Dose-limiting toxicities were evaluated in the first 28 days of treatment. Neoaantigen burden was assessed from pre-treatment tumor biopsy, as available, by next-generation sequencing. Fcγ receptor genotyping was assessed by real-time PCR. Immunophenotyping of peripheral blood mononuclear cells collected pre- and post-treatment were analyzed by flow cytometry.

**COSIBELIMAB, AN ANTI-PD-L1 ANTIBODY: PRELIMINARY SAFETY AND EFFICACY RESULTS FROM A GLOBAL, MULTICOHORT PHASE 1 CLINICAL TRIAL**

**Background** Cosbelimab is a high affinity, fully-human IgG1 monoclonal antibody that directly binds to programmed death ligand-1 (PD-L1) and blocks its interaction with the programmed death receptor-1 (PD-1) and B7.1 receptors to restore an anti-tumor immune response. Cosbelimab has the additional benefit of a functional Fc domain capable of inducing antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity against tumor cells. Study CK-301–101 is a global, multicenter, multicohort trial that is enrolling patients (pts) with select advanced cancers, including pivotal cohorts of pts with metastatic and locally advanced cutaneous squamous cell carcinoma (cSCC) and a cohort of pts with previously untreated advanced non-small cell lung cancer (NSCLC).
Methods Eligible pts were aged ≥18 years with an Eastern Cooperative Oncology Group performance status of 0–1. The cSCC cohorts enrolled pts with histologically confirmed metastatic or locally advanced cSCC not amenable to local therapy. The NSCLC cohort enrolled previously untreated NSCLC pts with advanced disease and a PD-L1 tumor proportion score of at least 50%. Pts received a fixed dose of 800 mg cosibelimab administered intravenously every two weeks. Anti-tumor activity was assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and were conducted every 8 weeks for the initial 32 weeks on study, and every 12 weeks thereafter.

Results As of August 2020, 114 pts (73M/41F, median age 66 years) with diverse tumor types have been enrolled and treated with cosibelimab. Among these pts, 103 (90%) experienced ≥1 treatment-emergent adverse event (AE), 42 (37%) experienced a grade ≥3 AE, and 6 (5%) experienced a grade ≥3 drug-related AE. The most common AEs were fatigue (25%), anemia (21%), rash (18%) and nausea (16%) and the most common drug-related AEs were fatigue (15%) and rash (14%). In 42 cSCC pts evaluable for response, ORR based on investigator assessment of tumor response was 55% (95% confidence interval [CI]: 39, 70), including 5 (12%) complete responses, with 20/23 (87%) responses ongoing and 10 responses ≥6 months in duration as of data cutoff. In 25 NSCLC pts evaluable for response, ORR based on investigator assessment was 44% (95% CI: 24, 65), with 5/11 (45%) responses ongoing and 10 responses ≥6 months in duration.

Conclusions Cosibelimab has a predictable and manageable safety profile and demonstrated robust clinical activity in cSCC and NSCLC pts, including durable complete and partial responses. Updated results will be presented.

Trial Registration NCT0312404

Ethics Approval The study was approved by an appropriate ethics committee for each participating institution. Informed consent was obtained for all subjects.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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400 COUPLEDCAR T TECHNOLOGY FOR TREATING THYROID CANCER

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Background Chimeric antigen receptor modified T cells (CAR T) have demonstrated remarkable clinical efficacy in the treatment of B cell malignancies and multiple myeloma. Significant challenges restrict their application across solid tumors due to multiple obstacles, including the lack of robust in vivo CAR-T cell expansion and persistence, the immunosuppressive tumor microenvironment, and tumor escape due to heterogeneous tumor cell composition with a potential loss of the targeted tumor antigen. To address these difficulties, we generated CAR T cells using a novel CoupledCAR® technology. Specifically, we engineered CoupledCAR T cells with lentiviral vectors encoding an anti-thyroid stimulating hormone receptor (TSHR) CAR molecule. Immunohistochemistry (IHC) results showed that TSHR was highly expressed in thyroid cancer cells making it an ideal tumor-specific target antigen. In vitro co-culture experiments showed that TSHR CAR T cells specifically recognized and subsequently killed TSHR-positive tumor cells. Animal model experiments showed that TSHR CAR T cells inhibited the proliferation of TSHR-positive tumor cells.

Methods We designed a ‘CoupledCAR’ lentivirus vector containing a single-chain variable fragment (scFv) targeting human TSHR. The lentivirus was produced by transfecting HEK-293T cells with ‘CoupledCAR’ lentiviral vectors and viral packaging plasmids. Patient’s CD3 T cells were cultured in X-VIVO medium containing 125U/mL interleukin-2 (IL-2), and transduced with ‘CoupledCAR’ lentivirus at certain MOI. Transduction efficiency and was evaluated at 7 to 9 days after ‘CoupledCAR’ lentivirus transduction, and quality controls for fungi, bacteria, mycoplasma, chlamydia, and endotoxin were performed. After infusing, serial peripheral blood samples were collected, and the expansion and the cytokine release of CART cells were detected by FACs and QPCR. The evaluation of response level for patients were performed at month 1, month 3, and month 6 by PET/CT.

Results To evaluate the clinical safety and efficacy of anti-TSHR CoupledCAR T cells on refractory or relapsed thyroid cancer, we treated refractory/relapsed post-thyroidectomy thyroid cancer patients according to an IRB approved protocol. We treated two patients using anti-TSHR CoupledCAR T cells and observed the rapid expansion of CAR T cells and enhanced the killing of tumor cells. One patient’s best response was complete remission, and the other was near complete remission.

Observations and Results: Patient 1: Male, 64Y, Papillary Thyroid Carcinoma. In May 2017, Thyroid cancer was diagnosed, bilateral total thyroidectomy, and right cervical lymph node dissection were performed in Jun 2018, followed by iodine 131 isotope therapy. In December 2018, bilateral multiple cervical lymph nodes were enlarged, especially on the right side. In February 2019, right neck lymphadenectomy was performed. Patient 2 Female, 60Y, Thyroid Carcinoma. In Aug 2013, a ‘double lobectomy of the thyroid gland’ was performed. From Oct 2013 to Jan 2014, she received iodine 131 isotope therapy. In Sep 2014, she was diagnosed with iodine-resistant thyroid cancer. From Sep to Jan 2016, 5 cycles of chemotherapy were performed. In Jun 2016, she enrolled in the Anlotinib experimental group. In Mar 2019, multiple metastases in both lungs and multiple enlarged lymph nodes in the mediastinum were observed. Observations and Results: Patient 1: One month after infusion (M1), the patient was evaluated as PR: lymph node metastasis became undetectable and the size of the thoracic paratracheal tumor nodules decreased significantly. Three months after infusion (M3), the patient was evaluated as CR, and the tumor tissue was substantially smaller than M1. Patient 2: M1, the patient was evaluated as PR (Partial Response): the tumor volume in the right lower lobe of the lung was reduced by approximately 67.51% (decreased from 65.5 mm to 42.39 mm). Three months after infusion (M3), compared with that before, the tumor volume was reduced by approximately 73.54% and SUV max value decreased from 14.9 to 2.8, therefore, the patient was evaluated as nCR (near complete remission).

Conclusions We show that TSHR is a good target for treating thyroid cancer, and our anti-TSHR CoupledCAR T cells are safe and effective for treating thyroid cancer. Recruitment is ongoing to evaluate the safety and efficacy of our Coupled-CAR T cells. Further, since our CoupledCAR® technology is a
Background In spite of advances made in the management of patients with HER2-expressing or -driven solid tumors, there remains a significant unmet need for novel approaches to improve patient outcomes. Intratumoral delivery of antitumor antibodies and immunostimulatory adjuvants such as toll-like receptor (TLR)7/8 agonists has been shown to activate tumor resident antigen-presenting cells (APCs), driving uptake, processing, and presentation of tumor neoantigens to T cells that mediate antitumor immunity. BDC-1001 is delivered systemically and has demonstrated superior preclinical biology. This novel ISAC consists of an investigational biosimilar of the humanized monoclonal antibody trastuzumab chemically conjugated to a TLR7/8 agonist with a non-cleavable linker. BDC-1001 activates human myeloid APCs in addition to retaining antibody-mediated effector functions such as antibody-dependent cellular cytotoxicity/phagocytosis (ADCC/ADCP). Studies in trastuzumab-resistant xenograft models and syngeneic tumor models indicate that HER2-targeted ISACs elicit potent and durable immune-mediated antitumor efficacy, leading to complete tumor regression in a TLR- and Fc receptor-dependent manner.1 2 Importantly, BDC-1001 did not induce interstitial lung disease, cytokine release syndrome, or thrombocytopenia in non-human primate studies. A four-part phase 1/2, first-in-human trial will evaluate preliminary antitumor activity of BDC-1001 in combination with an immune checkpoint inhibitor (Part 4). Secondary objectives will evaluate pharmacokinetic parameters and pharmacodynamic biomarkers in tumor tissue and in peripheral blood associated with drug exposure. These exploratory studies will help elucidate the mechanism of action and seek to identify biomarkers associated with BDC-1001 biological activity with or without immune checkpoint inhibitor. This current study is generally recruiting patients.

Results N/A

Conclusions N/A

REFERENCES


Background TGFβ1 is a key mediator of primary resistance to PD1 (programmed cell death protein 1) pathway blockade. SRK-181 is a high-affinity, fully humanized antibody that selectively binds to latent TGFβ1 and inhibits its activation on suppressive immune cells as well as within tumor stroma. Preclinical data demonstrated that selective inhibition of latent TGFβ1 with SRK-181 overcomes primary anti-PD-1 resistance and has an improved safety profile compared to broad inhibition of the TGFβ pathway.

Methods The DRAGON trial is a multi-center, open-label, Phase 1, first-in-human (FIH), dose-escalation, and dose expansion study to evaluate the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and efficacy of SRK-181 administered by IV infusion every 3 weeks (q3w) alone and in combination with an anti-PD-(L)-1 in adult patients with locally advanced or metastatic solid tumors. The study is divided into 3 parts: Part A1, a single agent dose escalation, will determine the maximum tolerated dose (MTD) or maximum administered dose (MAD) of SRK-181 as a single agent. Part A2, a combination dose escalation, will determine the MTD or MAD of SRK-181 in combination with anti-PD-(L)-1 therapy and the RP2D of the combination treatment for use in Part B. Part B, the dose expansion, will enroll parallel cohorts of patients with non-small cell lung cancer, urothelial...
carcinoma, melanoma, or other advanced solid tumors, to confirm the tolerability of the RP2D and to evaluate the anti-tumor activity of SRK-181 in combination with an anti-PD-(L)-1 therapy. Patients in Part A2 and Part B will have previously received anti-PD-(L)-1 therapy and considered non-responders to anti-PD-(L)-1 therapy alone. Patients will receive SRK-181 alone or in combination with anti-PD-(L)-1 until disease progression, unacceptable toxicity, or other reasons for study discontinuation. Safety, PK, PD and efficacy data will be collected and monitored throughout the study. PD effects will be assessed by measuring modulation of tumor immune cells and TGFβ pathway within the tumor microenvironment.

Results
As of the data cutoff date of May 4, 2020, 62 patients were included in the safety and efficacy analysis sets. Fifty percent (n=31) of patients had at least one progression, intolerance, or consent withdrawal.

Background
Enhancement of antitumor immunity through inhibition of the checkpoint PD-1 receptor has been effective in the treatment of many malignancies. AMG 404 is a monoclonal antibody (mAb) targeting PD-1. This phase 1, open-label, multicenter first-in-human study (NCT03853109) will evaluate the safety, tolerability, pharmacokinetics, and efficacy of AMG 404 monotherapy in adult patients with advanced solid tumors.

Methods
The primary study endpoint is dose-limiting toxicity (DLT) and safety; key secondary endpoints include pharmacokinetic parameters, objective response rate (assessed Q8W), duration of response, and progression-free survival. Key inclusion criteria include histologically or cytologically proven metastatic or locally advanced solid tumors not amenable to curative treatment with surgery or radiation for which standard therapies have been exhausted or not available. Prior anti-PD-(L)-1 or other checkpoint inhibitors were not allowed. Five dose-finding cohorts, including 2 expansion cohorts, ranged from 3–20 patients each. AMG 404 was given until disease progression, intolerance, or consent withdrawal.

Results
As of the data cutoff date of May 4, 2020, 62 patients received at least 1 dose of AMG 404 and were included in the safety and efficacy analysis sets. Fifty percent were men, 72% had ECOG 1 performance status, median age was 62 years (range: 28–83), and 42% had ≥3 lines of prior anticancer therapy. Median AMG 404 exposure was −3 months (maximum: −12 months). No DLTs were observed. Treatment-related adverse events (TRAEs) were reported for 29 patients (47%); those reported for ≥2 patients were fatigue (n=7); hypothyroidism (n=6); increased blood thyroid stimulating hormone and nausea (n=4 each); increased aspartate aminotransferase, decreased appetite, and pyrexia (n=3 each); and increased alanine aminotransferase, arthralgia, diarrhea, and increased weight (n=2 each). AEIs leading to withdrawal of AMG 404 were reported for 3 patients (5%); all were serious and considered to be not related to AMG 404. Sixteen (26%) patients died on study; no deaths were considered related to AMG 404. Preliminary pharmacokinetic results were consistent with those of other therapeutic anti-PD-1 mAbs. Three patients had a confirmed partial response (pancreatic cancer, clear cell cancer, and pleomorphic sarcoma); an additional 4 patients had one scan with a partial response and are pending a confirmatory scan (clear cell renal carcinoma, undifferentiated nasopharyngeal carcinoma, sarcomatoid carcinoma of unknown primary, and colon cancer).

Conclusions
AMG 404 is tolerable at the tested doses with no DLTs reported. All observed TRAEs are consistent with other anti-PD-1 therapies. Encouraging anti-tumor activity has been observed in heavily pretreated patients. The study is continuing enrollment into additional cohorts.

Trial Registration
NCT03853109

Ethics Approval
The study was approved by the Ethics Board of each institution involved in this study and can be produced upon request.
administered A+T, and with HNSCC administered A+P are also reported as of 30 June 2020. 

**Results** Fifty-five pts enrolled into this portion of the study. Twelve patients with ≥2L GC received A+T+ram+pac and were evaluated for safety. No DLTs were reported, and the ALX148 maximum administered dose was 15 mg/kg QW. Out of the 9 pts who experienced any adverse event, 7 pts reported treatment-related adverse events (TRAE). The most common TRAEs were low grade diarrhea, fatigue, pruritus and rash (each n=2,170). Nine of the 12 pts were response-evaluable and reported a 66% ORR with 6PR and 3SD (including one ongoing near PR, 19.6%). Three patients with 1L HNSCC were administered A+P+5FU+platinum. No DLTs were reported and accrual to 15 mg/kg QW continues. Three pts experienced any AE, none were treatment-related. Of 3 evaluable patients with HNSCC, 2PR and 1SD were reported. Initial ALX148 combination PK and CD47 target occupancy are similar to that of single agent administration. Response duration and survival follow-up of 19 pts with HER2-positive GC administered A+T (2nd or later-line; 21% ORR) and of 10 pts with checkpoint inhibitor naïve HNSCC administered A+P (2nd or later-line; 40% ORR) will be reported. Results of all cohorts will be updated at time of presentation.

**Conclusions** Initial data suggests the myeloid checkpoint inhibitor, ALX148, is well tolerated in combination with the above anticancer antibodies, T-cell checkpoint inhibitor, and cytotoxic chemotherapy regimens with early anticancer signals in GC and HNSCC that compare favorably with historic controls. No MTD has been reached in any combination to date and accrual to chemotherapy combination regimens is ongoing.

**Trial Registration** ClinicalTrials.gov identifier NCT03013218

**Ethics Approval** The study was approved by all participating institutions’ Ethics and/or Review Boards

**REFERENCES**


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**Background** CDX-1140 is an agonist anti-CD40 mAb selected to optimize systemic exposure and hence tumor microenvironment (TME) ingress. CDX-1140 activity may be enhanced by combining with CDX-301 (recombinant Flt3L), a dendritic cell growth factor, or with pembrolizumab, an anti-PD-1 mAb.

**Methods** Patients with advanced solid or hematologic (Part 1 only) tumors are enrolled. Part 1 dose-escalation results have been presented (SITC 2019). In Part 2, CDX-1140 dose-escalation (0.09–1.5 mg/kg q4w) is in combination with CDX-301 (75 mcg/kg sc QD x 5 for 2 cycles). In Part 3, CDX-1140 dose-escalation (0.72–1.5 mg/kg q3w) is in combination with pembrolizumab 200 mg q3w. Part 1 and 2 expansion cohorts are dosed at the CDX-1140 MTD, 1.5 mg/kg q4w. Part 3 expansion cohorts are planned. Peripheral blood and tumor biomarkers analysis are ongoing.

**Results** 92 patients have been treated (Part 1 n=57, Part 2 n=31, Part 3 n=4). Part 1 expansion cohorts in SCCHN (n=7) and RCC (n=5) are fully enrolled. Part 2 dose-escalation completed to the highest CDX-1140 dose and a SCCHN expansion cohort is ongoing. Part 3 dose-escalation recently initiated. Safety data is available for 23 and 10 patients at the MTD in Part 1 and 2, respectively. In general, the safety profiles were similar, with arthralgia (32% vs. 50%), pyrexia (44% vs 50%), fatigue (30% vs. 50%), chills (39% vs. 40%), vomiting (30% vs. 20%), nausea (26% vs 40%), myalgia (22% vs. 30%), increased ALT (22% vs. 20%), and increased AST (22% vs. 30%) being the most common drug related AEs at the MTD in Part 1 and 2, respectively. Most AEs were low grade. Across all cohorts, cytokine release syndrome (CRS) (G2 n=4, G3 n=2) occurred in 6 (Part 1 n=2; Part 2 n=4) and pneumonitis (G3) occurred in 5 (Part 1 n=4; Part 2 n=1) patients. Immune activation in the TME consistent with CD40 agonism and increases serum inflammatory cytokines were observed. Evidence of anti-tumor activity/clinical benefit include SD (n=13), tumor cavitation (n=2) and a uPR in solid tumors. A patient with follicular lymphoma has an ongoing durable complete metabolic response.

**Conclusions** The CDX-1140 MTD dose of 1.5 mg/kg, a dose level expected to provide good systemic exposure and TME penetration, is generally well tolerated alone and with CDX-301. Transaminitis and CRS have generally been low grade and infrequent. A cohort combining CDX-1140 with chemotherapy will be initiated in patients with previously untreated metastatic pancreatic adenocarcinoma.

**Trial Registration** NCT03329950

**Ethics Approval** The study was approved by the following: Providence St. Joseph Health IRB, approval number MOD2020001128; WIRB, approval number 1188814 (Hauke, Gabrail, Bordoni & Gordon); University of Pennsylvania IRB, approval number UPPC 18917; Mount Sinai School of Medicine IRB, approval number 18-00202; Memorial Sloan Kettering Cancer Center IRB, approval number 18-225A; Houston Methodist IRB, approval number MOD00000836.

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CDX527–01, A PHASE 1 DOSE-ESCALATION AND PHARMACODYNAMICS/PHARMACOKINETICS STUDY OF THE PD-L1×CD27 BISPECIFIC ANTIBODY CDX-527 IN PATIENTS WITH ADVANCED MALIGNANCIES

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Background CD27 ligation and PD-1 blockade elicit complementary signals mediating T cell activation and effector function. CD27 is constitutively expressed on mature T cells and the interaction with its ligand, CD70, plays key roles in T cell costimulation leading to activation, proliferation, enhanced survival, maturation of effector capacity, and memory. The PD-1/PD-L1 pathway plays key roles in inhibiting T cell responses. Pre-clinical studies demonstrate synergy in T cell activation and anti-tumor activity when combining a CD27 agonist antibody with PD-(L)1 blockade, and clinical studies have confirmed the feasibility of this combination by demonstrating safety and biological and clinical activity. CDX-527 is a novel human bispecific antibody containing a neutralizing, high affinity IgG1k PD-L1 mAb (9H9) and the single chain Fv fragment (scFv) of an agonist anti-CD27 mAb (2B3) genetically attached to the C-terminus of each heavy chain, thereby making CDX-527 bivalent for each target. Pre-clinical studies have demonstrated enhanced T cell activation by CDX-527 and anti-tumor activity of a surrogate bispecific compared to individual mAb combinations, and together with the IND-enabling studies support the advancement of CDX-527 into the clinic.

Methods A Phase 1 first-in-human, open-label, non-randomized, multi-center, dose-escalation and expansion study evaluating safety, pharmacokinetics (PK), pharmacodynamics (PD), and clinical activity of CDX-527 is ongoing. Eligible patients have advanced solid tumor malignancies and have progressed on standard-of-care therapy. Patients must have no more than one prior anti-PD-1/L1 for tumor types which have anti-PD-1/L1 approved for that indication and no prior anti-PD-1/L1 for tumor types that do not have anti-PD-1/L1 approved for that indication. CDX-527 is administered intravenously once every two weeks with doses ranging from 0.03 mg/kg up to 10.0 mg/kg or until the maximum tolerated dose. The dose-escalation phase initiates with a single patient enrolled in cohort 1. In the absence of a dose limiting toxicity or any ≥ grade 2 treatment related AE, cohort 2 will enroll in a similar manner as cohort 1. Subsequent dose-escalation cohorts will be conducted in 3+3 manner. In the tumor-specific expansion phase, up to 4 individual expansion cohort(s) of patients with specific solid tumors of interest may be enrolled to further characterize the safety, PK, PD, and efficacy of CDX 527. Tumor assessments will be performed every 8-weeks by the investigator in accordance with iRECIST. Biomarker assessments will include characterizing the effects on peripheral blood immune cells and cytokines, and for the expansion cohorts, the impact of CDX-527 on the tumor microenvironment.

Results N/A

Conclusions N/A

Trial Registration NCT04440943

Ethics Approval The study was approved by WIRB for Northside Hospital, approval number 20201542

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PRELIMINARY SAFETY, PHARMACOKINETICS/PHARMACODYNAMICS, AND ANTITUMOR ACTIVITY OF XMAB20717, A PD-1 1 CTLA-4 BISPECIFIC ANTIBODY, IN PATIENTS WITH ADVANCED SOLID TUMORS

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Background XmAb20717 is a humanized bispecific monoclonal antibody that simultaneously targets PD-1 and CTLA-4. We report preliminary data from an ongoing, multicenter, Phase 1 study investigating the safety/tolerability, pharmacokinetics/pharmacodynamics, and clinical activity (RECIST 1.1) of XmAb20717 in patients with selected advanced solid tumors.

Methods A 3+3 dose-escalation design was used to establish a maximum tolerated (MTD)/recommended dose for evaluation in parallel expansion cohorts, including melanoma, renal cell carcinoma, non-small cell lung cancer (NSCLC), prostate cancer, and a basket of tumor types without an FDA-approved checkpoint inhibitor (CI; n≤20 each). XmAb20717 was administered as an infusion on Days 1 and 15 of each 28-day cycle.

Results As of 08 Jul 2020, 109 patients had been treated (table 1), and 30 were continuing treatment. In escalation, 6 dose levels (0.15–10.0 mg/kg) were evaluated (n=34); an MTD was not established. Expansion cohorts were initiated at 10 mg/kg (n=72), and a 15 mg/kg escalation cohort was added (n=3). T-cell proliferation was noted in peripheral blood at doses as low as 3 mg/kg and was highest at 10 mg/kg. At this dose, consistent proliferation of CD8+ and CD4+ T cells was observed, indicative of dual PD-1 and CTLA-4 checkpoint blockade (figure 1). Paired pre- and post-dosing biopsies showed increased intratumoral T-cell infiltration and IFN-γ response signatures following treatment. Grade 3/4 treatment-related adverse events (TRAEs) reported for ≥3 patients included rash (13%), transaminase elevations (7%), lipase increased (4% [2% with amylase increased]), and acute kidney injury (3%), all considered immune-related. There were 2 Grade 5 TRAEs: immune-mediated pancreatitis (in the pres-
ence of pancreatic metastases) and immune-mediated myocarditis (Grade 4) that contributed to respiratory failure. A complete response was reported as the best overall response for 1 patient (melanoma); partial responses were reported for 5 patients (2 melanoma, 2 NSCLC, 1 ovarian). The objective response rate was 13% overall and 21% at 10 mg/kg (6/46 and 6/29 evaluable patients, respectively). All responders had prior CI exposure. Responses were observed only at 10 mg/kg and, within the 10 mg/kg group, appeared to correlate with higher peak serum concentration and area under the curve.

Conclusions XmAb20717 induced T-cell proliferation in peripheral blood consistent with dual-checkpoint blockade. Preliminary data indicate XmAb20717 was generally well-tolerated and associated with evidence of antitumor activity in CI-pretreated patients with various types of advanced solid tumors.

Trial Registration NCT03517488

Ethics Approval The study was approved by each institution’s IRB.

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PHASE I, FIRST-IN-HUMAN TRIAL EVALUATING BI 1387446 (STIMULATOR OF INTERFERON GENES [STING] AGONIST) ALONE AND COMBINED WITH BI 754091 (ANTI-PROGRAMMED CELL DEATH [PD]-1) IN SOLID TUMORS

Background Activation of the STING pathway in intratumoral immune cells leads to increased type I interferon production, promoting recruitment and priming of T-cells against tumor antigens, and providing anti-tumor activity.1 Intratumoral administration of STING agonists has resulted in notable therapeutic activity in animal models.1 STING agonists have also shown clinical activity in patients, which was more pronounced when combined with an anti-PD-1 antibody.2,3 BI 1387446 potently and highly selectively activates the STING pathway; BI 754091 is a humanized IgG4 anti-PD-1 monoclonal antibody. Intratumoral administration of BI 1387446 resulted in tumor regression, and enhanced the activity of anti-PD-1 therapy in syngeneic tumor models.4

Methods NCT04147234 is a first-in-human, Phase I, open-label, multicenter trial of BI 1387446 in patients aged ≥18 years with advanced, unresectable and/or metastatic malignant solid tumors. Patients (up to ~122) will be enrolled from ~six sites across Europe and the USA. The main objectives are to characterize safety and determine the maximum tolerated dose (MTD) for BI 1387446 ± BI 754091. BI 1387446 will be administered intratumorally at increasing doses as monotherapy in Arm A, and in combination with BI 754091 (240 mg every three weeks, intravenously) in Arm B. In both arms, BI 1387446 will be administered in superficial lesions. In a potential third arm, Arm C, BI 1387446 will be administered in deep/visceral lesions in combination with intravenous BI 754091. Dose escalation will be guided by a Bayesian Logistic Regression Model with overdose control. For trial eligibility, patients must have exhausted standard treatment options, have ≥1 tumor lesion suitable for injection, ≥1 additional tumor lesion amenable to biopsy, and ECOG performance status of 0/1. Treatment will continue until progressive disease, unacceptable toxicity, other withdrawal criteria, or a maximum treatment duration of 34 cycles (for cycle 19 and onwards, administration of BI 1387446 is applicable for patients with a partial response), whichever occurs first. Primary endpoints are the MTD based on number of dose-limiting toxicities (DLTs), and number of patients with DLTs in the MTD evaluation period. Secondary endpoints are objective response based on RECIST 1.1, and best percentage change from baseline in size of target and injected lesions. Paired pre- and post-treatment biopsies of injected- and non-injected lesions and peripheral blood will be collected to assess pharmacodynamic changes associated with treatment. The trial is currently open for recruitment.

Results N/A

Conclusions N/A

Acknowledgements Medical writing assistance, supported financially by Boehringer Ingelheim, was provided by Steven Kirkham, of GeoMed, an Ashfield company, part of UDG Healthcare plc, during the preparation of this abstract.

Trial Registration NCT04147234

Ethics Approval Not applicable.

Consent Not applicable.

REFERENCES


A PHASE I TRIAL OF TALIMOGENE LAHERPAREPVEC FOR THE TREATMENT OF PERITONEAL SURFACE MALIGNANCIES (TEMPO)

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Abstract 407 Figure 1 Mean change from baseline in percentage of Ki67+ T-cell expression in peripheral blood during first two cycles of XmAb20717
Background Peritoneal surface dissemination (PSD) of gastrointestinal and ovarian cancers carries a poor prognosis. Although cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy has emerged as a treatment option for this patient population, only a minority of patients benefit from this approach. This finding highlights the need for novel approaches to this disease. Previous data have shown that the local treatment of orthotopic tumors in syngeneic murine models with the oncolytic virus talimogene laherparepvec (TL) converts ‘cold’ immunosuppressive tumor microenvironments into ‘hot’ immune microenvironments that support the regression of tumors. We hypothesize that intraperitoneal (IP) delivery of TL will be safe and tolerable and demonstrate clinical activity in patients with PSD of gastrointestinal (GI) and ovarian cancers.

Methods We are conducting the TEMPO Trial (NCT03663712), a non-randomized, open-label Phase I trial of IP TL in patients with stage IV PSD from GI or ovarian tumors enrolled at University of Illinois College of Medicine at Chicago, Duke Cancer Institute, and Wake Forest University School of Medicine. There will be two stages in this study, a Dose Escalation Cohort, and a Dose Expansion Cohort. In the Dose Escalation Cohort, three subjects will be enrolled at the starting dose of 4 × 106 PFU, and the dosing will continue in a standard ‘3+3’ dose escalation scheme. If the starting dose is tolerated, higher doses of 4 × 107 and 4 × 108 PFU will be evaluated. Once the MTD is determined, six subjects will be enrolled in the Dose Expansion Cohort at the MTD. All subjects will be dosed with IP TL once every two weeks for up to 4 doses (in addition to the initial seroconversion dose). The primary objective is to evaluate the toxicity profile. The statistical analyses will be only descriptive and performed on the intent to treat, per protocol, and safety populations. We hypothesize that IP TL leads to coordinated interactions between resident peritoneal innate and adaptive immunity. We will delineate these interactions by evaluating peritoneal exudates to assess a) treatment-related changes in peritoneal cytokine levels using multiplex cytokine analysis and b) resident peritoneal immune cell phenotype and function with flow cytometry methods. Plasma and urine fluid samples will be analyzed for viral load.

Results N/A

Conclusions This study will test the safety, tolerability, and preliminary clinical activity of IP TL; the results will be relevant to inform future investigations of local oncoimmunotherapies in patients with PSD, a highly unmet need population that currently has limited therapeutic options.

Acknowledgements N/A

Trial Registration Registered at clinicaltrials.gov- https://clinicaltrials.gov/ct2/show/NCT03663712. The identifier is NCT03663712

Ethics Approval This study was approved by the Institutional Review Boards at the University of Illinois College of Medicine at Chicago, Duke Cancer Institute, and Wake Forest University School of Medicine.

Consent N/A

REFERENCES

1. N/A

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Abstracts
NOVEL INTRATUMORAL AGENT, INT230–6 INDUCES CANCER CELL DEATH, INCREASES TUMOR INFILTRATES AND RESULTS IN DURABLE BENEFIT ALONE OR IN COMBINATION WITH PembroliZUMAB IN REFRACTORY PATIENTS

1Anthony El-Khoueiry, 1 Jacob Thomas, 1 Anthony Olzanski, 1Nilofor Azad, 1 Lewis Bender, 1 Ivan Walters, 2 Gile Whalen, 2 Diana Hanna, 3 Matthew Ingham, 3 Lillian Su, 1 University of Southern California, Los Angeles, CA, USA; 2 Fox Chase Cancer Center, Philadelphia, PA, USA; 3 Johns Hopkins, Chevy Chase, MD, USA; 4 Intensity Therapeutics, Westport, CT, USA; 5 University of Massachusetts, Worcester, MA, USA; 6 Columbia University, New York, NY, USA; 7 Princess Margaret Cancer Center, Toronto, ON, Canada

Background: INT230-6 is a novel formulation of cisplatin and vinblastine with an amphiphilic cell penetration enhancer that has been shown to enhance dispersion of the drug throughout tumors and allow diffusion into cells when given intratumorally. In preclinical models, INT230-6 has resulted in cell engagement with strong synergy when combined with checkpoint inhibitors.

Methods: This phase 1/2 study evaluated Q2week injections of INT230-6 x 5 dosed by tumor volume alone or with 200 mg pembrolizumab IV q3 weeks. Eligible patients had advanced malignancy refractory to standard therapy with an injectable tumor.

Results: Sixty subjects (median 3 prior therapies (range 0–6)) were enrolled (53 monotherapy, 7 combo). Median age was 60 (42–85). 19 different cancer types were accrued with breast cancer and sarcoma being the most frequent. Over 200 deep tumor injections were administered at doses of up to 172 ml of INT230-6 (86 mg of CIS, 17 mg of Vin). PK analysis revealed <5% of the drugs were measured in systemic circulation, indicative of minimal systemic exposure. There was no dose limiting toxicity. The most frequent monotherapy drug related AE reported were: injection-site pain 58%, nausea 37%, fatigue 33%, and vomiting 27% with only 18% of subjects experiencing a grade 3 AE (no grade 4 or 5). Rates were comparable for the single agent INT230-6 and the combination with pembrolizumab. In the overall monotherapy cohort, patients completing all 5 doses of INT230-6 over 56 days (n=16), the median overall survival has not yet been reached. After a median followup of 408 days. In the 5 evaluable patients who received the pembrolizumab combination, the median TTP has not been reached with a median follow up of 6 mo. Paired biopsies (pre, 1 month) were available in 10 monotherapy patients and revealed a median of 63% reduction in viable cancer cells on H&E (30% had no viable cancer) that was also associated with qualitative decreases in Ki67, increases of CD4 and CD8 T-cells and reduction in FoxP3 Tregs. Despite receiving only 2 month of monotherapy, short half lives of the active agents, and no subsequent therapy, 8 injected tumors continued to regress past 1 year.

Conclusions: INT230-6 is well tolerated when administered intratumorally alone or in combination with pembrolizumab. Pharmacodynamic assessments provide proof of concept that this drug can reduce viable cancer cells and increases CD4/CD8 T-cell infiltrates leading to durable clinical benefit off treatment.

Trial Registration: NCT 03058289

Ethics Approval: The study was approved by USC, Princess Margaret Cancer Center, Fox Chase, UMass, Columbia, and Johns Hopkins Institution’s Ethics Board

Consent: Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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FIRST-IN-HUMAN PHASE I/IIA TRIAL TO EVALUATE THE SAFETY AND INITIAL CLINICAL ACTIVITY OF DUOBODY®-PD-L1×4–1BB (GEN1046) IN PATIENTS WITH ADVANCED SOLID TUMORS

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Background: Agonistic 4-1BB monoclonal antibodies were preclinically validated as promising cancer immunotherapies, both as monotherapy and as potentiators of the activity of PD-(L)1–blocking agents. However, toxicity and a narrow therapeutic window have hampered their clinical development. DUOBody-PD-L1×4–1BB, a first-in-class, bispecific, next-generation checkpoint immunotherapy, was designed to overcome these limitations by activating T cells through conditional 4-1BB costimulation, while simultaneously blocking the PD-L1 axis. We present preliminary data from the ongoing, first-in-human, open-label, phase I/IIa trial of DUOBody-PD-L1×4–1BB in advanced solid tumors (NCT03917381).

Methods: During dose escalation, patients with metastatic or unresectable solid tumors not eligible for standard therapy received flat-dose DUOBody-PD-L1×4–1BB (25–1200 mg) intravenously every 3 weeks until disease progression or unacceptable toxicity. Primary endpoints were dose-limiting toxicities (DLTs) and adverse events (AEs). Secondary endpoints included pharmacokinetic parameters and antitumor activity (RECIST 1.1). Pharmacodynamic biomarkers and antitumor activity (iRECIST) were assessed as exploratory endpoints.

Results: As of June 22, 2020, 61 patients were enrolled (median age: 59 years). The most common cancer types were colorectal (19.7%), ovarian (14.8%), pancreatic (9.8%), and NSCLC (9.8%). Patients had previously received a median (range) of 4 (1–15) treatment cycles; Cmax was observed shortly after the end of infusion (mean T½: 10.3 days). Maximum tolerated dose was not reached; 6 patients experienced DLTs. The most common (≥10%) treatment-related AEs (all grades; grades 3–4) were transaminase elevation (24.6%; 9.8%), hypothyroidism (16.4%; 1.6%), and fatigue (13.1%; 1.6%). Treatment-related grade-3 transaminase elevations decreased upon corticosteroid administration; no treatment-related bilirubin increases or grade-4 transaminase elevations occurred. Disease control, including stable disease at first assessment and partial responses in triple-negative breast cancer, ovarian cancer, and immune checkpoint inhibitor (ICI)–pretreated NSCLC, occurred in 40/61 patients (65.6%). Pharmacologic activity, as measured by modulation of adaptive immunity mediators, was observed across a broad range of dose levels. Peripheral proliferating (Ki67+) CD8+ effector memory T cells and serum interferon-gamma levels showed maximum induction relative to baseline (p=0.01) 8 days following treatment.
Conclusions DuoBody-PD-L1×4-1BB demonstrated biologic activity and a manageable safety profile. Encouraging early clinical activity across different dose levels was observed in a heavily pretreated population with advanced solid tumors, including those resistant to prior immunotherapy or typically less sensitive to ICIs. Expansion cohorts of patients for whom DuoBody-PD-L1×4-1BB treatment could be relevant and biologically sound have started enrollment. Updated data will be presented.

Acknowledgements The authors thank Manish Gupta, Lei Pang, and Thomas Breuer at Genmab AS; Alice Bexon, Alexander Muik, and Friederike Gieseke at BioNTech SE; and Zuzana Jirakova (formerly at BioNTech SE) for their valuable contributions. This trial was funded by Genmab AS and BioNTech SE.

Trial Registration ClinicalTrials. gov; trial number: NCT03917381

Ethics Approval This trial is undertaken following full approval of the final protocol, amendments, informed consent form, applicable recruiting materials, and subject compensation programs by the Independent Ethics Committee/Institutional Review Board.

Consent Written informed consent, in accordance with principles that originated in the Declaration of Helsinki 2013, current ICH guidelines including ICH-GCP E6(R2), applicable regulatory requirements, and sponsor policy, was provided by the patients.

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GEN-009, A PERSONALIZED NEOANTIGEN VACCINE, ELICITS ROBUST IMMUNE RESPONSES IN INDIVIDUALS WITH ADVANCED OR METASTATIC SOLID TUMORS

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Background ATLAS™ is a cell-based bioassay that utilizes a cancer patient’s own monocyte-derived dendritic cells and CD4+ and CD8+ T cells to screen their mutanome and identify neoantigens that elicit robust anti-tumor T cell responses, as well as, deleterious InhibigensTM.1 GEN-009, a personalized vaccine comprised of 4–20 ATLAS-identified neoantigens combined with Hiltonol®, harnesses the power of neoantigen-specific T cells to treat individuals with solid tumors. The safety and efficacy of GEN-009 is being assessed in a phase 1/2a clinical trial (NCT03633110).

Methods A cohort of 15 adults with solid tumors were enrolled in the study. During the screening period, patients received standard of care PD-1-based immunotherapies appropriate for their tumor type. Subsequently, patients were immunized with GEN-009 with additional doses administered at 3, 6, 12, and 24 weeks. Peripheral blood mononuclear cells (PBMCs) were collected at baseline, pre-vaccination (D1), as well as 29, 50, 92, and 176 days post first dose. Vaccine-induced immunogenicity and persistence were assessed by quantifying neoantigen-specific T cell responses in ex vivo and in vitro stimulation dual-analyte fluorospot assays. Polymorphism of neoantigen-specific T cells was evaluated by intracellular cytokine staining. Additionally, potential correlations between the ATLAS-identified profile and vaccine-induced immunogenicity were assessed.

Results GEN-009 augmented T cell responses in 100% of evaluated patients, attributable to vaccine and not checkpoint blockade. Furthermore, neoantigen-induced secretion of IFNγ and/or TNFα by PBMCs, CD4+, and CD8+ T cells was observed in all patients. Responses were primarily from polyfunctional T EM cells and detectable in both CD4+ and CD8+ T cell subsets. Some patients had evidence of epitope spreading. Unique response patterns were observed for each patient with no apparent relationship between tumor types and time to emergence, magnitude or persistence of response. Ex vivo vaccine-induced immune responses were observed as early as 1 month, and in some cases, persisted for 176 days. Clinical efficacy possibly attributable to GEN-009 was observed in several patients, but no correlation has yet been identified with neoantigen number or magnitude of immune response.

Acknowledgements We are grateful to the patients and their families who consented to participate in the GEN-009-101 clinical trial.

Trial Registration NCT03633110

Ethics Approval This study was approved by Western Institutional Review Board, approval number 1-1078861-1. All subjects contributing samples provided signed individual informed consent.

REFERENCE

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ENHANCING T CELL THERAPY FOR PATIENTS WITH RELAPSED/REFRACTORY WILMS TUMOR

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Background Patients with relapsed or refractory Wilms tumor (WT) have poor prognoses with limited treatment options.1–3 Immunotherapy offers a promising alternative for targeted therapy but has been limited by immune evasion tactics.4–6 Adoptive cell therapy with patient-derived tumor-associated antigen-specific T cells (TAA-T) targeting 3 antigens (WT1, PRAME, and survivin) has the potential to overcome antigen loss. The objective of this phase I clinical trial is to determine the safety of administering TAA-T to patients with high-risk, relapsed/refractory solid tumors. Secondary objectives include determination of clinical efficacy and immunobiology following infusion.

Methods T cells expanded from patient peripheral blood are stimulated weekly with antigen presenting cells expressing an overlapping peptide library spanning the tumor antigens WT1, PRAME, and survivin. Following release testing, patients are infused with TAA-T on a dose escalation study, ranging from a dose of 1 x 10^7/m^2 (dose level 1) to 4 x 10^7/m^2 (dose level 3). Clinical and immunobiological studies performed post-infusion monitor for adverse effects and assess immune and disease responses.

Results Therapy with TAA-T was shown to be safe and well-tolerated in patients with high-risk solid tumors on this dose-escalation study. A total of 18 patients have been infused, with WT as the predominant diagnosis, accounting for 10 patients. We elucidated a dose-response relationship, with a prolonged median time to progression for patients treated on dose level 3 (recommended dose level [RDL]) compared to those on dose level 1 and 2 combined (5.2 vs 2.8 months, respectively) (figure 1). Patients demonstrated prolonged progression-free survival (PFS) compared to therapy received just prior to TAA-T (figure 2). Best response observed was stable disease. A majority of patients demonstrated improved anti-tumor immunity as evidenced by antigen spreading (figure 3).

Conclusions In long-term follow up, the 1-year progression-free survival (PFS) remains improved for patients treated at the recommended dose level compared to the PFS observed with therapy received immediately prior to TAA-T (29% vs 15%, respectively). Three patients are long-term (28–38 months) survivors without disease progression or further therapy. This unique immunotherapeutic has been well-tolerated without life-threatening cytokine release syndrome. To enhance TAA-T activity further in vivo, we will evaluate the safety of prescribed lymphodepletion prior to TAA-T infusion and assess anti-tumor immunity. We expect that lymphodepletion will enhance T cell persistence and expansion and recruit endogenous immune response with altered kinetics.

Ethics Approval The study was approved by the Children's National Hospital Institutional Review Board, approval number NCT02789228.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0414
Background Several immune checkpoint inhibitors (ICIs), represented by programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) antibodies, have been approved for treatment of various malignant tumors (including advanced esophageal cancer) worldwide, and previous studies confirmed that they can significantly improve overall survival.1 However, there has been limited research on the use of ICIs as neoadjuvant therapy for patients with esophageal cancer. Toripalimab is a humanized IgG4 monoclonal antibody that targets PD-1. The objective of this study is to evaluate the efficacy and safety of toripalimab plus chemotherapy as a neoadjuvant therapy regimen for treatment of patients with locally advanced esophageal squamous cell carcinoma (ESCC).

Methods This single-arm, single-center study enrolled patients with ESCC at clinical stage T2-T4/N0-N2/M0, who were eligible for radical resection and regional lymph node dissection. The patients received 2–3 cycles of toripalimab (240 mg d1, Q3W) in combination with nab-paclitaxel (260 mg/m² d1, Q3W) and carboplatin (AUC=5 d1, Q3W) before surgery. Preoperative evaluation was performed within 4 weeks after the last administration of chemotherapy. The primary endpoints were pathologic complete response (PCR) and major pathologic response (MPR), and the secondary endpoints were safety and feasibility of the neoadjuvant immunotherapy.

Results Seventeen patients diagnosed with ESCC at a pre-treatment clinical stage of T2-T4/N0-N2/M0 were included. After neoadjuvant therapy, 15 of 17 patients (88.2%) experienced downstaging and met the surgical criteria. Twelve patients (80.0%) underwent surgery without delay and 3 patients (20.0%) refused surgery. The tumors were completely removed in all 12 patients (R0 resection rate: 100%). Seven patients (58.3%) achieved MPR and 2 (16.7%) achieved PCR. The median post-surgical follow-up time was 4.5 months, and there were no recurrences. Treatment-related adverse events (TRAEs) of the neoadjuvant therapy were tolerable. Grade 3 or higher TRAEs occurred in 2 patients (11.8%), but these did not delay surgery.

Conclusions Toripalimab in combination with chemotherapy as neoadjuvant therapy showed promising anti-tumor activity with acceptable tolerance for locally advanced ESCC, as demonstrated by reducing the tumor burden, improving the R0 resection rate, reducing the postoperative recurrence rate, and no delays in surgery.

Acknowledgements N/A

Ethics Approval This study was approved by the Ethics Board of the Army Medical Center of the PLA, approval number 142(2018).

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

REFERENCE


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SQ3370–001 IS A MULTI-CENTER OPEN-LABEL PHASE I DOSE-ESCALATION STUDY TO TEST A NOVEL INTRATUMORAL AND SYSTEMIC APPROACH TO TREAT ADVANCED SOLID TUMORS

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Background Cancer immunotherapies have been very successful in recent times; however, they benefit only a subset of patients and have varying response rates across tumor types. Conversely, conventional chemotherapies are effective in a large group of patients, but have limited dosing capabilities, lack specificity, and often result in systemic adverse events. Here, we present SQ3370, a novel approach that activates doxorubicin (Dox) at the tumor site while avoiding systemic toxicities commonly associated with the therapy, and also potentially activates an immune response against tumors. SQ3370 is based on a local intratumoral injection of a pro-drug-capturing biomaterial (SQL70) followed by 5 daily systemic infusions of an attenuated form of Dox (SQP33). Mutually-reactive click chemistry groups in the 2 components allow the capture and release of active Dox at the tumor site. While conventional Dox is known to induce immune activation1 and enhance tumor responsiveness to checkpoint inhibitors,2 its benefit is limited by cumulative dose cardiotoxicity. We safely administered SQ3370 in dogs at 8.95-times the veterinary clinical dose of Dox with minimal side effects including cardiotoxicity and immunosuppression. In syngeneic mouse models, SQ3370 improved overall survival and induced a robust anti-tumor response against the biomaterial-injected lesion compared to conventional Dox. Surprisingly, SQ3370 also induced regression of the non-injected tumor and enhanced T-cell infiltration in both injected and noninjected tumors. We hypothesize that activating Dox at a local site with SQ3370 promotes activation of the native immune system against the tumor. Thus, SQ3370 represents a new therapeutic modality to treat solid tumors by using a drug with known efficacy, Dox, and expanding its therapeutic window. SQ3370 could potentially also benefit patients with widely disseminated or micro-metastatic lesions.

Methods SQ3370-001 (NCT04106492), the first-in-human Phase 1 study, is currently open in the United States and Australia to treat patients with advanced solid tumors. SQ3370-001 is enrolling patients ≥18 years of age with an injectable local or metastatic lesion, for which published data indicates responsiveness to anthracyclines. Patients must be relapsed or refractory following standard of care therapy and must not have received more than 225 mg/m² of Dox (or equivalent anthracycline). Each cycle will be for 21 days with no limit on total cycles. Primary objectives include determining the safety, tolerability, and recommended Phase 2 dose. Additional
objectives include assessment of the pharmacokinetic profile, preliminary efficacy per RECIST 1.1, and immune response.

**Results** N/A

**Conclusions** N/A

**Acknowledgements** The authors would like to thank the National Institutes of Health (NIH), the National Science Foundation (NSF), and Y Combinator.

**Ethics Approval** This study was approved by: 1. The Institutional Review Board (IRB) of Stanford University; eProtocol Number: 54928.2. The IRB of The University of Texas MD Anderson Cancer Center; IRB ID Number: 2020-0185_MOD0013. Western IRB, on behalf of The Angeles Clinic and Research Institute and Henry Ford Health System IRB Office; IRB Tracking Number: 20200758.4. Bellberry Limited Human Research Ethics Committee, on behalf of Royal North Shore Hospital and Chris O’Brien Lifehouse; Application Number: 2019-10-848.

**REFERENCES**


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**417**

**DESIGN AND RATIONALE OF A PHASE 1 STUDY EVALUATING AMG 256, A NOVEL, TARGETED, IL-21 RECEPTOR AGONIST AND ANTI-PD-1 ANTIBODY, IN PATIENTS WITH ADVANCED SOLID TUMORS**

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**Background** Checkpoint inhibitors are a promising therapy for patients with solid tumors; however, many patients require additional therapies to maximize clinical benefit or overcome resistance. 1 The type-1 cytokine interleukin-21 (IL-21) is a promising candidate for combination and has shown clinical activity in melanoma and renal cell cancer. 2 IL-21 has also shown improved efficacy when combined with anti-programmed death (PD-1)-1 antibodies in preclinical models. 3, 4 AMG 256 is a mutated IL-21 cytokine fused to an anti-PD-1 antibody to combine IL-21 pathway stimulation with checkpoint inhibition—a strategy that is designed to prime and extend the activity of cytotoxic and memory T cells and induce anti-tumor immunity. This first-in-human (FIH) study will assess safety, tolerability, and estimated dosing of AMG 256 monotherapy in patients with advanced solid tumors.

**Methods** This is a FIH, multicenter, non-randomized, open-label, phase 1 study (NCT04362748) of AMG 256 in patients with advanced solid tumors. The planned sample size is approximately 100 patients in two parts: part 1 will evaluate safety, tolerability, pharmacokinetics (PK), pharmacodynamics, and determine the maximum tolerated dose (MTD), part 2 will evaluate the MTD determined in part 1 to further characterize the safety profile and preliminary tumor response. AMG 256 will be delivered by intravenous (IV) infusion. Enrollment criteria include adults with life expectancy of > 3 months, ECOG performance status ≤ 2, histologically or cytologically confirmed metastatic or locally advanced solid tumors not amenable to curative treatment with surgery or radiation, and at least one measurable lesion ≥ 10 mm that has not undergone biopsy within 3 months of screening scan. Exclusion criteria include primary brain tumor, untreated or symptomatic brain metastases, currently receiving treatment in another investigational device or drug study, or less than 28 days since ending treatment on another investigational device or drug study, history of solid organ transplantation or major surgery within 28 days of study day 1, live vaccine therapy within 4 weeks prior to study day 1, and active infection requiring oral or IV therapy. The primary endpoints are incidence of dose-limiting toxicities and adverse events, MTD, and recommended phase 2 dose. Secondary objectives will evaluate PK parameters, preliminary antitumor activity (objective response, duration of response, progression-free survival, disease control rate, duration of stable disease, overall survival), and immunogenicity of AMG 256 via incidence of anti-AMG 256 antibodies.

**Results** N/A

**Conclusions** N/A

**Acknowledgements** * The authors thank the investigators, patients, and study staff who are contributing to this study. The study was sponsored and funded by Amgen Inc. * Medical writing support was provided by Christopher Nosala (Amgen Inc.).

**Trial Registration** NCT04362748

**Ethics Approval** The study was approved by all institutional ethics boards.

**REFERENCES**


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**418**

**A PHASE 1, DOSE ESCALATION AND DOSE EXPANSION STUDY OF SQZ-PBMC HPV AS MONOTHERAPY AND IN COMBINATION WITH ATEZOLIZUMAB IN HLA-A*02+ PATIENTS WITH HPV16+ RECURRENT, OR METASTATIC SOLID TUMORS**

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**Background** SQZ-PBMC-HPV is a therapeutic cancer vaccine created with Cell Squeeze®, a proprietary cell-engineering system. SQZ-PBMC-HPV is a novel cancer vaccine generated from peripheral blood mononuclear cells (PBMC) squeezed with HPV16 E6 and E7 antigens, resulting in delivery into the cytosol. The resulting antigen presenting cells (APCs) provide enhanced antigen presentation on MHC-I to potentially
elicit robust, antigen-specific CD8+ T cell responses. Importantly, SQZ-PBMC-HPV are neither genetically modified nor immune effector cells. Studies in MHC-I knockout mice demonstrated that activation of antigen specific CD8+ tumor infiltrating lymphocytes (TILs) was a direct effect of cytosolic antigen delivery to PBMCs. In the murine TC-1 tumor model, tumor regression correlated with an influx of HPV16-specific CD8+ TILs. In vitro studies with human volunteer PBMCs demonstrated that each subset is capable of inducing CD8+ T cell responses. The Phase 1 study includes a significant biomarker program to investigate whether pharmacodynamic effects observed in non-clinical studies correlate with potential clinical benefit. Immune genomic and pharmacodynamic endpoints include EliSpot assays to measure frequency of interferon gamma secreting cells, as well as quantification and characterization of TILs and tumor microenvironment. In addition, various cytokine responses and circulating cell-free HPV16 DNA levels in plasma are measured.

Methods SQZ-PBMC-HPV-1 (NCT04084951) is open for enrollment to HLA A*02+ patients with HPV16+ recurrent, locally advanced or metastatic solid tumors and includes escalation cohorts for monotherapy and in combination with atezolizumab. After initial demonstration of safety, the study assesses dose effect by testing different cell dose levels, the effect of prolonged antigen priming in Cycle 1 [APC administration on Day 1 only compared to Days 1 and 2 (double priming)] and the impact of treatment duration to identify the optimal dose regimen. The cycle length is 3 weeks, and patients will receive SQZ-PBMC-HPV for up to 1 year or until available autologous drug product is exhausted. Atezolizumab will be administered for up to 1 year. Eligible patients including but not limited to anal, cervical and head and neck tumors will undergo a single leukapheresis at the study site. The manufacturing process includes a maturation step and takes less than 24 hours. The vein-to-vein time for the 1st administration is approximately one week. Patients must have a lesion that can be biopsied with acceptable clinical risk and agree to have a fresh biopsy at Screening and on study. A lesion that can be biopsied with acceptable clinical risk and agree to have a fresh biopsy at Screening and on study. A Study Safety Committee is in place. No formal statistical hypothesis testing will be performed.

Results N/A

Conclusions N/A

Trial Registration NCT04084951

Ethics Approval The study is registered on clinicaltrials.gov was approved by the Ethics Board of all institution listed as recruiting.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0418

PHARMACODYNAMIC BIOMARKERS DEMONSTRATE T-CELL ACTIVATION IN PATIENTS TREATED WITH THE ORAL PD-L1 INHIBITOR INCBO86550 IN A PHASE 1 CLINICAL TRIAL

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Background Pharmacological blockade of the PD-1:PD-L1 interaction with monoclonal antibodies (mAbs) has shown durable clinical responses and overall survival benefit in a variety of malignancies.1 2 Importantly, the most meaningful responses have been associated with enhancement of the anti-tumor effector functions of T cells as evidenced by increased peripheral T-cell proliferation, infiltration of T cells in tumors, together with increased expression of key interferon-γ (IFNγ) pathway genes, including CXCL9, CXCL10, and granzyme B in both biopsy and peripheral blood samples.3 4 To date, available therapies targeting this pathway are mAbs, but the potential advantages of a small molecule, orally administered, direct antagonist of PD-1:PD-L1 binding have led to the development of INCBO86550. INCBO86550 is being evaluated in a phase 1 study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics in patients with solid tumors. This preliminary report describes peripheral pharmacodynamic activity.

Methods Peripheral blood was collected at baseline and at multiple time points posttreatment from 16 patients treated with INCBO86550 QD (100, 200 mg) or BID (200, 400 mg). Pharmacodynamic assessments included binding of drug to PD-L1 and secretion of cytokines, IL-2 and IFN-γ with ex vivo restimulation. Measurement of downstream pharmacodynamic effects included evaluation of immune activation markers on peripheral blood cells by flow cytometry and measurement of a panel of interferon-related cytokines in plasma.

Results Following INCBO86550 treatment, the ex vivo stimulation of whole blood from patients showed a dose-related reduction of up to 85% in free PD-L1 on cells after 2 hours and increases as high as 3-fold of interleukin-2 secretion after 6 hours. Increases in the proliferation of circulating T cells, as measured by Ki-67, were dose-related and as high as 2.5-fold posttreatment. Plasma concentrations of CXCL9 and CXCL10 increased following INCBO86550 treatment by 1.3- and 1.4-fold, respectively. A dose-related 1.2-fold increase in the plasma concentration of soluble target (PD-L1) and a 3.4-fold increase in IFN-γ was also observed posttreatment. Other proteins related to T-cell function, including but not limited to granzyme B, granzyme H, and LAG3, also increased following drug treatment.

Conclusions These results indicate that oral administration of INCBO86550 provides dose-related pharmacodynamic T-cell activation similar to data reported for PD-(L)1 mAbs and evidence that INCBO86550 is biologically active in blocking PD-1:PD-L1 interactions, leading to T-cell proliferation and activation in patients. This trial continues to evaluate the intratumoral pharmacodynamic activity, safety, and efficacy of INCBO86550.

Ethics Approval The study was approved by institutional review boards or independent ethics committees of participating institutions.

REFERENCES


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Background An unmet need exists for novel therapies that produce deep and durable responses in more patients with metastatic melanoma (metMEL). Encouraging clinical activity was observed with the CD122-preferential IL-2 pathway agonist bempegaldesleukin (BEMPEG) plus nivolumab (NIVO) in first-line metMEL in the phase 1/2 PIVOT-02 trial (NCT02983045), leading to FDA Breakthrough Therapy Designation. We present updated clinical results from PIVOT-02 in first-line metMEL, and biomarkers of response.

Methods 41 patients with previously untreated stage IV melanoma (known PD-L1 status by immunohistochemistry; 28–8 PharmDx) received ≥1 dose of BEMPEG (0.006 mg/kg) plus NIVO (360 mg) q3wks; 38 patients were efficacy-evaluable (≥1 post-baseline tumor scan). Primary endpoints were safety and objective response rate (ORR; RECIST v1.1; BICR); other endpoints included PFS, OS and biomarkers. Polymicrobial strength index (PSI) of circulating lymphocytes (determined using single-cell cytokine analysis [Isoplexis]) and eosinophil count (determined from hematologic analysis) at baseline and Cycle1-Day8 were analyzed using the median cut-off for correlations with ORR and PFS. Biomarkers, including CD8+ tumor infiltrating lymphocytes (TIL) and interferon-gamma (IFNg) gene expression profile (GEP), were measured in baseline tumor biopsies and analyzed for correlation with ORR and PFS.

Results At median follow-up of 25.7 months (May 2020), ORR by BICR was 53% (20/38 patients). Complete response occurred in 13/38 patients (34%): 23% PD-L1-negative (<1% tumor cell expression); 41% PD-L1-positive (≥1% tumor cell expression). Further deepening of response was observed, with 17/38 patients (45%) achieving 100% reduction in target lesions and a 79% median reduction from baseline in tumor size (previously 62%). Median time to response and time to complete response was 2.0 and 7.9 months, respectively. Median PFS and OS were not reached. 2-year OS rate was 77% (95% CI: 60–87; ITT). Safety was consistent with previous reports. IFNg GEP and CD8+ TIL in baseline tumor biopsies were significantly associated with ORR and PFS. Analysis of Cycle1-Day8 blood samples demonstrated significant increases in CD4+PSI, CD8+PSI, and eosinophils from baseline. Increased CD8+PSI was significantly associated with higher ORR and PFS; increased eosinophils were significantly associated with higher ORR.

Conclusions BEMPEG plus NIVO was well tolerated in first-line metMEL, with durable and further deepening of responses, regardless of baseline PD-L1 status. At 25.7 months' follow-up, mPFS and mOS were not reached. Early on-treatment (Day 8) increases in CD8+PSI and eosinophils in blood were identified as non-invasive biomarkers of response that are detectable well before clinical measures of response. A phase 3 trial evaluating BEMPEG plus NIVO in first-line metMEL is enrolling (NCT03635983).

Trial Registration NCT02983045

Ethics Approval The study was approved by the institutional review board of each participating site.

REFERENCE


Abstracts
Background

RP1 is an enhanced potency oncolytic HSV encoding a fusogenic protein (GALV-GP R-) and GM-CSF that has previously demonstrated tolerable safety and tumor regression alone and with nivolumab in patients with a number of tumor types. Updated data from the phase 1 expansion with nivolumab, melanoma phase 2 (enrollment complete) and further 125 patient anti-PD1 refractory cutaneous melanoma cohorts will be presented (NCT03767348). Enrollment of a second RP1 cohort; and activation of a cohort of anti-PD1 refractory and other difficult to treat melanomas, and in patients with CSCC.

Methods

Stage IIIb-IV melanoma patients for whom anti-PD-1 was indicated or who were refractory to prior anti-PD-1 alone or in combination with anti-CTLA-4, were enrolled. NMSC patients were anti-PD1 naïve. Patients received ≤8 doses of RP1 (≤10 mL/visit Q2W; first dose 10⁶ PFU/mL then 10⁷ PFU/mL) with nivolumab (240 mg IV Q2W for 4 months then 480 mg IV Q4W up to 2 years) from the second RP1 dose.

Results

As of 24th June 2020, 36 melanoma and 16 NMSC patients had been enrolled with follow up of <1–17 months. Of the melanoma patients, 16 previously anti-PD1 treated cutaneous (8 also prior anti-CTLA-4), 8 anti-PD1 naïve cutaneous, 6 mucosal, and 6 uveal. Of the NMSC patients, 10 had squamous cell (CSCC), 3 had a basal cell, 1 had Merkel cell carcinomas, and 2 had angiosarcoma. Treatment emergent adverse events (TEAEs) remain consistent with phase 1, with RP1 side effects generally of Grade 1/2 constitutional-type symptoms, with no exacerbation of the side effects expected for nivolumab. At the data cut-off, 5 previously anti-PD1 treated (4 also anti-CTLA-4) cutaneous melanoma patients, 4 anti-PD1 naïve cutaneous melanoma patients, two mucosal melanoma patients (one anti-PD1 refractory) and one uveal melanoma patient (ipi/nivo refractory) have achieved response (WHO criteria for uveal). For NMSC, for the 13 patients with >8 weeks follow up, one of two angiosarcoma patients and seven of eight CSCC patients (5 CR) have achieved response (CSCC ORR 87.5%; CR rate 62.5%, including of uninjected visceral disease). Tumor biopsies in patients continue to routinely show immune activation, including robust recruitment of CD8+ T cells, reversal of T cell exclusion, and increased PD-L1 expression. Treatment remains ongoing, and current data will be presented.

Conclusions

RP1 and nivolumab have continued to be well tolerated, with continued promising anti-tumor activity in patients with skin cancers, including those with anti-PD1 refractory and other difficult to treat melanomas, and in patients with CSCC.
Methods AST-008-102 is an ongoing Phase 1b/2 study (NCT03684785). The Phase 1b dose escalation stage examined intratumoral (IT) cavrotolimod at doses of 2, 4, 8, 16, and 32 mg in combination with pembrolizumab in patients with advanced solid tumors. Cavrotolimod was dosed once weekly for 8 weeks and once every 3 weeks thereafter. The Phase 2 dose expansion stage is examining cavrotolimod 32 mg IT in combination with IV pembrolizumab for the treatment of advanced Merkel cell carcinoma (MCC) and in combination with IV cemiplimab for the treatment of advanced cutaneous squamous cell carcinoma (CSCC). Both cohorts are enrolling patients with documented progression of disease on PD-1/L1 blockade. This analysis provides interim results of the Phase 1b stage.

Results In the Phase 1b stage, 20 patients were enrolled across all planned dose levels. No dose-limiting toxicities, grade (G)4 toxicities, or treatment-related serious adverse events (AEs) were observed. The most common AEs were injection site reactions (ISRs) and flu-like symptoms. All treatment-related AEs were < G3 except agitation and ISR (1 each). At data cutoff, ORR is 21% (4 of 19 evaluable patients) in a heterogeneous population with solid tumors. All 4 responders (2 melanoma and 2 MCC patients) have ongoing responses, with duration of response exceeding 52 weeks in 2 patients. Three of 4 responders had disease progression on PD-1 blockade at the time of enrollment, and one patient had a prior response to PD-1 blockade, but subsequently relapsed off therapy. Regression of both injected and noninjected lesions was observed. Gene expression analyses demonstrated increased IT infiltration by cytotoxic immune cells in both injected and noninjected tumors. The highest dose (32 mg) was selected for the Phase 2 stage.

Conclusions IT administration of cavrotolimod appears to be safe and well tolerated in combination with pembrolizumab. Durable responses have occurred in patients previously experiencing progressive disease on PD-1 blockade.

Trial Registration NCT03684785

Ethics Approval The study was approved by Institutional Review Boards of Dana-Farber Cancer Institute (IRB #18-584), John Wayne Cancer Institute (WIRB #20183064), University of Miami (IRB #20180957), University of Iowa (IRB #201810763), University of Cincinnati (WIRB #20183064), University of Washington (WIRB #20183064), MSKCC (IRB #20-174), UC San Francisco (WIRB #20183064), U Colorado (WIRB #20183064), Northwestern (IRB #STU00211083), U Arizona (WIRB #20183064), UC Irvine (WIRB #20183064), U Pitt (WIRB #20183064), and Washington University (WIRB #20183064).

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**INVESTIGATION OF WNT LIGAND SIGNALING REGULATORS AS A PREDICTOR OF ANTI-PD-1 RESPONSE IN METASTATIC MELANOMA**

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**Background** Responses to anti-PD-1 antibodies (aPD1) have changed the therapeutic landscape of metastatic melanoma, however predictive biomarkers of resistance are lacking. Beta-catenin pathway activation has been inversely correlated with tumor-infiltrating T lymphocytes in melanoma as well as several other solid tumors.¹ However, activating mutations involving this pathway are rare, implying that the modulation of upstream Wnt ligand/Fzd receptor (Wnt/Fzd) signaling could be a critical regulator of anti-tumor immunity. Indeed, expression of certain Wnt ligands has been associated with inferior responses to checkpoint inhibitor immunotherapy in metastatic melanoma patients.² In addition, we have further found tumor-derived paracrine and autocrine Wnt ligand signaling to drive dendritic cell tolerization and to be associated with escape from aPD1 therapy in transgenic mouse models.³ No studies to date have focused on the impact of the various regulators and components of proximal Wnt/Fzd receptor signaling on resistance to aPD1 therapy in melanoma patients. We therefore developed a unique Wnt/Fzd pathway panel using Nanostring technology to examine alterations in Wnt ligands, their receptors, and regulators as a predictor of aPD1 resistance.

**Methods** To test whether this panel could identify aPD1 resistant patients, Nanostring analysis was performed on archival FFPE tissue specimens of 12 responding (R) and 12 nonresponding (NR) metastatic melanoma patients (pts) taken prior to aPD1 monotherapy. Response was assessed radiographically by week 12 RECIST criteria.

**Results** Several components of both canonical and non-canonical Wnt ligand signaling, including regulators of autocrine/paracrine signaling, were upregulated in aPD1 NR pts
Abstracts

MK-3475-U02: PHASE 1/2 STUDY OF INVESTIGATIONAL AGENTS WITH OR WITHOUT PEMBROLIZUMAB VERSUS PEMBROLIZUMAB MONOTHERAPY IN MELANOMA


Methods MK-3475-U02 is a phase 1/2, rolling arm, multicenter, open-label, adaptive design study to evaluate the safety and efficacy of investigational agents with or without pembrolizumab or pembrolizumab alone for the treatment of melanoma. Patients will be enrolled in 1 of the 3 substudies. Substudy 02A will include patients with programmed death-1 (PD-1)–refractory melanoma (progressed after ≥2 doses of anti-PD-1/programmed death ligand-1 (PD-L1) therapy) randomized equally to treatment arms evaluating ≥1 investigational agent(s) with or without pembrolizumab. Enrollment is planned for up to ~100 patients per arm. Substudy 02B will include patients with unresectable stage III or stage IV melanoma not amenable to local therapy. Patients will be randomized 2:1 to combination (≥1 investigational agent(s) with or without pembrolizumab) or monotherapy (pembrolizumab alone) stratified by baseline lactate dehydrogenase status (normal/elevated) and prior adjuvant therapy with a PD-1 inhibitor (yes/no). Enrollment is planned for ~90 patients in the combination arm and ~45 in the control arm. Substudy 02C will include patients with stage IIIIB/IIIC/IIID melanoma who are candidates for neoadjuvant therapy. Patients will be randomly assigned to combination (≥1 investigational agent(s) with or without pembrolizumab) or monotherapy (pembrolizumab alone). Surgical resection will be performed 6 weeks after the first dose of neoadjuvant study intervention. Enrollment is planned for ~25 patients in combination and ~15 in the pembrolizumab monotherapy arms. Treatment will continue for up to 2 years (up to 1 year neoadjuvant/adjuvant therapy for substudy 02C), until disease progression, unacceptable toxicity, or study discontinuation. The primary end points include safety (adverse events and study intervention discontinuations) for all 3 substudies; objective response rate by blinded independent central review per Response Evaluation Criteria in Solid Tumors 1.1 for substudies 02A and 02B, and pathologic complete response (pCR) as assessed by central review of the pathology results for substudy 02C. Secondary end points include duration of response for substudies 02A and 02B, and recurrence-free survival, near pCR, and pathological partial response rates for substudy 02C.

Results N/A

Conclusions N/A

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Trial Registration NCT04305041, NCT04305054, NCT04303169

Ethics Approval The study protocol and all amendments were approved by the relevant Institutional Review Board or ethics committee at each study site. All patients provided written informed consent to participate in the clinical trial.

Consent N/A

REFERENCE

N/A

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A259
A PHASE 1B/2 STUDY OF CABOZANTINIB IN COMBINATION WITH PEMBROLIZUMAB IN ADVANCED MELANOMA

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Background In the United States, melanoma is the fifth leading cancer in men and the seventh in women. Immunotherapy has improved antitumor activity and survival. Overall response rate (ORR) with single agent PD-1 inhibitor is 35%, and 55% with the combination of PD-1/CTLA-4 inhibitors but with significant grade 3–4 toxicity.1 2 Cabozantinib inhibits multiple receptor tyrosine kinases, including c-MET and vascular endothelial growth factor receptor 2 (VEGFR2), and has been shown to have immunomodulatory effects in vitro and in murine models.3 In addition, c-Met has been found to induce overexpression of PD-L1.4 We hypothesize that combination treatment with these two drugs has the potential to improve response rate in metastatic or recurrent melanoma, without significant regimen-limiting toxicities.

Methods This trial in progress is an open-label, single center Phase 1b/2 study of the combination of cabozantinib and pembrolizumab in patients with advanced melanoma. Eligible patients have stage IV or recurrent/metastatic inoperable melanoma, treatment naive for immunotherapy. Prior BRAF and MEK inhibitor is allowed in metastatic setting. Exclusion criteria includes those with ocular or mucosal melanoma or uncontrolled CNS metastases. The trial is currently recruiting. The phase 1b study is based on a 3+3 design with a fixed dose combination of cabozantinib (40, 20 and 60 mg), administered orally daily. The primary endpoint of the phase 1b study is safety of the combination in metastatic melanoma patients. The phase 2 study will be conducted in two stages to evaluate the preliminary efficacy of combination cabozantinib and pembrolizumab, with up to a total of 44 subjects. The study will be terminated early if five or fewer subjects respond in the first stage; otherwise, additional subjects will be accrued. The primary endpoint is ORR. The secondary endpoints are disease control rate (DCR), duration of DCR, time to response, progression-free survival and overall survival. Exploratory endpoints include assessing biomarkers as a measure of clinical efficacy.

Results N/A

Conclusions N/A

Trial Registration NCT03957551

Ethics Approval The study was approved by The University of Iowa’s Institutional Review Board, approval number 201904712.

REFERENCES

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Background Previous findings from the MASTERKEY-265 phase 1b study showed that the combination of T-VEC and pembrolizumab was well tolerated and produced a high complete response (CR) rate of 43% in patients with advanced melanoma. The 3-year progression-free survival (PFS) and overall survival (OS) rates at that time were 53.6% and 71%, respectively. Here, we report the results of the long-term follow-up efficacy analyses.

Methods The MASTERKEY-265 phase 1b trial (NCT02263508) was an open-label, single-arm study that enrolled patients who had unresectable, stage IIIB-IVM1c melanoma with injectable, measurable lesions and no prior systemic treatment. T-VEC was administered intravenously at the time of disease progression or intolerance on HHI therapy.

Results As of the data cutoff (Mar 2, 2020), all 21 patients enrolled were off treatment; 6 died and 15 are in long-term follow-up. The median follow-up time was 58.6 months (range: 1.4–61.6). The CR rate remained 43% (9/21 patients). Twelve of the 13 responders (92.3%) are still in follow-up efficacy analyses.

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Ethics Approval The study protocols and all amendments were approved by the institutional review board at each participating study site. The study was conducted in accordance with the principles of the Declaration of Helsinki and with Good Clinical Practice guidelines as defined by the International Conference on Harmonization. All patients provided written informed consent before enrollment.

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Background Fifty percent of patients with uveal melanoma (UM) develop metastatic disease, surviving 6–12 months from metastatic diagnosis. Liver-directed therapies, immunotherapy, targeted therapy and chemotherapy have limited activity. Lymphocyte activation gene 3 (LAG-3) is an immune checkpoint receptor associated with decreased T-cell effector function and tumor escape. Preclinical models have shown that dual inhibition of LAG-3 and PD-1 blockade generates synergistic anti-tumor activity. In uveal melanoma, CD8+ T cells express the checkpoint receptor LAG3 to a greater extent than PD1 or CTLA4. This recent discovery nominates LAG3 as a potential candidate for checkpoint inhibitor immunotherapy in UM.

Methods This is an open-label, single arm, single site investigator-initiated phase II study. Based on Simon two-stage mini-max design, 13 patients will be enrolled in Stage 1. If at least one response is noted, the study will proceed to Stage 2 and enroll additional 14 patients. The null hypothesis will be rejected if 4 or more responses are observed among 27 patients. This design achieves 5% type I error and 80% power when the true ORR is 20%. Main eligibility criteria includes patients with biopsy proven metastatic uveal melanoma, previously untreated with PD-1, CTLA4 and/or LAG3 blocking antibodies and in good performance status. Enrolled patients will be treated in the outpatient setting. Nivolumab 480 mg will be mixed in the same bag with relatlimab 160 mg and administered intravenously over 60 minutes every 4 weeks until disease progression or intolerable toxicity for up to 24 months. The primary endpoint is best objective response rate (ORR). Secondary endpoints include disease control rate (DCR), progression-free survival (PFS), overall survival (OS), median duration of response (mDOR), and adverse events.

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(AEs). Correlative studies will evaluate pre- and post-treatment characteristics of T cells in the tumor microenvironment and blood.

Results N/A

Conclusions N/A

Ethics Approval The study was approved by the University of Miami Sylvester Cancer Center PRMC #20200847

Consent N/A

REFERENCES

PROSPECTIVE, RANDOMIZED TRIAL OF THE TUMOR LYSATE, PARTICLE ONLY VACCINE COMPARED TO THE TUMOR LYSATE, PARTICLE-LOADED, DENDRITIC CELL VACCINE TO PREVENT RECURRENCE FOR RESECTED STAGE III/IV MELANOMA

Background The autologous tumor lysate, particle-loaded, dendritic cell (TLPLDC) vaccine is safe and effective in improving 24 and 36-month disease-free survival (DFS) in patients (pts) with resected stage III/IV melanoma who completed the primary vaccine series. The tumor lysate, particle only (TLPO) vaccine has been developed to accelerate production by omitting DC isolation and ex vivo loading in favor of in vivo phagocytosis of the TL-loaded particles. We are currently conducting a randomized and double-blind trial of the TLPO vs TLPLDC to improve DFS and overall survival (OS) in patients with resected late stage melanoma.

Methods Patients with stage III/IV melanoma who were clinically disease-free after standard of care therapies were randomized to receive TLPO vs TLPLDC (2:1) as a continuation of the phase IIb trial comparing TLPLDC vs placebo (2:1). For the TLPLDC vaccine, autologous TL was loaded into yeast cell wall particles (YCWP) which were then phagocytized by isolated autologous DC ex vivo. For the placebo DC were loaded with empty YCWP. For TLPO, the autologous TL-loaded YCWP were coated with a chemoattractant and injected intradermally for in vivo phagocytosis. Some patients in the TLPLDC arm received G-CSF prior to DC harvest to minimize blood draw (60 mL instead of 120 mL without G-CSF). For all arms, six vaccine/placebo doses were administered intradermally at 0, 1, 2, 6, 12, and 18 mos. Data was analyzed by an intention-to-treat (ITT) analysis for DFS and OS by the Kaplan-Meier method and compared by log-rank test.
Results 63 pts were randomized to TLPO (n=43) vs TLPLDC (n=20). The TLPO cohort contained more females and received less chemotherapy (0% vs 10%), but otherwise were comparable. There were no differences in DFS (p=0.948) or OS (p=0.779) between the two vaccines (figures 1&2). Comparing the TLPO pts to all other pts in the phase IIb trial [TLPLDC+G-CSF (n=57), TLPLDC-G-CSF (n=46), and placebo (n=41)] the TLPO arm had improved DFS compared to placebo (p=0.019) and TLPLDC-G-CSF (p=0.001), but roughly equivalent to the TLPLDC-G-CSF arm (p=0.276) (figure 3). A similar trend was seen in OS analysis, though differences were not statistically significant (figure 4).

Conclusions TLPO and TLPLDC vaccines (without the use of G-CSF) improve DFS in patients with resected stage III/IV melanoma compared to placebo. The TLPO vaccine may offer additional advantages via reduced cost and vaccine production time. TLPO should be closely considered for further clinical trials.

Trial Registration NCT02301611: Phase IIIB TL + YWCP + DC in MelanomaTLPLDC IND#16101TLPO IND#17274

Ethics Approval This study was approved by WIRB; protocol #20141932

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432 3-YEAR RESULTS OF THE PHASE 2 RANDOMIZED TRIAL FOR TALIMOGENE LAHERPAREPVEC (T-VEC) NEOADJUVANT TREATMENT PLUS SURGERY VS SURGERY IN PATIENTS WITH RESECTABLE STAGE IIIB-IVM1A MELANOMA

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Background Neoadjuvant immunotherapies and targeted therapies for advanced melanoma are an active area of investigation. This is the first clinical trial of an approved oncolytic viral immunotherapy as a neoadjuvant treatment in advanced melanoma and the largest randomized controlled neoadjuvant trial including all types of resectable regional metastases to date. Previously published 2-year primary analysis results reported improved recurrence-free survival (RFS, HR 0.66, P=0.038) and overall survival (OS, HR 0.49, P=0.050) for neoadjuvant T-VEC plus surgery vs immediate surgery in resectable stage IIIB-IVM1a melanoma patients. Here, we report the 3-year interim analysis results.

Methods Patients with resectable stage IIIB-IVM1a melanoma and ≥1 injectable cutaneous, subcutaneous, or nodal lesions were randomized 1:1 to receive 6 doses/12 weeks of neoadjuvant T-VEC then surgery (Arm 1) vs immediate surgical resection (Arm 2). T-VEC was administered until surgery, no remaining injectable tumors, or intolerance. RFS was defined as time from randomization to the first of local, regional, or distant recurrence, or death, where patients who did not receive surgery were imputed as events at baseline. Key secondary and exploratory endpoints include safety, an RFS sensitivity analysis that censored events at the start of subsequent anticancer therapy, OS, and event-free survival (EFS), defined as time from randomization to disease progression that precludes surgery, or local, regional or distant recurrence post-surgery, or death from any cause, whichever occurs first. All P values are descriptive.

NCT02211131

Results As of April 30, 2020, median follow-up for all patients was 41.3 months. For Arm 1 vs. Arm 2, the 3-year KM estimates of RFS were 46.5% vs. 31.0% (HR 0.67, P=0.043). In the RFS sensitivity analysis that removed the potential effect of subsequent anticancer therapy on RFS, the 3-year Kaplan-Meier (KM) estimates of RFS were 49.1% for Arm 1 and 22.9% for Arm 2 (HR 0.60, P=0.022). The 3-year KM estimates of EFS were 50.3% for Arm 1 and 32.7% for Arm 2 (HR 0.58, P=0.015). For OS, the 3-year KM estimates were 83.2% for Arm 1 and 71.6% for Arm 2 (HR 0.54, P=0.061). No new safety signals were detected.

Conclusions At 3-year follow up, we continued to observe improved RFS and OS and observed improved EFS with neoadjuvant TVEC plus surgery compared with surgery alone. These results build upon the prior 2-year results to support the treatment effect of neoadjuvant TVEC on advanced resectable melanoma. The final analysis will occur at 5 years.

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Trial Registration NCT02211131

Ethics Approval The study was approved by all institutional ethics boards.

REFERENCE

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433 TALIMOGENE LAHERPAREPVEC (T-VEC) IN COMBINATION WITH IPILUMIMAB (IPI) VERSUS IPI ALONE FOR ADVANCED MELANOMA: 4-YEAR INTERIM ANALYSIS OF A RANDOMIZED, OPEN-LABEL, PHASE 2 TRIAL

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Background This is the first randomized trial evaluating an oncolytic virus with an immune checkpoint inhibitor in
advanced melanoma. Improved objective response rate (ORR) was observed for T-VEC plus IPI compared to IPI alone (39% vs. 18%; OR 2.9; 95% CI, 1.5–5.5; P = 0.002). At 3-year follow-up, median OS was not reached in either arm (HR, 0.85; 95% CI, 0.55–1.32; P = 0.480). Here we present 4-year interim analysis results including BRAF V600 mutation subgroup analysis.

Methods Patients with unresectable or metastatic (IIIB-IV) melanoma were randomized 1:1 to receive T-VEC plus IPI or IPI alone. T-VEC was injected day 1, week 1, at 106 PFU/mL, followed by 108 PFU/mL on day 1, week 4, and Q2W thereafter. IPI (3 mg/kg) was given Q3W starting day 1, week 6, up to 4 doses, for T-VEC arm; day 1, week 1 for IPI alone. Response was assessed per immune-related response criteria (irRC) Q12W until disease progression. The primary endpoint was ORR; key secondary endpoints were overall survival (OS), progression-free survival (PFS), durable response rate (DRR), and safety (NCT01740297).

Results A total of 198 patients (98 combination, 100 IPI alone) were randomized. As of February 25, 2020, median follow-up was 48.3 months for combination and 35.7 months for IPI alone. DRR improved for combination vs. IPI (33.7% vs. 13.0%; OR 3.4; 95% CI, 1.7–7.0; P = 0.001). Median PFS was 13.5 months with combination and 6.4 months with IPI (HR 0.81; 95% CI, 0.57–1.15; P = 0.23). Median OS was not reached for combination and was 50.1 months for IPI (HR 0.82; 95% CI, 0.54–1.25; P = 0.36). For combination, 47 (48.0%) patients received subsequent anti-cancer therapy vs. 64 (64.0%) for IPI; median time from randomization to first subsequent therapy was 27.7 months and 8.3 months, respectively. In subgroup analysis, patients without BRAF V600 mutation (63% combination, 60% IPI) improved DRR and PFS for combination vs. IPI alone (DRR: 33.9% vs. 5.0%; median PFS: 18.0 months vs. 4.5 months); BRAF V600 mutation positive patients (36% combination, 34% IPI) were similar between arms (DRR: 34.3% vs. 26.5%; median PFS: 4.2 months vs. 6.4 months). No additional safety signals observed in follow-up.

Conclusions The improved PFS and DRR for the combination arm at 4-year follow-up indicates continued benefit of combination therapy. Patients receiving IPI alone were more likely to receive subsequent anti-cancer therapy in a shorter time. Subsequent anticancer therapies may confound OS analysis. The BRAF mutant post-hoc analysis requires further mechanistic investigation.

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Trial Registration NCT01740297

Ethics Approval The study was approved by all institutional ethics boards.

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434 UPDATED CLINICAL DATA FROM THE MELANOMA EXPANSION COHORT OF AN ONGOING PH1/1B STUDY OF EGANELISIB (FORMERLY IPI-549) IN COMBINATION WITH NIVOLUMAB

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Background Eganelisib is a first-in-class, oral, selective PI3Kγ inhibitor. Preclinically, eganelisib reprograms macrophages/myeloid derived suppressor cells (MDSCs) from an immune-suppressive to an immune-activating phenotype and enhances efficacy of checkpoint inhibitors. Efficacy of eganelisib + nivolumab in patients with advanced melanoma resistant to immediate prior anti-PD(L)1 therapy is presented.

Methods IPI-549-01 (NCT02637531) evaluates eganelisib in advanced solid tumors, as monotherapy and in combination with nivolumab. The combination expansion dose was eganelisib 40 mg QD PO + nivolumab 240 mg Q2W IV. Combination expansion cohorts include unresectable stage III/IV melanoma patients resistant to immediate prior anti-PD(L)1 therapy. Safety, preliminary clinical activity, PK, and correlative study of blood and tumor biopsy samples were mandated.

Results As of June 1, 2020, 180 patients were treated with eganelisib + nivolumab including 40 with melanoma. The most common (>20% of patients) treatment-emergent adverse events in patients treated with eganelisib + nivolumab (N = 180) were fatigue (34.4%), increased AST (30.0%), increased ALT (26.7%), nausea (25.0%), pyrexia (25.0%), anemia (22.8%), decreased appetite (20.6%), and cough (20.6%). 85 (47.2%) patients experienced at least 1 treatment-emergent serious adverse event (SAE) and 19 (10.6%) had a treatment-related SAE. There was no toxicity unique to the melanoma cohort, and no treatment-related death as assessed by investigators. Preliminary data from the melanoma cohort show that in the efficacy-evaluable population which includes all patients (n=39) who had at least 1 post-baseline response assessment or discontinued treatment due to disease progression, the overall response rate (ORR, ie. CR [complete response] or PR [partial response]) per RECIST v1.1 is 7.7%, the disease control rate (DCR, ie. CR, PR, or SD [stable disease]) is 35.9%, and the clinical benefit rate (CBR, ie. CR, PR, or SD of at least 24 weeks from first treatment) is 17.9%, per RECIST v1.1. For patients that received ≤ 2 lines of prior systemic therapy (n=19), the ORR is 15.8%, the DCR is 52.6%, and the CBR is 36.8%. In total, there are 3 patients with PR (duration of response 4–12 months) and 4 with SD > 6 months’ treatment duration. Translational data evaluating blood MDSCs, cytokines, and proliferation of previously exhausted CD8 memory T-cells as well as changes in immune cell infiltrates from tumor biopsies will be presented.

Conclusions Eganelisib + nivolumab demonstrates an acceptable safety profile and clinical activity in patients with melanoma who were resistant to immediate prior anti-PD(L)1 therapy. Updated clinical and translational data will be presented.
A PHASE II TRIAL OF NIVOLUMAB PLUS AXITINIB IN PATIENTS WITH ANTI-PD1 REFRACTORY ADVANCED MELANOMA

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Background Immuno-therapy has changed the treatment landscape for melanoma, although many patients (pts) do not respond to treatment. While there are likely multiple mechanisms of resistance at play, one key mechanism is the generation of an immunosuppressive and metabolically harsh tumor microenvironment (TME). This is likely the result of an altered angiogenic pattern along with dysregulated metabolism of the tumor itself, which leads to hypoxia. CD8+ tumor infiltrating lymphocytes (TIL) isolated from tumors with high oxidative metabolism have an exhausted phenotype and decreased functionality (decreased IFN-γ and TNF-α production). Thus, TIL may be blunted due to failure to meet their metabolic needs. Vascular endothelial growth factor (VEGF) is a critical mediator of angiogenesis and is overexpressed in many solid tumors, including melanoma. Axitinib has high inhibitory activity for VEGF receptors 1, 2, and 3. In a preclinical B16 melanoma model, we found that anti-PD1 plus axitinib provided an improved and durable response compared to monotherapy with either agent. We hypothesize that by modulating angiogenesis, axitinib will reduce intra-tumoral hypoxia and resultant T cell dysfunction, which will re-sensitize melanoma to anti-PD1 therapy.

Methods This is an investigator-initiated, phase II trial of nivolumab plus axitinib for pts with unresectable stage III or IV melanoma who have progressed on prior anti-PD1 therapy with or without concomitant anti-CTLA4. Prior treatment with BRAF/MEK inhibitors is permitted. Pts with brain metastases are permitted if they are asymptomatic and have stable disease 2 weeks after CNS-directed treatment. Pts will receive nivolumab 480 mg IV every 4 weeks and axitinib PO 5 mg twice daily for up to two years or until progression or unacceptable toxicity. Timing of biopsies is reported in figure 1, with an optional biopsy at progression. Pts will receive an oral dose of pimonidazole 0.5 mg/m² before each biopsy to permit in vivo evaluation of intra-tumoral hypoxia. Primary endpoint: overall response rate (ORR). Secondary endpoints: safety, progression-free survival, overall survival, and correlational analyses (evaluation of hypoxia in the TME, TIL function, immune phenotype, and tumor cell metabolism). Statistical analysis includes Simon’s minimax two-stage design. The null hypothesis is that the true ORR is 10%, tested against a one-sided alternative of 25% or higher. N=31 patients with a type I error rate of 0.08 and power 0.81 when the true response rate is 0.25.

Results N/A

Conclusions N/A

References

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Combination immunotherapies

RATIONAL SEQUENCING OF IMMUNE-ONCOLOGY THERAPIES ACHIEVES DURABLE RESPONSE AND IMMUNOLOGIC MEMORY

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Background Oncologically-sound standard of care therapy often indicates ablation of draining lymphatic basins to eradicate repositories of metastatic disease. However, emerging cancer immunotherapies often necessitate intact secondary lymphoid organs to achieve maximum effect. Therefore, multimodal immune-oncology (IO) therapeutic approaches introduce an inherent paradox into the clinical management of the cancer patient: how to reconcile the clinical benefit of lymphatic ablation with the destruction of an indispensable immune organ.

Methods Here, we leverage a novel preclinical model of tobacco-signature head and neck squamous cell carcinoma (HNSCC) to examine the impact of lymphatic ablation on the efficacy of immunotherapy and to identify sequences of therapy that maximize durable response without compromising oncologically-sound standard of care therapy.

Results We show that cervical lymphatic ablation in tumor bearing animals abolishes the response to CTLA-4 blockade by eradicating lymph-node associated conventional dendritic cells and restricting CD8 T cell priming and subsequent tumor infiltration. By modelling recurrent HNSCC, we find that upfront, elective cervical lymphatic ablation eliminates the tumor response to adjuvant CTLA-4 blockade in contrast to a lymphatic-sparing approach, which preserves sensitivity to CTLA-4 blockade. In the neoadjuvant setting, we show that delayed, but not early, cervical lymphatic ablation leads to durable response after CTLA-4 blockade. Lastly, we demonstrate that a successful tumor response to CTLA-4 blockade begets long-lasting immunologic memory, resistant to delayed cervical lymphatic ablation.

Conclusions Collectively, this work addresses an inherent paradox in the delivery of combination IO therapy, informs

Abstract 435 Figure 1  Study schema


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optimal sequencing of multimodal therapy and affords a premise for the introduction of CTLA-4 blockade into the clinical management of HNSCC.

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437 ADENOVIRUS IL-12 AND DOCETAXEL IN COMBINATION WITH ANTI-PD1 AS AN EFFECTIVE TREATMENT STRATEGY FOR TNBC

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Background In 2020, over 42,000 women in the US are expected to die from Breast Cancer (BC). Triple Negative Breast Cancer (TNBC), a subtype defined by lack of estrogen receptor (ER), progesterone receptor (PR) and HER2 amplification, account for 15-20% of all BC. TNBC is more prevalent in pre-menopausal African-American and Hispanic women. Currently, chemotherapy is the standard of care for TNBC. Unfortunately, despite the high rate of initial response to neo-adjuvant chemotherapy, TNBC have higher rates of distant recurrence, and few (less than 30%) of the patients survive more than 5 years. Even though this subtype express high levels of PD-L1, the response to checkpoint inhibitor therapy have been modest. We hypothesized that the induction of cell death (Docetaxel) coupled with an immuno-activated milieu (locally injected adv.IL-12) would prime the tumor to respond to Anti-PD1 therapy. In this study, we investigated the effects of initially treating TNBC with a single dose of Docetaxel and adv.IL-12, followed by Anti-PD1 in syngeneic models.

Methods Syngeneic E0771 and 4T1 cell lines were injected into the mammary fat pad of C57BL/6, and Balb/c mice respectively. On day 0, mice in the Triple Combo group received a single dose (20 mg/kg) of Docetaxel and an intratumoral injection (1.25 × 109) of mAdv.II-12 (a replication defective adenoviral vector containing mouse IL-12 cDNA under the transcriptional control of Rous sarcoma virus long terminal repeat) (provided by Dr. Chen), followed by IP injection Anti-PD1 (InVivoMab anti-mouse PD-1 CD279) on days 3,5,7,10,12, and 14. The other groups, received single therapy following the same procedure. On day 19, Tumor Infiltrating Lymphocytes (TILS) were isolated by Ficoll gradient and submitted for immuno-phenotyping by CyTOF analysis to the HMRI ImmunoMonitoring Core, in addition, tumor lysates were used to measure cytokine expression using Millipore Sigma’s Milliplex MAP Mouse Cytokine/Chekomine Magnetic Beads panel (cat: MCYTMA7-70K). Survival status over time, as well as tumor volume (measured every 3 days) were monitored in both models.

Results Triple combination inhibited tumor growth in the 4T1 model while significantly delaying E0771 tumor progression. Triple Combo (TC) group had significantly higher number of TILS in both models, while the phenotype and cytokine expression significantly differed. In 4T1, TC increase the infiltration of both CD8 and CD4 effector cells, while significantly decreasing neutrophils. The levels of G-CSF, Rantes were significantly upregulated in this model, while pro-tumorigenic cytokines such as IL-6, LIF, IL-1b, and anti-inflammatory cytokines such as IL-9 and IL-10 were downregulated. In E0771, only effector, and IFN-g producing CD8 levels were increased in TC group. Although TC treated animals survived an average of 18 days more than single Doc treated animals, levels of IL-6, IL-1b, LIF, KC, TNFα and VEGF levels were higher at the end of the study.

Conclusions Ad.II-12 plus Docetaxel followed by Anti-PD1 therapy appears to only be beneficial to a specific subgroup of TNBC. We are actively studying the molecular difference between the two models used in this study, as well as investigating the clinical relevance of these markers using our extensive repertoire of PDXs in a humanized mouse model.

Ethics Approval The study was approved by the Houston Methodist Research Hospital IACUC committee AUP: 0320-0023

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438 SYNERGY BETWEEN SEA-CD40 AND CHEMOTHERAPEUTICS DRIVES CURATIVE ANTITUMOR ACTIVITY IN PRECLINICAL MODELS

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Background CD40 is a co-stimulatory receptor of the TNF receptor superfamily expressed on antigen presenting cells (APCs). Antibodies targeting CD40 may have antitumor therapeutic benefit by driving innate immune cell activation that supports generation of antigen-specific T cell responses. Multiple CD40-directed antibodies are in clinical development in both solid and hematologic indications and differ according to immunoglobulin isotype, affinity to CD40, and differential FcγR-binding. SEA-CD40 is an agnostic nonfucosylated, humanized IgG1 monoclonal antibody directed against CD40. SEA-CD40 is distinct from other CD40 targeted agents in clinical development as it binds with increased affinity to FcγRIIa resulting in enhanced effector function and CD40 agonism. This unique composition of SEA-CD40 could amplify immune stimulation and antitumor activity relative to other CD40-directed therapeutics.

Methods Effective immunity requires the presence of diverse antigens to drive generation of distinct antigen-specific memory T cells. SEA-CD40 in many ways works like a vaccine as it can increase active acquired immunity against endogenous tumor antigens. A potential limiting factor for maximal SEA-CD40 antitumor activity across multiple tumor types may be the limited level and diversity of tumor-associated antigens within the tumor microenvironment (TME). Chemotherapeutic agents drive tumor cell death resulting in the release and increase of tumor antigens locally within the TME. Combining chemotherapeutic agents with SEA-CD40 could facilitate robust antigen release and amplified presentation of those antigens to CD8+ T cells. Antitumor activity and immune cell changes of SEA-CD40 in combination with chemotherapeutic agents was evaluated in vitro and in vivo using human CD40 transgenic mice.

Results In preclinical mouse models, SEA-CD40 combined with chemotherapeutic agents to drive robust anti-tumor activity. The nature of the chemotherapeutic agent influenced immune cell activation within the tumor microenvironment (TME) and extent of combatability with SEA-CD40. Preclinical assessment indicates that chemotherapeutics which induce immunogenic cell death (ICD) combine with SEA-CD40 to increase curative activity compared to non-ICD-inducing chemotherapeutics. The preferred partnership of SEA-CD40 with ICD-inducing agents, such as a monomethyl auristatin E (MMAE) antibody-drug conjugate, increased curative antitumor
activity in mouse models. The combination of SEA-CD40 and chemotherapeutic agents with a T cell targeted anti-PD1 antibody could deepen and extend these anti-tumor responses.

**Conclusions** These data support continued clinical evaluation of SEA-CD40 in combination with chemotherapeutic agents and potentially in the future MMAE based ADCs. A phase 1 clinical trial is actively enrolling (NCT02376699) and includes a cohort in pancreatic cancer assessing the combination of SEA-CD40, gemcitabine, nab-paclitaxel, and pembrolizumab.

**Ethics Approval** Studies with human samples were performed according to institutional ethics standards. Animal studies were approved by and conducted in accordance with Seattle Genetics Institutional Care and Use Committee protocol #SGE-029.

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**DUAL MODES OF ACTION FOR ANTI-TIM-3 ANTIBODY MBG453 IN MYELODYSPLASTIC SYNDROMES (MDS) AND ACUTE MYELOID LEUKEMIA (AML): PRECLINICAL EVIDENCE FOR IMMUNE-MEDIATED AND ANTI-LEUKEMIC ACTIVITY**

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**Background** TIM-3 is expressed on leukemic stem cells (LSCs) and blasts in AML. 1, 2 and TIM-3 expression on MDS blasts correlates with disease progression. 3 Functional evidence for TIM-3 in AML was established with an anti-TIM-3 antibody which inhibited engraftment and development of human AML in immuno-deficient murine hosts. 4 TIM-3 promotes an autoimmune stimulatory loop via the TIM-3/Galectin-9 interaction, supporting LSC self-renewal. 4 In addition to its cell-autonomous role on LSCs/blasts, TIM-3 also has a critical role in immune system regulation, in adaptive (CD4+ and CD8+ T effector cells, regulatory T cells) and innate (macrophages, dendritic cells, NK cells) immune responses. 5 MBG453 is a high-affinity, humanized anti-TIM-3 IgG4 antibody (Ab) (stabilized hinge, S228P), which blocks the binding of TIM-3 to phosphatidylserine (PtdSer). Recent results from a multi-center, open label phase Ib dose-escalation study (NCT03066648) in patients with high-risk MDS and no prior hypomethylating agent therapy evaluating MBG453 in combination with decitabine demonstrated encouraging preliminary efficacy with an overall response rate of 58%. 6 MBG453 combined with azacitidine also showed encouraging response rates. 7 Preclinical experiments were undertaken to define the mechanism of action of the hypomethylating agent and anti-TIM-3 combination.

**Methods** THP-1 cells (a human monocytic AML cell line) were pre-treated with decitabine and co-cultured with anti-CD3 activated healthy human donor peripheral blood mononuclear cells (PBMCs) in an Incucyte-based assay to measure cell killing. The ability of MBG453 to mediate antibody-dependent cellular phagocytosis (ADCP) was measured by determining the phagocytic uptake of an engineered TIM-3-overexpressing Raji cell line in the presence of MBG453 by phorbol 12-myristate 13-acetate (PMA)-activated THP-1 cells. Patient-derived AML xenograft studies were undertaken in immune-deficient murine hosts to evaluate the combination of decitabine and MBG453.

**Results** MBG453 was determined to partially block the TIM-3/Galectin-9 interaction in a plate-based MSD (Meso Scale Discovery) assay, supported by a crystal structure of human TIM-3. 8 Pre-treatment of THP-1 cells with decitabine enhanced sensitivity to immune-mediated killing in the presence of MBG453. MBG453 was determined to mediate modest ADCP, relative to controls. MBG453 did not enhance the anti-leukemic activity of decitabine in patient-derived xenograft studies in immuno-deficient hosts.

**Conclusions** Taken together, these results support both direct anti-leukemic effects and immune-mediated modulation by MBG453. Further studies are ongoing to determine: (1) whether MBG453 can mediate physiologically relevant ADCP of TIM-3-expressing leukemic cells; and (2) the potential of MBG453 to impact the autocrine feedback loop of TIM-3/Galectin-9.

**Ethics Approval** The human tissue used in these studies was under the Novartis Institutes of BioMedical Research Ethics Board IRB, Approval Number 201252867.

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**ACTIVITY AND SAFETY OF CAMRELIZUMAB, AN ANTI-PD-1 IMMUNE CHECKPOINT INHIBITOR, FOR PATIENTS WITH ADVANCED NON-SMALL-CELL LUNG CANCER**

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**Background** Effective options are limited for patients with non-small-cell lung cancer (NSCLC) whose disease progresses after first-line chemotherapy. Camrelizumab is a potent anti-PD-1 monoclonal antibody and has shown promising activity in NSCLC. We assessed the activity and safety of camrelizumab for patients with previously treated, advanced NSCLC patients with negative oncogenic drivers.

**Methods** Patients who progressed during or following platinum-based doublet chemotherapy were enrolled. All patients received camrelizumab(200 mg)every 3 weeks or in combination with chemotherapy until loss of clinical benefit. The primary endpoint was objective response rate (ORR), other
endpoints included disease control rate (DCR), progression-free survival (PFS) and safety.

**Results** Between Aug 5, 2019, and Jun 19, 2020, we enrolled 29 patients, 25 patients were available evaluated, ORR and DCR was 36% (9/25) and 92% (23/25), respectively. 25 of 29 patients were still receiving the treatment, the median PFS was not yet achieved. Compared with those without reactive cutaneous capillary endothelial proliferation (RCCEP), patients with RCCEP had higher ORR (60% vs. 28.6%). Treatment-related adverse events (AEs) occurred in 69.0% of patients (all Grade), and the most common were RCCEP (37.9%), pneumonitis (6.9%), and chest congestion (6.9%). Treatment-related grade 3 to 4 adverse events occurred in 10.3% of patients.

**Conclusions** In patients with previously treated advanced NSCLC, camrelizumab demonstrated improved ORR and DCR, compared with historical data of the 2nd line chemotherapy, with a manageable safety profile. While patients with RCCEP derived greater benefit from camrelizumab. Further studies are needed in large sample size trials.

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**OUTCOMES OF PATIENTS WITH METASTATIC RENAL CELL CARCINOMA WITH INTERMEDIATE- OR POOR-RISK SYMPTOMATIC DISEASE WHO RECEIVED THEIR FIRST CYCLE OF NIVOLUMAB AND IPILIMUMAB WHILE BEING HOSPITALIZED**

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**Background** Nivolumab plus ipilimumab (nivo/ipi) is an approved therapy for patients with metastatic renal cell carcinoma (mRCC) who have intermediate- or poor-risk disease.1 Clinical factors that guide the selection of this regimen for patients with mRCC are urgently needed.

**Methods** We retrospectively analyzed medical records of patients with mRCC who were hospitalized because of cancer-related symptoms and received their first cycle of nivo/ipi in the inpatient setting. Clinical parameters including demographics, histology, clinical history, response and survival were collected. The 4-month survival probability, progression-free survival (PFS) and overall survival (OS) were calculated using Kaplan-Meier methods.

**Results** Between November 2017 and June 2020, 21 patients were identified that fit the search: 19 patients (91%) had poor-risk disease based on the International metastatic Renal Cell Carcinoma Database Consortium (IMDC) risk score; 17 patients (81%) had ≥4 risk factors; 9 patients (43%) had sarcomatoid features on histology. Shortness of breath (28%) and abdominal pain (19%) were the two most common reasons for hospitalization. Partial response was achieved in 14% (3/21) of patients. Median PFS for all patients was 1.7 months (95% CI 0 – 3.9); median OS for all patients was 1.7 months (95% CI 0 – 4.2); the 4-month survival probability was 36% (95% CI 25% - 47%) (figure 1).

**Conclusions** In this retrospective study, patients with mRCC who have intermediate- or poor-risk disease and are hospitalized for cancer-related symptoms derive little clinical benefit from nivo/ipi when started in the inpatient setting. Alternative more effective systemic therapies should be considered for these patients.

**Acknowledgements** We would like to thank the software developers at the Department of Genitourinary Medical Oncology at MD Anderson Cancer Center and the informatics analysts from the Department of Pharmacy Quality Improvement and Analytics at MD Anderson Cancer Center.

**Trial Registration** N/A

**Ethics Approval** This study was approved by the Institutional Review Board of MD Anderson Cancer Center, approval number PA16-0736.

**Consent** N/A

**REFERENCE**


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**ICT01, AN ANTI-BTN3A MAB THAT ACTIVATES VG9VD2 T CELLS, PLUS INTERLEUKIN-2: A POTENT AND PROMISING COMBINATION FOR CANCER IMMUNOTHERAPY**

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**Background** gdT-cells are attractive targets for cancer immunotherapy given their strong cytolytic and pro-inflammatory...
cytokine secretion activities, and the association between tumor infiltration and positive prognosis. ImCheck Therapeutics is developing ICT01, an anti-human butyrophilin-3A (BTN3A/CD277) mAb specifically activating g9d2 T-cells in a phosphoantigen (pAg)-independent manner. ICT01 is currently in a Phase 1/2a study in solid and hematologic tumors (NCT04243499). IL-2 has been shown to expand g9d2 T-cells in vitro and in non-human primates in presence of pAg. We wanted to characterize the proliferative effects of combining ICT01 with IL-2 on g982 T-cells as an approach to potentiating g9d2 T-cell mediated cancer immunotherapy.

**Methods** g9d2 T-cell activation and expansion was assessed in vitro in human PBMCs treated with ICT01+IL-2, and in vivo, in the blood of immunocompromised NCG mice engrafted with 20 × 10⁶ human PBMCs and treated with ICT01 (single IV dose, 5 mg/kg on Day 1) +IL-2 (0.3MIU/kg IP on Day 1–4). A dose-ranging ICT01 (single IV dose, 1 or 5 mg/kg on Day 1)+IL-2 combination (1 MIU SC QD on Days 1–5) study was conducted in cynomolgus monkeys.

**Results** In PBMCs cultures in vitro, ICT01 selectively activated g9d2 T-cells and IL-2 significantly enhanced ICT01-mediated g9d2 T-cell proliferation, this compartment reaching >50% of T-cells after 8 days of treatment versus ~10% with ICT01 alone. This was confirmed in vivo in mice models. Flow cytometry analysis of mice blood revealed a 5.5-fold increase in human g9d2 T-cell number in the combination groups compared to ICT01 or IL-2 alone treated animals, with g9d2 T-cell frequency reaching ~35% of the CD3+ T-cell compartment. In Cynomolgus, a specific expansion and activation of peripheral g9d2 T-cells from ~1–2% at baseline to up to 30% of T cells 7 days post ICT01 administration was observed. No ICT01 effect was observed on other immune cells. Histopathological examinations revealed a trend towards higher numbers of g9d2 T-cells in several organs in ICT01+IL-2 treated monkeys. There was no evidence for a systemic cytokine release syndrome at any time point. Adverse effects with variable severity were observed, most of them being reversible and commonly associated with IL-2 alone, and not reported in the IND-enabling GLP toxicity study with ICT01 monotherapy at doses up to 100 mg/kg.

**Conclusions** These results demonstrate the ability of ICT01 +IL-2 combination to trigger profound g982 T-cell activation and expansion, suggesting that the clinical combination of ICT01 with a lymphoproliferative cytokine (e.g., IL-2) may be a novel therapeutic approach for cancer patients.

**Ethics Approval** Pseudonymized samples isolated from healthy volunteers: whole blood by ImCheck Therapeutics under the agreement n° 7173 between ImCheck Therapeutic SAS and Elfedas, pseudonymizing volunteers: whole blood by Immunecheck Therapeutics under the agreement n° 7173 between Immunecheck Therapeutic SAS and EFS PACA (Etablissement Français du Sang Provence-Alpes-Côte d’Azur)

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**AN IMMUNOTHERAPY TRIO IN ADVANCED HNSCC FOR COORDINATED B AND T CELL ANTIGEN RESPONSE**

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**Background** Outcomes for recurrent or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC) are dismal and responses to anti-PD-1 appear best in tumors with PD-1+ T cells in proximity to PD-L1+ cells, arguing that improved outcome is associated with a pre-existing anti-cancer immune response. Based on this, we hypothesize that vaccines which prime and/or expand T cells to a spectrum of antigens overexpressed by HNSCC combined with T cell agonists, like anti-GITR, that provide costimulatory signals will improve the anti-PD-1 response rates. We have developed a cancer vaccine, DPV-001, that contains more than 300 proteins for genes overexpressed by HNSCC, encapsulated in a CLEC9A-targeted microvesicle and containing TLR/NOD agonists and DAMPs. Recently, we reported that combining anti-GITR + vaccine + anti-PD-1 augmented therapeutic efficacy in a preclinical model and now plan a phase 1b trial of this combination in patients with advanced HNSCC.

**Methods** Sera from patients receiving DPV-001 as adjuvant therapy for definitively treated NSCLC, were analyzed for IgG responses to human proteins by MAP bead arrays and results compared to TCGA gene expression data sets for HNSCC. HNSCC cell lines were evaluated by RNASeq and peptides were eluted from HLA, analyzed by mass spectroscopy and correlated against MAP bead arrays and TCGA data sets. Tumor-reactive T cells from a vaccinated patient were enriched and expanded, and used in cytokine release assay (CRA) against autologous NSCLC and partially HLA matched allogeneic HNSCC cell lines.

**Results** Patients receiving DPV-001 (N=13) made 147 IgG responses to at least 70 proteins for genes overexpressed by HNSCC, encapsulated in a CLEC9A-targeted microvesicle and containing TLR/NOD agonists and DAMPs. Recently, we reported that combining anti-GITR + vaccine + anti-PD-1 augmented therapeutic efficacy in a preclinical model and now plan a phase 1b trial of this combination in patients with advanced HNSCC.

**Conclusions** Recent observations from our lab and others have correlated IgG Ab responses with T cell responses to epitopes of the same protein. Based on the data summarized above, we hypothesize that we have induced T cell responses against a broad spectrum of shared cancer antigens that are common among adenoocarcinomas and squamous cell cancers. Our planned clinical trial will vaccinate and boost the induced responses by costimulation with anti-GITR and then sequence in delayed anti-PD-1 to relieve checkpoint inhibition. MAP
bead arrays and the peptide library generated above will be used to assess anti-cancer B and T cell responses.

Ethics Approval

The original clinical trial was approved by the Providence Portland Medical Center IRB, approval # 13-046. The proposed clinical trial has not yet been reviewed by the IRB.

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444 MHC-I SKEWING IN MUTANT CALRETICULIN-POSITIVE MYELOPROLIFERATIVE NEOPLASMS IS COUNTERED BY HETEROCLITIC PEPTIDE CANCER VACCINATION

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Background The majority of JAK2V617F-negative myeloproliferative neoplasms (MPN) have disease-initiating frameshift mutations in calreticulin (CALR) resulting in a common novel C-terminal mutant fragment (CALRMUT), representing an attractive source of neoantigens for cancer vaccines. However, studies have shown that CALRMUT-specific T cells are rare in CALRMUT MPN patients, but the underlying reasons for this phenomenon are unknown.

Methods In this study, we examine class-I major histocompatibility complex (MHC-I) allele frequency in CALRMUT MPN patients from two independent cohorts and observed that MHC-I alleles that present CALRMUT neopeptiopes with high affinity are under-represented in CALRMUT MPN patients. We speculate that this is due to an increased chance of immune-mediated tumor rejection by individuals expressing one of these MHC-I alleles such that the disease never clinically manifests. As a consequence of this MHC-I allele restriction, we reasoned that CALRMUT MPN patients would not efficiently respond to cancer vaccines composed of the CALRMUT fragment, but could do so when immunized with a properly modified CALRMUT heteroclitic peptide vaccine approach.

Results We found that heteroclitic CALRMUT peptides specifically designed for CALRMUT MPN patient MHC-I alleles efficiently elicited a cross-reactive CD8+ T cell response in human PBMC samples otherwise unable to respond to the matched weakly immunogenic CALRMUT native peptides. We also modeled this effect in mice and observed that C57BL/6J mice, which are unable to mount an immune response to the human CALRMUT fragment, can mount a cross-reactive CD8+ T cell response against a CALRMUT-derived peptide upon heteroclitic peptide immunization and this was further amplified by combining the heteroclitic peptide vaccine with blockade of the immune checkpoint molecule PD-1.

Conclusions Together, our data underscore the therapeutic potential of heteroclitic peptide-based cancer vaccines in CALRMUT MPN patients.

Ethics Approval Approval was obtained for the use of patient-derived specimens and access to clinical data extracted from patient charts by the Institutional Review Boards at Memorial Sloan Kettering Cancer Center, the Dana-Farber Cancer Institute and the Massachusetts General Hospital, as well as by the Danish Regional Science Ethics Committee. Mouse experiments were performed in accordance with institutional guidelines under a protocol approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee.

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445 BLOCKADE OF THE INHIBITORY COLLAGEN RECEPTOR LAIR-1 WITH NC410, A LAIR-2FC FUSION PROTEIN, ENHANCES ANTI-TUMOR ACTIVITY OF THE BIFUNCTIONAL FUSION PROTEIN BINTRAFUSP ALFA

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Background LAIR-1 is an immune inhibitory receptor expressed on several immune cell types including activated T cells, B cells, NK cells, macrophages, and dendritic cells. The ligands for LAIR-1 contain collagen-like domains which are commonly found in extracellular matrix collagens and complement component C1q. In numerous cancer types, including gastric, colon, ovarian, bladder, and others, upregulation of collagens has been shown to enhance tumor growth, metastases, and invasion while actively suppressing antitumor immunity. Although humans produce a natural, soluble decoy, LAIR-2, that competes with LAIR-1 for binding of collagen domains, excess LAIR ligands in the tumor often result in an immune suppressive environment.

Methods Here, we report on a novel immunotherapy approach which combined NC410, a LAIR-2-Fc fusion protein capable of blocking LAIR-1 signaling, and bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of the human transforming growth factor β receptor II (TGF-βRII) or TGF-β ‘trap’ fused via a flexible linker to the C-terminus of each heavy chain of an IgG1 antibody blocking programmed death ligand 1 (anti-PD-L1).

Results We demonstrate that the combination of NC410 and bintrafusp alfa more effectively controls in vivo tumor growth of the collagen rich MC38 colon carcinoma compared to either monotherapy. We hypothesize that this potent antitumor immune response is propagated through the synergy of activated tumor infiltrating lymphocytes and a repolarization of macrophages towards a tumoricidal phenotype. MC38 tumors treated with the combination of NC410/Bintrafusp alfa contained higher numbers of infiltrating CD4+ and CD8+ T cells and higher numbers of CD38+ and MHCII+ M1 polarized macrophages.

Conclusions This study highlights the synergy of reshaping the large suppressive myeloid cell populations often present in tumors with activation of adaptive T-cell immune responses dampened by checkpoint inhibition. The results also provide the rationale for the future evaluation of this combination therapy in the clinic.

Acknowledgements Bintrafusp alfa was kindly provided by EMD Serono under a CRADA with the NCI.

Trial Registration N/A

IMMUNOPEPTIDOME CHANGES MEDIATED BY A NOVEL ERAP1 INHIBITOR LEADS TO TUMOR GROWTH INHIBITION

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Background Clinical data demonstrates increased antigen presentation diversity is a key factor in determining response rates to checkpoint inhibitors. 1 In addition to tumour mutational burden/microsatellite instability, increased HLA heterozygosity and HLA evolutionary diversity are non-overlapping factors recently identified to further diversify the immunopeptidome and improve clinical response to checkpoint therapies. 2

Endoplasmic reticulum aminopeptidase 1 (ERAP1) is an enzyme that trims peptides loaded into classical and nonclassical class I MHC molecules. 4 Ablation of mouse ERAP modifies the immunopeptidome, resulting in improved immunogenicity, generation of CD8 T cell responses and tumor growth inhibition. 5 Recently identified selective small molecules potently inhibit ERAP1 across key species and haplotypes. 6 We report the further profiling of lead candidate ERAP1 inhibitors in human primary T cell in vitro assays and in vivo tumor models in mice.

Methods Human cancer cell lines treated with ERAP1 inhibitors in vitro or in vivo in xenograft mouse models were assessed by immunopeptidomics 9 to profile peptide repertoire changes. Novel or upregulated peptides were also tested in human immunogenicity assays. FACs analysis of T cells stimulated with Tyrosinase mRNA transduced human dendritic cells ± ERAP1 inhibition was to assess T cell repertoire changes. ERAP1 inhibitor and anti PD-1 mAb combination was assessed in syngeneic mouse tumor models to investigate tumor growth inhibition and PD end-points (e.g. IHC).

Results Analysis of human cervical, lung, colorectal and melanoma cell lines carrying distinct HLA haplotypes demonstrates a consistent and profound effect of ERAP1 inhibition on the immunopeptidome. Novel and upregulated cancer associated antigens identified in association with multiple different HLA-A and B alleles stimulate IFNγ production in primary naïve human T cell immunogenicity assays. The impact of ERAP1 inhibition on the T cell repertoire to the melanoma antigen tyrosinase is ongoing. The combination of ERAP1 inhibitor and anti PD-1 mAb led to significant tumor growth inhibition in the CT26 syngeneic mouse tumor model that correlated with increased infiltration of T cells to the tumor. Further PD end-points to be analysed include immune gene array and TCR Vbeta repertoire.

Conclusions Grey Wolf ERAP1 inhibitors significantly modify the immunopeptidome both in vitro and in vivo across a broad range of HLA and tumor types. Combination of these inhibitors with anti PD-1 leads to significant T cell infiltration and tumor growth inhibition. Thus, ERAP1 mediated modulation of the immunopeptidome has the potential to drive anti tumor T cell responses and be a transformative immunotherapy.

REFERENCES

VISTA TARGETING REMODELS THE TUMOR MICROENVIRONMENT TO OVERCOME ADAPTIVE RESISTANCE

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Background VISTA is a negative checkpoint regulator prominently expressed in the TME of a wide variety of cancers. In a preclinical model of colorectal cancer, monotherapy of small tumors (40 mm3) with anti-VISTA results in markedly slowed tumor growth. Mice bearing significantly larger tumors (600 mm3) are resistant to anti-PD-1 and anti-CTLA4 treatment and all mice die following treatment, indicating checkpoint resistance. Inclusion of anti-VISTA leads to complete rejection of 50% of tumors.

Methods The underlying therapeutic mechanisms of leading to enhanced anti-tumor immunity in both models was investigated by high-dimensional scRNAseq of the CD45+ immune infiltrate of tumors 10 days after treatment initiation.

Results In both models, anti-VISTA treatment stimulated several pathways involving myeloid activation and antigen-presentation. Multi-spectral imaging of anti-VISTA treated tumors supported increased antigen presentation, and suppression assays showed that the myeloid infiltrate was less suppressive to T cells. Transcriptional analysis of tumor-specific CD8 T cells showed that anti-VISTA therapy induced T cell pathways highly distinct from the anti-exhaustion effects of anti-PD-1 therapy.

Conclusions These data document the unique and complementary impact of targeting VISTA in contrast to PD-1 and CTLA-4 in both the myeloid and T cell lineages. These mechanistic insights strongly support the use of anti-VISTA to
overcome the checkpoint resistance seen in contemporary treatments involving PD-1.

**Ethics Approval** All mouse studies described in this work were carried out in accordance with the principles of the Guide for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee of Dartmouth College, NH, USA (protocol 2012).

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![Abstracts](Abstracts)

**444** DISCOVERY OF CLINICAL CANDIDATE IK-175, A SELECTIVE ORALLY ACTIVE AHR ANTAGONIST

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**Background** Aryl Hydrocarbon Receptor (AHR) is a transcription factor that regulates the activity of multiple innate and adaptive immune cells subsequent to binding to a diverse set of endogenous and exogenous ligands. One such endogenous AHR ligand is kynurenine, generated from the precursor tryptophan by indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase 2 (TDO2). Binding of kynurenine to AHR leads to a net immunosuppressive tumor microenvironment. In addition, increased levels of serum kynurenine are associated with resistance to checkpoint inhibitors. Given that kynurenine can be generated by both IDO1 and TDO2 and that AHR is activated by multiple other endogenous ligands, AHR inhibition provides a novel and ideal approach to overcome immunosuppression in a broad range of cancers.

**Methods** We sought to identify an orally active AHR antagonist as an immunomodulatory agent for the treatment of solid tumors. Lead optimization efforts identified IK-175 as an AHR antagonist with a favorable ADME and pharmacokinetic profile in preclinical species.

**Results** IK-175 inhibits AHR activity in rodent and human cancer cell lines as well as human and nonhuman primate primary immune cells, with concentration dependent effects on AHR target gene expression and cytokine release. IK-175 is inactive in a broad panel of kinases, receptors, and transporters. Orally administered IK-175 dose-dependently blocks ligand-stimulated-AHR activation of Cyp1a1 transcription in liver and spleen, demonstrating on-target in vivo activity in mice. IK-175 alone and in combination with an anti-PD-1 antibody demonstrates significant antitumor activity in syngeneic mouse models of colorectal cancer (CT26.WT) and melanoma (B16-IDO1). In addition, IK-175 in combination with liposomal doxorubicin demonstrates antitumor activity in syngeneic mouse models of colorectal cancer (CT26.WT and MC38).

**Conclusions** These studies provide rationale for targeting AHR in cancer patients. Ikena will evaluate the anti-tumor activity of IK-175 as a single agent in cancers with activated AHR and in combination with other therapies. Overall, our data demonstrates that IK-175 is a selective orally active AHR antagonist that inhibits tumor growth and reverses immune suppression in mouse tumors models. IK-175 is currently being evaluated in a Phase 1 clinical trial in patients with advanced solid tumors and urothelial carcinoma (Clinicaltrials.gov NCT04200963).

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**449** NEOADJUVANT CYCLIC DINUCLEOTIDES COMBINED WITH INTERLEUKIN-2 AND ANTI-PD-1 ANTI-BODY LIMIT LUNG METASTASIS OF ORTHOTOPIC BREAST TUMORS THROUGH PROLONGED NK CELL ACTIVATION

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**Background** Cyclic dinucleotides (CDN) – agonists of stimulator of interferon genes – can initiate potent anti-tumor immunity by activating antigen presenting cells which prime CD8+ T cells. Recent studies have also highlighted CDN activation of NK cells via IL-15 in T cell-resistant tumors. Thus far, limited analysis has been made of the impact of CDN-based therapies on cancer metastasis. We employed a surgical resection model of metastatic mammary carcinoma to examine the effects of surgery – a predominant breast cancer intervention – and lung metastasis on neoadjuvant therapy with CDNs combined with other clinically-relevant immunotherapies including IL-2 and anti-PD-1.

**Methods** 4T1-luciferase cells were inoculated in the mammary fat pad, palpable tumors were treated with immunotherapy starting eight days later, any remaining primary tumor was surgically resected on day 17, and metastases were monitored by luciferase imaging. Combinations of intratumoral bisphosphonate 2’3’-c-di-AMP (CDN), intraperitoneal insulin-like growth factor (IGF), albumin-IL2 fusion protein (Alb-IL2), and i.p. anti-PD-1 were tested in this model by measuring primary tumor growth and monitoring overall survival. CD8+ T cells, CD4+ T cells, or NK cells were depleted using anti-CD8 (2.43), anti-CD4 (GK1.5), and anti-asialo-GM1 antibodies, respectively, administered i.p. every 3 days beginning one day prior to treatment initiation. Immunophenotyping of primary tumors and lungs was conducted at several timepoints after starting therapy.

**Results** In mice bearing orthotopic 4T1-luciferase tumors, administration of three doses of CDN resulted in no cures in the absence of surgical resection. When administered prior to surgical resection CDN monotherapy yields a 20% cure rate and enhanced median overall survival compared to untreated mice (median survival 44.5 days vs 38 days, p=0.0026). Combination of CDN with Alb-IL2 and anti-PD-1 substantially improved survival, with 60% of mice surviving long-term. Through cellular depletions we determined that neither CD8+ nor CD4+ T cells were required for efficacy in this neoadjuvant therapy model, while NK cell depletion decreased survival rate by approximately 50%. Lung immunophenotyping of CDN/Alb-IL2-anti-PD-1-treated mice revealed a near doubling of the absolute NK cell count compared to untreated controls. More strikingly, lung infiltrating NK cells in the CDN/Alb-IL2-anti-PD-1 cohort exhibited prolonged granzyme B production compared to CDN monotherapy (6.24x higher after 6 days) and Alb-IL2 monotherapy (2.5x higher after 6 days) cohorts.

**Conclusions** Our findings suggest that combining intratumoral CDN with systemic Alb-IL2 and anti-PD-1 can delay the growth of primary breast tumors and limit metastatic outgrowth in the lungs. Efficacy is attributed to sustained cytotoxicity of NK cells.

**Ethics Approval** All mouse experiments were approved by MIT’s Committee on Animal Care, protocol #0720-070-23.

**REFERENCES**
Background rhIL-7-hyFc is a hybrid Fc-fused recombinant human interleukin-7 (NT-I7; efineptakin-alfa) with enhanced bioactivity. In a previous study, we found that a systemic administration of rhIL-7-hyFc induced antitumor effect by increasing CD8+ T cells in the tumor microenvironment. rhIL-7-hyFc monotherapy increased not only PD-1+ tumor-reactive but also intratumoral PD-1+ bystander CD8+ T cells. Therefore, we hypothesized that the activation of PD-1+ bystander T cells in tumors would enhance the antitumor activity of rhIL-7-hyFc. Here we evaluated the antitumor effect of combination therapy with rhIL-7-hyFc and a bispecific antibody (bsAb), anti-PD-L1xCD3, targeting both a tumor-associated antigen (PD-L1) and a T-cell stimulatory antigen (CD3e).

Methods In vitro cell culture. For analysis of T cell activation and cytotoxicity, splenocytes were isolated from PD-L1 knockout (KO) mice and co-cultured with either wild type (MC-38WT) and PD-L1-depleted (MC-38SPD-L1) tumor cells in the presence of bsAb for 48 hours. In vivo treatment. Tumor-bearing mice were treated subcutaneously (s.c.) with 1.25 mg/kg of rhIL-7-hyFc. An indicated dose of bsAb was daily treated intravenously (i.v.) or intratumorally (i.t.) route starting from 3 days after the rhIL-7-hyFc treatment for a total 5 times.

Preparation of tumor-infiltrating cells. Tumor tissues were harvested after 7 days of rhIL-7-hyFc treatment. Single-cell suspensions were prepared through mechanical separation followed by collagenase D and DNase I treatment.

Results Anti-PD-L1xCD3 bsAb induced the PD-L1-specific activation and cytotoxicity of CD8+ T cells in vitro (figure 1). rhIL-7-hyFc combined with a systemic administration of bsAb enhanced antitumor responses, although loss of body-weight was shown with high-dose bsAb combination (figure 2). The combination of rhIL-7-hyFc with a systemic administration of bsAb increased not only the frequency of CD8+ T cells in tumors but also the PD-1+ bystander CD8+ T cells with enhanced expression of a Granzyme B (figure 3). Intratumoral administration of high-dose bsAb enhanced antitumor response of rhIL-7-hyFc without body-weight loss (figure 4).

Abstract 450 Figure 1 (a) PD-L1 expression levels on each cell line. (b) Splenocytes isolated from PD-L1 KO mice were co-cultured with indicated tumor cells (E:T = 20:1) in the presence of bsAb. Expression levels of activation markers, such as CD69 and CD25, on the CD8+ T cells were analyzed by flow cytometry. (c) Cytotoxicity against tumors was analyzed in the presence of bsAb. Cytotoxicity was calculated using the formula: [1 - live target cells(sample)/live target cells(control)] × 100

Abstract 450 Figure 2 (a-b) Mice bearing MC-38 tumors were treated with different doses of bsAb (i.v.) as indicated in (a) (n = 5 per group). (b) Shown are mean tumor growth curves (left) and body-weight changes (right). (c-d) Mice bearing MC-38 tumors were treated either 1.25 mg/kg of rhIL-7-hyFc (s.c.), indicated doses of bsAb (i.v.), or combination of each therapy as indicated in (c). In the case of combination therapy with 1 ug bsAb, mice were treated only for the first 3 doses of bsAb because of body-weight loss (n = 5–7 per group). (d) Shown are mean tumor growth curves (left) and body-weight changes (right). Arrows indicate the dosing of bsAb. Data are represented as mean ± SEM. Statistical significance was analyzed by two-way ANOVA with bonferroni’s multiple comparisons for (b and d).

Abstract 450 Figure 3 (a) Experimental scheme for the analysis of tumor-infiltrating T cells (n = 4 per group). (b) Frequencies of CD8+, CD4+Foxp3+ T helper (Th), and CD4+Foxp3- T regulatory (Treg) cells among CD45+ cells. (c) Frequencies of CD4+Foxp3+ Treg cells among CD4+ T cells. (d) The ratio of CD8+ T cells to Treg cells. (e) Frequencies of PD-1+ cells among CD8+ T cells. (f) Frequencies of Granzyme B (GzmB) expressing cells among PD-1+ or PD-1- CD8+ T cells. Data are represented as mean ± SD. Statistical significance was analyzed by one-way ANOVA with bonferroni’s multiple comparisons. \*P<0.05; \**P<0.01; \***P<0.001

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Conclusions The combination treatment of anti-PD-L1×CD3ε bsAb with rhIL-7-hyFc enhances antitumor efficacy. Both systemic and intratumoral administration of bsAb with rhIL-7-hyFc augments antitumor effects, and intratumoral administration induced less weight loss than systemic administration. The activation of PD-1+ bystander CD8+ T cells in tumors by the combination of bsAb and rhIL-7-hyFc suggests that antitumor response may be partially mediated by the targeted activation of bystander CD8+ T cells. Our results serve as a proof-of-concept that the combination of rhIL-7-hyFc, a strong T cell amplifier, with bsAb, a tumor-targeted T-cell stimulator, would be a promising strategy for cancer immunotherapy.

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Ethics Approval This study was approved by POSTECH institutional animal care and use committee; approval number POSTECH-2020-0057.

Results BEMPEG/NKTR-262 resulted in significantly improved survival compared to BEMPEG/RT. BEMPEG/NKTR-262 efficacy was NK and CD8+ T cell-dependent, while BEMPEG/RT primarily relied on CD8+ T cells. Response to BEMPEG/NKTR-262 was characterized by a significant expansion of activated CD8+ T cells (GzmA+, Ki-67+, ICOS+, PD-1+) in the blood, which correlated with reduced tumor size (p<0.05). In the tumor, NKTR-262/BEMPEG induced higher frequencies of GzmA+ CD8+ T cells exhibiting reduced expression of suppressive molecules (PD-1+, TIM-3+), compared to BEMPEG/RT. Indeed, CD8+ T cells isolated from BEMPEG/NKTR-262-treated tumors had greater cytolytic capacity than those from BEMPEG/RT-treated mice. CD8+ T cell expansion (blood) and activity (tumor) depended upon the initial NK response, as neither occurred in the absence of NK cells. BEMPEG/NKTR-262 uniquely induced the expansion of early and high effector NK cells.

Conclusions Combining BEMPEG with NKTR-262 lead to an early and robust NK cell expansion not observed in the BEMPEG/RT combination. The improved tumor regression and survival was dependent on the NKTR-262 driven expansion of NK cells. A clinical trial of BEMPEG/NKTR-262 for patients with metastatic solid tumors is in progress (NCT03435640).

Background Previously, we demonstrated that radiation therapy (RT) combined with Bempegaldesleukin (BEMPEG; NKTR-214), a first-in-class CD122-preferential IL-2 pathway agonist, led to enhanced anti-tumor efficacy through a T cell-dependent mechanism. However, we observed only modest systemic responses to BEMPEG/RT across several murine tumor models. Therefore, we explored alternative approaches to improve systemic tumor-specific immunity. We evaluated whether intratumoral NKTR-262, a polymer-modified toll-like receptor (TLR) 7/8 agonist, combined with systemic BEMPEG treatment resulted in improved tumor-specific immunity and survival compared to BEMPEG combined with RT. We hypothesized that BEMPEG/NKTR-262 immunotherapy would promote synergistic activation of local immunostimulatory innate immune responses followed by systemic adaptive immunity to significantly improve tumor regression and overall survival.

Methods Tumor-bearing mice (CT26; EMT6) received BEMPEG (0.8 mg/kg; iv), RT (12 Gy x 1), and/or intratumoral NKTR-262 (0.5 mg/kg). Flow cytometry was used to evaluate CD4+ and CD8+ T cell activation status in the blood and/or tumor (7 days post-treatment) and NK cell activity in the tumor (1, 3 days post-treatment). The contribution of specific immune subsets was determined by depletion of CD4+, CD8+, or NK cells. CD8+ T cell activity was determined in vitro by tracking apoptosis in an Incucyte assay. Data are representative of 1–2 independent experiments (n=5–14/group) and statistical significance was determined by 1-way ANOVA (p-value cut-off of 0.05).
interferon genes agonists (STINGa) were shown to induce a potent type I interferon response in preclinical studies. The intratumoral administration of STINGa, to promote tumor inflammation, was shown to result in a protective spontaneous immune response in several murine tumor models. However, the encouraging preclinical results are not supported by recent clinical data, challenging the efficacy of unspecific monotherapy. As it is more and more clear that an effective cancer immunotherapy will require the combination of different treatment strategies, we investigate here the efficacy of combining KISIMA™ cancer vaccine with STINGa treatment.

**Methods** Mice were vaccinated with subcutaneous (s.c.) injection of KISIMA™ vaccine combined with s.c. administration of STINGa. Safety and immunogenicity were assessed by measuring temperature, serum cytokines and the peripheral antigen-specific response. Anti-tumoral efficacy as well as in depth monitoring of TILs and tumor microenvironment modulation were assessed following therapeutic vaccination in a HPV16 E6 and E7 expressing TC-1 cold tumor model.

**Results** Combination treatment was well tolerated and promoted the development of circulating antigen-specific CD8 T cells. In TC-1 tumor bearing mice, KISIMA™ therapeutic vaccination resulted in the infiltration of both antigen-specific CD8 and CD4 T cells within the tumor, as well as a switch of tumor associated macrophages polarization toward the more inflammatory type 1. Combination therapy further increased the tumor microenvironment modulation induced by KISIMA™ vaccine, promoting the polarization of inflammatory Thelper 1 CD4 T cells and increasing the effector function of antigen-specific CD8 T cells. The profound modulation of the tumor microenvironment induced by combination therapy enhanced the beneficial effect of KISIMA™ vaccination, resulting in a prolonged tumor control.

**Conclusions** Combination of KISIMA™ cancer vaccine with systemic STINGa treatment induces the development of a potent, tumor-specific immune response resulting in a profound modulation of the TME. As check-point inhibitor (CPI) therapy is ineffective on poorly infiltrated tumors, combination with therapies able to highly enhance tumor infiltration by T cells could expand CPI indications.

**Ethics Approval** The study was approved by the Canton of Geneva Ethic Board, under the license number GE165/19

**REFERENCE**


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**453**

**NOVEL COMBINATION IMMUNOTHERAPY FOR BOOSTING AND PRIMING IMMUNE RESPONSES IN PANCREATIC CANCER: STRONG ANTI-TUMOUR EFFECTS WITH INTERLEUKIN-15 AND CD40 AGONIST TREATMENT**

**Background** With the poorest 5-year survival of all cancers, improving treatment for pancreatic cancer is one of the biggest challenges in cancer research. In this era of combination immunotherapies, we sought to explore the potential of combining both priming and activation of the immune system. To achieve this, we combined a CD40 agonist with interleukin-15 and tested its potential in pancreatic cancer.

**Methods** Two different mouse models of pancreatic cancer were used to assess the potential of this combination regimen. Therefore, effects on tumour growth kinetics and survival were charted. Differential effects on immune signatures was investigated using RNA sequencing. Functional immune subset involvement was tested using different immune depletion experiments and multicolour flow cytometry in different relevant immune sites. Immune memory was checked using rechallenge experiments.

**Results** We demonstrated profound reduction in tumour growth and increased survival of mice with the majority of mice being cured when both agents were combined, including an unprecedented dose reduction of CD40 agonist without losing any efficacy (fig 1). RNA sequencing analysis showed involvement of natural killer cell and T cell mediated anti-tumour responses and the importance of antigen-presenting cell pathways. This combination resulted in enhanced infiltration of tumours by both cytotoxic T cells and natural killer cells, as well as a striking increase in the ratio of CD8+ T cells over T regulatory cells. We also observed a significant increase in numbers of dendritic cells in tumour draining lymph nodes, particularly CD103+ dendritic cells with cross-presentation potential. A critical role for CD8+ T cells and involvement of natural killer cells in the anti-tumour effect was highlighted. Importantly, strong immune memory was established, with an increase in memory CD8+ T cells only when both interleukin-15 and the CD40 agonist were combined.

**Conclusions** We demonstrated profound synergistic anti-tumour effects upon combination of CD40 agonist and interleukin-15 treatment in mouse models of pancreatic cancer. This preclinical data supports initiation of a first-in-human clinical trial.
with this combination immunotherapy strategy in pancreatic cancer.

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454 ONC LYTIC PARAINFLUENZA VIRUS 5 VECTOR ENHANCES NATURAL KILLER CELL KILLING OF LUNG TUMOR CELLS IN 2D AND 3D SPHEROID CULTURES

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Background Natural killer (NK) cells are innate immune cells with natural cytotoxicity towards both tumor cells and virus infected cells. We have developed a particle-based method for in vitro specific expansion of NK cells that yields highly cytotoxic NK cells (PM21-NK cells). There is intense interest in the use of novel oncolytic viruses with the potential to synergize with immune cells to kill tumor cells. Here we have tested the hypothesis that infection with a tumor-selective cytopathic Parainfluenza virus 5 (PIV5-P/V) vector will enhance PM21-NK cell-mediated killing of lung cancer cells in both 2-dimensional (2D) and 3-dimensional (3D) cultures.

Methods In 2D cultures, live cell time-lapse imaging, flow cytometry and luminescence-based methods were used to assess the killing efficiency of PM21-NK cells against A549 lung tumor cells infected with PIV5-P/V. Blocking antibodies were used to evaluate different NK cell activating receptors involved in recognition of infected tumor cells. IncuCyte live cell imaging system was used to assess real time killing of 3D lung spheroids by a combination of NK cells and PIV5-P/V virus. Z-stack spheroid images were captured using Keyence microscope.

Results In 2D cultures, PM21 NK cells efficiently kill A549 cells that have been infected with P/V CPI- virus and enhance the overall rate of killing compared to uninfected cell targets. Antibody blocking showed that the viral Hemagglutinin-Neuraminidase (HN) glycoprotein and NK cell receptors NKP30, NKP46 and NKG2D were involved in PM21-NK cell recognition of PIV5-P/V infected A549 cells. In 3D cultures of A549 tumor spheroids, PIV5-P/V infection was limited to the outer layer of the spheroid, with restricted spread of the infection to inner compartments. However, addition of PM21-NK cells to PIV5-P/V-infected spheroids resulted in killing of not only the infected surface of the spheroid but continued to the uninfected cells located at the center of the spheroid.

Conclusions Our data support the potential of combining oncolytic virotherapy along with PM21-NK cell adoptive therapy against lung cancer.

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455 IMPACT OF EPHB4 AND PD-1 TREATMENT ON IMMUNE INFILTRATE IN ADVANCED BLADDER CANCER

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Background Bladder cancer is the fourth most common cancer in American men with chances of 1 in 27 developing this form of cancer. Despite the progress in treating these patients with immunomodulatory agents, the vast majority of patients remain refractory to therapeutic intervention. EphB4 and EphrinB2 are induced in the tumor vasculature and modulate immune response within the tumor microenvironment. Intervention blocking Ephrin and PD-1/PD-L1 pathway has shown promising data in preclinical models. These data form the basis of clinical investigation of combined therapy in bladder cancer and other tumor types.

Methods Preclinical mouse models were treated with decoy soluble EphB4 and tumor infiltrating immune cells were profiled by RNA expression analysis post-treatment and compared to control treated mice. Next, patients were treated with soluble Eph4B in combination with anti-PD1 therapy, biopsies were obtained prior to and during the course of treatment. Biopsies were used for analysis of localized protein and RNA expression by GeoMx Digital Spatial Profiling (DSP). DSP analysis focused on tumor rich regions of interest (ROIs), adjacent stromal immune populations and microniches around vascular sites, with emphasis on sites where CD45+ T-cells were observed to be surrounding capillaries within and surrounding the tumor, presumably from extravasation.

Results In preclinical mouse models, EphB4 was found to induce several inflammatory pathways as a monotherapy including key immunomodulatory checkpoints such as PD1, PDL1, PDL2. Similarly, patients enrolled in this study were observed to have elevated T-cell infiltration in primary and secondary tumor sites, resulting in tumor mass reduction in post-treatment observations. DSP between matched samples discovered interesting differences in T-cell populations between both protein and mRNA expression. We observed evidence of tumor-debulking by decreased expression of epithelial markers such as Pan-cytokeratin and S100B within tumor ROBs, and increased infiltration within these ROIs measured by immune cell markers such as CD3 and CD163. Additionally, we observed increased GZMA expression post-treatment in peri-vascular regions suggestive of higher ongoing response by cells entering the tumor microenvironment. Additional analysis of localized RNA expression provided further support for activation of inflammatory cascades in post-treatment samples.

Conclusions These discoveries provide insights into the mechanism of action of EphB4 combination therapy in bladder cancer, providing support for a role of EphB4 acting as an adjuvant for PD1 therapy. Our results highlight the ability of EphB4 to activate the immune system both in preclinical models and in key structures within the tumor microenvironment during combination therapy.

Trial Registration NA

Ethics Approval The studies were approved by USC IRB Protocol 4B 15-11 and IACUC Protocol 20570.

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456 IMPACT OF ANGIOTENSIN II PATHWAY INHIBITION ON TUMOR RESPONSE TO ANTI PD(L)1 BASED THERAPY

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Background Angiotensin II (Ang II) has been shown preclinically to increase VEGF and TGF-β expression through AT1 receptor signaling but to decrease VEGF and TGF-β through AT2. Thus, we hypothesized that the ang II pathway might have a role in carcinogenesis and immune evasion and selectively inhibiting AT1 via angiotensin receptor blockers (ARBs)
would enhance responses in combination with PD(L)1 blockade.

Methods We pooled data on 597 patients with advanced solid tumors on 20 prospective anti-PD(L)1 based trials. Fisher’s exact tests were used to compare objective response rates (ORR) and complete response rates (CRR) in patients receiving ARBs or ACE inhibitors (ACEi) to those in patients not receiving ARBs nor ACEi. Log-rank tests and Kaplan-Meier curves were used to compare overall survival (OS) in these same groups. Data were analyzed in tumor types where at least 5 patients were taking ARBs or ACEi. Multiple logistic regression and Cox regression analyses were performed to assess the effect of ARBs on ORR/CRR and OS respectively.

Results In total, 597 patients with dozens of tumor types were pooled. Of these, 71 were taking ARBs and 82 were taking ACEi. Three tumor types had at least 5 patients taking ARBs: bladder, ovarian and prostate. ARB use was associated with improvement in ORR (77.8% vs 30.2%; p=0.019), CRR (55.6% vs 9.3%; p=0.005) and OS (median: not reached vs 14.2 months (95% CI: 7.1–22.0 months; p=0.005) in patients with bladder cancer (n=52), but not ovarian nor prostate cancer. On multi variable analysis, ARB use remained associated with improved ORR, CRR and OS in patients with bladder cancer. Five tumor types had at least 5 patients taking ACEi: prostate, ovarian, colorectal, cervical and bladder. For all five, no benefit was seen in ORR, CRR nor OS with ACEi use (all p>0.10).

Conclusions ARB use was associated with improvement in ORR, CRR and OS in patients with urotheelial or bladder cancer receiving anti PD(L)1 based therapy. No benefit was seen with ARBs in prostate or ovarian cancer nor with ACEi in any tumor type evaluated. The associated benefit seen in bladder cancer with ARBs but not ACEi may be due to selective AT1 blockade by ARBs versus dual AT1/AT2 blockade by ACEi. This data is hypothesis generating and further study is needed to determine if selective AT1 inhibition can improve outcomes when combined with anti PD(L)1 based therapy in bladder cancer and other tumor types.

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457 INTRATUMORAL INTERLEUKIN-12 ADMINISTERED AFTER CRYOABLATION DOES NOT IMPROVE SURVIVAL IN MULTIPLE BILATERAL MURINE MODELS

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Background Clinically, cryoablation is used to treat certain early stage prostate, liver, and kidney tumors in addition to bone and soft tissue sarcomas. However, for late-stage cancers, ablation is only an auxiliary step before complete resection. This leaves a gap of patients with advanced and inoperable tumors, where up to 90% of all pancreatic, and 80–85% of all prostate cancers are unresectable at diagnosis.1 2 Because cryoablation can release large amounts of antigen, it is uniquely capable of not only treating advanced, unresectable tumors, but also may induce an in situ vaccination response when combined with the appropriate immunotherapy. Previously, our results in single primary tumor models indicated that the addition of interleukin-12 (IL-12) to cryoablation (cryo) improved tumor burden and survival. We hypothesized that intratumoral injection of IL-12 after cryo would activate a strong T cell response and induce systemic immunity in bilateral tumor models.

Methods Panc02 cells were purchased from ATCC; MC38 and MB49 cells were acquired from the NIH. Female C57BL/6 mice were purchased from Jackson Laboratory. For primary tumor implantation, 1 × 10⁶ Panc02 cells and 3 × 10⁶ MC38 cells were injected subcutaneously (s.c.) in the right flank. For rechallenge, the same dose of cells was implanted on the left flank of cured mice. For bilateral models, in both the MB49 and MC38 models, 3 × 10³ and 1.5 × 10³ cells were injected s.c. in the right and left flanks respectively on the same day. For the Panc02 model, 1 × 10⁶ cells were implanted s.c. on both the right and left flanks on the same day. Tumor volume was calculated as 0.5*a*b² given the perpendicular long (a) and short (b) dimensions. Tumors measuring between 150–300 mm³ were cryoablated with three cycles of freeze/thaw using the Visual-ICETM Cryoablation System (Boston Scientific). The dose of IL-12 was 1 µg/mouse in 1.5% (w/v) chitosan acetate (CS) dissolved in DPBS, and then injected intratumorally within an hour after cryoablation. For the anti-PD-1 and isotype antibodies (BioXCell, clone: RMP1.14). 300µg was injected intraperitoneally every 3 days starting on the day of cryoablation for a total of 4 doses.

Results In the bilateral MB49 mouse bladder cancer model, the median survival for the cryo alone group was 20 days post treatment (p.t.) compared to 23 days p.t. for cryo + CS/IL-12, which was not significant, and 12 days for the untreated control group. In the bilateral Panc02 model, the median survival for both the cryo alone and cryo + CS/IL-12 groups was the same at 20.5 days p.t., compared to 10 days p.t. for the untreated control. In the bilateral MC38 model, the addition of anti-PD-1 to cryo + CS/IL-12 did not significantly improve survival compared to isotype + cryo + CS/IL-12, with a median survival of 24 days p.t. and 16 days p.t. respectively (p=0.53, Log-rank test) (figure 1). However, addition of anti-PD-1 did significantly delay ascosatal tumor growth up to 500 mm³ when compared to the isotype + cryo + CS/IL-12 (p=0.0398, Unpaired t test) (figure 2). Finally, the addition of IL-12 worsens memory in the MC38 model, where 100% of rechallenged cryo alone mice
were protected (5/5) compared to only 43% protected of the cryo + CS/IL-12 group (3/7).

Conclusions Conclusions: While cryoablation in combination with immunotherapy has the potential to treat advanced, unresectable primary tumors and distant untreated tumors, the addition of a single injection of IL-12 is not enough to induce a strong abscopal effect. Furthermore, it may actually worsen the establishment of effector memory cells. The addition of anti-PD-1 only slows abscopal tumor growth. Future work is needed to better characterize the anti-tumor mechanisms that mediate this effect.

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Abstract 458 Figure 1 Effect of MHC class I expression on response to RT and combination immunotherapy (IT-IC, anti-CTLA4, anti-CD40, CpG). A) Increased MHC class I expression in 9464D-GD2 derived tumors did not alter tumor growth or survival following treatment. B) Increased MHC class I expression did not alter immune subsets following treatment of 9464D-GD tumors with radiation and combination immunotherapy. Increased numbers of CD8+ and CD4+ T-cells was observed with both moderate and absent MHC class I expression. T regulatory cells were also effectively depleted in both treated groups.
achieved by depleting monoclonal antibodies and confirmed by flow cytometry. T-cell receptor deficient (TCR KO) mice were used to confirm findings in T-cell depletion experiments. 9464-D GD-2 parental cells have low MHC-I expression; subclones with low and moderate MHC Class I expression were obtained by flow cytometry sorting and the impact of MHC class I expression on immune cell infiltration and survival was assessed.

Results The effectiveness of RT and combination immunotherapy was not significantly reduced by NK or T cell depletion, and TCR KO mice had similar tumor growth and survival to mice that underwent T-cell depletion. Moderate MHC class I expression did not slow tumor growth or improve survival in mice bearing 9464-D GD-2 tumors (over those with low MHC-I) following treatment. Moderate MHC class I expression also did not alter individual immune cell subsets in treated tumors (figure 1). Overall, increased infiltration of CD8 T-cells, CD4 T-cells, and depletion of T regulatory cells was observed in all treated tumors (p<0.05).

Conclusions Treatment with RT and combination immunotherapy (IT-1C, anti-CTLA4, anti-CD40, CpG) may act through mechanisms that are MHC class I, NK-cell, and T-cell independent. Further investigation of the role of innate immunity and myeloid subsets in this scenario is warranted.

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Methods To test this hypothesis, BALB/c mice were injected with TSA mammary carcinoma cells and treated with RT (8Gy X3) and daily subcutaneous injections of IL-15 (5μg). Tumors were excised at day 18 and analyzed by immunostaining for NKP46+ cells on tumor sections and flow cytometry after tumor dissociation.

Results The number of intra-tumoral NKP46+ NK cells was significantly higher (p<0.005) in mice treated with IL-15 as compared to control. Whereas RT itself had no effect, it further increased NK cell numbers above what was achieved by IL-15 alone (p<0.05). In addition, tumor infiltrating NK cells expressed higher levels of CD137/4-1BB, an effect largely driven by IL-15. Finally, NK cells depletion by anti-<span class="caps">CD</span>16 GM1 before initiation of the treatment abrogated the enhanced cDC1 infiltration in tumors of mice treated with RT + IL-15, and the therapeutic effect of the combination.

Conclusions Our results strongly suggest a role for NK cells in the anti-tumor immune response induced by the combination of RT and IL-15. We are currently working to confirm the role of NK cells by using a complementary approach of engineering TSA cells to overexpress CLEC2D/Clr-b, the ligand for the inhibitory NKR-P1 NK receptor (3,4). Data obtained will improve current knowledge about the interaction of RT with IL-15 and support a rationale strategy for translation to the clinic.

REFERENCES
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Improving specific targeting of tumors through bispecific SNIPER antibodies

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Background Disialoganglioside 2 (GD2) is expressed on neuroblastomas as well as melanomas, small cell lung cancers, and sarcomas. Anti-GD2 mAb (Dinutuximab) can be used to treat these cancers and is part of the standard care for neuroblastoma. While GD2 is expressed minimally on most normal tissues, it is expressed on some nerve cells, and anti-GD2 treatment can cause neurotoxicity. A separate tumor antigen, B7H3, is overexpressed on multiple tumor types, including those listed above, with minimal expression on most normal cells and no expression on nerve cells. We developed a bispecific SNIPER antibody, INV721, to simultaneously target these 2 tumor antigens, with one arm specific to GD2 and the other arm to B7H3. The individual Fab arms targeting GD2 and B7H3 are each low to moderate affinity, such that INV721 will only bind with high affinity when both arms bind to their antigens on the same cell, resulting in high-specificity of the SNIPER to tumor cells.

Methods INV721 binding to GD2/B7H3-expressing tumors was confirmed by flow cytometry, as well as in tumor-bearing mice injected with 89Zr-labeled to monitor in vivo biodistribution via positron emission tomography imaging. Antibody-dependent cellular cytotoxicity (ADCC) testing of INV721 was performed on human neuroblastoma and melanoma cell lines with an Incucyte spheroid-killing assay. In vivo efficacy studies were carried out in mice bearing GD2/B7H3-expressing melanoma tumors to test our in situ vaccine (ISV) regimen, which included testing combinations of external beam radiation therapy (RT, 12Gy) + INV721 (40 μg/dose) ± IL2 (75K U/dose). Results INV721 showed binding by flow cytometry to tumors that express both GD2 and B7H3 but minimal binding to cells that don’t express both antigens. 89Zr-IN721 showed elevated and persistent accumulation in the tumor with minimal uptake in normal tissues. Incucyte spheroid-killing assays revealed that INV721 was capable of ADCC. The ISV combination of RT+INV721+IL2 was capable of curing mice bearing ~57 mm3 melanoma tumors (12/12 mice tumor free), with >70% of these mice exhibiting long-term immune memory.

Conclusions INS721 binds to cells that express both GD2 and B7H3, and these preliminary studies show that INV721 is effective in our ISV regimen at curing mice bearing tumors that express these antigens. We are continuing our efforts to determine if INV721 is associated with less pain than Dinutuximab. The goal of this SNIPER-antibody is to enhance the tumor-specific delivery of therapeutic mAbs, which may decrease toxicity and improve efficacy for cancers expressing both GD2 and B7H3.

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Single agent immunotherapy response in patients with head and neck squamous cell carcinoma with prior history of radiation therapy

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Background Locally advanced head and neck squamous cell carcinomas (HNSCC) are treated with multidisciplinary approach which includes radiation therapy. Immunotherapy with nivolumab or pembrolizumab is used for platinum refractory disease. We analyzed the association of radiation treatment patterns and immunotherapy responses HNSCC.

Methods We performed a retrospective analysis at University of New Mexico Comprehensive Cancer Center for patients with diagnosis of HNSCC treated at our institution between 2011 and 2020 with immunotherapy agent’s nivolumab and pembrolizumab. Our cohort included 21 patients with previous history of definitive radiation therapy for HNSCC who received immunotherapy for recurrent disease, as part of adjuvant treatment as either front-line or second line therapy. In terms of response, patients were divided into responders (R) and non-responders (NR). Responders were defined as the presence of partial remission in initial imaging or stable disease for a period of six months or longer.

Results Of our 21 patients, 10 patients were R and 11 patients were NR. p16 positivity was 6 (60%) in R vs 3 (27%) in NR. 8 patients in R group (80%) and 10 patients in NR group (91%) had prior platinum based chemotherapy concurrent with radiation or for recurrence as salvage
chemotherapy. All patients had radiation therapy prior to immunotherapy for adjuvant or for definitive treatment. Time from last day of radiation treatment to start of immunotherapy was 47 months in R group while it was 9 months in NR group (P<0.05). (Figure 1) There was no difference in time from radiation to immunotherapy depending on the P16 status. Immunotherapy was stopped after completing 2 years of immunotherapy for 3 patients. 2 of those patients resumed immunotherapy due to progression, and continue to have response after resuming treatment. One of these patients received SBRT to lung nodule after resuming immunotherapy.

Conclusions Immunotherapy with single agent PD-L1 inhibitor is used for platinum refractory disease in HNSCC, however response rates are low. Our study shows that the patients who had early recurrence and received immunotherapy closer to definitive radiation therapy had lower response rate. Therefore we need further studies to investigate changes in immune micorenvironment with radiation therapy for better immune targeting of patients with early recurrence after radiation treatment.

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METASTATIC GASTRIC CANCER PATIENT BENEFITING FROM COMBINED RADIO-IMMUNOTHERAPY TREATMENT DISPLAYED SUSTAINED ANTI-NY-ESO-1 SPECIFIC T CELLS AND EXPRESSED IMPORTANT IMMUNO-MODULATORY MARKERS


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Background Combined radio-immunotherapy is currently being investigated to treat cancer patients. Anti-PD-1 immunotherapy offers the prospect of long-term disease control in solid tumors. Radiotherapy has the ability to promote immunogenic cell death leading to the release of tumor antigens, increasing infiltration and activation of T cells. NY-ESO-1 is a cancer-testis antigen expressed in 20% of advanced gastric cancers and known to induce humoral and cellular immune responses in cancer patients. We report on the dynamic immune response to the NY-ESO-1 antigen and important immune-related biomarkers in a metastatic gastric cancer patient treated with radiotherapy combined with anti-PD-1 pembrolizumab antibody.

Methods Our patient was an 81-year-old male diagnosed with locally advanced unresectable MMR-deficient gastric cancer having progressed to a metastatic state under a second line of systemic treatment consisting of an anti-PD-1 pembrolizumab antibody. The patient was subsequently treated by local radiotherapy administered concomitantly with anti-PD-1, with a complete response on follow-up radiologic assessment. Disease control was sustained with no further therapy for a period of 12 months before relapse (figure 1).

Results We have identified an NY-ESO-1-specific IFN-? secretion from the patients T cells that was significantly increased at response (****p<0.0001) (figure 2). A novel promiscuous immunogenic NY-ESO-1 peptide P39 (P153-167) restricted to the 4 patient’s HLA-DQ and HLA-DP alleles was identified. Interestingly, this peptide contained the known NY-ESO-1-derived HLA-A2-02:01(P157-165) immunogenic epitope. We have also identified a CD107+ cytotoxic T cells subset within a specific CD8+/HLA-A2-NY-ESO-1 T cell population that was low at disease-progression, markedly increased at disease-resolution and significantly decreased again at disease-re-progression (figure 3). Finally, we identified 2 groups of cytokines/chemokines. Group 1 contains 5 cytokines (IFN-?, TNF-a, IL-2, IL-5 and IL-6) that were present at disease progression, significantly downregulated at disease resolution and dramatically upregulated again at disease re-progression. Group 2 contains 4 biomarkers (Perforin, sFAS, MIP-3a and CXCL-11/ITAC) that were present at disease progression, significantly upregulated at disease resolution and dramatically downregulated again at disease re-progression (figure 4).
Conclusions Combined radio-immunotherapy can enhance specific T cell responses to the NY-ESO-1 antigen that correlates with beneficial clinical outcome of the patient.

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Trial Registration NOT applicable

Ethics Approval The study was approved by the Institutional Review board committee of Hamad Medical Corporation, Doha, Qatar.

Consent The patient signed an informed consent form to carry out the study and to publish the data.

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464  CLONAL REPLACEMENT OF TUMOR-INFLITRATING CD8+ T CELLS BY INDUCTION AND ACTIVATION OF TUMOR-RESIDING BATF3-DEPENDENT DENDRITIC CELLS

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Background The ability of cancer cells to ensure T-cell exclusion from the tumor microenvironment (TME) is a significant mechanism of resistance to anti-PD-1/PD-L1 therapy. Evidence indicates crucial roles of Batf3-dependent conventional type 1 dendritic cells (cDC1s) for inducing antitumor T-cell immunity. However, strategies to maximize the engagement of cDC1s into such ‘immune cold tumors’ remain elusive. Using multiple syngeneic orthotopic mouse models of tumors resistant to anti-PD-L1-therapy, we hypothesized that in situ induction and activation of tumor-residing cDC1s overcomes poor T-cell infiltration.

Methods We utilized three mouse non-T cell-inflamed tumor models that are refractory to anti-PD-L1 therapy (AT-3, B16 and 4T1), and evaluated the efficacy of the combinatorial therapeutic regimen, in situ immunomodulation (ISIM) comprised of intratumoral administration of Fms-like tyrosine kinase 3 receptor ligand (Flt3L) to mobilize cDC1s to the TME, local radiotherapy (RT) to promote immunogenic death of cancer cells and maturation of DCs, and peritumoral CD40/toll-like receptor 3 (TLR3) agonists administration to activate antigen-loaded cDC1s for priming and expansion of tumor-specific CD8+ T cells.

Results Intratumoral administration of Flt3L increased the number of CD103+ DCs in the TME, and RT induced upregulation of CD40 and CD86 in the tumor-residing CD103+ DCs. In situ CD40/TLR3 stimulation facilitated trafficking of CD103+ DCs carrying tumor-associated antigens (TAA) to the tumor draining LN (TDLN), and generation of tumor-specific CD8+ T cells in TDLNs, indicating cross-presentation of TAA. Consequently, ISIM triggered infiltration of tumor-specific stem-like Tcf1+CD8+ T cells into the TME, mediated rapid regression of untreated distant and primary tumors, and rendered poorly T cell-infiltrated tumors responsive to PD-L1 blockade in multiple mouse tumor models. Moreover, T-cell receptor (TCR) sequencing of TILs revealed that ISIM

Abstract 463 Figure 2  Enzyme-linked ImmunoSpot assay for IFN-g production by T cells from patient’s peripheral blood mononuclear cells

Abstract 463 Figure 3  Phenotyping and functional characterization of patient’s T cells from peripheral blood

Abstract 463 Figure 4  Differential expression of cytokines/chemokines in patient’s plasma
facilitated the infiltration of novel clones in the TME. Importantly, serial ISIM further reshaped the TCR repertoires in the TME which had been destined to become resistant to anti-PD-L1 therapy, and rendered tumors continuously responsive to anti-PD-L1 therapy, resulting in durable complete responses and establishment of tumor-specific immunological memory.

**Conclusions** Taken together, ISIM not only increased CD8+ T-cell infiltration but also reshaped the intratumoral TCR repertoires. These findings provide insights into the utility of an in situ combinatorial immunotherapeutic regimen for overcoming resistance to anti-PD-L1 therapy due to tumor-mediated mechanisms of immune cell exclusion.

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**Background** Radiotherapy (RT) in combination with CTLA-4 inhibition (CTLA4i) can expand and activate T-cells to reject tumors in both mice and some patients with tumors unresponsive to CTLA4i alone. However, only a subset of patients achieves long-term control of metastatic disease. Similar responses to RT+CTLA4i are seen in the 4T1 mouse model of triple negative breast cancer (TNBC), making it an ideal model to interrogate the interaction between RT and CTLA4i, and identify barriers to its effectiveness.

**Methods** Mice were inoculated in one or both flanks with 4T1 cells. In some experiments one tumor was removed for RNA/TCR sequencing. The METABRIC dataset was used to associate gene expression signatures with patient survival. In some experiments, RT+CTLA4i was combined with PD-1, LAG-3, or CD40 Abs.

**Results** RT, alone and with CTLA4i, increased the TCR repertoire clonality and density of activated T cells in the tumors (figure 1A-G). In untreated tumors, Gzmb+Prf1+Lag3+Pdl1+Cd8+ T cells (cluster 0) were most common. CTLA4i `unlocked’ Ifng+Cd40lg+Cd4+ T cells (cluster 2) while RT favored expansion/persistence of Cd8+ T cell clusters. In tumors of mice treated with RT+CTLA4i activated Treg cells (cluster 1) were decreased and Ifng+Cd40lg+Cd4+ T cells (cluster 2) increased. Relatively among CD8+ T cells, Ifng+Tnf+Cd8+ (cluster 4) was expanded at the expense of cluster 0 (figure 2A-F). Gene signatures defining clusters 0, 2, and 4 were associated with improved survival in the METABRIC TNBC patient cohort using a multivariate model (figure 2G-H). In mice, AH1-tumor antigen-specific CD8+ T cells occupied different transcriptional states, with a shift to cluster 4 in mice treated with RT+CTLA4i (figure 2I), suggesting that multiple functional T cell states are required for tumor rejection. Based on the T cell phenotypes expanded by RT+CTLA4i, antibodies to PD-1, LAG-3, and CD40 were tested for the ability to enhance RT+CTLA4i therapy. Only CD40-agonist improved significantly tumor control (figure 3A-B).

**Conclusions** Altogether, these results revealed that RT and CTLA4i have complementary effects and besides driving T cells into tumors shape CD4 and CD8 T cell functional differentiation towards subsets that are associated with improved treatment outcomes.
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Abstract 465 Figure 2 RT+CTLA-4i increased tumor infiltration by Gzm-b+Prf1+Lag3+Pd1+Cd8+, Ifng+Cd40lg+Cd4+, and Ifng+Tnf+Cd8+ T cells in 4T1 tumors. (A) Design of experiment enabling single cell analysis of T cells infiltrating 4T1 tumors. (B) Based on gene expression levels, the T cells were divided into 17 clusters (indicated by colors) and visualized in 2D using UMAP dimensionality reduction algorithm. (C) Gene expression levels of selected high-level T cell markers. (D) Table with main phenotype, key genes representative for each cluster, and the distribution of T cells from each condition falling into the different clusters. (E) Proportion of Cd4+ and Cd8+ T cells for the different treatment groups. (F) The expression of cluster-specific gene signatures in bulk 4T1 tumors for clusters 0, 2, and 4. (G) Survival curves and (H) treatment groups. (I) The positioning of the all AH1-dextramer+ Cd8+ T cell clones within the UMAP plot. Color annotate cluster. (Abbreviations) tx, treatment; RT, radiation therapy; CTLA4, CTLA-4 Ab; TCR CDR3, T cell receptor annotate cluster. (Abbreviations) RT, radiation therapy; CTLA4, CTLA-4 Ab; CD40, anti-CD40 Ab; mm3, cubic millimeter; d, days

survival in patients. Unexpectedly, inhibition of checkpoint receptors expressed by a large CD8 T cells cluster did not further improve responses to RT+CTLA4i, whereas agonistic CD40 therapy did, suggesting new therapeutic strategies.

Acknowledgements Grant support: R01CA198533

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Abstract 465 Figure 3 Agonistic CD40 treatment improves RT+CTLA-4 therapy. Individual tumor growth curves for untreated, RT+CTLA4, or RT+CTLA-4+CD40 treated mice. Color annotate group. *, **, *** and **** indicate p-values < 0.05, 0.01, 0.001, and 0.0001, respectively, calculated using a linear mixed-effects model. (A) and (B) represent two individual experiments. (Abbreviations) RT, radiation therapy; CTLA4, CTLA-4 Ab; CD40, anti-CD40 Ab; mm3, cubic millimeter; d, days


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467 ANTI-EGFR ANTIBODY ADDED TO ONGOING ANTI-PD-1 ANTIBODY TREATMENT FOR METASTATIC CUTANEOUS SQUAMOUS CELL CARCINOMA OF THE FACE: TWO CASE REPORTS

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Background Recurring cutaneous squamous cell carcinoma (SCC) remains an area of high unmet medical need. While anti-PD-1 antibodies are now approved for this diagnosis, more than half the patients will need more effective treatments, supporting the development of new or combination regimens.1–3 Weekly cetuximab targets EGFR and has anti-tumor immunogenic properties that could complement anti-PD-1 immunotherapy. Cetuximab is being evaluated in combination clinical trials.1–3 Panitumumab also targets EGFR but is felt to function as a signal transduction inhibitor with weaker anti-tumor immunogenic properties; however, this medication is dosed every two weeks rather than weekly and has a relatively favorable toxicity profile.2

Methods Two consecutive elderly patients with significant comorbidities presented with a performance status of ECOG 3 and rapidly progressive recurrent cutaneous SCC of the face. The patients were presented treatment with an anti-PD-1 antibody, with an option - were there an inadequate palliative response - to include an EGFR antibody provided tolerance was acceptable. The first cycle of pembrolizumab 2 mg/kg or nivolumab 3 mg/kg, respectively, escalating in both cases to flat dosing once it was apparent that tolerance was acceptable. The first cycle of panitumumab (6 mg/kg), when needed to be invoked, was administered solo between two cycles of PD-1 inhibitor, then every two weeks while the PD-1 inhibitor continued every two - four weeks.

REFERENCES


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467 ANTI-EGFR ANTIBODY ADDED TO ONGOING ANTI-PD-1 ANTIBODY TREATMENT FOR METASTATIC CUTANEOUS SQUAMOUS CELL CARCINOMA OF THE FACE: TWO CASE REPORTS

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REFERENCES

Results A 78 year old women with significant cardiac disease and a St Jude tissue aortic valve, had undergone prior surgeries and radiation therapy for her recurring SCC of the face followed then by major resection, parotidectomy, flap reconstruction, and supraomohyoid neck dissection; only two weeks after the latter surgery, she presented with over 20 new in-radiation field metastases (see photo below). A 90 year old woman with emphysema on home oxygen and living in a facility presented with diffuse local recurrence 4 months after orbital exenteration, parotidectomy, neck dissection, and flap. Both patients’ tumors were characterized: PD-L1 (clone E1L3N) 2% and 10%, respectively; scant peritumoral or intratumoral lymphocytes; tumor mutation burden high (33 and 30 mutations per megabase, respectively); epidermal growth factor receptor (EGFR) high (3+ by IHC, but with no gene mutations detected in EGFR, kras or nras; microsatellite stable. In the 78 yo woman, after two cycles of pembrolizumab, the – 5 mm pink nodules grew further to up to 3 cm with facial erythema, edema, sealing the eye closed. Only by criteria was this not considered pseudoprogression. Pembrolizumab was integrated between cycles 2 and 3, resulting in a dramatic abrupt response: the masses became centrally necrotic, flaking, pouring off her face with prompt resolution in edema and complete response (CR) within 2 months - now lasting over 18 months - a period during which pembrolizumab and panitumumab were continued for 27 and 26 cycles respectively). Her major toxicity was diffuse erythema involving ~ 30% of her torso; this resolved early on with triamcinolone 0.1% cream. She also developed scabs in her uninvolved scalp - some where other squamous and basal carcinomas had previously been resected and these all healed slowly (see photo), suggesting we were preventing similar future cancers from emerging in these areas. Similarly, the 90 yo woman achieved only a mixed response to nivolumab over 3 months with shrinking level V neck node but continued stubborn diffuse disease over her face and into the exenteration field. When panitumumab was added, however, there was clear improvement (See photo). With each of eight cycles, prolific crusting/scabbing would occur, shed, and reoccur, some in areas of the face without visible tumor, Mild acneiform rash and mild hypomagnesemia were readily managed. Her performance status and appetite improved and she gained back 14 pounds. After only 6 months, with pathologically confirmed CR, treatment had to be held because she was restricted to her assisted living facility in the midst of the COVID-19 pandemic. Now after a year, the remaining scabs are largely gone (see photo).

Conclusions The excellent tolerance of multiple cycles of outpatient combination treatment in these two consecutive patients with the same diagnosis, coupled with the observed durable anti-tumor clinical activity lasting now over a year - all support further exploration of panitumumab in combination with anti-PD-1 antibody treatment. A randomized trial would be needed to establish whether outcomes are truly better with the combination. Deciding on hyperprogression vs pseudoprogression while getting anti-PD-1 antibody treatment remains a challenge. Laboratory studies would evaluate how such specific signal transduction inhibition by panitumumab might interfere with immune suppressive mechanisms in metastases, rendering them more sensitive to an induced anti-tumor cellular immune response by an anti-PD-1 antibody. Finally such combination treatment should help reduce the need for increasingly cosmetically and functionally altering surgeries.

Ethics Approval ‘Per our Hartford Health Care IRB, case series of three or less patients does not constitute research.’

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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468 ENHANCERS AND REPRESSORS OF IMMUNOTHERAPY: TRANSITIONAL PERSPECTIVES ON GENE-MEDIATED CYTOTOXIC IMMUNOTHERAPY IN GLIOBLASTOMA

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Background Gene-mediated cytotoxic immunotherapy (GMCI) is a local tumor immunotherapy that uses aglalimagine besadenovec (a non-replicating serotype 5 adenovirus, expressing HSV1 thymidine kinase) with the prodrug ganciclovir to induce DNA strand breaks (DSB), leading to immunogenic tumor cell death and intratumoral immune cell invasion. Here we investigate potential repressors and enhancers of GMCI’s effectiveness. GMCI is currently in clinical trials in combination with immune checkpoint blockade in glioblastoma. Thus we set out to identify potential areas to improve this approach for future application. Dexamethasone is used in symptomatic treatment of glioma patients, although it is known to cause immune suppression. However, the influence of dexamethasone on the efficacy of GMCI has not been explored. In contrast, DNA damage response inhibitors like ATRi in vivo, the combination of ATR with GMCI led to an increase in long-term surviving animals (65.7%) compared to GMCI (50%) and proved to be highly significant compared to the untreated control (p=0.0022).

Conclusions Our data suggest that dexamethasone may decrease the efficacy of immunotherapy for glioma through impaired T cell function: this emphasizes the need in identifying alternatives to dexamethasone to prevent attenuated responses in immunotherapies. The combination of GMCI with ATRi however points to additional therapeutic benefit through enhanced cytotoxic efficacy, improved immunogenicity in vitro and increased long-term survival in vivo, making it a promising future therapy for the treatment of glioblastoma.

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Background We have previously described ATRC-101, a fully human, engineered IgG1 antibody binding a tumor-restricted ribonucleoprotein (RNP) complex as its target. ATRC-101 is currently under evaluation in the clinic as a monotherapy for solid tumors. Following target engagement, ATRC-101 functions in an Fc-mediated fashion to deliver the target to the innate immune system, which modifies the tumor microenvironment and generates an adaptive immune response involving CD8+ T cells leading to anti-tumor activity in syngeneic mouse models. Binding of ATRC-101 appears restricted to malignant tissues in both mouse models and human, across a range of cancer histologic phenotypes, including carcinomas that are known candidates for anti-PD-1 treatment. In the EMT6 mouse model, representing a T cell-excluded phenotype in which anti-PD-1 agents display limited activity, ATRC-101 monotherapy was uniformly vigorous with persistent anti-tumor memory. When co-administered at a lower dose with anti-PD-1, the combination of therapy demonstrated a robust and heightened anti-tumor response relative to either agent dosed as monotherapy at similar concentrations.

Methods To gain insight into the mechanisms that contribute to the anti-tumor effect with combination therapy, in vivo experiments in the EMT6 syngeneic mouse model were performed to determine temporal and spatial patterns of infiltrates and assessed tumors by using whole exome sequencing following administration of ATRC-101 vs. vehicle control. Within naive human tumor samples, coincident immunoreactivities of ATRC-101 and PD-L1 were also characterized.

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Results In mice treated with ATRC-101, analysis by immunofluorescence revealed a significant increase in the percentage of PD-1 reactive T cells within the tumor microenvironment. Elevated transcripts for PD-L1 also were detected in tumors from mice administered ATRC-101 vs baseline levels or vehicle control. When human tumor tissues were characterized for coincident expression of these targets, a high prevalence of ATRC-101 immunoreactivity was noted in both PD-L1 reactive and non-reactive tumor cores. Across multiple indications, ATRC-101 immunoreactivity was apparent in > 50% of PD-L1+ cores.

Conclusions In situ studies suggest the target of ATRC-101 may co-localize with PD-L1, and in vivo studies indicate that ATRC-101 administration increases PD-L1 transcripts and PD-1-positive infiltrates in mouse tumor. Altogether, our data support studies to combine ATRC-101 with agents targeting PD-1 in the clinical treatment of solid tissue malignancies.

Acknowledgements We acknowledge the significant effort and contributions of our colleagues from the clinical, in vivo pharmacology, translational sciences, in vitro pharmacology, and cell biology groups. This includes Mark Armanini, Erin Brosey, Chantia Carroll, Sean M. Carroll, Nicole Haaser, Benjamin Haugen, Dongkyoon Kim, Beatriz Millare, Yann Chong Tan, Danhui Zhang, and Patricia Zano.

Trial Registration NCT04244552

Ethics Approval The study was approved by WIRB (Western Institutional Review Board) on Jun 11, 2013. The WIRB study number is 20130121.

REFERENCE

TARGETING PAN-TUMOR ASSOCIATED ANTIGEN B7H3 VIA COMBINATION OF TRI-SPECIFIC KILLER ENGAGER AND OFF-THE-SHELF NK CELL THERAPY ENHANCES SPECIFICITY AND FUNCTION AGAINST A BROAD RANGE OF SOLID TUMORS

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Background B7H3 is a tumor associated antigen (TAA), found on numerous malignancies including prostate, lung, and breast cancers. High levels of B7H3 expression are correlated with late stage disease and poor prognosis. Furthermore, B7H3 is minimally expressed on normal tissue, making it an ideal TAA for broad cancer treatment strategy. We developed a tri-specific killer engager (TriKETM) consisting of a nanobody anti-B7H3, CD16, IL-15, and nanobody anti-B7H3 joined by flexible linker regions (camB7H3 TriKE) (figure 1A). The combination of B7H3 TriKE with an off-the-shelf NK cell therapy presents an appealing therapeutic strategy for the treatment of solid tumors with decreased risk of toxicity in allogeneic settings compared to T-cell derived products.

Methods An anti-B7H3 nanobody was developed via biopanning and cloned into a TriKE vector. TriKE was produced in Expil293 cells and affinity purified using poly-His tag. NK cells were co-incubated with cell lines exhibiting a range of B7H3 expression and with 3nM of camelid B7H3 TriKE or control. We have previously derived NK cells expressing high affinity non-cleavable hnCD16, CD38 KO, and IL-15/IL-15R fusion from clonal master engineered iPSC lines. Engineered iNK cells were tested in conjunction with the TriKE. A repeated measures ANOVA was used for statistical comparisons as noted in figure legends

Results Engineered iNK cells co-incubated with camB7H3 TriKE and C4-2 prostate cells significantly increased degranulation (CD107a) and cytokine production (IFN-gamma) compared to controls (figure 1B, P<0.05, n=3). camB7H3 TriKE directly bound C4-2 cells with an estimated EC50 of approximately 3nM. camB7H3 TriKE increased percentages of engineered iNK cells dividing robustly (3 or more times) compared to corresponding IL-15 doses at 3 nM (figure 1D, P<0.001, n=3). Furthermore, camB7H3 TriKE enhanced cytotoxic activity of engineered iNK cells against a variety of tumor cells in 2D and spheroid format independent of cytokine support (figure 1E-F). Engineered iNK cells incorporating an anti-B7H3 chimeric antigen receptor (CAR) is also being developed and will be discussed.

Conclusions camB7H3 TriKE dramatically increases function and activation on endogenous NK cells as well as engineered iNK cell, which can be adoptively transferred to patients with a broad range of cancers, including prostate cancer. TriKE
activity was potent across a broad concentration spectrum and corresponded directly with B7H3 target expression. These studies represent the proof-of-concept of a novel pairing of off-the-shelf, engineered iNK cells with B7H3-directed pan-cancer engager molecules (TriKEs and CARs) to enhance specificity, persistence and anti-tumor function.

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471 PANCREATIC CANCER THERAPY BASED ON COMBINATION OF DNA VACCINATION AND PI3KGAMMA INHIBITION

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Background Pancreatic ductal adenocarcinoma (PDA) is the 4th leading cause of cancer mortality in developed countries, with one of the poorest prognoses among all cancers. Although 10–15% of patients are candidates for gross total surgical resection, recurrence is frequent, and the overall 5-year survival rate is around 8%. Using a proteomic approach, we have identified alpha-Enolase 1 (ENO1) as PDA-associated antigens. We have shown that ENO1 DNA vaccination efficiently prolongs survival of engineered mice that spontaneously develop PDA (both KC and KPC mice). Recently, we have demonstrated that PI3K gamma play a critical role in PDA by driving the recruitment of myeloid derived suppressor cells into tumor tissues and it’s genetic or pharmacologic inhibition effectively inhibits PDA progression and metastasis. In this study we assessed the hypothesis that targeting myeloid derived suppressor cells, via pharmacological PI3Kgamma inhibition, synergizes with ENO1 DNA vaccination by inducing a strong and sustained immune response.

Methods KPC mice were vaccinated 4 times with ENO1 starting at 4 weeks of age; 2 weeks after the last immunization mice were treated with the PI3Kgamma inhibitor TG100-115 (2.5 mg/kg), for further two weeks. At sacrifice neoplastic lesions, immune infiltrate, T and B cell response were analyzed.

Results Mice that received ENO1 and TG100-115 therapy showed a significant decrease in tumor size compared to both ENO1 and PBS treated mice. Moreover, the analysis of pancreas tissues indicated that combined therapy induced an increase of Granzyme B in both ENO1 and ENO1+TG100-115 mice. We observed an increased number of CD8 and F4/80 cells and a decrease of FoxP3, CD31 and NG2 cells compared to control mice. In embedded pancreas tissues of treated mice. We observed an increase of Granzyme B in both ENO1 and ENO1+TG100-115 and a down modulation of genes involved in fibroblast and stellate cell activation suggesting a modulation of microenvironment in the combined therapy group.

Conclusions Treatment with ENO1 plus TG100-115 is able to reduce tumor size in pancreas, increase immune cell infiltration and modulate stroma cell compartment, making the therapy a suitable approach for PDA treatment.

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Ethics Approval All animal experiments were approved by the University of Torino, Italian Ministry of Health and performed in accordance with EU laws in the animal facility of the Molecular Biotechnology Center (MBC). Reference no: 378/2015-PR and 597/2019-PR.

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472 IMMUNOPEP-INFORMED SEQUENCE FOR FOCUSED ULTRASOUND-TARGETED MCD47 BLOCKADE CONTROLS GLIOMA

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Background The natural disease course for glioblastoma (GB) entails invariably grim outcomes for patients. Phagocytic immunotherapies, such as CD47 blockade (e.g. mCD47), have recently demonstrated promise for GB therapy. However, their efficacy is challenged by presence of the blood brain and tumor barriers (BBB/ BTB). Transient disruption of the BBB/BTB via focused ultrasound (FUS) and circulating microbubbles (MB) holds promise for improving therapeutic outcomes in the context of mCD47. However, critical questions regarding the optimal protocol for therapeutic antibody delivery with FUS remain. We herein leverage immuno-PET imaging to spatiotemporally map [89Zr]-mCD47 delivery across the BBB/BTB with FUS in an orthotopic GB model. We then use these insights to design a combinatorial paradigm for mCD47 delivery with repeat FUS BBB/BTB-D.

Methods MRI-guided FUS BBB/BTB-D was performed in the presence of systemically circulating MBs in mice with ortho-topically implanted GL261 tumors. Mice received i.v. [89Zr]-mCD47 either without FUS, immediately prior to FUS [FUSPRE] or following FUS [FUSPOST]. Subsequently, mice underwent serial PET/CT imaging followed by terminal ex vivo assessment of antibody biodistribution. A therapeutic paradigm was then executed, wherein GL261-bearing mice received i.v. mCD47 (8 mg/kg) either as monotherapy or in combination with FUS BBB/BTB-D over three sessions spaced three days apart. Overall survival was monitored and tumor outgrowth was tracked via serial contrast-enhanced MRI.

Results Contrast-enhanced MRI confirmed BBB/BTB-D in GL261 tumors (figure 1A). However, PET/CT imaging revealed a lack of tumor-preferential [89Zr]-mCD47 uptake with or without FUSPRE, suggesting that neither condition improved antibody penetration over that in naïve brain (figure 1B-C). Remarkably, FUSPOST conferred superlative [89Zr]-mCD47 uptake at the site of BBB/BTB-D, boasting between 4.3- to 6.7-fold more uptake relative to other groups (figure 1C). This elevation in uptake was sustained over the time points assessed (0–72 hours post-FUS) (figure 1C-D). Using these insights, we evaluated a rational paradigm (figure 2A) combining mCD47 with repeat FUSPOST BBB/BTB-D (figure 2B-C) for glioma therapy. FUS-mediated delivery of mCD47 across the BBB/BTB significantly constrained tumor outgrowth (figure 2D-E) and enhanced survival (figure 2F) in GL261-bearing mice.
Conclusions
Taken together, our findings suggest that mCD47 delivery with FUS BBB/BTB-D is a promising therapeutic strategy for GB. For myriad ongoing pre-clinical and clinical evaluations of FUS-mediated immunotherapy delivery, these findings generate timely and compelling insights regarding impact of injection timing on antibody penetrance in brain tumors. This study underscores the outstanding potential role of immuno-PET imaging for rational design and monitoring of response to FUS immunotherapy approaches.

Acknowledgements
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Ethics Approval
This study was prospectively reviewed and approved by the University of Virginia Animal Care and Use Committee.

Consent
N/A

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Abstract 472 Figure 1
Immuno-PET monitoring of [89Zr]-mCD47 delivery
(A) Representative contrast-enhanced T1-weighted MR images of GL261 tumor-bearing brain pre- and post-FUS. Enhancement in the right cerebral hemisphere on pre-FUS imaging indicates baseline barrier disruption induced by the presence of a brain tumor. Expansion of the enhanced region on post-FUS imaging reflects effective FUS-mediated BBB/BTB disruption. (B) Representative axial decay-corrected PET/CT images for each experimental group on Day 14. White arrows denote region of visibly elevated radioactivity at the tumor site targeted with FUS. (C) Whole brain standardized [89Zr]-mCD47 uptake values (SUVs) extracted from serial static PET/CT images obtained between days 14 and 16 post-implantation (0 to 2 days post-[89Zr]-mCD47 injection), % ID/mL = % injected dose per mL. **p<0.01, ****p<0.0001 vs. group(s) indicated. Significance assessed by RM mixed effects model implementing restricted maximum likelihood method, followed by Tukey multiple comparison correction. (D) Tumor-drug exposure for [89Zr]-mCD47 in naïve brain or GL261 tumors, based on integration of SUVs from decay-corrected PET/CT images collected between 0 and 48 hours after BBB/BTB-D and/or [89Zr]-mCD47 injection. Significance assessed by one-way ANOVA followed by Tukey multiple comparison correction. ****p<0.0001 vs. all other groups

Abstract 472 Figure 2
Therapeutic impact of FUS-mediated mCD47 delivery
(A) Overview of experimental design for evaluating mCD47 delivery to orthotopically implanted GL261 tumors in the context of repeat BBB/BTB-D with FUS. Mice received i.v. mCD47 (8 mg/kg) either alone (FUS-) or following BBB/BTB-D at 0.4 MPa (FUS+). (B) Axial contrast-enhanced T1-weighted MR images of murine GL261 tumors pre- and post-FUS. (C) Mean greyscale intensity (MGI) of contrast enhancement pre- and post-FUS over three separate BBB/BTB-D sessions conducted every three days. Calculated as fold change over contralateral brain. Mean ± SD. **p=0.0023. Significance assessed by RM 2-way ANOVA followed by Sidak’s multiple comparison test. (D) Contrast-enhanced T1-weighted MR images of GL261 tumors on days 14, 17 and 20 post-implantation. ? = image excluded due to poor quality. (E) GL261 tumor outgrowth quantified based on serial MR imaging. Mean ± SD. *p=0.0010. Significance assessed by RM mixed-effects model implementing restricted maximum likelihood method, followed by Sidak’s multiple comparison test. (F) Kaplan-Meier curve depicting overall survival of GL261-bearing mice. n=5–6 mice per group. *p=0.0008. Significance assessed by log-rank (Mantel-Cox) test

MAVRILIMUMAB, A HUMAN MONOCLONAL ANTIBODY TARGETING GM-CSFRα, INHIBITS POLARIZATION TO MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS) THAT EXPRESS PD-L1 AND RESTORES T-CELL PROLIFERATION IN VITRO

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Background
Myeloid-derived suppressor cells (MDSCs) accumulate in the blood and tumor microenvironment (TME) and suppress anti-tumor immune responses.1 Cancer cells express the granulocyte-macrophage colony-stimulating factor (GM-CSF), which drives MDSC differentiation and function.2 3 4 It is upregulated in several cancers, including mesothelioma, pancreatic and colorectal, and it is linked to higher levels of intra-tumoral MDSCs and poorer overall survival.2 4 5 In
animal models, knockdown of GM-CSF in pancreatic epithelium or pancreatic mesenchymal stem cells inhibits tumorigenesis, reduces intra-tumor MDSCs and enhances CD8+ T cell accumulation.\textsuperscript{6} \textsuperscript{7} \textsuperscript{8} Therefore, targeting the GM-CSF receptor alpha (GM-CSFRe) on MDSCs is an attractive strategy to restore anti-tumor immunity. Mavrilimumab is a clinical stage fully human monoclonal antibody that blocks GM-CSFRe. It has demonstrated efficacy and acceptable safety profile in patients with rheumatoid arthritis, and it’s currently undergoing investigation in phase II studies in giant cell arteritis and in patients with severe COVID-19 pneumonia and hyper-inflammation (NCT03827018, NCT04397497, respectively). The present study investigates its potential as a therapeutic strategy to target MDSCs in the TME as an adjuvant to immunotherapy.

Methods Cancer cell supernatants were collected when cells reached confluency. Human GM-CSF was measured by ELISA. Healthy donor CD14+ monocytes were incubated (± mavrilimumab) with cancer cell supernatants for either 3 or 6 days followed by phenotypic analysis (CD14, CD33, HLA-DR, CD11b, CD206, CD80, PD-L1, Arginase-1) by flow cytometry. On day 3, autologous CD3+ T cells were stimulated with CD3/CD28 and IL-2 and co-cultured with putative MDSCs for 5 days. T-cell proliferation was evaluated by measuring carboxyfluorescein succinimidyl ester (CFSE) dilution in CD4+ and CD8+ T cells by flow cytometry.

Results GM-CSF is expressed in the supernatant of cancer cell lines (HCT116, SW-480, Panc-1, Capan-1). Human monocytes cultured with conditioned medium from colorectal carcinoma (SW-480) or pancreatic adenocarcinoma (Capan-1) show downregulation of HLA-DR, increased expression of PD-L1, Arg-1, CD206, and can suppress T-cell proliferation in vitro. Similarly, peripheral blood monocytes purified from pancreatic cancer patients suppress T-cell proliferation ex vivo. Notably, Mavrilimumab inhibits the polarization of healthy donor monocytes to M-MDSCs and restores T-cell proliferation.

Conclusions Targeting of GM-CSFRe with mavrilimumab may alleviate the pro-tumorigenic and immunosuppressive functions of MDSCs in the TME. Future clinical studies should evaluate whether targeting of the GM-CSFRe in combination with immune checkpoint inhibitors is a viable therapeutic option to bolster their efficacy.

Ethics Approval The study was approved by the Institute of Immunology and Immunotherapy, University of Birmingham, UK Ethics Board. Healthy volunteer human material was obtained from commercial sources and approved by Stemex-Institutional Review Board (IRB).

REFERENCES


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INCIDENTAL FINDING OF COLORECTAL CANCER IN A COVID-19 PATIENT, FOLLOWED BY DEEP PROFILING OF SARS-COV-2-ASSOCIATED IMMUNE LANDSCAPE AND TUMOUR MICROENVIRONMENT

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Background Reports suggest that cancer patients may be more vulnerable to COVID-19, with increased disease severity and higher mortality rate.1–3 Although this is likely multifactorial, the exact pathogenesis has not been clearly elucidated. Studies have shown increased ACE2 expression in tumours as compared to normal tissues,4–5 thereby providing increased viral binding. Moreover, other mechanisms of cancer immunotherapy including treatment- and disease-related immunosuppression and functional exhaustion have been reported in patients with concomitant cancer and COVID-19; contributing to greater COVID-19 disease severity.6–8 There is still much to be revealed about the interplay between COVID-19, cancer and the immune system. These insights will give us greater understanding of the immunopathological processes underlying COVID-19 in cancer patients and their clinical relevance.

Methods A 45-year-old South Asian male diagnosed with COVID-19, with incidental discovery of stage II T3N0 caecal adenocarcinoma was consented for our study. The patient had experienced mild symptoms throughout the course of the disease, and underwent laparoscopic right hemicolectomy 10 days after recovery from COVID-19. His blood, lymph nodes, normal tissue and tumour samples were obtained for further analysis (figure 1). Multiplex immunohistochemistry was performed to understand SARS-CoV-2-associated tumour immune microenvironment. Moreover, to simulate ex vivo SARS-CoV-2 infection, dissociated cells from blood, lymph nodes, and tissue samples were stimulated with SARS-CoV-2 peptides or control for 16 hours. This was followed by 25-colour flow cytometry analysis for immune markers and cytokines. We then compared unstimulated with stimulated cells to study SARS-CoV-2-elicited immune response.

Results Multiplex immunohistochemistry demonstrated upregulated expression of ACE2 in the tumour as compared to adjacent normal tissue, whilst SARS-CoV-2 was detected only in adjacent normal tissue but not within the tumour (figure 2). We also observed SARS-CoV-2 in other organs such as appendix and lymph nodes; and the presence of tertiary lymphoid structure, abundant T cells and NK cells within the proximity of the tumour (figure 2). Additionally, upon stimulation with SARS-CoV-2 peptides, we successfully elicited SARS-CoV-2-specific CD4+ T cells expressing immune markers such as granzyme B, TNF-α and IFN-γ (figure 3). Deep profiling of
the samples is on-going with single-cell sequencing and digital spatial profiling.

Conclusions We believe this is the first report of immune profiling of in situ tumour microenvironment in a cancer patient with COVID-19. Our findings showed the presence of viral proteins in several tissues despite negative swab test result, and the ability to elicit ex vivo SARS-CoV-2-specific T cell responses through peptide stimulation experiments.

Ethics Approval This study was approved by Centralised Institutional Review Board of SingHealth; approval number 2019/2653.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

REFERENCES

Methods Evaluation of AK119 activity to bind to the CD73 antigen was performed by using ELISA, Fortebio, and FACS assay. The activity of AK119 to inhibit enzymatic activity of CD73 was evaluated by cell-based enzyme assay; and the activity of AK119 to induce internalization of CD73 and enhance CD69 and CD83 expression on B cells were performed by using FACS assays. We also investigated the potential of AK119 to promote immunoglobulin production from human B cells.

Results AK119 could effectively bind to human CD73 with high affinity, which is comparable or superior to 10.3AA, a leading anti-CD73 antibody, in protein-based assays. AK119 inhibited CD73 enzymatic activity on MDA-MB-231 cells (IC50 AK119, 27.60 nM; IC50 10.3AA, 15.99 nM) and U87-MG cells (IC50 AK119, 0.2448 nM; IC50 10.3AA, 0.0691 nM), with a higher maximal inhibition rates of 108.26% in MDA-MB-231 cells and 96.24% in U87-MG cells compared with 10.3AA (77.02% and 75.77%, respectively). AK119 effectively induced CD73 internalization in MDA-MB-231 cells and U87-MG cells, and the internalization rate of CD73 was 60.75% and 82.39%, respectively; for 10.3AA, the internalization rate was 50.53% and 73.65%, respectively. Moreover, AK119 could stimulate approximately 4–5 fold up-regulation of CD69 (figure 1A) and CD83 (figure 1B) that are markers of B cell activation; and, AK119 significantly promoted IgG production from B cells.

Conclusions In summary, in comparison to a leading CD73 antibody currently in clinical trial, AK119 demonstrated more complete CD73 inhibition; and more dramatic B cell activation and antibody production. Thus, AK119 presented desirable preclinical activities. The safety and pharmacokinetics of single ascending doses of AK119 will be evaluated in healthy volunteers in an upcoming Phase 1, First-in-Human study (NCT04516564).

Trial Registration NCT04516564

REFERENCES

**Tingting Zhong, 1Zhaoliang Huang, Xinghua Pang, 1Na Chen*, 1Konyew Kwok, 1Chris Wynne, 1Adan Konpa, 1Xiaoying Jin, 1Yu Xia, 1Maelwel Zhongmin Wang, 1Baiyong Li, 1Yu Xia. 1Akeso Biopharma Co., Ltd., Zhongshan city, China; 2Christchurch Clinical Studies Trust Ltd., Christchurch, New Zealand**

Background CD73, an ecto-5′-nucleotidase involved in ATP metabolism, converts AMP into adenosine. ATP could induce CD73, an ecto-5′-nucleotidase involved in ATP metabolism, converts AMP into adenosine.5 Inhibition of CD73 was evaluated by cell-based enzyme assay; and the activity of AK119 to induce internalization of CD73 and enhance CD69 and CD83 expression on B cells were performed by using FACS assays. We also investigated the potential of AK119 to promote immunoglobulin production from human B cells.

Methods Evaluation of AK119 activity to bind to the CD73 antigen was performed by using ELISA, Fortebio, and FACS assay. The activity of AK119 to inhibit enzymatic activity of CD73 was evaluated by cell-based enzyme assay; and the activity of AK119 to induce internalization of CD73 and enhance CD69 and CD83 expression on B cells were performed by using FACS assays. We also investigated the potential of AK119 to promote immunoglobulin production from human B cells.
DEEP LEARNING TO DRIVE COVID-19 RAPID DRUG REPURPOSING

Sarah Kolitz *, Jason Kim, Jenny Zhang, Yoonjeong Cha, Sailaja Battula, Rebecca Kusko, Rajaraman Krishnan, Benjamin Zeskind, Howard Kaufman. Immuneering Corp., Cambridge, MA, USA

Methods Fluent is a quantitative structure–activity relationship (QSAR) deep learning-based platform that evaluates small molecule drug binding to protein targets. All drug structures from the FDA approved library were evaluated for binding to the ACE2 receptor and re-filtered for preferential ACE2 vs. ACE1 receptor binding. Top hits were evaluated for specificity by predicting binding across the human proteome and filtered by evaluating rankings from each of two models along with average ranks and combined scores from both models. The drugs were then evaluated for classification, potential availability and prioritized for in vitro validation. Selected compounds were screened using a high-throughput SARS-CoV-2 cell-based assay as described previously (Jonsson et al. J Biomol Screen 2007 12: 33. DOI: 10.1177/1087057106296688). Plates are quality-controlled in each run using Z score and CV statistics. Positive controls consisting of cells only and negative controls consisting of virus were used to normalize the data. Individual drugs are added to each plate at a single dose with at least four doses tested. For titer reduction assays, VeroE6 cells are infected with virus at MOI of 0.1 for one hour to promote adsorption. After two days, the supernatant is harvested and the amount of virus in each well is measured using TCID50 or plaque assay.

Results We identified 25 top drugs that were predicted to bind to ACE2 receptors and could theoretically block SARS-CoV-2 cell entry. Of these drugs, we prioritized 12 drugs for validation covering multiple pharmacologic classes and after assessing drug availability (table 1). They included an ALK/EGFR inhibitor, JAK inhibitor, two electrolyte channel inhibitors, an antibiotic, and several anti-viral drugs, ACE inhibitors and anticoagulants. Validation studies are in progress and viral inhibition and titer reduction data will be presented.

Conclusions Our data show that machine learning platforms can be used to rapidly identify existing drugs that may have activity against SARS-CoV-2 infection. This hybrid computational and experimental approach enables rapid discovery of drugs for clinical testing against COVID-19 and other emerging human diseases.

Acknowledgements We would like to thank Dr. Colleen Jonsson and Dr. Jeremy Smith at Oak Ridge National Laboratories and the University of Tennessee Regional Biocontainment Laboratory for assistance with in vitro validation studies.

Trial Registration N/A

Reference


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Background There is an urgent need for a vaccine with efficacy against SARS-CoV-2. We hypothesize that peptide vaccines containing epitope regions optimized for concurrent B cell, CD4+ T cell, and CD8+ T cell stimulation would drive both humoral and cellular immunity with high specificity, potentially avoiding undesired effects such as antibody-dependent enhancement (ADE) (figure 1). Leveraging methods initially developed for prediction of tumor-specific antigen targets, we combine computational prediction of T cell epitopes, recently published B cell epitope mapping studies, and epitope accessibility to select candidate peptide vaccines for SARS-CoV-2 (figure 2).

Methods SARS-CoV-2 HLA-I and HLA-II ligands were predicted using multiple MHC binding prediction software. T cell vaccine candidates were further refined by predicted immunogenicity, viral source protein abundance, sequence

Abstract Figure 1 Summary of combination CD4+/CD8+ T cell and B cell SARS-CoV-2 peptide vaccine. Humoral immunity (blue dashed box) is targeted through B cell and HLA-II epitopes, aimed at viral neutralization while avoiding non-neutralizing and ADE promoting targets. Cellular immunity (red dashed box) is targeted through HLA-I and HLA-II epitopes, aimed to clear virally infected cells.
conservation, coverage of high frequency HLA alleles, and co-localization of CD4+/CD8+ T cell epitopes. B cell epitope regions were chosen from linear epitope mapping studies of convalescent patient serum, filtering to select regions with surface accessibility, high sequence conservation, spatial localization near functional domains of the spike glycoprotein, and avoidance of glycosylation sites. Using murine compatible T/B cell epitopes, vaccine studies were performed with downstream ELISA/ELISpot to monitor immunogenicity.

Results

We observed distribution of HLA-I (n = 2486) and -II (n = 3138) ligands evenly across the SARS-CoV-2 proteome, with significant overlap between predicted human and murine ligands (figure 3). Applying a multivariable immunogenicity model trained from IEDB viral tetramer data (AUC 0.7 and 0.9 for HLA-I and -II, respectively), alongside filters for entropy and protein expression resulted in 292 CD8+ and 616 CD4+ epitopes (figure 4). From an initial pool of 58 B cell epitope candidates, three epitope regions were identified (figure 5). Combining B cell and T cell analyses, alongside manufacturability heuristic, we propose a set of SARS-CoV-2 vaccine peptides for use in subsequent murine studies and clinical trials (figure 6). Preliminary murine studies demonstrate evidence of T and B cell activation (figure 7).

Abstract 478 Figure 2  Summary of B cell and CD4+/CD8+ epitope prediction workflows. Pathways are colored by B cell (blue), human T cell (black), and murine T cell (red) epitope prediction workflows. Color bars represent proportions of epitopes derived from internal proteins (ORF), nucleocapsid phosphoprotein, and surface-exposed proteins (spike, membrane, envelope).

Abstract 478 Figure 3  Landscape of SARS-CoV-2 MHC ligands. (A&B) Selection criteria for (A) HLA-I and (B) HLA-II SARS-CoV-2 HLA ligand candidates. Scatterplot (bottom) shows predicted (x-axis) versus IEDB (y-axis) binding affinity, with horizontal line representing 500 nM IEDB binding affinity and vertical line representing corresponding predicted binding affinity for 90% specificity in binding prediction. Histogram (top) shows all predicted SARS-CoV-2 HLA ligand candidates. (C) Landscape of predicted HLA ligands, showing nested HLA ligands comprising HLA-I and -II ligands with complete overlap (top), and LOESS fitted curve (span = 0.1) for HLA-III ligands by location along the SARS-CoV2 proteome (bottom). Red track represents SARS epitopes identified in literature review with sequence identity in SARS-CoV-2. Predicted HLA ligands with conserved sequences to this literature set are represented in the lollipop plot with a red stick. (D) Summary of total number of predicted HLA-III ligands and nested HLA ligands. (E) Summary of nested HLA ligand coverage by protein, with raw counts (left) or counts normalized by protein length (right). (F) Summary of murine/human MHC ligand overlap. (G) Distribution of population frequencies among predicted HLA-I, -II, and nested HLA ligands.

Abstract 478 Figure 4  Prediction of SARS-CoV-2 T cell epitopes. (Top) Summary of predicted (left) and IEDB-defined (right) SARS-CoV-2 HLA ligands, showing proportions of each derivative protein. (Middle) Funnel plot representing counts of HLA-I (red text), HLA-II (blue text), and nested HLA (violet text) ligands along with proportions of HLA-I (top bar) and HLA-II (bottom bar) alleles at each filtering step. (Bottom) Summary of CD8+ (red, top), CD4+ (blue, bottom), and nested T cell epitopes (middle) after filtering criteria in S, M, and N proteins. Y-axis and size represent the population frequency of each CD8+ and CD4+ epitopes. Middle track of diamonds represents overlaps between CD8+ and CD4+ epitopes, showing the overlap with greatest population frequency (size) for each region of overlap. Color of diamonds represents the proportion of overlap between CD4+ and CD8+ epitope sequences.
Conclusions

A peptide vaccine targeting B cells, CD4+ T cells, and CD8+ T cells in parallel may prove an important part of a multifaceted response to the COVID-19 pandemic. Adapting methods for predicting tumor-specific antigens, we presented a set of peptide candidates with high overlap for T and B cell epitopes and broad haplotype population coverage, with validation of immunogenicity in murine vaccine studies.

Acknowledgements

The authors appreciate funding support from University of North Carolina University Cancer Research Fund (AR and BGV), the Susan G. Komen Foundation (BGV), the V Foundation for Cancer Research (BGV), and the National Institutes of Health (CCS, 1F30CA225136). We would like to thank members of the #DownWithTheCrown Slack channel for helpful discussion and feedback.

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Abstract 478 Figure 5 Selection of SARS-CoV-2 B cell epitope regions. (A) SARS-CoV-2 linear B cell epitopes curated from epitope mapping studies. X-axis represents amino acid position along the SARS-CoV-2 spike protein, with labeled start sites. (B) Schematic for filtering criteria of B cell epitope candidates. (C) Spike protein amino acid sequence, with overlay of selection features prior to filtering. Polymorphic residues are red, glycosites are blue, accessible regions highlighted in yellow. The receptor binding domain (RBD), fusion peptide (FP), and HR1/HR2 regions are outlined. (D) Spike protein functional regions (RBD, FP, HR1/2) amino acid sequences, with residues colored by how many times they occur in identified epitopes. Selected accessible sub-sequences of known antibody epitopes, highlighted in purple outline. (E) S protein trimer crystal structure with glycosylation, with final linear epitope regions highlighted by color

Abstract 478 Figure 6 T cell and B cell vaccine candidates. (A) 27mer vaccine peptide sets selecting for best CD4+, CD8+, CD4+/CD8+, and B cell epitopes with HLA-I, HLA-II, and total population coverage. (B) Unified list of all selected 27mer vaccine peptides. Vaccine

Abstract 478 Figure 7 Immunogenicity of murine-compatible peptide vaccines. (A) ELISA result: peptides derived from three B cell vaccine candidate regions were coated on peptide capture plates, either in combination by overlapping core epitopes (1-2 and 3-4) or alone (5). (B) ELISpot results: splenocytes from animals vaccinated against predicted B cell epitopes (1–5) or measles peptide control (M; adapted from Obeid et al. 1995). Each point represents the average of technical triplicates, background subtracted against no-peptide control. (A&B) Colors represent adjuvant used for vaccination. P-values shown above each graph represent pair-wise Mann-Whitney u-test

Conclusions

A peptide vaccine targeting B cells, CD4+ T cells, and CD8+ T cells in parallel may prove an important part of a multifaceted response to the COVID-19 pandemic. Adapting methods for predicting tumor-specific antigens, we presented a set of peptide candidates with high overlap for T and B cell epitopes and broad haplotype population coverage, with validation of immunogenicity in murine vaccine studies.

Acknowledgements

The authors appreciate funding support from University of North Carolina University Cancer Research Fund (AR and BGV), the Susan G. Komen Foundation (BGV), the V Foundation for Cancer Research (BGV), and the National Institutes of Health (CCS, 1F30CA225136). We would like to thank members of the #DownWithTheCrown Slack channel for helpful discussion and feedback.

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A LYMPH-NODE TARGETED AMPHIPHILE VACCINE INDUCES POTENT CELLULAR AND HUMORAL IMMUNITY TO SARS-COV-2

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Background

The SARS-CoV-2 pandemic’s public health, economic, and social impacts mandate urgent development of effective vaccines to contain or eradicate infection. To that end, we evaluated a novel amphiphile (AMP) vaccine adjuvant, AMP-CpG, composed of diacyl lipid-modified CpG, admixed with the SARS-CoV-2 Spike-2 receptor binding domain (Spike RBD) protein for immunization (ELI 005) in two mouse
models. AMP immunogens are efficiently delivered to lymph nodes, where innate and adaptive immune responses are generated.

Methods Female, 6 to 8-week-old C57BL/6J and BALB/c mice and 37-week-old C57BL/6J mice received two or more doses of benchmark (alum or CpG) or AMP-modified vaccines, comprised of Spike RBD protein and AMP-CpG adjuvant, subcutaneously injected into the tail base in two-week intervals. Antigen was dose spaced to determine if AMP-CpG would maintain the immune response. Cellular immune responses were determined via ELISPOT analysis of IFNγ production by splenocytes, intracellular cytokine staining of peripheral blood and lung-resident T-cells, and flowcytometric bead array analysis of Th1/2/17 cytokines. Humoral immune responses were determined via blood serum ELISAs to determine sera antibody binding titers, and pseudoviral neutralization assays for comparison to human convalescent serum.

Results Compared to alum, AMP immunization induced 29-fold higher antigen-specific T cells which produced multiple Th1 cytokines and trafficked into lung parenchyma. Antibody responses favored Th1 isoforms (IgG2b, IgG3) and potently neutralized Spike-2-ACE2 receptor binding, with titers >100-fold higher than the natural immune response from convalescent COVID-19 patients; responses were maintained despite 10-fold dose-reduction in Spike antigen. Both cellular and humoral immune responses were preserved in aged mice.

Conclusions ELI-005 exhibits the qualities of an optimal SARS-CoV-2 vaccine, which should (1) induce robust and durable CD8+ and CD4+ T cell responses, (2) elicit high magnitude neutralizing antibodies, (3) produce Th1 bias in the elicited antibody and T cell responses, (4) potentially expand pre-existing cross-reactive T cells, (5) enable dose-sparing of required immunogens to improve the speed and cost of broad vaccination campaigns, and (6) be efficacious in elderly populations. These advantages merit clinical translation to SARS-CoV-2 and other protein subunit vaccines.

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480 PRELIMINARY EVALUATION OF A NOVEL CORONAVIRUS VACCINE (CORVAX) USING ELECTROPORATION OF PLASMID DNA ENCODING A STABILIZED PREFUSION SARS-COV-2 SPIKE PROTEIN ALONE OR WITH TRANSFECTION OF PLASMID IL-12

1Shawn Jensen*, 2Christopher Twitty, 3Christopher Paustian, 4Madelein Laws, 5Glenna McDonnell, 6Keith Wegmann, 7Tasem Moudgil, 8Michael Alentouls, 9Nia Han, 10Kellei Malloy Foerster, 11David Canton, 12Jack Lee, 13Blanca Nguyen, 14John Rodriguez, 15Kim Jaffe, 16Brian Piening, 17Carlo Bluco, 18Daniel O’Connor, 19Walter Urba, 20Rom Leidner, 21Traci Hilton, 22Hong-Ming Hu, 23Bernard Fox, 24Earle A. Chiles Research Institute, Prov, 25Portland, OR, USA; 26Oncotarget, 27Bulova Health Sciences, Bostom, MA, USA; 28Dana-Farber Cancer Institute, Boston, MA, USA; 29Massachusetts General Hospital, Boston, MA, USA; 30Brigham and Women’s Hospital, Boston, MA, USA

Background SARS-CoV-2 (CoV2) has precipitated a global pandemic and the effectiveness of standard vaccine strategies to induce potent and persistent immunity to CoV2 is in question, particularly for the elderly. This problem is not dissimilar to what we have struggled with in our quest to induce immunity to cancer antigens, where vaccine-induced anti-cancer immune responses can be weak. Here, we describe a novel vaccine approach which leverages electroporation (EP) of a plasmid encoding a prefusion stabilized CoV2 spike protein (CORVax). As IL-12 has been shown to augment the efficacy of immunotherapy in aged mice,1 we have initiated studies to evaluate if plasmid IL-12 (TAVO™) can similarly augment anti-CoV2 immune responses in young mice and have planned studies in aged animals.

Methods A prefusion stabilized CoV2 spike plasmid expression vector was constructed, a master cell bank generated and clinical-grade plasmid manufactured. C57BL/6 and BALB/c were vaccinated via intramuscular (IM) and/or intradermal (ID) injection followed immediately by EP of plasmids encoding the CoV2 spike protein with or without plasmid-encoded murine IL-12 on days 1 and 14 or 21. Mice were followed for >120 days to assess safety. Splenocytes and serum were harvested at different time points to interrogate virus-specific cellular responses as well anti-spike IgG1/IgG2 antibody titers. A surrogate viral neutralization test (sVNT) assessed serum blockade of soluble hACE2R binding to immobilized CoV2 spike.

Results Preliminary data shows that EP of CORVax alone or combined with IL-12 was safe. EP of CORVax was able to elicit anti-Spike IgG antibodies (IC50 = 1/2112), as well as IgG antibodies targeting the receptor binding domain of the Spike protein (IC50 = 1/965) approximately 40 days after the booster vaccination. In 2 of 2 experiments, CORVax combined with IL-12 significantly (P<0.0001) increased the sVNT titers at 2 months, but this benefit was lost by 3 months.

Conclusions Early preclinical data shows that EP of CORVax can induce IgG responses to CoV2 Spike and the receptor binding domain (RBD) as well as apparent viral neutralizing activity. The addition of IL-12, at least transiently, increased sVNT titer. We plan to investigate alternate vaccine boosting strategies while extending these studies into aged animals and initiate a clinical trial in the near future.

REFERENCES


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481 IMPACT OF COVID-19 ON CANCER PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITORS

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Background There are conflicting data regarding the vulnerability of cancer patients receiving immune checkpoint inhibitors (ICIs) to COVID-19 infection.1–3 In addition, immune-related adverse events (irAEs) driven in part by cytokine dysregulation could parallel the cytokine storm implicated in COVID-19. We sought to evaluate the impact of COVID-19 infection on irAEs and mortality in cancer patients receiving ICIs.

Methods We performed a retrospective matched cohort study of 25 patients receiving ICIs within one year of a confirmed COVID-19 diagnosis between March 20, 2020 and June 3, 2020 at the Dana-Farber Cancer Institute/Mass General Brigham network. Cases were matched 1:1 with controls withoutICI use based on age, sex, and use of non-ICI anti-cancer
therapy within 6 months prior to COVID-19 diagnosis. The primary outcome was death due to COVID-19, and potential covariates (patient comorbidities, concomitant medications, ICI therapy, other anti-cancer therapy) were explored using multivariable logistic regression models.

Results We reviewed the records of 611 patients with prior ICI use who were evaluated at our institutions. The final study population included 25 patients who tested positive for COVID-19. The median age was 72 years (range 45–83) and 11 patients (44%) were female (table 1). Seven of 25 (28%) patients on ICIs died from COVID-19 compared to 9 of 25 (36%) controls (figure 1). In multivariable analysis, determinants of mortality included age (OR 1.14, 95% CI 1.03–1.27) and chronic obstructive pulmonary disease (OR 12.26, 95% CI 1.76–85.14), while concomitant statin use was protective against mortality (OR 0.08, 95% CI 0.01–0.63). After adjusting for age, sex, and anti-cancer therapy, ICI use was not associated with increased risk for COVID-19 death (OR 0.36, 95% CI 0.07–1.87, figure 2). Two patients experienced persistent irAEs (hypophysitis) and one patient had new onset irAE (hypothyroidism) during their COVID-19 course. Patients with ICI use presented with significantly higher platelet (p = 0.017) and D-dimer (p = 0.037) levels compared to controls (figure 3A). Elevated troponin levels (p = 0.01) were associated with COVID-19 death in patients using ICI but not in controls (figure 3B).

Conclusions In our study, ICI use was not associated with increased risk of COVID-19 related death. We observed low rates of new or persistent irAEs within our small sample. The potential protective effect of statin therapy and predictive role of laboratory biomarkers warrants further investigation. Our findings are promising for the continuation of immunotherapy in cancer patients with COVID-19.

Acknowledgements K.T. and A.N.B. contributed equally. N.R. L. and O.E.R. contributed equally. The authors would like to acknowledge the DFCI Oncology Data Retrieval System (OncDRS) for the aggregation, management, and delivery of

Abstract 481 Table 1 Demographic, clinical and treatment characteristics of cancer patients treated with or without immune checkpoint inhibitors (ICI) before testing positive for COVID-19

<table>
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<td>14 (56)</td>
<td>6 (24)</td>
<td></td>
</tr>
<tr>
<td>Atherosclerotic</td>
<td>8 (32)</td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>9 (36)</td>
<td>6 (24)</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>7 (28)</td>
<td>6 (24)</td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI ≥ 30)</td>
<td>6 (24)</td>
<td>4 (16)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (8)</td>
<td>10 (40)</td>
<td></td>
</tr>
<tr>
<td>ICI received in last year</td>
<td>3 (12)</td>
<td>6 (24)</td>
<td></td>
</tr>
<tr>
<td>Antidepressant</td>
<td>1 (4)</td>
<td>4 (16)</td>
<td></td>
</tr>
<tr>
<td>Dihydropyridine</td>
<td>1 (4)</td>
<td>4 (16)</td>
<td></td>
</tr>
<tr>
<td>Spironolactone</td>
<td>1 (4)</td>
<td>4 (16)</td>
<td></td>
</tr>
<tr>
<td>Novothrombo</td>
<td>1 (4)</td>
<td>4 (16)</td>
<td></td>
</tr>
<tr>
<td>Penileprostaquin</td>
<td>1 (4)</td>
<td>6 (24)</td>
<td></td>
</tr>
<tr>
<td>Other anti-cancer therapy in past 6 months (cases)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>9 (36)</td>
<td>10 (40)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-cancer therapy in past 6 months (controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>9 (36)</td>
<td>10 (40)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time between last dose of anti-cancer therapy and COVID-19 diagnosis</td>
<td>20 (80-250)</td>
<td>22 (0-118)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

This study included 25 cancer patients with prior ICI use (case) and 25 cancer patients without prior ICI use (controls).

a For cases, thoracic cancers include 5 squamous cell, 4 adenocarcinoma, and 1 non-small-cell lung cancer, not otherwise specified (NOS). For controls, 1 case of small cell lung cancer.

b For cases, skin cancers include 3 melanoma and 2 non-melanoma skin cancers (basal cell carcinoma and squamous cell carcinoma)

c For cases, gastrointestinal cancers include 1 esophageal, 1 liver, 1 colon, and 1 gastric cancer. For controls, 1 liver, 4 colon, 1 gastric, and 1 pancreatic cancer.

d For cases, hematologic cancers include 1 acute myeloid leukemia (AML). For controls, 1 breast, 1 AML, 1 chronic myeloid leukemia (CML), 1 acute lymphoblastic leukemia (ALL) 1 myelodysplasia, 3 B-cell lymphoma, 1 T-cell lymphoma, 1 multiple myeloma.

e For cases, other cancer types include 4 oropharyngeal, 1 breast. For controls, 2 prostate, 1 ovarian, 1 thyroid, 1 bladder, 1 renal, and 1 brain cancer.

f Including coronary artery disease (CAD), congestive heart failure (CHF), anemia, atrial fibrillation, and cardiac disease not otherwise specified (NOS)

ICI = Immune checkpoint inhibitor, COPD = Chronic obstructive pulmonary disease, BMI = Body mass index

Abstract 481 Figure 1 COVID-19 outcomes among 25 cases and 25 controls

Flow graphic demonstrating the hospitalization status, highest level of care, and final COVID-19 outcomes among the two patient cohorts (25 patients with prior ICI use and 25 controls). Complications are defined as patients who continue to require supplemental oxygen after discharge

Abstract 481 Figure 2 Predictors of COVID-19 mortality in multivariable analysis

Odds ratios for the impact of baseline patient characteristics on COVID-19 mortality, including age, sex, use of non-ICI anti-cancer therapy (chemo-targeted) in the past 6 months, ICI therapy in the past year, active statin use, and COPD. Odds ratios were calculated by multivariable logistic regression. Error bars represent 95% confidence intervals (CI). The x-axis is on a log10 scale. The odds ratio for age is per increasing year. (*) p-value < 0.05
the clinical and operational research data used in this project. The content is solely the responsibility of the authors.

Ethics Approval This project was approved by the Partners Healthcare Institutional Review Board (#2020P000851).

REFERENCES


COVID-19 IN PATIENTS WITH LUNG CANCER RECEIVING IMMUNOTHERAPY. A REPORT FROM AN SPANISH ACADEMIC CENTER

David Viñal*, Laura Gutiérrez, Julia Villamayor, Oliver Higuera, Dario Sanchez Cabrero, Javier De Castro. Hospital Universitario La Paz, Madrid, Spain

Background COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared pandemic in March 2020. We know that patients with cancer represent a high risk population. Lung cancer have an already damage lung that may affect the evolution and outcomes of these patients.1–3 The aim of this study is to report the characteristics and outcomes of patients with lung cancer receiving immunotherapy and SARS-CoV-2 infection.

Methods We retrospectively collected sociodemographic and clinical data from patients with lung cancer and COVID-19 diagnosis who were admitted to La Paz University Hospital (Madrid, Spain) from March 1 to May 7, 2020. Survival analysis was performed using the Kaplan-Meier method and log-rank test. Hazard ratios and corresponding 95% confidence intervals were estimated with the use of Cox proportional-hazards regression models.

Results A total of 29 patients were included. Baseline characteristics are depicted in table 1. Non-small-cell lung cancer (NSCLC) was reported in 93% of the patients and 69% were at advanced stage at the time of COVID-19 diagnosis. Eighty-two percent of the patients were admitted to the hospital and 75% received experimental therapy for COVID-19, including hydroxychloroquine (HCQ) (N=9), HCQ plus azithromycin (N=11) or lopinavir/ritonavir (N=2). A total of 12 patients developed acute distress respiratory syndrome (ADRS) at a median time of 7 days from COVID-19 diagnosis. ADRS was managed with steroids in 75% of the patients. Thirteen (44.8%) deaths were reported, 11 of them were considered to be COVID-19 related. Death occurred at a median time of 8 days. In the univariate analysis, diabetes mellitus, respiratory failure at the time of admission, presence of multilobar infiltrates and SDRA were associated with death.T wenty-two patients were on systemic treatment, of whom 10 patients were receiving immunotherapy alone (N=7) or in combination with chemotherapy (N=3) at the time of COVID-19 diagnosis. No significant association with the development of ADRS (P=0.38) or death (P=0.41) was found between patients on immunotherapy versus other systemic therapies. Overall survival was not reached in the immunotherapy group vs 14 days in patients on other systemic therapies (P=0.25), see figure 1.
Background Coronavirus disease 2019 (COVID-19) is a new pandemic disease caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The C5a anaphylatoxin and its receptor C5aR1 (CD88) play a key role in the initiation and maintenance of several inflammatory responses, by recruiting and activating neutrophils and monocytes in the lungs.

Methods We provide a longitudinal analysis of immune responses, including immune cell phenotyping and assessments of the soluble factors present in the blood and broncho-alveolar lavage fluid (BALF) of patients at various stages of COVID-19 severity: paucisymptomatic, pneumonia and acute respiratory distress syndrome (ARDS)

Results We report an increase in soluble C5a levels proportional to COVID-19 severity and high levels of C5aR1 expression in blood and pulmonary myeloid cells, supporting a role for the C5a-C5aR1 axis in the pathophysiology of ARDS. Avdoraliab, an anti-C5aR1 therapeutic monoclonal antibodies (mAbs) prevented C5a-mediated human myeloid cell recruitment and activation, and inhibited acute lung injury (ALI) in human C5aR1 knockin mice.

Conclusions These results support the evaluation of avdoraliab to block C5a-C5aR1 axis as a mean of limiting myeloid cell infiltration in damaged organs and preventing the excessive lung inflammation and endothelialitis associated with ARDS in COVID-19 patients

Acknowledgements The Explore COVID-19 IPH group, the Explore COVID-19 Marseille Immunopole group.

Ethics Approval Human study protocol was approved by the Committee for the Protection of Persons Ile-de-France III – France (#2020-A00757-32). Animal experiments were approved by the ministere de l’enseignement superieur, de la recherche et de l’innovation – France (APAFIS#25418-2020051512242806 v2).

Data sharing, handling, and access

483 ASSOCIATION OF COVID-19 INFLAMMATION WITH ACTIVATION OF THE C5A-C5AR1 AXIS

1 Olivier Demaria, 2 Julien Carwell, 3 Nassima Chouaki Benmansour, 1 Joanna Fares, 1 Luciana Batista, 1 Marie-Laure Tribout, 1 Ariane Morel, 1 Sabrina Carpenter, 1 Romain Remarck, 1 Agnès Represa, 1 Frédéric Vély, 1 Mikael Ebbo, 1 Nicolas Schleinitz, 1 Robert Zerbib, 1 Yannis Morel, 1 Eric Vivier, 1 Olivier Demaria*. 1 Innove Pharma, Marseille, France; 2 Hôpital de la TIMONE, Marseille Cedex 5, France; 3 Hôpital d’Instruction des Armées Laveran, Marseille, France

Background Single-cell sequencing technology has opened an unprecedented ability to interrogate cancer. It reveals significant insights into the intratumoral heterogeneity, metastasis, therapeutic resistance, which facilitates target discovery and validation in cancer treatment. With rapid advancements in throughput and strategies, a particular immuno-oncology study can produce multi-omics profiles for several thousands of individual cells. This overflow of single-cell data poses formidable challenges, including standardizing data formats across studies, performing reanalysis for individual datasets and meta-analysis.

Methods N/A

Results We present BioTuring Browser, an interactive platform for accessing and reanalyzing published single-cell omics data. The platform is currently hosting a curated database of more than 10 million cells from 247 projects, covering more than 120 immune cell types and subtypes, and 15 different cancer types. All data are processed and annotated with standardized labels of cell types, diseases, therapeutic responses, etc. to be instantly accessed and explored in a uniform visualization and analytics interface. Based on this massive curated database, BioTuring Browser supports searching similar expression profiles, querying a target across datasets and automatic cell type annotation. The platform supports single-cell RNA-seq, CITET-seq and TCR-seq data. BioTuring Browser is now available for download at www.bioturing.com.

Conclusions N/A
RAFT: A FRAMEWORK TO SUPPORT RAPID AND REPRODUCIBLE IMMUNO-ONCOLOGY ANALYSES

Steven Vensko*, Benjamin Vincent, Dante Bortone. University of North Carolina, Chapel Hill, NC, USA

Abstract 485 Figure 1 Example RAFT Usage

Users define their required inputs, build their analysis, and run their analysis using the RAFT command-line interface. The metadata from the analysis can then be shared through a RAFT package with collaborators or interested third-parties in order to reproduce or expand upon the initial results.

Abstract 485 Figure 2 End-to-end RAFT

RAFT supports end-to-end analysis development through a ‘project’ structure. Users link local required files (e.g. FASTQs, references or manifests) into their appropriate/raft subdirectory. (1) Projects are initiated using the raft init-project command which creates and populates a project-specific directory. (2–3) Users then load required metadata (e.g. sample manifests or clinical data) and references (e.g. alignment references) into the project using the raft load-metadata or raft load-reference commands, respectively. (4) Modules consisting of tool-specific and topical workflows are cloned from a collection of remote repositories into the project using raft add-step. (5) Specific processes and workflows from previously loaded modules are added to the analysis (main.nf) through raft add-step. Users can then modify main.nf with their desired parameters and execute the workflow using raft run-workflow. (6) Additionally, RAFT allows an iterative approach where results from RAFT can be analyzed and modified through RStudio and re-run through Nextflow.

establish RAFT’s ability to support reproducibility by running locally on laptop computers, on multiple research compute clusters, and on the Google Cloud Platform.

Conclusions

The RAFT platform shows promising capabilities to support rapid and reproducible research within the field of immuno-oncology. Several features remain in development and testing, such as incorporation of additional immunogenomics feature modules such as variant/fusion detection and HLA/peptide binding affinity estimation. Other functionality in development will enable collaborators to use remote Git repository hosting (e.g. GitHub or GitLab) to jointly and iteratively modify an analysis.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0485
standards to facilitate sharing and analysis of these repertoire data through the AIRR Data Commons (ADC).

**Methods** The iReceptor Gateway (www.ireceptor.org) implements the AIRR Data Commons as a network of federated repositories which facilitates data queries and advanced analyses. Secure data repositories, single cell immune profiling, and RNA gene expression and more detailed cell phenotype data for Systems Immunology are being added by the iReceptor Plus consortium, funded by Canadian Institutes of Health Research (CIHR) and the EU Horizon 2020 program.

**Results** As of August 2020, the iReceptor Gateway provides access to 2.7 billion receptor sequences, from 2779 repertoires, and 46 studies; these include 3 B-cell and 10 T-cell cancer studies. These can be queried for specific CDR3 sequences, in order to test whether particular sequences are public (occurring in multiple patients) or private (only found in a few individuals). These can also be queried for specific ‘metadata’, e.g. ‘find all repertoires from studies of ovarian cancer.’ The Gateway aggregates these repertoire data for further analysis by sophisticated AIRR-seq algorithms on HPC resources.

**Conclusions** Analysis of aggregated AIRR-seq data through the iReceptor Gateway has great potential to revolutionize many aspects of cancer immunotherapy. The FDA has already approved the use of AIRR-seq data for monitoring clonal expansion as a diagnostic tool in MRD (minimal residual disease). Sequences from tumor specific clones provide targets for monoclonal antibodies in anti-checkpoint therapy and CAR-T cell approaches. Several studies have shown that AIRR-seq data provide biomarkers that partition patients into responders/non-responders and predict those who may exhibit adverse reactions to novel cancer immunotherapies. This potential will be realized as more researchers adopt the AIRR Community standards for sharing and analyzing AIRR-seq data, resulting in more efficient biomedical research and improved patient care.

**Acknowledgements** Funded by the European Union’s H2020 Research and Innovation Programme under Grant Agreement No. 823821 and Canadian Institutes of Health Research (CIHR)

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**Education and treatment management**

487 REASONS FOR NOT TESTING FOR BIOMARKERS IN NON-SMALL CELL LUNG CANCER: A REGIONAL COMPARISON OF PATIENTS IN THE US AND EUROPE

Nikita Sharma, Mahalechumy Krishnam, Ayse Levent*.
IpsoS, Parsippany, NY, USA

**Background** The growth in the number of targeted therapies available for the treatment of solid tumors has placed biomarker testing at the heart of clinical practice, especially for non-small lung cancer (NSCLC). Guidelines such as those by the American Society of Clinical Oncology and the European Society for Medical Oncology, recommend that all advanced NSCLC patients be tested for EGFR, ALK, ROS-1 and PD-L1 and that further markers (such as BRAF and KRAS) be included in larger panels. Despite these guidelines, oncolologists do not always test NSCLC patients for these biomarkers. This study explores the reasons for not testing and compares these across the US, France, Germany, UK, Italy and Spain (collectively EU5) by examining real-world usage data.

**Methods** Between September and November 2019, a panel of oncologists (n=65 in US and n=235 in EU5) were asked to report on their practices relating to biomarker testing for 1,110 NSCLC patients through the submission of online, de-identified charts detailing testing for EGFR, ALK, ROS-1, PD-L1, BRAF, KRAS/NRAS, MET, RET, dMMR/MSI, TMB and NTRK. We collected data on 11,116 instances where biomarkers were skipped and recorded physicians’ reasons for not testing (selected from a pre-coded list).

**Results** Of the reasons provided for not testing in the US (n=2,114) and EU5 (n= 9,002), waiting for progression was selected the most (27% and 25%, respectively). Lack of data regarding clinical utility (18% and 16%) and patients not meeting criteria (13% and 17%) were mentioned next as the top reasons for not testing across both regions. Compared to the US, EU5-based physicians had higher mentions of patients not meeting criteria (17% vs. 13%), tests not being reimbursed (7% vs. 5%) and treatment costs not being reimbursed (6% vs. 4%). The full distribution of reasons is shown in table 1 below.

<table>
<thead>
<tr>
<th>Abstract 487 Table 1 Reasons for not testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reason</td>
</tr>
<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Waiting for progression</td>
</tr>
<tr>
<td>Lack of data regarding clinical utility</td>
</tr>
<tr>
<td>Patients not meeting criteria</td>
</tr>
<tr>
<td>Treatment costs not being reimbursed</td>
</tr>
<tr>
<td>Cost of testing</td>
</tr>
<tr>
<td>Treatment was not discussed</td>
</tr>
<tr>
<td>Did not meet criteria for treatment</td>
</tr>
<tr>
<td>Reason was not known</td>
</tr>
</tbody>
</table>

**Conclusions** Despite recommendations in guidelines, physicians in the US and EU5 often forgo testing to wait until after progression, because of a perceived lack of clinical utility or because they deem the patient ineligible for testing. While individual countries differ on their approaches to testing - some are more cost sensitive (UK, France) while others are more discerning as to which patients are eligible for testing (Germany) - a concerted effort is needed to educate physicians on the clinical utility of biomarker testing.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0487
events combination BRAF/MEK inhibitors and ICI (triplet therapy) are being evaluated to optimize outcomes. With several trials due to report, oncologists need education to stay up-to-date on the available data and contextualize this potential treatment option.

**Methods** An online continuing education (CME) activity consisting of a multi-media 30-minute video panel discussion explored the rationale, available clinical trial data, and future directions of triplet therapy for the treatment of advanced BRAF-mutated melanoma. Educational effect was assessed using a repeated pairs pre-assessment/post-assessment study design and compared the pre- and post-assessment responses. A chi-square test was used to identify differences between pre- and post-assessment responses. Effect size was calculated using Cramer’s V test by determining the strength of the association between the activity and the outcomes (V = 0.16 – 0.26 is considerable and V > 0.26 is extensive). P values were calculated and those < 0.05 were considered statistically significant. Data from oncologist participants were collected between 12/23/2019 through 2/26/20.

**Results** Participation in education resulted in statistically significant improvements and noticeable educational effect for oncologists (n=49; p < 0.05, V = 0.136). • 39% of pre-assessment questions were correctly answered increasing to 52% post-assessment • 15% of oncologists had a measurable improvement in confidence regarding the rationale for the use of triplet therapy in advanced melanoma. Significant improvement in knowledge regarding clinical trial data in triplet therapy was observed (33% vs. 55%; p < 0.05, V = 0.203)

**Conclusions** This online, interactive, expert-led, CME-certified educational activity resulted in significant gains in oncologist knowledge and confidence regarding triplet therapy in the management of melanoma. These results demonstrate the effectiveness of on-demand education but also highlight an ongoing need for education on this topic as further data becomes available.

**Acknowledgements** This educational initiative was supported through educational grants from Novartis Pharmaceuticals Corporation and Genentech

**REFERENCE**


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**THE IMPACT OF EDUCATION ON NOVEL CONCEPTS IN ADJUVANT MELANOMA: A CLOSER LOOK AT HIGH RISK STAGE II DISEASE**

**Background** Adjuvant therapy for patients with melanoma is currently recommended for patients with stage III disease with either immune checkpoint inhibitors or combination dabrafenib/trametinib based on BRAF-status. Adjuvant treatment demonstrates improvement in recurrence-free survival and overall survival. However, risk models suggesting that patients with stage IIIB/IIC disease may have a higher risk of recurrence than patients with stage IIIA disease have prompted exploration into the use of adjuvant therapy in this patient subgroup as well. With several ongoing trials due to report, oncologists need education to stay up-to-date on the available data and contextualize this potential treatment option to implement therapy at the earliest point of clinical benefit to patients while also collaborating with surgical teams for optimal care planning.

**Methods** An online continuing education (CME) activity consisted of a multi-media 30-minute video panel of a medical oncologist and surgical oncologist discussing the rationale, available clinical trial data, and future directions of adjuvant therapy for the treatment of patients with stage II melanoma. Educational effect was assessed using a repeated paired pre-assessment/post-assessment study design and compared the pre- and post-assessment responses. A chi-square test was used to identify differences between pre- and post-assessment responses. Effect size was calculated using Cramer’s V test by determining the strength of the association between the activity and the outcomes (V = 0.16 – 0.26 is considerable and V > 0.26 is extensive). P values were calculated and those < 0.05 were considered statistically significant. Data from 65 oncologists and 138 surgeons are represented here through 8/12/2020.

**Results** Participation in education resulted noticeable educational effects for both oncologists (p < 0.01, V = 0.143) and surgeons (p = 0.001, V = 0.114): Statistically significant improvements in knowledge and competence were also seen regarding: -Knowledge regarding the rationale for adjuvant therapy in stage II diseaseo Oncologists: 46% pre; 69% post, p < 0.01o Surgeons: 24% pre; 36% post, p < 0.05 -Competence utilizing patient and tumor characteristics to identify potential candidates for adjuvant therapy in stage II diseaseo Oncologists: 52% pre; 77% post, p < 0.01o Surgeons: 29% pre; 43% post, p < 0.05 -Increase in confidence was also observed for coordinating with the multidisciplinary team to augment surgical care with potential systemic adjuvant treatment for eligible patientso 22% improvement for oncologistso 19% improvement for surgeons

**Conclusions** This online, interactive, multi-media, expert-led, CME-certified educational activity resulted in significant gains in oncologist and surgeon knowledge and competence with improvements in confidence regarding the role of adjuvant therapy in the management of high risk stage II melanoma and recommending clinical trials for eligible patients. These results demonstrate the effectiveness of education, especially in online and on-demand formats and those requiring cross-discipline collaboration, and also highlights an ongoing need to further educate on this topic.

**Acknowledgements** This educational initiative was supported through independent educational grants from Bristol Myers Squibb.

**REFERENCE**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0489

**AN IMMUNO-ONCOLOGY CENSUS: ASSESSMENT OF CLINICIAN KNOWLEDGE AND EDUCATIONAL NEEDS IN 2020**

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**REFERENCE**

Background The landscape for clinician education in immunology (IO) has changed dramatically since the first approval of an immune checkpoint inhibitor (ICI) in 2011. Educational initiatives have had to evolve with the multitude of new IO approvals and indications, as well as continuous integration of these therapies in patient care. As such, an IO census survey was administered and analyzed to better assess the current knowledge and educational needs of the oncology care team at the start of a new decade in IO.

Methods In June 2020, the Association of Community Cancer Centers launched an online survey to its membership of multidisciplinary oncology providers. The survey included questions related to demographic information, current IO practices, and top priorities and challenges in IO. In August 2020, an interim, descriptive analysis was conducted on complete survey responses (n=38).

Results At the time of interim analysis, survey respondents represented the full multidisciplinary cancer care team (e.g., advanced practice providers [18%], pharmacists [16%], medical and surgical oncologists [14%]), as well as diverse practice settings (e.g., community cancer program [28%], physician practice [20%]). In addition, the majority (67%) of respondents treated more than 20 patients per week with immunotherapies across most cancer types. When assessing familiarity with IO agents, most respondents were ‘moderately familiar’ or ‘extremely familiar’ with ICIs (26% and 53%, respectively). However, many respondents were ‘not at all familiar’ or only ‘slightly familiar’ with chimeric antigen receptor (CAR) T-cell therapy (5% and 42%, respectively) and bispecific antibody therapies (16% and 42%, respectively). The top challenges (i.e., ‘very challenging’ or ‘extremely challenging’) for respondents included the expansion of indications for IO agents (45% and 11%, respectively), coordinating care with non-oncology providers (29% and 18%, respectively), and financial toxicity (32% and 26%, respectively). Regarding future education topics, respondents expressed most interest (i.e., ‘very interested’ or ‘extremely interested’) in biomarker and molecular testing (71% and 18%, respectively), patient access, advocacy, and financial impact (61% and 24%, respectively), and evidence, data, and publication updates (55% and 26%, respectively).

Conclusions These interim results from a representative cohort strongly indicate that clinicians desire more clinical and operational support on use of IO agents and associated testing, easing patients’ and programs’ associated financial strain, and coordinating care across specialties. Additional analysis will focus on if/how respondents’ specific disciplines or practice settings influence the results.

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DEVELOPING EDUCATIONAL MATERIALS ABOUT IMMUNOTHERAPY FOR PATIENTS AND THEIR CAREGIVERS

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Background As the use of immunotherapy as treatment for cancer patients continues to expand, it is important that patients and caregivers have access to relevant educational and community resources to support them in making informed decisions and receiving optimal care.1 To help meet these needs, the Cancer Support Community (CSC) designed Frankly Speaking About Cancer (FSAC): Immunotherapy. The intent of FSAC: Immunotherapy is to act as a patient education resource that offers information about immunotherapy, side effects, psychosocial impacts, and patient-provider communication. It is critical to gather stakeholder feedback when developing such programs to ensure all information and resources are appropriate and useful to the target audience. To achieve this, CSC worked with patients and caregivers to get feedback and refine the FSAC: Immunotherapy educational materials.

Methods In June 2020, CSC facilitated a virtual discussion board with cancer patients that have received immunotherapy (N = 8) and their caregivers (N = 2). Participants were asked to talk through and provide feedback on two booklets: FSAC: Immunotherapy and FSAC: Immunotherapy & Lung Cancer. Participants reviewed the booklets and answered open-ended questions about clarity and completeness of information. Sample points of discussion focused on their comprehension and perception of information regarding immunotherapy, immunotherapy options, side effects, and decision-making.

Results Qualitative analysis of discussion board responses revealed that while participants judged most of the content to be clear and informative, they desired more information about differences between immunotherapy types, technical terms, and cost. Specific requests included: Explain how types of immunotherapies differ from one another. Provide information on oncolytic vaccines and how they work. Clarify if immunotherapy can be used in adjuvant treatment or just in metastatic disease. Add information about costs associated with immunotherapy treatment and common practices in health insurance reimbursement. Add information about how is immunotherapy administered.

Conclusions Patients and caregivers provide valuable perspectives to those creating educational resources. Incorporating these stakeholder voices can increase the effectiveness of materials and should continue throughout the resource development processes. Regarding implementation, CSC distributes the booklets at no charge to cancer patients and caregivers via its internal network of almost 50 Cancer Support Communities and Gilda’s Clubs worldwide, the CancerSupportCommunity.org webpage, and partner patient advocacy groups. We also promote these materials to the medical community and allow them to order/download it, at no charge, to help patients undergoing immunotherapy treatment and their caregivers.

Acknowledgements This project was supported by grants from Bristol Myers Squibb, Lilly, EMD Serono, and Pfizer.

Ethics Approval This study was conducted under IRB-exempt protocols [category 45 CFR 46.101(b) 2].

REFERENCE


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0491
Immune cell biology

INTEGRATION OF HIGH DIMENSIONAL DATASETS IN AN IMMUNOCOMPETENT MAMMARY MOUSE MODEL REVEALS PATHWAYS OF TOLERANCE AND RESISTANCE TO IMMUNE CHECKPOINT BLOCKADE

1Lin Ma, 2San-Hua Mao, 3Mary Helen Barcellos-Hoff, 4Jade Moore*. 1University of California, San Francisco, San Francisco, CA, USA; 2Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Background Checkpoint inhibitors can induce robust and durable responses in a subset of patients. Extending this benefit to more patients could be facilitated by better understanding of how interacts with immune cells with the tumor microenvironment, which is a critical barrier to control both local and systemic disease. The composition and pattern of the immune infiltrate associates with the likelihood of response to immunotherapy. Inflamed tumors that exhibit a brisk immune cell infiltrate are responsive, while those in which immune cells are completely or partially excluded are not. Transforming growth factor β (TGFβ) is immunosuppressive and associated with the immune excluded phenotype.

Methods Using an immune competent mammary tumor derived transplant (mTDT) model recently developed in our lab, exhibits inflamed, excluded or deserts immune infiltrate phenotypes based on localization of CD8 lymphocytes. Using whole transcriptome deep sequencing, cytof, and PET-CT imaging, we evaluated the tumor, microenvironment, and immune pathway activation among immune infiltrate phenotypes.

Results Three distinct inflamed tumors phenotypes were identified: ‘classically’ inflamed characterized by pathway evidence of increased CD8+ T cells and decreased PD-L1 expression, inflamed tumors with pathways indicative of neovascularization and STAT3 signaling and reduced T cell mobilization, and an inflamed tumor with increased immunosuppressive myeloid phenotypes. Excluded tumors were characterized by TGFβ gene expression and pro-inflammatory cytokine signaling (e.g. TNF, IL1β), associated with decreased leukocytes homing and increased immune cell death of cells. We visualized and quantified TGFβ activity using PET-CT imaging of 89Zr-fresolimumab, a TGFβ neutralizing antibody. TGFβ activity was significantly increased in excluded tumors compared to inflamed or desert tumors, which was supported by quantitative pathology (Perkin Elmer) of its canonical signaling target, phosphorylated SMAD2 (pSMAD2), pSMAD2 was positively correlated with PD-L1 expression in the stroma of excluded tumors. In contrast, in inflamed tumors, TGFβ activity positively correlated with increased F4/80 positive macrophages and negatively correlated with expression of PD-L1. CyTOF analysis of tumor and spleen immune phenotypes revealed increased trafficking of myeloid cells in mice bearing inflamed tumors compared to excluded and deserts.

Conclusions The immunocompetent mTDT provides a model that bridges the gap between the immune landscape and tumor microenvironment. Integration of these high-dimensional data with further studies of response to immunotherapies will help to identify tumor features that favor response to treatment or the means to convert those that are unresponsive.

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TIRED AND HUNGRY: A POTENTIAL ROLE FOR CD47 IN T CELL EXHAUSTION

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Background Multiple suppressive mechanisms within the tumor microenvironment are capable of blunting anti-tumor T cell responses, including the engagement of inhibitory receptors expressed in tumor-associated, exhausted CD8+ T cells, such as programmed cell death protein 1 (PD-1), T-cell immunoglobulin-mucin-domain-containing-3 (TIM-3), lymphocyte activation gene 3 (LAG-3), 2B4 (also known as CD244), and T cell immunoreceptor with Ig and ITIM domains (TIGIT). 1, 2 While immune checkpoint blockade therapies aimed at reinvigorating T cell effector function have demonstrated their clinical effectiveness, 3, 4 not all patients demonstrate long-term disease control. 5 The refractory nature of terminally differentiated, exhausted CD8+ T cells to be reinvigorated by PD-1 blockade is one potential cause. 6–8 This limitation warrants the need to explore modulatory pathways that potentially program T cells toward exhaustion.

Methods Single cell-RNA sequencing (scRNA-seq) data derived from the tumor-infiltrating lymphocytes (TILs) of melanoma patients 9 were used for transcriptomic analysis and flow cytometry results were used to quantify protein levels in TILs. Murine B16-F10 (B16) melanoma model was used for both in vitro and in vivo studies. TCR-transgenic Pmel-1 and OT-1 transgenic mice, as well as CD47−/− (knockout, KO) mice were purchased from the Jackson Laboratory to generate CD47+/+ (wild-type, WT), CD47+/− (heterozygote, HET) mice with Pmel-1 or OT-1 background. For T cell co-transfer studies, Rag-deficient mice or C57BL/6j mice with sub-lethal irradiation (600cGy) were used as recipients. Naïve TCR-transgenic CD47 WT and CD47 HET CD8+ T cells were labelled, mixed in a 1:1 ratio for co-transfer experiments.

Results Flow cytometry analysis of human melanoma TILs found a strong upregulation of CD47 expression in tumor-associated, exhausted CD8+ T cells. We confirmed that CD47 transcription is significantly elevated among CD8+ T cells with a phenotype consistent with exhaustion using scRNA-seq results of TILs derived from melanoma patients. 9 Our study in murine B16 melanoma model confirms our finding in melanoma patients. 7 Our study specifically address the role of CD47 in anti-tumor CD8 effector function, we conducted T cell co-transfer studies and found that CD8+ T cells with lower copy number of CD47 (CD47 HET) significantly outnumbered the co-transferred CD47 WT CD8+ T cells within the tumor, exhibiting an enhanced effector function and less exhausted phenotype. Our study demonstrates a potentially novel role for CD47 in mediating CD8+ T cell exhaustion.

Conclusions CD47 expression in CD8+ T cells programs T cells toward exhaustion.

Ethics Approval All mice were maintained in microisolator cages and treated in accordance with the NIH and American Association of Laboratory Animal Care regulations. All mouse procedures and experiments for this study were approved by the MSKCC Institutional Animal Care and Use Committee (IACUC).

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Basal Cell Carcinoma Demonstrates a T-Cell Immune Cell Profiling Across Solid Tumor Types

Abstracts

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Basal Cell Carcinoma Demonstrates a T-Cell Exclusion Immune Phenotype in Contrast to Other Anti-PD-1 Therapy Responsive Cutaneous Malignancies

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Background Basal cell carcinoma (BCC) is considered an immunogenic tumor based on the high tumor mutational burden (TMB), increased incidence in immunocompromised patients, and responsiveness to immunomodulators, a toll-like receptor agonist therapy. However, anti-PD-1 immunotherapy response rates in patients with advanced BCC appear less than that seen with other advanced cutaneous malignancies. Molecular profiles of BCC tumors were analyzed to determine immune phenotypes and resistance mechanisms in comparison to other anti-PD-1 therapy responsive cutaneous malignancies.

Methods Next generation sequencing on DNA (NGS; NextSeq and Novaseq), PD-L1 immunohistochemistry (SP-142 and 28–8 antibody clones, cutoff >5% tumor staining) and mRNA gene expression level (Whole Transcriptome Sequencing, NovaSeq) data from BCC (N=69), melanoma (N=914), and cutaneous squamous cell carcinoma (SCC) tumors (N=165) at Caris Life Sciences (Phoenix, AZ) were analyzed. Tumor mutational burden (TMB) was calculated by counting all non-synonymous missense mutations that had not been previously described as germline alterations. Microenvironment cell population counter was used to estimate cell population abundance in the TME. Gene set enrichment analysis (GSEA) was performed on transcriptomes. Statistical significance was set at P value or false discovery rate (FDR) < 0.05.

Results Of the 69 BCC tumors with NGS data, the most frequent mutations were in PTCH1 (82%), P53 (73%) and ARID1A (42%); additional relevant mutations included SMO (18%), JAK1 (9%), PI3KCA (6%), APC (4%), and CTNNB1 (3%). TMB was significantly greater in BCC compared to melanoma (median 30.5 vs 12 mut/Mb, P<0.0001) and similar to SCC (median 29.5 mut/Mb, P=0.9389). PD-L1 positivity was 1/23 (4%) in BCC, 215/831 (26%) in melanoma, and 81/147 (56%) in SCC. Interferon gamma and T-effector immune gene analyses showed significantly lower expression in BCC compared to melanoma and SCC (e.g., IFNg TPM=0.26 (BCC) vs 0.65 and 0.58 (melanoma, SCC, both P<0.01). BCC demonstrated the lowest CD8 T-cell fractions and the highest neutrophil and cancer associated fibroblast (CAF) fractions compared to melanoma and SCC. Angiogenesis and TGF-beta gene sets were enriched in BCC compared to melanoma (NES=1.5, FDR=0.046 and NES=1.35, FDR=0.055, respectively), but not compared to SCC (NES=0.90, FDR=0.57 and NES=0.94, FDR=0.60, respectively).

Conclusions While BCC tumors demonstrated a high TMB, a markedly lower level of adaptive anti-tumor immunity in comparison to other cutaneous malignancies was observed. T-cell exclusion mechanisms mediated through CAFs and desmoplasia, with upregulation of TGF-beta and angiogenic signaling, may play a role. Further investigation into abrogation of these mechanisms is warranted to develop improved anti-PD-1 based therapies for BCC.

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Immune Cell Profiling Across Solid Tumor Types by Mass Cytometry Reveals Tumor Enrichment of PD-1+/LAG-3+ CD8 Memory T Cells That Exhibit Tumor-Reactive Yet Dysfunctional Features

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Background Characterization of human immune responses by profiling immune cells from patients is critical for the successful development of immuno-oncology agents and is useful to understand mechanism-of-action, identify pharmacodynamic/response biomarkers, and guide patient selection strategies. Extensive immune cell heterogeneity necessitates comprehensive high parameter immunophenotyping to yield these actionable insights.

Methods Cytometry by time-of-flight (CyTOF) was performed on homogenates from commercially procured tumors (n=28) and matched PBMCs (n=7) from patients with various solid tumors (colon (n=10), endometrial (n=9), kidney (n=4), liver (n=2), skin (n=1), lung (n=1), and gastro-intestinal (n=1)). Two antibody panels, recognizing a total of 18 lineage and 31 target proteins, were used to profile marker expression among the major lymphocyte and myeloid lineages. Data were analyzed using manual gating and non-linear dimensionality reduction (tSNE and UMAP), and expression was measured by frequency (% gate) and arschnihh-transformed median ion counts. Pairwise Wilcoxon Rank Sum tests were performed on arschnihh-transformed median ion counts to determine statistically significant differences in marker expression, and P values were adjusted using Benjamini-Hochberg correction (p<0.05 considered statistically significant). Cell subpopulation percentages were compared using unpaired two-sided T-tests. Sample populations with less than 150 events were excluded from
LONGITUDINAL IMMUNE PROFILING REVEALS UNIQUE MYELOID AND T CELL PHENOTYPES ASSOCIATED WITH SPONTANEOUS IMMUNOEATING IN A NOVEL PROSTATE TUMOR MODEL

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Background The theory of cancer immunoediting, which describes the dynamic interactions between tumors and host immune cells that shape the character of each compartment, is foundational for understanding cancer immunotherapy. Few models exist that facilitate in-depth study of each of the three canonical phases of immunoediting: elimination, equilibrium, and escape. Here, we perform high dimensional longitudinal immune profiling of NPK-C1, a transplantable prostate tumor model that recapitulates the three phases of immunoediting spontaneously in immunocompetent C57BL/6 animals.

Methods We generated a 28-color immune phenotyping panel to interrogate the NPK-C1 microenvironment using a Cytek Aurora spectral flow cytometer. We analyzed NPK-C1 tumors on days 10, 15, 20 and 24 post-implantation, representing elimination, equilibrium, early escape, and late escape phases, respectively. These data were analyzed by both traditional gating and with an optimized dimensionality reduction and unsupervised clustering workflow. We additionally performed in vivo depletion studies of T cell and granulocyte subsets at early and late time points to determine if these bulk populations are required for immunoediting during elimination and equilibrium/escape.

Results Matched samples revealed enrichment of effector memory (EM) and central memory (CM) CD8 T cells in tumors compared to PBMCs, as expected. EM cells represented on average 63.36% of the CD8 T cells in tumors vs 30.31% in PBMCs (p=0.0067), and CM cells 12.11% vs 5.58% respectively (p=0.1558). Non-linear dimensionality reduction mapping of these CD8 EM and CM cell subtypes among tumors displayed an activated but potentially dysfunctional phenotype, characterized by substantially higher expression of multiple coinhibitory receptors (PD-1, LAG-3, TIM-3, TIGIT) and activation markers (HLA-DR, ICOS) compared to PBMCs. Among these cells, a PD-1+/LAG-3+ subset, observed in 17/28 TIL samples, expressed TIM-3, TIGIT, HLA-DR, and ICOS at significantly higher levels compared to other PD1/LAG3 expression subsets. Interestingly, CD137 (4-1BB), a marker of potentially tumor-reactive cells, is expressed predominantly in PD-1+ memory CD8 T cells, with the most intense expression levels observed in the PD-1+/LAG-3+ subset.

Conclusions The present results provide insight into the relative (co)expression of potentially targetable immunological pathways, and suggest a biological basis for informing approaches to combination checkpoint inhibition therapy.

Acknowledgements We thank Paul Fischer for his contributions in acquiring the CyTOF data and performing initial data QC and analysis.

Ethics Approval This study was approved by Bristol Myers Squibb’s Global Data Repository (Biological Assessment of Risk (BAR) number EVL_2020_12339). Samples were provided by Discovery Life Sciences (CA), MT Group (CA), Avaden BioSciences (WA), or BioOptions (CA). All patients gave written informed consent at the time of sample collection according to the IRB protocols of each provider.

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498 DOWNREGULATION OF CD5 IN CD8+ T TUMOUR-INFILTRATING LYMPHOCYTES ASSOCIATES WITH INCREASED LEVEL OF ACTIVATION AND EXHAUSTION

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Background CD5, a member of the scavenger receptor cysteine-rich superfamily, is a marker for T cells and a subset of B cells (B1a). CD5 associates with T-cell and B-cell receptors and impair TCR signaling1 2 and increased CD5 is an indication of B cell activation. Furthermore, CD5 levels on CD8+ T cell splenocytes were significantly increased after TCR/CD3 stimulation using ex vivo treatment with anti-CD3/anti-CD28 MAbs compared to non-stimulated CD8+ T splenocytes.3 Previous studies have shown a correlation between CD5 and anti-tumour immunity where CD5 knockout mice inoculated with B16F10 melanoma cells had delayed tumour growth compared to wild type mice.4 In tumour-infiltrating lymphocytes (TILs) isolated from lung cancer patients, CD5 levels were negatively correlated with anti-tumour activity and tumour-mediated activation-induced T cell death,5 suggesting that CD5 could impair activation of anti-tumour T cells. However, the correlation between CD5 level expression and T cell activation and exhaustion in the tumour microenvironment and in peripheral organs is ill-defined and requires further investigation.

Methods We determined CD5 levels in T cell subsets in different organs in mice bearing syngeneic 4T1 breast tumour
homografts and assessed the relationship between CD5 and increased CD69 and PD-1 (markers of T cell activation and exhaustion) by flow cytometry.

**Results** We report that T cell CD5 levels were higher in CD4 + T cells than in CD8 + T cells in 4T1 tumour-bearing mice, and that high CD5 levels on CD4+ T cells were maintained in peripheral organs (spleen and lymph nodes). However, both CD4+ and CD8+ T cells recruited to tumours had reduced CD5 compared to CD4+ and CD8+ T cells in peripheral organs. In addition, CD5highCD4+ T cells and CD5highCD8 + T cells from peripheral organs exhibited higher levels of activation and associated exhaustion compared to CD5lowCD4 + T cell and CD5lowCD8 + T cell from the same organs. Interestingly, CD8 + T cells among TILs and downregulated CD5 were activated to a higher level, with concomitantly increased exhaustion markers, than CD8+CD5+ TILs.

**Conclusions** Thus, differential CD5 levels among T cells in tumours and lymphoid organs can be associated with different levels of T cell activation and exhaustion, suggesting that CD5 may be a therapeutic target for immunotherapeutic activation in cancer therapy.

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**Ethics Approval** This study was approved by the Animal Use Subcommittee of the University of Western Ontario

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**AT1636, A COLON CANCER SURVIVOR-DERIVED ANTIBODY RECOGNIZES A PREVIOUSLY UNIDENTIFIED TRUNCATED, O-MANNOSYLATED 70KDA VARIANT OF E-CADHERIN**

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**Background** Colorectal cancer (CRC) associated with Lynch syndrome is characterized by an abundance of infiltrating lymphocytes. To study whether tumor-specific antibodies with therapeutic potential can be isolated from these patients, the B-cell repertoire from a patient with Lynch syndrome who recovered from a stage IV colon carcinoma was screened. Here we describe an antibody, AT1636 that recognizes a previously unidentified O-mannosylated 70kDa form of E-cadherin. The intercellular interactions by E-cadherin on tumor cells have for long been recognized as protective in cancer metastasis, and deregulation of E-cadherin is a hallmark for epithelial-mesenchymal transition (EMT).

**Methods** The study protocol was approved by the Medical Ethical Committee of the Academic Medical Centre, Amsterdam, The Netherlands (NL42718.018.12). AIMM’s BCL6 and Bcl-XL immortalization method1 was used to interrogate the human antibody repertoire. From a carrier of a pathogenic gene variant in the MSH6 gene diagnosed with stage IV CRC and liver metastasis that had been treated with avastin, capecitabine and oxaliplatin, peripheral-blood memory B cells were obtained 9 years after last treatment. Antibodies-containing supernatant of cultured B-cells were screened for binding to 3 different CRC cell lines (DLD1, LS174T and COLO205) and absence of binding to fibroblast by flow cytometry. A high-affinity variant of AT1636 (AT1636YN) was sorted from the original AT1636, AID-expressing B-cell clone.2

**Results** Antibodies that demonstrated differential binding to CRC cells were characterized and targets recognized by such antibodies were identified using immunoprecipitation and mass-spectrometry. One of the antibodies, AT1636, recognized a previously unidentified O-mannosylated 70kDa E-cadherin variant (ECV). Although the 70kDa ECV is found in full-length E-cadherin expressing cells, tumor-specific binding of AT1636 is dependent on the O-mannosylation pattern in the antibody epitope on ECV. Using shRNA knock-down AT1636 binding was shown to depend on the transmembrane O-mannosyltransferase targeting cadherins 3 (TMTC3).3 In accordance, coexpression of TMTC3 and E-cadherin in tumor cells is predictive for AT1636 binding. In addition, we observed that (over)expression of ECV results in a strong de-adhesive, EMT-like phenotype. Although AT1636 by itself is not able to induce ADCC, the CD3-bispecific antibody (single-chain UCHT1) AT1636 format specificity killed CRC cell lines.

**Conclusions** The AT1636 antibody retrieved from a patient with Lynch syndrome binds a previous unidentified cancer-specific O-mannosylated 70kDa form of E-cadherin. This variant might play a role in tumor-cell invasion and metastasis. More importantly, we provide a rationale to advance AT1636 based therapeutics for treatment of CRC.

**Ethics Approval** The study protocol was approved by the Medical Ethical Committee of the Academic Medical Centre, Amsterdam, The Netherlands (NL42718.018.12)

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**P2RX7 AGONIST TREATMENT BOOSTS THE ABILITY OF IL-12-ACTIVATED CD8+ T CELLS TO INFILTRATE AND CONTROL MURINE MELANOMA**

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**Background** Extracellular adenosine triphosphate (eATP) is a ‘danger signal’ used to sense cellular damage, and recognized by purinergic receptors in mammals. Among those
VISTA REGULATES THE DIFFERENTIATION AND SUPPRESSIVE FUNCTION OF MYELOID-DERIVED SUPPRESSOR CELLS
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Background V-domain immunoglobulin suppressor of T cell activation (VISTA) is a B7 family inhibitory immune checkpoint protein and is highly expressed on myeloid cells and T cells. VISTA acts as both an inhibitory ligand when expressed on antigen-presenting cells and a receptor when expressed on T cells. Our recent study has shown that VISTA is a myeloid cell-specific immune checkpoint and that blocking VISTA can reprogram suppressive myeloid cells and promote T cell-stimulatory tumor microenvironment. In this study, we further demonstrate that VISTA blockade directly alters the differentiation and the suppressive function of myeloid-derived suppressor cells (MDSC).

Methods Flow cytometry was performed to examine VISTA expression on MDSCs in multiple murine tumor models including the B16BL6 melanoma model, MC38 colon cancer model, and the KPC pancreatic cancer models. To examine the role of VISTA in controlling the differentiation and suppressive function of MDSCs, we cultured wild type (WT) and VISTA.KO bone marrow progenitor cells with GM-CSF and IL-6 to induce BM-derived MDSCs.

Results Our preliminary results show that VISTA is highly expressed on M-MDSCs in B16BL6, MC38 and KPC tumors. In BM-derived MDSCs, VISTA deletion significantly altered the signaling pathways and the differentiation of MDSCs. Multiple inflammatory signaling pathways were downregulated in VISTA KO MDSCs, resulting in decreased production of cytokines such as IL1 and chemokines such as CCL2/4/9, as well as significantly impaired their ability to suppress the activation of CD8+ T cells. The loss of suppressive function in VISTA KO MDSCs is correlated with significantly reduced expression of iNOS. To validate the results from BM-MDSCs, we sorted CD11b+CD11c-Ly6C+Ly6G-M-MDSCs and CD11b+CD11c-Ly6G+ G-MDSCs from B16BL6 tumor tissues and tested the ability of a VISTA-blocking mAb to reverse the suppressive effects of tumor-derived MDSCs. Our results show that blocking VISTA impaired the suppressive function of tumor-derived M-MDSC but not G-MDSCs.

Conclusions Taken together, these results demonstrate a crucial role of VISTA in regulating the differentiation and function of MDSCs, and that blocking VISTA abolishes MDSC-mediated T cell suppression, thereby boosting.

Ethics Approval All in vivo studies were reviewed and approved by Institutional Animal Care and Use Committee (Approval number 2019-2142).

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THE CO-EXPRESSION OF TWO IMMUNE COMPLEX MOLECULES, VISTA AND TIGIT, DEFINE A DYSFUNCTIONAL CYTOTOXIC T CELL SUBSET

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Background Cancer immunotherapies have proven, over the last decade, to be of extreme importance for long term survival of patients. Specifically, immune checkpoint inhibitors such as anti-CTLA-4 and anti-PD-1/PD-L1 have had tremendous clinical success treating many cancers including melanoma, lung, breast, colon, and bladder cancer. The low response rate (~30%) of these drugs suggest a mechanism of resistance within the tumor microenvironment, and it demonstrates the immense need to study and develop alternative routes to long-term anti-tumor immunity. V-domain Immunoglobulin Suppressor of T-cell Activation (VISTA) has been shown to be a suppressive molecule in the tumor microenvironment in preclinical models and VISTA’s expression is correlated with poor patient outcome across several cancers. T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) is a functional receptor expressed on NK cells and T cells that contributes to a suppressive tumor microenvironment by acting on T cells, NK cells, and antigen presenting cells.

Methods We use flow cytometry to visualize single cell expression of VISTA and TIGIT on CD8+ tumor infiltrating lymphocytes in pre-clinical models. Functional studies include cytotoxic assays as well as intracellular cytokine staining after cell sorting.

Results Here we show the expression of these two immune checkpoint molecules, VISTA and TIGIT, across several pre-clinical models, and how their co-expression subsets a distinctly dysfunctional population of cytotoxic T cells.

Conclusions Our data provides foundation to study the rejuvenation of this subset of T cells to restore cytotoxic function and therefore, anti-tumor immunity.

Trial Registration NA

Ethics Approval All in vivo studies were reviewed and approved by Institutional Animal Care and Use Committee (Approval number 2019-2142).

Consent NA

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TUMOR INfiltrATING LYMPHOCyTES IN SOFT TISSUE SARCOMAS UPRegulate THE EXHAUSTion MARKER TIGIT AND ARE REINIGORATED BY IL-15 STiMULATIoN AND TIGIT BLOCKADE

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Background Although the presence and activity of tumor infiltrating lymphocytes (TILs) have been shown to be important factors for survival and response to immunotherapy for multiple cancer types, the benefits of immunotherapy in soft tissue sarcomas (STS) have been limited, and novel approaches are needed. In this study, we sought to characterize the phenotype and function of tumor infiltrating natural killer (NK) and T cells in STS patients and to evaluate clinically relevant strategies to augment TIL function.

Methods Using both prospectively collected blood and tumor tissue from STS patients undergoing surgical resection (n = 21) and archived specimens (n = 45), we performed flow cytometry and immunohistochemistry to evaluate the extent of peripheral and intratumoral CD3-CD56+ NK and CD8+ T cell phenotype and function as predictors of outcome. We also analyzed TCGA data and the peripheral blood of dogs with spontaneous osteosarcoma receiving inhaled IL-15 on a clinical trial to evaluate the association of CD3-NKp46+ NK and CD8+ T cell activation as well as TIGIT upregulation with outcome. Finally, we stimulated patient PBMCs and TILs ex vivo with IL-15 and a novel human anti-TIGIT antibody to assess the impact of combination therapy on NK and T cell phenotype and function. Parametric and non-parametric statistical tests were used where appropriate. Univariate and multivariate survival analyses were performed by Cox proportional hazards models.

Results Compared to peripheral expression, intratumoral NK and T cells showed an activated and exhausted phenotype by CD69 and TIGIT, respectively. Ex vivo TIL stimulation with IL-15 further increased markers of activation and function including CD69, Ki67, IFNγ, and granzyme B, while increasing expression of exhaustion marker TIGIT. Analysis of a retrospective STS cohort and TCGA STS gene expression confirmed the association of TILs with improved prognosis. Dogs with metastatic osteosarcoma receiving inhaled IL-15 exhibited upregulation of activation markers and TIGIT. Analysis of a retrospective STS cohort and TCGA STS gene expression confirmed the association of TILs with improved prognosis. Dogs with metastatic osteosarcoma receiving inhaled IL-15 exhibited upregulation of activation markers and TIGIT. Analysis of a retrospective STS cohort and TCGA STS gene expression confirmed the association of TILs with improved prognosis. Dogs with metastatic osteosarcoma receiving inhaled IL-15 exhibited upregulation of activation markers and TIGIT. In vitro, IL-15 and TIGIT blockade of both peripheral and intratumoral NK cells increased cytotoxicity against sarcoma cell lines and increased expression of degranulation marker CD107a compared to IL-15 alone.

Conclusions TILs are associated with improved survival in STS, and tumor infiltrating NK and T cells show features of both increased activation and increased exhaustion. Tumor infiltrating NK and T cells respond to IL-15 stimulation, but simultaneously further upregulate TIGIT with the combination of IL-15 and TIGIT blockade showing greatest cytotoxic effects. Overall, our data suggest that the combination of IL-15 and TIGIT blockade is a promising clinical strategy in STS.

Ethics Approval All experiments involving human and canine patients were approved by the respective Institutional Review Boards at the University of California, Davis, Schools of Medicine (Protocol #218204-9) and Veterinary Medicine (IACUC #20179).

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Methods C57BL/6 mice were given a subcutaneous injection of B16F10 melanoma cell line (1 X 10^6 cells). Mice were given intraperitoneal injection of alpha-galactosylceramide (a-GalCer, a ligand for iNKT cells; 2 microgram/injection) on day +1, +5, +10, +15 and +20. NK cells, Gr1+ cells and F4/80+ macrophages in mice were depleted using cell-specific antibodies. The growth of tumors was monitored, and immune cells were characterized using flow cytometry and immunofluorescence staining. Student’s t-test and one-way ANOVA were used for statistical analysis.

Results Our results showed that intratumoral NK T cells had significantly low expression of CD25, CD69, CD122, and IFN-gamma receptor molecules and produced lower inflammatory cytokines (IFN-gamma, TNF-alpha, and GM-CSF) as compared to splenic NK T cells. The soluble factor produced by B16F10 cells reduces the expression of these cytokines and cytotoxic receptor in vitro on the NK T cells purified from the spleen. Treatment of tumor-bearing mice with a-GalCer significantly increased the IFN-gamma-producing NK T cells, CD8+ T cells, and effector Th1 cells in secondary lymphoid organs, and tumors, also significantly reduced the tumor growth. Furthermore, a-GalCer treatment significantly increased the iNOS^-CD206^- M1-macrophages and reduced the iNOS^+CD206^- M2-macrophages in the spleen and tumor. The depletion of F4/80^- macrophages prevented the a-GalCer-induced reduction of tumor growth.

Conclusions Our results showed that tumor produced soluble factors alter the phenotype of NK T cells. Activation of NK T cells with a-GalCer promotes the M1-macrophages, and effector CD8^+ T cells, Th1 cells in the secondary lymphoid organs and tumor microenvironment. This finding suggests that activation of NK T cells may provide an effective anti-tumor response.

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Ethics Approval All the procedures performed in the experiments involving mice were in accordance with the ethical standards of (NCCS) Institutional Ethics Committee of Animals Usage (Approval ID: EAF/B-166/2011 and EAF/B-256/2016).

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harnessing the immune system as a major therapeutic strategy for the treatment of patients with osteosarcoma.

Methods 66 osteosarcoma tissue specimens were stained and analyzed by immunohistochemistry. Tumor-infiltrating lymphocytes (TILs) from 25 specimens were profiled by functional multiparameter flow cytometry (MFC). Distinct regions from 16 pulmonary metastases (PMs) were microdissected, and RNA was extracted to perform comparative transcriptomic studies. Clinical follow-up (median 24 months) was available from resection.

Results Digital image analysis of immunohistochemistry demonstrated significantly higher infiltrating immune cells in the PMs compared to primary bone tumors, concentrated at the tumor-normal lung ‘PM interface’ region, and elevated expression of multiple immune checkpoint molecules at the PM interface (figure 1). MFC confirmed the increased expression of the immune checkpoint molecules programmed cell death 1 (PD-1, p<0.01) and lymphocyte activation gene 3 (LAG-3, p<0.01), as well as the activation marker IFN-γ (p<0.05) in CD8+ TILs. Gene expression profiling provided further evidence for the presence of TILs with expression of activation markers and inhibitory immune checkpoint molecules at the PM interface compared to the PM interior (figure 2). A strong M2 macrophage signature was present in both regions. Further analysis revealed that genes related to neutrophil and myeloid cell chemotaxis and known to be associated with polymorphonuclear myeloid-derived suppressor cells were highly expressed at the PM interface, along with genes for multiple subsets of dendritic cells (figure 3). Expression of PD-L1,

Abstract 506 Figure 1 Immunohistochemistry of osteosarcoma pulmonary metastases
A. H&E with demarcation of tumor-normal lung interface (center green line) and area quantified as the ‘PM interface’ (outer green lines).

Pulmonary metastases demonstrate a higher concentration of immune cells (CD3 p<0.001, CD8 p<0.001, CD163 p<0.01) and PD-1 (p<0.001)/PD-L1 (p<0.05) at the PM interface.

B. H&E with demarcation of PM interface as above. Pulmonary metastases demonstrating increased staining of TIM-3 (p<0.01), LAG-3 (p<0.01) and IDO1 (p<0.0001) at the PM interface (no significant concentration of CSF1R at PM interface).

Abstract 506 Figure 2 Activated/exhausted lymphocyte signatures at PM interface
A. Heatmap displaying significant genes that contribute to leading-edge of core enrichment subset via Gene Set Enrichment Analysis (GSEA) demonstrating higher expression of immune regulatory molecules at the PM interface compared to the PM interior. Expression levels were converted into heatmaps and colors quantitatively correspond to fold changes. FDR=GSEA false-discovery rate q-value.

B. Heatmap illustrating coefficients of xCell analysis shows higher expression of markers of cytotoxicity and activation, as well as multiple checkpoint molecules, at the PM interface, with evidence that they are being contributed chiefly by T cells. Intensity represents xCell coefficient, which corresponds to the amount that a particular region (PM interior or PM interface) or cell population (T cells, B cells, or myeloid cells) contributes to the expression of a specific gene.

Abstract 506 Figure 3 Genes related to dendritic cells and MDSCs at PM interface
A. By GSEA, genes associated with multiple subclasses of antigen-presenting dendritic cells are significantly upregulated at the PM interface (cDC1=conventional type 1 dendritic cell; cDC2=conventional type 2 dendritic cell; pDC=plasmacytoid dendritic cell; moDC=monocyte-derived dendritic cell). FDR=GSEA false-discovery rate q-value.

B. Heatmap shows heightened expression of cytokines, chemokines and endothelin transcripts associated with development, recruitment and maintenance of PMNs and granulocytic MDSCs at the PM interface compared to the PM interior.

Abstract 506 Figure 4 Markers of immune TME at PM interface correlate with PFS
A. Hazard ratios for immunohistochemistry markers at the PM interface as they relate to PFS. For absolute count biomarkers (CD3, CD8, Foxp3, PD-1, CD163, and LAG-3) the unit is per 100 cells, and for percentage biomarkers (PD-L1, CSF1R, TIM-3, and IDO1) the unit is per 1%. B. Hazard ratios for gene sets at the PM interface as they relate to PFS. NS=p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
HIGH DIMENSIONAL FLOW CYTOMETRY ANALYSIS IN NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA PREDICTS PATIENTS OUTCOMES

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Abstract 507 Figure 1 UMAP embedding of T cells in CR, NR, at diagnosis (BM_DG) and after chemotherapy (BM_post), HC colored by T cell state (resting, activated, terminal differentiated), overlaid with a contour plot

Abstract 507 Figure 2 Boxplots showing the differential cluster abundance and adjusted p-values for CR, NR, at diagnosis (BM_DG) and after chemotherapy (BM_post), HC in the three different T cell states (resting, activated, terminal differentiated)

Background We have previously characterized phenotypic and transcriptional profile of CD8+ T cells in acute myeloid leukemia (AML) and their differences between responders vs. nonresponders to chemotherapy.1 Goal of ongoing work was to further probe uniqueness of AML in sculpting CD8+ T cell responses and the plasticity of their signatures upon chemotherapy response.

Methods We first examined the cumulative expression of multiple inhibitory receptors (IRs) (detected by 2 different panels) on CD8+ T cells and created an IR-score which summarizes the relative amount of PD-1, Tim3, KLRG1, 2B4, CD160, CD57, and BTLA-positive CD8+ T-cells in relation to the well-characterized maturation states of CD8+ T cells. Serial bone marrow samples from 33 newly diagnosed AML patients with well-annotated clinical data (21 complete responders (CR) and 12 nonresponders (NR) to chemotherapy) and 11 healthy controls (HC) were analyzed. Finally, using custom made R code, we performed dimensionality reduction, clustering, and pseudotime analysis.

Results The IR-score discriminated NR and CR (p = 3e-02, AUC 0.84) after treatment with CD57 and KLRG1 accounting for most of this difference (p = 2e-02, AUC = 0.79). Next we investigated CD8+ T cell populations that best correlated with response to chemotherapy. FlowSOM revealed seven major clusters: naive and naive-like, CD28+KLRG1+ activated-effector, CD28+KLRG1+PD1+ dysfunctional, PD1 +CD57+ senescent effector-memory and two clusters of terminally differentiated CD45RA+KLRG1+ cells. Since the activation and differentiation states accounted for most of the subpopulation variability, we grouped the clusters into resting (naive, naive-like), activated (activated-effector, dysfunctional), and terminally differentiated cells (senescent effector-memory, terminally differentiated). UMAP developmental trajectories and differential abundance testing showed increased frequency of activated cells at diagnosis (p-adj = 2.9e-05) and of resting cells after treatment (p-adj = 1.3e-02) in CR, while terminally differentiated T cells prevailed in NR (p-adj = 5.3e-08) after treatment (figures 1 and 2).

Conclusions The increased number of functional activated T cells at diagnosis and the persistence of a naive/naive-like reservoir at the time of response is a signature associated with achievement of CR. Lack of response (NR) correlates with accumulation of the terminally differentiated and senescent cells in the bone marrow. These results uncover an intertwined relationship between skewing of T cell differentiation and clinical response to chemotherapy. The data provide rationale to either remove senescent or augment activity of
 naïve/naïve-like T cells as a strategy to reinforce antileukemia immunity.

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508 **DIFFERENT NEOANTIGEN EXPRESSION PATTERNS IMPACT THE STRENGTH OF ANTI-TUMOR IMMUNE RESPONSES**

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**Background** Many cancer immunotherapies depend on the ability of cytotoxic CD8+ T cells to recognize neoantigens on MHCI complexes to effectively eliminate tumor cells. However, patient response following immunotherapy is highly variable, with recent work suggesting that neoantigen expression patterns may impact patient response. Specifically, it was observed that the immune response is dampened when neoantigens are expressed only by a subset of tumor cells (heterogeneous expression). To study why anti-tumor immunity is reduced in a heterogeneous setting we developed a transplant murine tumor model engineered to express neoantigens in a heterogeneous pattern or homogenously.

**Methods** A curated list of neoantigens with varying predicted MHCI binding affinities was used to establish an array of cell lines expressing at one to three neoantigens. The lines were inoculated subcutaneously in immunocompetent mice as mixtures (heterogenous) or as a single line (homogenous) to study the resulting immune response. Tumors were harvested at days 7, 10 and 14 and flow cytometry analysis was used to phenotype infiltrating immune populations, including antigen-specific CD8+ T cells. ELISpot assays were performed using splenocytes from the same timepoints to determine the frequency of antigen-specific T cells in the periphery.

**Results** Compared to neoantigens predicted to bind weakly to MHCI, neoantigens predicted to bind strongly elicited robust expansion of antigen-specific T cells in the periphery and tumors expressing these antigens alone exhibited greater numbers of tumor infiltrating T cells. Homogenous expression of two neoantigens was found to enhance anti-tumor immunity by increasing the frequency of tumor-reactive T cells. Further, homogenous expression of two neoantigens induced protective immunity against antigens, including those that failed to be controlled when expressed alone.

**Conclusions** Using our novel reductionist tumor model, our results suggest that a more robust response against weak antigens could be induced if a response against a strong, highly immunogenic neoantigen is mounted simultaneously. This observation has direct implications for the design of neoantigen vaccines either as mono- or combination immunotherapies, especially in the setting of a heterogeneous neoantigen expression pattern.

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509 **POTENT AND SELECTIVE INHIBITION OF AXL RECEPTOR TYROSINE KINASE FOR THE TREATMENT OF CANCER**

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**Background** AXL receptor tyrosine kinase (AXL) is a transmembrane protein that is over-expressed in a variety of cancer and immune cells. AXL signaling has been implicated in creating an immunosuppressive tumor microenvironment (TME) through both tumor-intrinsic and immunomodulatory mechanisms promoting resistance to various therapies. AXL is highly expressed on a subset of immune cells, including DCs, NK cells and M2 macrophages as well as fibroblasts, which contribute to a blunted anti-tumor response. Consistent with these observations, AXL is strongly associated with increased infiltration of macrophages, exhausted NK and T-cells, as well as significantly increased CD73 expression in multiple cancer types in TCGA. Additionally, AXL expression is strongly and significantly correlated with epithelial-mesenchymal transition (EMT), which further generates an immunosuppressive TME and promotes resistance to immune, targeted and chemotherapies. High expression of AXL is also strongly associated with poor survival in NSCLC, pancreatic, breast, head & neck, stomach, colorectal, ovarian & prostate adenocarcinomas, especially in the metastatic setting. AXL inhibitors that exhibit high potency in both biochemical (IC <5nM) and cell-based (IC <25nM) assays in addition to good selectivity against closely related kinases MER and TYRO3 (>90x and >25x fold selectivity, respectively) as well as other kinases involved in downstream signaling such as PI3K have been developed. Initial studies in animal models indicate a favorable pharmacokinetic profile and anti-tumor efficacy.

**Methods** Compound inhibition potency against the kinase activity of AXL and other kinases was determined by detecting phosphorylated substrate using homogeneous time-resolved fluorescence (HTRF). Binding affinity of inhibitor to intracellular AXL kinase was determined by monitoring displacement of a competitive fluorescent tracer using an AXL NanoBRET assay. Recombinant Gas6, cancer cell lines, whole blood or isolated cells from healthy donors were used to determine the reduction in AXL-mediated signaling in-vitro. PK/PD and anti-tumor effects of selected AXL inhibitors were evaluated in murine models.

**Results** AXL is highly expressed on a subset of immune cells, including DC’s, NK cells and M2 macrophages as well as fibroblasts, which contribute to a blunted anti-tumor response. Consistent with these observations, AXL is strongly associated with increased infiltration of macrophages, exhausted NK and T-cells, as well as significantly increased CD73 expression in multiple cancer types in TCGA. Additionally, AXL expression is strongly and significantly correlated with epithelial-mesenchymal transition (EMT), which further generates an immunosuppressive TME and promotes resistance to immune, targeted and chemotherapies. High expression of AXL is also strongly associated with poor survival in NSCLC, pancreatic, breast, head & neck, stomach, colorectal, ovarian & prostate adenocarcinomas, especially in the metastatic setting. AXL inhibitors that exhibit high potency in both biochemical (IC <5nM) and cell-based (IC <25nM) assays in addition to good selectivity against closely related kinases MER and TYRO3 (>90x and >25x fold selectivity, respectively) as well as other kinases involved in downstream signaling such as PI3K have been developed. Initial studies in animal models indicate a favorable pharmacokinetic profile and anti-tumor efficacy.

**Conclusions** AXL is a promising therapeutic target involving both immunomodulatory and tumor-intrinsic mechanisms. AXL inhibition reduces the immunosuppressive TME, enables activation of an anti-tumor immune response and renders tumors more susceptible to previously resistant therapies. Highly potent and selective AXL inhibitors have been designed, displaying biological profiles superior to those of less-selective molecules currently advancing through clinical development.

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Abstracts


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510 HELPING THE KILLERS: INNOVATIVE CANCER IMMUNOTHERAPY HARNESSING QUASI-UNIVERSAL TUMOR ANTIGEN-SPECIFIC CD4 T CELLS

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Background While cancer immunotherapy has mainly focused on exploiting CD8 T cells given their role in the direct elimination of tumor cells, increasing evidence highlights the crucial roles played by CD4 T cells in anti-tumor immunity. However, their very low frequency, the lack of robust algorithms to predict peptide binding to MHC class II molecules and the high polymorphism of MHC class II molecules render the study and use of circulating tumor antigen-specific CD4 T cells challenging. In this regard, the HLA-DRB3*02:02 gene encoding an HLA allele that is expressed by half of the Caucasian population, offers a way to identify CD4 T cell-defined tumor antigens with broad cancer patient coverage.

Methods Here, we aim to identify, isolate and functionally characterize ‘quasi-universal’ human tumor antigen-specific HLA-DRB3*02:02-restricted CD4 T cells in cancer patients. Using an algorithm we recently developed in house, tumor-associated antigenic peptides binding to this allele are identified. We have generated a large collection of HLA-DRB3*02:02-restricted CD4 T cell clones of different tumor-antigen specificities. We will perform in vitro co-cultures of CD4 T cell clones with tumor cells to measure cytokine secretion, their tumor cell killing and their phenotypic profile (PD-1, TIM3, TIGIT, 4-1BB, CD40L, LAG3, VISTA, OX40). We will sequence and clone the TCR of the most promising candidates for adoptive cell transfer therapy. Lastly, we will directly evaluate the presence of these cells ex-vivo and longitudinally monitor them in patients.

Results N/A

Conclusions Together, these results should contribute valuable targets for coordinated CD4 and CD8 T cell-based immunotherapy of cancer.

REFERENCE

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511 TUMOR IMMUNE MICROENVIRONMENT IN ADULT MICE ASYNCHRONOUSLY CROSS-FOSTERED AS PUPS

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Background Immune responses to cancer are highly variable and influenced by genetic and environmental factors.1 Syngeneic tumor models in mice with intact immune systems are required to study anti-tumor immune responses but are unable to adequately model varied immune responses. Classically, different mouse strain backgrounds have been used to compare different immune responses to cancer immunotherapy, but this approach is limited by the inability to administer identical tumor cell lines, keeping constant the tumor while experimentally varying the immune response. Proper establishment of the immune system begins in early life and is regulated by environmental cues from maternal breast milk and the developing microbiota. To disrupt these cues prior to weaning, newborn pups can be cross-fostered to dams that delivered their litters asynchronously, either 2 weeks earlier or later, a model referred to as asynchronous cross-foster (ACF).2 We previously demonstrated that ACF can profoundly skew the immune profile of genetically identical offspring.3 Young ACF mice exhibited enhanced Th2 immunologic skewing and reduced peripheral tolerance in response to antigen, which resulted from impaired development of peripherally-induced regulatory T cells (pTreg). Adult mice that underwent ACF also exhibited altered systemic cytokine expression even in the absence of immunologic stimuli, suggesting that ACF has lasting impact on the immune system. Because peripheral tolerance and immune skewing directly impact anti-tumor immunity,4 we hypothesized that ACF would also impact the immune response to tumor growth.

Methods To measure impact of ACF on tumor growth and tumor infiltration, we introduced EL4 lymphoma cells into 7-week-old mice with the following foster schemes: conventionally reared mice, 1-day-old pups cross-fostered with 10-day post-partum dam (ACF1 to ppd10), and 13-day-old pups cross-fostered with 1-day post-partum dams (ACF13 to ppd1). Immune infiltration at tumor endpoint was measured using flow cytometry.

Results EL4 tumor growth was increased in ACF mice compared to conventionally-reared controls. Further, the immune infiltrate at endpoint was altered, with ACF mice having fewer natural killer (NK) cells, dendritic cells, and activated cytotoxic CD8+ T cells in the tumor microenvironment.

Conclusions Our observations support the hypothesis that ACF impacts tumor growth and immune infiltration. Future directions include phenotyping the immune infiltrate with finer resolution, the study of additional tumor models, and investigation of the effects of ACF on spontaneous tumor incidence and immunotherapy efficacy. Development of this novel model could provide valuable insight into early life factors that influence anti-tumor immunity.

Ethics Approval The study was approved by Mayo Clinic’s IACUC approved all uses in this study, approval number A00004845.

REFERENCE
TERMINALLY EXHAUSTED CD8+ T CELLS POTENTIATE CD26 ENZYMATIC ACTIVITY MODULATES EFFICIENT EPIGENETIC, AND TRANSCRIPTIONAL DYSFUNCTION. 4

ABSTRACT

TERMINALLY EXHAUSTED CD8+ T CELLS POTENTIATE THE TOLERGENIC TUMOR MICROENVIRONMENT AS FUNCTIONAL SUPPRESSORS

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Background Blockade of co-inhibitory ‘checkpoint’ molecules, PD-1 and CTLA-4, has induced impressive clinical responses in advanced tumors; yet only in a subset of patients.1–3 Limited success with checkpoint blockade therapy suggests other cell extrinsic or intrinsic mechanisms may be dampening an effective immune response. Cytotoxic CD8+ T cells (CTL) encountering chronic antigen and metabolic restriction can differentiate to a terminally exhausted state (Texh), marked by hyporesponsiveness and metabolic, epigenetic, and transcriptional dysfunction.4–8 While enrichment of this population in tumor is a negative prognostic factor,9–10 it remains unclear whether Texh are simply non-functional or instead possess tolerogenic or suppressive properties. Transcriptional profiling of tumor-infiltrating PD-1int (progenitor exhausted) CTL versus PD-1hiTIM-3+ (terminally exhausted; Texh), reveals that exhausted cells express a pattern of genes associated with immune suppression. We hypothesize that Texh potentiate the suppressive microenvironment of solid tumor by autoregulation and inhibition of local immune responses.

Methods T cell populations were isolated from murine melanoma-B16-F10 or a lab-generated melanoma clone of the spontaneous BREF/PTEN model—by expression of inhibitory receptors and assayed in tandem in microsuppression assays. Murine melanoma clones with inhibited oxidative metabolism were generated by CRISPR-Cas9 deletion and validated for ablated mitochondrial respiration by extracellular flux analysis. Enforced expression of CD39 in effector T cells was attained by murine retroviral vector delivery.

Results When sorted directly from tumor, PD-1hiTIM-3+ Texh, but not progenitor exhausted PD-1int CTL, induce marked suppression of T cell effector responses, comparable to Foxp3+ Treg from the same environment. Expression of the ectonucleotidase, CD39, is uniquely expressed in Texh and increases as T cells differentiate towards exhaustion. Genetic deletion of CD39 in Texh eliminates the regulatory phenotype of tumor-infiltrating Texh and enforced CD39 expression on effector T cells can inhibit T cell receptor signaling and downstream function. CD39 expression correlates with exposure to hypoxia and Texh sorted from tumors engineered to be less hypoxic displayed a significant loss of suppressive capacity. Our data suggest that tumor hypoxia enforces Hif1α-dependent expression of CD39 which depletes extracellular ATP contributes to generation of immunosuppressive adenosine, and has been previously associated with terminal exhaustion.11–13

Conclusions Our data support a model that as CTL progress to terminal exhaustion, hypoxic exposure enforces the upregulation of CD39, providing Texh a mechanism to suppress proinflammatory processes. These findings suggest Texh are not solely dysfunctional but rather are deleterious to antitumor immunity and may need to be drastically reprogrammed or deleted in order to alleviate immunosuppressive functions.

REFERENCES


CD26 ENZYMATIC ACTIVITY MODULATES EFFICIENT MIGRATION OF ADAPTIVELY TRANSFERRED T CELLS TO SOLID TUMORS

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Background The inadequate ability of adaptively transferred T cells to eradicate solid tumors limits their use in treatments for patients afflicted with those cancers. Efforts to improve ACT for solid tumors aim to identify strategies that poise T cells for optimal response. We have previously identified a specific subset of CD4 T cells which express high levels of the ubiquitous ectoenzyme dipeptidyl peptidase-4 (DPP-4), also known as CD26, that produce a tremendous antitumor response in solid tumor models. We therefore sought to investigate the importance of CD26 on T cells destined for ACT.

Methods We adaptively transferred tumor specific CD26+ T cells into melanoma tumor-bearing CD26− mice, and continuously blocked the CD26 enzymatic activity of the donor cells in vivo with sitagliptin, an established competitive inhibitor of CD26.

Results Tumors in sitagliptin-treated mice eventually reached study endpoint, while tumors untreated mice were regressed for 130+ days. Tumor infiltration of donor cells and host CD8 and CD4 cells was diminished with sitagliptin treatment. A 32-plex cytokine array of blood plasma revealed a diminished profile of cytokines and chemokines, indicating that the inflammatory response of the T cells was dampened with sitagliptin treatment. Further experiments characterized the ability of CD26+ T cells to respond to tumor trafficking signals with...
a transwell migration assay and found that sitagliptin treatment significantly impaired their migratory capacity. However, sitagliptin did not impair the ability of T cells to functionally respond to antigen.

Conclusions These data suggest that the enzymatic activity of CD26 is important for the ability of T cells to migrate to the tumor site in order to mount an effective antitumor response. Further investigations into the mechanism behind the role of CD26 are ongoing.

Ethics Approval This study was approved by the Medical University of South Carolina’s IACUC, protocol #00488

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514 DISTURBED MITOCHONDRIAL DYNAMICS REWIRE THE EPIGENETIC PROGRAM FOR CD8+ TIL EXHAUSTION

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Background Cancer immunotherapy, including checkpoint blockade and adoptive transfer of tumor-reactive T cells, represents a paradigm shift in the treatment of malignancies in recent years, and yields remarkable responses by reawakening anti-tumor immunity in established tumors. Nevertheless, a significant portion of patients are refractory to cancer immunotherapies, which may be in part due to the persistent impairment of anti-tumor effector functions in T cells, a phenomenon referred to as T cell exhaustion. Emerging evidence reveal that alterations in global chromatin accessibility and de novo DNA methylation patterns are keys events to drive development of T cell exhaustion under chronic antigenic stresses. However, it remains elusive how T cells engage epigenetic reprogramming to orchestrate exhausted state.

Methods Here, we examined the mitochondrial fitness in CD8 + TILs with mitoTrackers.

Results We found that tumor-infiltrating tumor-reactive T cells with accumulation of damaged mitochondria, characterized by increased mitochondrial mass but reduced mitochondrial membrane potential and cristae, display more severe exhausted phenotypes, including decreased proliferation capacity, reduced cytokine production and up-regulation of co-inhibitory receptors. The accumulation of damaged mitochondria is in part due to the deficiency of mitophagy machinery. Importantly, we found that the accumulation of dysfunctional mitochondria is correlated to the specificity and affinity of antigen, and also supported by the PD-1 expression. Moreover, the combination of glucose deprivation, hypoxia and TCR signaling in vitro can drastically weaken T cell immunity with the accumulation of dysfunctional mitochondria as seen in TILs previously. Furthermore, T cells with accumulation of damaged mitochondria, generated artificially by Oligomycin A and Mdivi-1, also exhibit persistent exhaustion features. Ultimately, supplementation with nicotinamide riboside enhances T cell mitochondrial fitness and improved responsiveness to anti-PD-1 treatment.

Conclusions Taken together, our study suggests that mitochondrial fitness is pivotal for T cell-mediated immunity and the accumulation of dysfunctional mitochondria could result in exhaustion phenotypes in T cells. And our findings also provide pillars for better harnessing T cell immune responses with metabolic regulations for immunotherapy.

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515 METABOLIC REPROGRAMMING OF ANTITUMOR CD8+ T CELL IMMUNITY

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Background Adoptive cell transfer (ACT) therapies are successfully used in the clinic; however, a large fraction of patients remains unresponsive. The limited efficacy of this therapy is due, in part, to the terminally differentiated state of transferred T cells, which limits their proliferation and long-lasting antitumor response. Memory CD8+ T cells display specific phenotypic and functional characteristics endowing them with the ability to provide a more robust and long-lasting antitumor immune response than their terminally differentiated counterparts. The development and fitness of memory T cells was recently shown to be associated with specific metabolic pathways.

Methods We aimed to metabolically reprogram CD8 + T cells in order to generate fitter memory-like T cells prior to ACT.

Results We have found that pharmacological inhibition of the metabolic enzyme isocitrate dehydrogenase 2 (IDH2) during the priming of CD8+ T cells led to an increased memory formation and to an enhanced tumor growth inhibition upon ACT into melanoma tumor-bearing mice. Interestingly, IDH2 inhibition was associated with increased histone methylation and acetylation. We show that these histone modifications were required to induce the observed memory phenotype.

Conclusions These results suggest a novel strategy to promote stable memory T cell differentiation by epigenetic processes induced by metabolic reprogramming during T cell priming. These findings might be exploited to optimize ACT immunotherapy against cancer.

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516 CASPASE-8 REGULATED SENESCENCE AS AN IMMUNE CHECKPOINT IN T LYMPHOCYTES FOR ADOPTIVE CELL THERAPY

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Background The development of immunotherapies holds great promise for the treatment of refractory infections and cancer. Current approaches, although effective in many settings, have limitations that prevent their widespread use. Hence, several aspects require improvements, including the re-wiring of T-cell fates and function. T-cell dysfunction is central to the persistence of several chronic viral infections and the progression of malignancies. Upon activation, T cells can follow several paths of differentiation, leading to terminal effector differentiation and/or exhaustion which are widely recognized as dysfunctional features limiting human immune competence. Furthermore, dysfunctional features induced during laboratory-based manipulations of T-cell products prior to adoptive cell transfer has a determining effect on outcomes. Similarly, repeated antigen encounters after transfer in vivo favors the development of T-cell dysfunction. However, the nature and underlying mechanisms of T-cell dysfunction are still incompletely understood.

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**Methods** Combining genomics, phenotypic and functional analyses in various physiologically and clinically relevant settings, we investigated the key factors leading to T-cell dysfunction. Specifically, we evaluated the impact of repeated stimulations using CD3/CD28-coated beads or antigen-loaded dendritic cells in human T-cell long term cultures, and BCMA-expressing cells for anti-BCMA CAR T cells. We also examined mouse antigen-specific T cells during chronic lymphocytic choriomeningitis virus (LCMV) infection as well as datasets obtained from circulating T cells from acute myeloid leukemia (AML) patients.

**Results** We identified telomere-independent cellular senescence as a central aspect of exhausted PD-1-expressing T cells following repeated stimulations. Mechanistically, it is associated with the induction of p16INK4a. Additionally, we found that cellular senescence features are partly regulated by the activation of caspase-8, through a non-apoptotic function of this molecule not previously described in T cells.

**Conclusions** We thus conclude that caspase-8 may regulate the balance between apoptosis and proliferation by protecting T cells from cellular senescence. Senescence-associated mechanisms may be seen as key players in T-cell dysfunction occurring following repeated stimulations and as such should be considered as novel immune checkpoints impeding the success of T-cell adoptive immunotherapy in humans.

**Ethics Approval** This study was approved by the local Maisonneuve-Rosemont Hospital research ethics authorities and participants’ informed consent was obtained (CÉR2020-2141 and CÉR13030).

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**REGULATORY T CELL FUNCTIONAL IDENTITY IS SUSTAINED BY A GLUCOSE:LACTATE AXIS THAT IS EXPLOITED IN THE TUMOR MICROENVIRONMENT**

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**Background** Regulatory T (Treg) cells are vital for preventing autoimmunity but are a major barrier to robust cancer immunity as the tumor microenvironment (TME) recruits and promotes their function. The deregulated cellular metabolism of tumor cells leads to a metabolite-depleted, hypoxic, and acidic TME. While the TME impairs the effector function of highly glycolytic tumor infiltrating CD8 T cells, Treg cell suppressive function is maintained. Further, studies of in vitro induced and ex vivo Treg cells reveal a distinct metabolic profile compared to effector T cells. Thus, it may be that the altered metabolic landscape of the TME and the increased activity of intratumoral Treg cells are linked.

**Methods** Flow cytometry, isotopic flux analysis, Foxp3 driven Cre-lox, glucose tracers, Seahorse extracellular flux analysis, RNA sequencing.

**Results** Here we show Treg cells display heterogeneity in terms of their glucose metabolism and can engage an alternative metabolic pathway to maintain their high suppressive function and proliferation within the TME and other tissues. Tissue derived Treg cells (both at the steady state and under inflammatory conditions) show broad heterogeneity in their ability to take up glucose. However, glucose uptake correlates with poorer suppressive function and long-term functional stability, and culture of Treg cells in high glucose conditions decreased suppressive function. Treg cells under low glucose conditions upregulate genes associated with the uptake and metabolism of the glycolytic end-product lactic acid. Treg cells withstand high lactate conditions, and lactate treatment prevents the destabilizing effects of high glucose culture. Treg cells utilize lactate within the TCA cycle and generate phosphoenolpyruvate (PEP), a critical intermediate that can fuel intratumoral Treg cell proliferation in vivo. Using mice with a Treg cell-restricted deletion of lactate transporter Slc16a1 (MCT1) we show MCT1 is dispensable for peripheral Treg cell function but required intratumorally, resulting in slowed tumor growth and prolonged survival.

**Conclusions** These data support a model in which Treg cells are metabolically flexible such that they can utilize ‘alternative’ metabolites present in the TME to maintain their suppressive identity. Further, our studies support the notion that tumors avoid immune destruction not only by depleting effector T cells of essential nutrients, but also by metabolically supporting regulatory T cells.

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**518** **EPIGENETIC DYSFUNCTION OF TERMINALLY EXHAUSTED TUMOR INFILTRATING T CELLS**

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**Background** Tumor-infiltrating CD8+ T cells have been characterized by their exhausted phenotype with decreased cytokine expression and increased expression of co-inhibitory receptors, such as PD-1 and Tim-3. These receptors mark the progression towards exhaustion from a progenitor stage (PD-1Low) to a terminally exhausted stage (PD-1+Tim-3+). While the epigenetics of tumor-infiltrating T cells are unique compared to naïve, effector, and memory populations, how the chromatin landscape changes during this progression has not been described.

**Methods** Using a low-input ChiP-based assay called Cleavage Under Targets and Release Using Nuclease (CUT&RUN), we profiled the histone modifications at the chromatin of tumor-infiltrating CD8+ T cell subsets to better understand the relationship between the epigenome and the transcriptome as TIL progress towards terminal exhaustion.

**Results** We have identified two epigenetic characteristics unique to terminally exhausted T cells. First, we found a substantial increase in the number of genes that exhibit bivalent chromatin marks, defined by the presence of both activating (H3K4me3) and repressive (H3K27me3) epigenetic modifications that inhibit gene expression. In contrast to stem cells which exhibit bivalency, bivalent genes in terminally exhausted T cells are not associated with plasticity and represent aberrant hypermethylation in response to tumor hypoxia. Secondly, we have also identified a unique set of enhancers, characterized by H3K27ac that do not drive gene expression. These enhancers are enriched for AP-1 transcription factors, whereas enhancers that correlate with gene transcription are enriched for nuclear receptor (NR) transcription factors. Intriguingly, while most AP-1 and NR transcription factors are not expressed in terminally exhausted cells, we found that Baf, an inhibitory AP-1 family member, and Nr4a2, a NR known to promote both exhaustion and modify chromatin were
Background Tumors evade T cell responses targeting them through the upregulation of tolerance-inducing mechanisms. One of the best characterized is that of PD-1/PD-1L engagement, that in healthy CD8+ T cells limits cytotoxic responses against self-antigens and that tumors employ to neutralize T cell attack. Antibody-based therapies aimed to block the PD-1/PD-1L axis have rendered notable results, but most patients eventually develop resistance. This failure is attributed to CD8+ T cells achieving an exhausted phenotype where recovery is hardly feasible. The dysfunctional phenotype of tumor-infiltrating T cells is largely triggered by the unbalance of diacylglycerol (DAG)- and Ca2+-regulated signals that results in alteration of the transcriptional T cell program. DAG kinase (DGK) ζ-dependent DAG consumption contributes to hypofunctional T cell states while DGKζ deficiency facilitates tumor rejection in mice without apparent adverse autoimmune effects. In spite of its therapeutic potential, little is known about DGKζ function in human T cells and there are not isoform-specific inhibitors targeting this DGK isoform.

Methods Here we used of a human triple parameter reporter (TPR) cell line to examine the consequences of DGKζ depletion in the transcriptional restriction imposed by PD-1 ligation. We also investigated the effect of DGKζ deficiency in the expression dynamics of PD-1, as well as the impact of the absence of this DGK isoform in the in vivo growth of a MC38 adenocarcinoma cell line.

Results We demonstrate that DGKζ depletion enhances DAG-regulated transcriptional programs, favoring IL-2 production and limiting PD-1 expression. Diminished PD-1 expression and enhanced expansion of cytotoxic CD8+ T cell populations is also observed even in the context of immunosuppressive milieus and correlates with the failure of MC38 adenocarcinoma cells to form tumors in DGKζ-deficient mice.

Conclusions Our results suggest the relevance of DGKζ as a therapeutic target on its own as well as a biomarker of CD8+ T cell dysfunctional states.

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Immune regulatory metabolites were also assessed. The performance of previously identified immune signatures as the Immunologic Constant of Rejection (2,3), which captures an active Th1/cytotoxic response associated with favorable prognosis and responsiveness to immunotherapy, was also checked within each tumor subtype.

**Results** We found 5 main modules, in agreement with results obtained in adult solid tumors: Wound Healing, TGF-B signaling, IFN-G signaling, Macrophages and Lymphocytes (figure 1). These 5 modules clustered pediatric patients into 6 immune subtypes S1-S6 with distinct survival (S2 vs S4, p=0.0044, adjusted for cancer type), S2 cluster has the best overall survival and characterized by low enrichment of wound healing signature, high Th1, low Th2 and high expression of HLA-A and HLA-C, while the opposite holds true for cluster S4 with the worst survival and highest enrichment of wound healing signature, high Th2, and low Th1. The S6 cluster is characterized by highest enrichment of lymphocyte signature, the highest expression of immune checkpoints accompanied by elevated expression of exhaustion markers, and an unpolarized immune response with high abundance of macrophages. Additionally, pan-cancer, the upregulation of WNT-Beta catenin pathway is associated with adverse outcome and lack of T-cell infiltration. In the per-cancer analysis, ICR is associated with better survival in osteosarcoma and with worse survival in Wilms’ tumors, similarly with what observed in adult kidney cancer despite the different embryological origin.

**Conclusions** We demonstrated that pediatric solid cancers can be classified according to their immune disposition, unveiling unexpected similarity with adults’ tumors. Immunological parameters might be explored to refine diagnostic and prognostic biomarkers and to identify potential immune responsive tumors. This is the first pan-cancer immunogenomic analysis in children.

**REFERENCES**


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### Abstracts

#### 521 IMMUNE REGULATORY METABOLITES IN HUMAN OVARIAN CANCER

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**Background** Immune regulatory metabolites are key features of the tumor microenvironment (TME), yet with a few exceptions, their identities remain largely unknown. Importantly, little is known about the heterogeneity of metabolites that are present or absent in specimens from human tumors and immune compartments.

**Methods** Here, we profiled tumor and T cells from tumor and ascites of patients with high-grade serous carcinoma (HGSC) to uncover the metabolomes of these distinct TME compartments. We devised a stringent and robust protocol to enrich cell populations from surgically resected samples in patients with HGSC. We conducted mass spectrometry-based analysis and developed machine learning tools to highlight novel metabolites that are present in different cellular lineages of the tumor.

**Results** Cells within the ascites and tumor had pervasive metabolic differences, with a striking enrichment in 1-methyl nicotinamide (MNA) in T cells infiltrating the tumor compared to ascites. Despite the elevated levels of MNA in T cells, the expression of nicotinamide N-methyltransferase, the enzyme that catalyzes the transfer of a methyl group from S-adenosylmethionine to nicotinamide, was restricted to fibroblasts and tumor cells. T cells treated with MNA stimulated secretion of the tumor promoting cytokine tumor necrosis factor alpha.

**Conclusions** Our studies provide the first catalogue of metabolites in patient-derived tumors and T cells. We found that MTE-derived MNA contributes to the immune modulation of T cells and represents a potential immunotherapy target to treat human cancer.

**Ethics Approval** This study was approved by the University of British Columbia and BC Cancer Research Ethics Board (H07-00463).

**Consent** Written informed consent was obtained from the patient to use the results of this study for educational purposes including publications. A copy of the written consent is on file and available for review by the Editor of this journal.

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A subset of mature neutrophils contains the strongest PMN-MDSC activity in blood and tissue of patients with head and neck cancer

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Background A high neutrophil-to-lymphocyte ratio in the circulation and high frequencies of tumor-associated neutrophils (TAN) in malignant tissue are associated with poor outcome and tumor progression in patients with cancer. It is hypothesized that immunosuppressive neutrophil activity (aka PMN-MDSC activity) contributes to this effect. In addition, this MDSC activity represents a major resistance mechanism in different types of immunotherapy. The exact cellular identity of human PMN-MDSC is still under debate. Improved immunomonitoring and functional characterization of MDSC is needed in order to exploit these cells as novel biomarkers and targets for combination immunotherapy.

Methods In this study, we sought to identify the neutrophil subset that contained the highest T cell suppressive activity. To this end, we purified different subsets of circulating neutrophils by FACS and performed multi-parameter immunofluorescence together with digital pathology on 2-D and 3-D tumor tissue samples.

Results We found that a population of circulating, mature, arginase 1+ neutrophils that co-purified with mononuclear cells in density gradients, most potently suppressed T cell function in multiple in vitro assays. These PMN-MDSC were also superior to monocytic MDSC in T cell suppression. Using a novel technology of tissue whole mount labeling, clearing and imaging we derived 3-D spatial maps of neutrophil – T cell interaction in human tumors. We found that T cells, which were conjugated to arginase 1+, myeloperoxidase + TAN, had significantly reduced expression of proliferation and cytotoxicity markers. In patients, frequent conjugation of T cells to those PMN-MDSC was associated with poor prognosis. In contrast to circulating PMN-MDSC, tissue PMN-MDSC expressed high amounts of LOX-1 (oxidized low density lipoprotein receptor 1) and a high intratumoral frequency of LOX-1+ PMN-MDSC was associated with poor survival.

Conclusions We identified and characterized PMN-MDSC activity in human cancer patients. Our findings will facilitate and improve MDSC immunomonitoring and MDSC targeting in combination therapies.
Abstract 524 Figure 1  After activation by antigen-presenting cells in the lymph nodes, viable CD8+ T cells express high levels of phosphatidylserine, which coincides with a highly proliferative and cytotoxic state. As they migrate towards tumors cells in the serous body cavities, they are sequestered by Tim-4+ resident macrophages which impede their anti-tumor cytotoxicity. Tim-4 abrogation can alleviate this sequestration and enhance anti-tumor immunity

PShigh CD8+ T cells expressed genes associated with cytotoxicity, activation/exhaustion, and proliferation, and mediated greater cytotoxicity. Mechanistic studies revealed that Tim-4 mediates sequestration of PShigh CD8+ T cells by macrophages which subsequently impedes CD8+ T cell cytotoxicity of tumor cells.

Conclusions We demonstrate that Tim-4+ resident macrophages impair anti-tumor CD8+ T cell immunity in the serous body cavities and Tim-4 blockade represents on a novel therapeutic strategy to overcome resistance to immune checkpoint blockade (figure 1).

Ethics Approval The retrospective clinical analysis was approved by Memorial Sloan Kettering Cancer Center IRB #06-107 and #16-1566. The human biospecimen analyses were approved by Memorial Sloan Kettering Cancer Center IRB #06-107 and 14-091.

REFERENCES

Abstract 525 Figure 1  KIT mutation and MS4A1/CD20 expression - overall survival

Low MS4A1/CD20 expression with concurrent KIT mutation is associated with poor overall survival
cell activity. KIT mutation was seen in 10 of 147 patients with high MS4A1/CD20 expression, and 10 of 135 patients with low MS4A1/CD20 expression. Overall survival was 15 months for the patients with KIT mutation and low MS4A1/CD20 expression, and significantly lower when compared with other groups despite low number of patients. (P<0.0001) (figure 1).

Conclusions B cells have significant role in immune response to tumor. Lower expression of MS4A1/CD20 is known to be associated with poor prognosis in melanoma and other solid tumors.3 We demonstrated that a concurrent KIT mutation in melanoma with lower expression of MS4A1/CD20 contributes to poor prognosis in melanoma. Therefore, this small subset of aggressive tumors may need combination strategies involving targeting driver pathways with a kinase and immune checkpoint inhibitor.

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INHIBIGENTM-SPECIFIC RESPONSES SUPPRESS ANTI-TUMOR IMMUNITY AND PROMOTE TUMOR GROWTH

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Background Personalized cancer immunotherapies can generate potent antitumor responses yet finding the right targets remains challenging. The ATLAS™ platform employs ex vivo functional screening of tumor mutations using autologous cells to identify patient-specific neoantigens. Stimulatory neoantigens are identified by upregulation of inflammatory cytokine secretion and can be employed in vaccines or cell therapies. Conversely, ATLAS also identifies inhibitory neoantigens (termed Inhibigens) that lead to cytokine downregulation, and in murine models accelerate tumor growth and abrogate the efficacy of otherwise-protective vaccines. Here we further explore Inhibigen mechanism of action in humans and mice including whether checkpoint inhibition (CPI) can ameliorate Inhibigen-accelerated tumor growth.

Methods Human and mouse ATLAS screens were performed as previously described.1 ATLAS-identified stimulatory or Inhibigen peptide vaccines were evaluated in a therapeutic B16F10 melanoma tumor model ± CPI. Immune responses were measured using ELISPOT, flow cytometry, and immunohistochemistry (IHC).

Results In the GEN-009 personalized neoantigen vaccine trial (NCT03633110), Inhibigens were observed in 92% of patients (N=39). Of total mutations screened, 16% (1.8 - 47.5%) were classified as Inhibigens, which were found more often in the CD4+ (mean 10.3%; 0.5 - 42%) versus CD8+ T cell subset (mean 6.1%; 1.2 - 23%). No relationship between Inhibigen-specific responses and tumor type or mutational burden were observed. To study the functional effects of Inhibigen vaccination in vivo, a B16F10 mouse melanoma model was employed. Inclusion of Inhibigen in an otherwise protective vaccine abrogated efficacy and correlated with decreased T cell responses to vaccine antigens as well as a global depression of T cell cytokine secretion. Early experiments suggest that these decreases are not due to MHC competition. In addition, administration of a therapeutic vaccine containing an Inhibigen led to reduced tumor infiltration of CD8+ T cells and myeloid populations. A corresponding increase of classical Tregs in the tumor or periphery was not observed. Surprisingly, preliminary data show combination therapy with anti-CTLA4 partially ameliorated Inhibigen-accelerated tumor growth but anti-PD1 provided no additional benefit.

Conclusions The nearly ubiquitous presence of Inhibigens in human cancer patients and the demonstrated pro-tumor effects in mice suggest that ATLAS-identified Inhibigens must be considered and omitted in the design of cancer immunotherapies. Furthermore, in mice, CPI co-administration has a modest (anti-CTLA4) or no (anti-PD1) effect on Inhibigen-accelerated tumor growth suggesting that Inhibigen profiling could guide CPI selection or predict clinical outcome. These data confirm the benefits of the ATLAS platform for neoantigen and Inhibigen identification.

Ethics Approval All animal studies were undertaken in conformity with the Cambridge, MA City Ordinance 1086 of the city’s Municipal Code and in accordance with the policies and protocols approved by Genocoea’s Institutional Animal Care and Use Committee (IACUC).

REFERENCE


TUMOR ORGANOID AND IMMUNE CELL CO-CULTURE SYSTEM POTENTIATES IMMUNO-ONCOLOGY DRUG DEVELOPMENT

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Background Patient-derived organoids (PDOs) are derived from adult epithelial stem cell with self-renewal, organisation and differentiation properties, reflecting the original 3D organ-like or tissue-like structure and morphology in vitro. PDOs also faithfully recapitulate the genetic modifications and phenotypical features of original tumors, making them an attractive preclinical models for oncology drug development. However, modeling the tumor microenvironment (TME) in vitro remains a challenge due to the lack of stromal and immune cells. In this study, we reconstituted component of the TME through co-culture of tumor organoids with various immune cells in vitro to assess the immune modulatory and tumor killing effects of immuno-oncology (IO) drug candidates such as therapeutic monoclonal antibodies, bispecific T cell engagers and CAR-T cells.

Methods Using the Hubrecht organoid technology (HUB) protocols we have established a biobank of tumor and normal organoids, which closely resemble the genetic and morphologic features of original organs from multiple different tissue types. This large and diverse biobank of organoids can act as...
surrogates for individual patients making them suitable for patient population studies including evaluating the response to IO drug candidates in vitro.

**Results** We co-cultured organoids expressing tumor associated antigen (TAA) of interest with bispecific T cell engagers and CAR-T cells recognizing the TAAs. Our data demonstrated antigen-specific T cell killing of tumor organoids and tumor antigen reactivity of bispecific antibody activated T cells and CAR-T. We engineered tumor organoids to express CD19 and a luciferase reporter gene and measured luciferase activity to monitor the growth and killing of tumor organoids by CD19 CAR-T cells. The luciferase activity in organoids reflected the killing efficiency in a very sensitive, robust and high through-put manner. Immune checkpoint molecules are differentially expressed on individual tumor organoids and we evaluated the potency of immune check blockade using tumor organoids cocultured with allogenic T cells. Killing of tumor organoids and T cell activation was enhanced by PD-1/PD-L1 blockade. We profiled the expression of immune checkpoint molecules on our banked tumor organoids which will provide a valuable resource to choose tumor models and cancer types for preclinical testing of IO drugs.

**Conclusions** In conclusion, we demonstrated the feasibility of in vitro patient-derived model system in the field of IO research using tumor organoid co-culture with immune cells, and their application in IO target and drug discovery.

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**528 SEXUAL DIMORPHISM IN MYELOID-DERIVED SUPPRESSOR CELLS PROMOTE GBM PROGRESSION IN FEMALES VIA IL-1B**

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**Background** A potently immunosuppressive tumor microenvironment facilitates progression of glioblastoma (GBM). Immunotherapies have had variable success in improving the outcome of GBM patients, suggesting that there is a need to gain insight into the mechanisms of immunosuppression. Our findings indicated that proliferating monocyteic MDSCs (mMDSCs) accumulate in tumors of male mice and patients, while female tumor-bearing mice had an increase in circulating granulocytic MDSC (gMDSC) frequency, and a high gMDSC gene signature correlated with worse outcome of female patients.

**Methods** To investigate the basis and prognostic value of sex differences in MDSC profile, we analyzed the role of sex hormones, determined gene expression signatures of MDSCs and preclinically tested the therapeutic benefit of candidate drugs predicted to be effective against individual MDSC subsets.

**Results** In line with the differential MDSC accumulation pattern, targeting the systemic gMDSCs with the anti-Ly6G neutralizing antibody extended the lifespan of female mice without affecting males. These differences were not driven by sex steroids, as castration or ovarietomy failed to alter MDSC subset accumulation patterns in GBM-bearing mice. Drug-prediction algorithms using the differential MDSC gene expression profiles predicted IL-1 inhibitors are effective against gMDSCs. Correspondingly, IL-1β was highly expressed in female but not male gMDSCs. Single-cell sequencing revealed that circulating but not tumor-infiltrating gMDSCs were the primary source of IL-1β and that its neutralization provided a female-specific survival advantage by reducing circulating gMDSCs. This was accompanied by declines in tumor infiltration of microglia, microglia activation status and tumor cell proliferation. In vitro, IL-1β inhibition reduced viability and expression of activation markers by primary microglia.

**Conclusions** These findings highlight a novel peripheral gMDSC-microglia IL-1β mediated communication axis in female GBM and indicate expression differences in MDSC subsets can be leveraged for improved immunotherapy efficacy in a sex-specific, precision medicine strategy.

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**529 CANCER CELLS EDUCATE NATURAL KILLER CELLS TO A METASTASIS PROMOTING CELL STATE**

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**Background** Metastatic disease drives breast cancer mortality. We recently discovered that leading cells at the invasive edge of mammary tumor organoids retain a conserved basal epithelial program defined by their expression of keratin-14 (K14), establishing K14 as a good marker of invasive breast cancer cells. K14-positive invasive cells also exhibit characteristics that make them targets of immunosurveillance by natural killer (NK) cells. While NK cells are key immune mediators in the control of metastasis, our understanding of the specific mechanisms behind this regulation and its eventual evasion by metastatic cells remains incomplete.

**Methods** We have developed a novel preclinical 3D co-culture assay to discover mechanisms behind interactions between K14 + invasive breast cancer cells and NK cells. Combined with in vivo assays of metastasis, we are able to determine how NK cells limit the early stages of metastasis and also how tumor cells can influence key NK cell properties.

**Results** In ex vivo co-culture assays of NK cells isolated from healthy mouse donors and mammary tumor organoids from MMTV-PyMT and C31T mouse models of breast cancer, we demonstrate that NK cells limit the early stages of metastasis. Antibodies to invasive K14 + cells were able to enhance the ability of NK cells to limit colony formation, suggesting antibody-dependent cell mediated cytotoxicity. Surprisingly, when isolated from tumor bearing mice, NK cells did not limit invasion and instead promoted colony formation. The in vivo adoptive transfer of NK cells from healthy donors prevents the progression of early lung metastatic seeds to macrometastases, while the adoptive transfer of cells isolated from tumor bearing donors promotes macrometastatic development. Transcriptomic analysis of reprogrammed NK cells demonstrate they have similar profiles to resting NK cells. This growth promoting phenotype can be reversed with antibodies targeting inhibitory cell surface receptors or the epigenome.
Conclusions Our ex vivo and in vivo data demonstrate that healthy donor NK cells can limit metastasis through the directed cytotoxicity against pioneering K14+ invasive cells. However, prolonged exposure to tumors reprogram NK cells from tumor killing to tumor promoting, specifically in promoting the outgrowth of macrometastases. Further, we can neutralize this effect using NK cell specific inhibitory antibodies and epigenetic modifiers. This is the first time inhibitory signaling on NK cells have been linked with a growth promoting phenotype. These data can provide insight into when the use of NK cell directed therapies can be used to treat or prevent clinically relevant metastatic disease.

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Background Natural killer (NK) cells are part of the innate immune system, but are capable of participating in both innate and adaptive immune responses due to their wide range of cytolytic activities, from degranulation, secretion of cytokines to antibody-dependent cell-mediated cytotoxicity. These are possible due to the cells’ ability to recognize self and non-self-entities via the net signal generated from their activating and inhibitory receptors upon engagement. TIM-3 is a part of the NK receptor repertoire, expressed commonly on different lymphocytes. In T cells, TIM-3 is established as an inhibitory marker. However, in NK cells, the role of TIM-3 could be agonistic or antagonistic to NK cytotoxicity based on the disease type and activation status, though limited information is known about its role in cancer and its correlation to NK cell effector functions.

Methods We measured TIM-3 expression upon activation of human NK cells under various conditions. NK cells were isolated from peripheral blood of healthy donors and expanded either in K562-based feeder media or feeder-free OpTmizerTM media. After expansion, they were co-cultured for 4 hours with patient-derived glioblastoma multiforme cells (GBM43) at effector:target ratios of 2.5:1 and 10:1. To evaluate the effect of TIM-3 expression on NK cells, 7AAD/CFSE killing assays, CD107a degranulation and IFNγ secretion assays were carried out while blocking TIM-3 with neutralizing antibodies. Bioinformatics analysis of GBM patient RNAseq data was carried out to correlate TIM-3 expression with in vivo function, and this analysis is supplemented by phenotyping TIM-3 on NK cells isolated from patient samples in order to infer the role of this receptor in GBM.

Results We found that TIM-3 was downregulated on OpTmizerTM -cultured NK cells once exposed to cancer targets, and this correlated to a decreased in NK killing capacity when compared to feeder media-cultured NK cells, where the downregulation was not observed. Culturing NK cells in different derivatives of both media suggested that a combination of serum and cytokines can induce TIM-3 expression change to cancer targets. Flow cytometric assays revealed that while degranulation remained the same, the decreased in cytotoxicity corresponded to a decrease in IFNγ secretion. In GBM patient datasets, TIM-3 expression correlates to high IFN-γ levels and associates with both pro- and anti-tumorigenic functions. Here, we report a new role of TIM-3 in modulating NK functionality by correlating its loss to a loss in NK cell effector functions, and how its expression can be modified by ex vivo activation.

Conclusions TIM-3 expression on NK cells can be induced by ex vivo expansion, and this change in expression could influence NK cytotoxicity and cytokine secretion. Our data suggested that TIM-3 is not necessarily an inhibitory marker in GBM, and more likely to be a status marker or an activation limiter, working in conjunction with other receptors to modulate NK cell anti-tumor responses.

Ethics Approval This study was approved by Purdue Intuition’s Ethics Board, approval number [1804020540].
lymphomas in vitro and in vivo in immunodeficient NSG mouse models.

**Conclusions** Collectively, these data identify promising combinations of AFM13 with cytokine-activated adult blood or cord blood NK cells against CD30+ hematologic malignancies, warranting clinical trials with these novel combinations.

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**SOCS3 DEFICIENCY BLOCKED AUTOPHAGY-DEPENDENT MYELOID DIFFERENTIATION OF EARLY-STAGE MYELOID-DERIVED SUPPRESSOR CELLS VIA THE MIR-155/C/EBPß/WNT AXIS**

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**Background** Early-stage myeloid-derived suppressor cells (eMDSCs) are a newly defined subset of myeloid-derived suppressor cells (MDSCs) that accumulate densely in tumors and potently promote tumor growth and metastasis by suppressing antitumor immune responses in vitro and in vivo. We previously identified a subset of eMDSCs in human breast cancer with a characteristic phenotype of Lin-HLADR-CD33+. We also found that SOCS3 deficiency and sustained activation of the JAK/STAT signaling pathway are critical molecular events coordinating the differentiation of eMDSCs, although the distinct molecular regulation has not been fully elucidated.

**Methods** Herein, we genetically constructed conditional SOCS3 knockout mice with SOCS3 deficiency specifically in the myeloid lineage (SOCS3MyeKO). We analyzed the number of eMDSCs in SOCS3MyeKO mice (eMDSCsSOCS3KO). To explore which pathways participated in dysfunctional eMDSC differentiation, we performed whole-genome RNA sequencing and miRNA microarray on CD11b+Gr-1+ cells, eMDSCsfl/fl and eMDSCsSOCS3KO to screen the potential regulatory ceRNA network in eMDSCsSOCS3KO. CD11b+Gr-1+ cells isolated from SOCS3fl/fl mouse spleens were used as mature myeloid cell controls. Furthermore, we applied a specific miR-155 antagonist and the autophagy agonist rapamycin to suppress tumor growth and eMDSC infiltration.

**Results** The transcriptome results and corresponding intervention experiment revealed that the differentiation block in eMDSCsSOCS3KO was caused by SOCS3 deficiency-mediated limited autophagy activation in an AMPK-independent manner. The results of miRNA microarray and RNA sequencing demonstrated that miR-155 overexpression and Wnt/ß-catenin pathway activation were involved in the SOCS3 knockout-mediated myeloid differentiation block and autophagy repression. Further experiments revealed that miR-155 was induced by activation of the STAT3/NK-ß pathway upon SOCS3 deficiency, which consequently activated the Wnt/ß-catenin pathway via targeting C/EBPß. Furthermore, applying a specific miR-155 antagonist or the autophagy agonist rapamycin efficiently suppressed tumor growth and eMDSC infiltration in vivo.

**Conclusions** Overall, these findings indicated that SOCS3 deficiency blocked autophagy-dependent myeloid differentiation of e-MDSCs via the miR-155/C/EBPß/Wnt axis, and thus targeted therapy against this pathway could be a potential therapeutic target in breast cancer.

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**CROSS-SPECIES IMMUNOGENOMIC ANALYSIS IDENTIFIES PATHWAYS OF CANINE NATURAL KILLER CELL RESPONSE TO CYTOKINE THERAPY, AND REVEALS CONVERGENCE OF ACTIVATED DOG AND HUMAN NATURAL KILLER TRANSCRIPTOMES**

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**Background** Natural killer (NK) cells are key effectors of the innate immune system, but major differences between human and murine NK cells impede translation. Outbred dogs offer an important link for NK-based cancer immunotherapy studies. We compared gene expression profiles of dog NK signatures in vitro and from a phase I clinical trial of inhaled IL-15, and analyzed dog, mouse and human NK cells using a novel orthologous transcriptome.

**Methods** We performed differential gene expression (DGE) using resting healthy donor CD5dim NK populations and following ex vivo activation using recombinant human (rh) IL-15 or co-culture with irradiated feeder cells. Eight dogs with naturally-occurring pulmonary metastases were enrolled on a Phase I clinical trial of inhaled rhIL-15 using a 3+3 cohort design with escalating doses of inhaled rhIL-15. Blood was collected from study dogs before, during, and after therapy. We compared DGE among healthy and cancer-bearing dogs and then across mouse, dog and human NK cells in resting and activated states using −7000 1:1 orthologous genes.

**Results** DGE revealed distinct transcriptional profiles between the ex vivo resting, IL-15 and co-cultured canine NK cells. Among treated patients, hierarchical clustering revealed that in vivo NK cell transcriptional signatures grouped by individual dog, and not amount of time exposed to treatment. PCA showed in vivo profiles of the clinical responders were distinctly separate from the non-responding patients (PC1 38%, PC2 12%). Patient in vivo NK cell transcriptional profiles most closely resembled those of ex vivo resting NK cells and not IL-15 treated or co-culture activated (PC1 43%, PC2 19%), likely reflecting key differences in activation. In cross-species analysis, PCA showed within-species spatial clustering of resting NK cells. After activation, variance between dog and human NK cells decreased, while variance between human and mouse NK cells increased (PC1 40%, PC2 28%).

**Conclusions** In this first transcriptomic sequencing of dog NK cells, we demonstrate distinct gene profiles of ex vivo activated NK cells from healthy donors compared to circulating NK cells from dogs receiving inhaled rhIL-15 on a clinical trial. Baseline in vivo NK cell profiles appear to predict response to therapy more than changes over time. We also show distinct gene profiles of NK cells across the most commonly used mouse, dog, and human NK populations, with convergence of dog and human NK cells after activation. By defining the canine NK cell DGE signatures, these data fill a gap in translational NK studies.

**Ethics Approval** The canine clinical trial study was approved by IACUC and Clinical Trials Review Board (Inhaled IL-15 Immunotherapy for Treatment of Lung Metastases, Protocol #20179).

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**Immune cell types**

533 Cross-species immunogenomic analysis identifies pathways of canine natural killer cell response to cytokine therapy, and reveals convergence of activated dog and human natural killer transcriptomes
Background Colorectal cancer is the most common gastrointestinal tract cancer, there are many factors which plays an important role on short and long term outcome prior to surgery and adjuvant therapy. For many decades, oncological factor has been state as the main of favorable outcome which could be evaluated by diseases free survival (DFS). Current study already evaluated the immune factor which has an important role on the progression on this colorectal cancer patients.

Methods We evaluated the colorectal cancer patients whose has been diagnosed as adenocarcinoma colon and rectal from operative specimens. The blood level of CRP, IL-6, IFN?, CB-8, IG G and IG M will be examined initially before the operative procedure done. All patients were stage III colorectal adenocarcinoma and adjuvant chemotherapy has been administered for six months period. The patients whose could not completed the adjuvant chemotherapy will be excluded from the study. The outcome of this study will be evaluated the 1 year disease free survival based on the abdominal CT Scan and chest x-rays.

Results There were 2 groups on this study, adults (< 60 years old) and elderly (>60 years old). 62 patients were included, 30 adults patients and 32 elderly patients has been evaluated for the immune profiles. We found the signifiance difference were on the level of CRP, IL-6,IFN?, CB-8, IG G and IG M (p < 0.05). All patients had R0 resection and completed the adjuvant chemotherapy. 5 patients in the adult colorectal cancer group has locoregional and distant metastases in the lung and liver after 1 year evaluation. On the contrary, we could achieved 1 year diseases free survival in the elderly patients (p < 0.05) respectively.

Conclusions Elderly colorectal cancer patients has better immune profiles and has better 1 year disease free survival.

Ethics Approval The study has approved by Ethical Committe of Health Study Faculty of Medicine Sebelas Maret University, Indonesia. Approval number: 21457/BD/2020

Consent All of the patients already have a consent for this study

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Background Primary B cells are an important target for investigation and transfection of B cells is considered difficult. Electroporation is a very useful technology for transfection but its application on B cells has been unsatisfactory with low efficiency and low viability. The first reason is the small size of B cells compared to cell lines and the second reason is the low abundance of B cells in human PBMC. Since we had previous experience with T cell electroporation, we sought to extend our knowledge on electroporation to B cells.

Methods Here we studied the B cell electroporation in PBMC samples and found that it is preferable to electroporate the B cells in the PBMC mixture and B cells can be purified after electroporation if necessary. In this fashion, the total cell number in electroporation is boosted by other cell types in the PBMC and it helps B cell electroporation. Furthermore, we studied expanded B cells and found that they have a larger size than unstimulated B cells and the size increase is correlated to a decrease in electroporation voltage, consistent with the electroporation principle that larger cells need a lower voltage.

Results When B cells are expanded, the electroporation efficiency is similar to common cell lines and it becomes easy to do gene expression or genomic modification.

Conclusions Our studies elucidated the mechanism of difference between unstimulated B cells and expanded B cells and could be useful in helping the research on B cells.

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waves of gene expression in GC B cells in HNSCC tumors, ultimately revealing a novel transitional state for GC B cells in the tumor microenvironment (TME).

Conclusions Understanding B cell function in human cancers and how different TMEs influence B cells and TLS are important for devising novel therapeutic options for cancer patients. Ultimately, development of therapeutics to enhance B cell responses in the TME should be prioritized as a compliment to T-cell mediated therapies.

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537 CONVENTIONAL TYPE 1 DENDRITIC CELLS AND NATURAL KILLER CELLS DEMONSTRATE STRONG CORRELATION TO T LYMPHOCYTE INFILTRATION IN CERVICAL CANCER TUMORS

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Background The ability of T cells to mediate anti-tumor immunity has been harnessed to develop successful immunotherapies in recent years. Although direct presentation of tumor antigens by tumor cells plays an important role in the effector function of cytotoxic T lymphocytes (CTLs), cross-presentation by professional antigen presenting cells such as dendritic cells (DCs) is vital for priming naive CD8+ T cells and developing a sustainable cytotoxic response. Natural killer (NK) cells within the tumor microenvironment (TME) recruit a specific population of DCs called conventional type 1 DCs (cDC1s) into the TME by secreting chemokines such as CCL5 and XCL1. However, these cells are very low in abundance and are characterized by the expression of numerous markers, making their detection in the tissue context challenging.

Methods Therefore, to interrogate the presence of cDC1 and NK cells in the TME and reveal their spatial relationship we utilized the highly sensitive and specific RNAscope Multiplex Fluorescence in situ hybridization (ISH) assay. Probes for XCR1, THBD, CLEC9A, and CCR5 were used to identify cDC1 cells within 4 cervical cancer tumors. These tumors were then assessed for the presence of NK cells by using specific marker probes such as CD56 and NCR1 and chemokines XCL1 and CCL5. Finally, CTLs were visualized to determine if there is a correlation between the presence of cDC1 and NK cells and CTL infiltration within the cervical cancer tumors.

Results Our results revealed a strong correlation between the presence of NK cells, cDC1 cells, and CTLs within 3 out of 4 cervical cancer samples. The NK cells showed expression of the chemokines XCL1 and CCL5, which are the ligands for XCR1 and CCR5 respectively, suggesting that the XCR1+/ CCR5+ cDC1 cells may have been potentially recruited by these NK cells. Regions high in dC1 and NK cells also showed significantly higher levels of CTL recruitment, as indicated by the presence of CD8+/IFNG+ T cells. Conversely, 1 of the 4 cervical cancer samples demonstrated relatively lower levels of NK cells which correlated with lower cDC1 cells and in turn lower CTL infiltration.

Conclusions In conclusion, by utilizing the RNAscope Multiplex ISH assay we were able to identify and visualize the spatial relationship between NK cells, CTLs, and cDC1 cells, a rare subset of DC cells that excel at presenting tumor antigens to T cells. Using this technology, it is possible to spatially interrogate the TME and detect specialized immune cells when assessing response to immunotherapies.

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538 HARPENNING CROSS-DRESSING DENDRITIC CELLS TO STRENGTHEN ANTI-TUMOR IMMUNITY

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Background Cytotoxic (CD8+) T-cells are required for tumor eradication and durable anti-tumor immunity. The induction of tumor-reactive CD8+ T-cells is predominately attributed to a subset of dendritic cells (DC) called Batf3-driven DC1, given their robust ability to present cross-antigens for T-cell priming and their role in effector T-cell recruitment. Presence of the DC1 signature in tumors correlates with improved survival and response to immunotherapies. Yet, most tumors with a DC1 infiltrate still progress, suggesting that while DC1 can initiate tumor-reactive CD8+ T-cell responses, they are unable to sustain them. Therefore, there is a critical need to identify and engage additional stimulatory DC subsets to strengthen anti-tumor immunity and boost immunotherapy responses.

Methods To identify DC subsets that drive poly-functional CD8+ T-cell responses, we compared the DC infiltrate of a spontaneously regressing tumor with a progressing tumor. Multicolor flow immunophenotyping and single-cell RNA-sequencing were used to profile the DC compartment of both tumors. IFNγ-ELISpot was performed on splenocytes to assess systemic tumor-reactive T-cell responses. Sorted DC subsets from tumors were co-cultured with TCR-transgenic T-cells ex vivo to evaluate their stimulatory capacity. Cross-dressing (in vivo/ex vivo) was assayed by staining for transfer of tumor-derived H-2b MHC complexes to B cells, which express the H-2b haplotype. Protective systemic immunity was assayed via contralateral flank tumor outgrowth experiments.

Results Regressor tumors were infiltrated with more cross-presenting DC1 than progressor tumors. However, tumor-reactive CD8+ T-cell responses and tumor control were preserved in Batf3–/– mice lacking DC1, indicating that anti-tumor immune responses could be induced independent of DC1. Through functional assays, we established that anti-tumor immunity against regressor tumors required CD11c+ DC and cGAS/STING-independent type-I-interferon-sensing. Single-cell RNA-sequencing of the immune infiltrate of regressor tumors revealed a novel CD11b+ DC subset expressing an interferon-stimulated gene signature (ISG+ DC). Flow studies demonstrated that ISG+ DC were more enriched in regressor tumors than progressor tumors. We showed that ISG+ DC could activate CD8+ T-cells by cross-dressing with tumor-derived peptide-MHC complexes, thereby bypassing the requirement for cross-presentation to initiate CD8+ T-cell-driven immunity. ISG+ DC highly expressed cytosolic dsRNA sensors (RIG-I/MDA5) and could be therapeutically harnessed by exogenous addition of a dsRNA analog to drive protective CD8+ T-cell responses in DC1-deficient mice.

Conclusions The DC infiltrate in tumors can dictate the strength of anti-tumor immunity. Harnessing multiple stimulatory DC subsets, such as cross-presenting DC1 and cross-dressing ISG+ DC, provides a therapeutic opportunity to enhance anti-tumor immunity and increase immunotherapy responses.
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539 HIGH DIMENSIONAL ANALYSIS OF THE HUMAN LYMPH NODE DURING MELANOMA PROGRESSION REVEALS SHIFTS IN MYELOID CONTENT THAT RELATE TO DIFFERENTIAL T CELL CONTENT

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Background The sentinel lymph node (SLN) in melanoma represents the crossroads of the initiation of effector T cell responses and of lymphatic metastasis of the primary tumor. As such, alterations in the human LN immune cell network during melanoma progression are of particular interest for the development of effective immunotherapeutic approaches for each stage of disease.

Methods We used mass cytometry (CyTOF) and multiparameter flow cytometry to characterize the alterations in the major immune populations in the human LN. We included LN derived from healthy donors (n=10), tumor-negative (SLN, n=7) and tumor-positive SLN (SLN+, n=3) and LN metastatic samples (n=4).

Results Our results show that melanoma progression in the LN is accompanied by increased relative frequencies of myeloid cells, B cells and NK cells whereas T cell rates are significantly decreased. More specifically, for the myeloid cells we observed a decrease in frequencies of migratory cDC subsets and of LN-resident cDC and macrophage subsets in the SLN accompanying early melanoma development and metastasis. In fully metastatic LN from patients with advanced melanoma, a clear predominance of inflammatory, monocyte-derived subsets was observed. Simultaneously with this shift in myeloid subsets, an increase in CD4+ Tregs and CD8+ effector T cell subsets became apparent with metastatic progression in the LN. Both Tregs and CD8+ effector T cells in LN metastases were further characterized by relatively high expression of PD-1 and TIGIT immune checkpoints.

Conclusions The changes observed in the myeloid compartment accompanying metastatic progression in melanoma-draining LN, were found to be related to the shifts in lymphocytic subsets and their differentiation and activation state. Our results provide insights into the steady-state immune characteristics of the healthy human LN and identify all the changes that accompany melanoma progression through the different stages and give important clues about possible therapeutic interventions, aiming at immune potentiation of possible SLN.

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540 TRANSCRIPTIONALLY DEFINED IMMUNE LANDSCAPE IN HUMAN GLIOMAS

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Background Brain immunity is largely myeloid cell dominated rather than lymphoid cells in healthy and diseased state including malignancies of glial origins called as gliomas.

Despite this skewed myeloid centric immune contexture, immune checkpoint and T cell based therapeutic modalities are generalizably pursued in gliomas ignoring the following facts i) T cells are sparse in tumor brain ii) glioma patients are lymphopenic iii) gliomas harbor abundant and highly complex myeloid cell repertoire. We recognized these paradoxes pertaining to fundamental understanding of constituent immune cells and their functional states in the tumor immune microenvironment (TIME) of gliomas, which remains elusive beyond a priori cell types and/or states.

Methods To dissect the TIME in gliomas, we performed single-cell RNA-sequencing on ~123,000 tumor-derived sorted CD45+ leukocytes from fifteen genomically classified patients comprising IDH-mutant primary (IMP; n=4), IDH-mutant recurrent (IMR; n=4), IDH-wild type primary (IWP; n=3), or IDH-wild type recurrent (IWR; n=4) gliomas (hereafter referred as glioma subtypes) and two non-glioma brains (NGBs) as controls.

Results Unsupervised clustering analyses delineated predominately 34-myeloid cell clusters (~75%) over 28-lymphoid cell clusters (~25%) reflecting enormous heterogeneity within and across glioma subtypes. The glioma immune diversity spanned functionally imprinted phagocytic, antigen-presenting, hypoxia, angiogenesis and, tumoricidal myeloid to classical cytotoxic lymphoid subpopulations. Specifically, IDH-mutant gliomas were predominantly enriched for brain-resident microglial subpopulations in contrast to enriched bone barrow-derived infiltrates in IDH-wild type especially in a recurrent setting. Microglia attrition in IWP and IWR gliomas were concomitant with invading monocyte-derived cells with semblance to dendritic cell and macrophage like transcriptomic features. Additionally, microglial functional diversification was noted with disease severity and mostly converged to inflammatory states in IWR gliomas. Beyond dendritic cells, multiple antigen-presenting cellular states expanded with glioma severity especially in IWP and IWR gliomas. Furthermore, we noted differential microglia and dendritic cell inherent antigen presentation axis viz, osteopontin, and classical HLAs in IDH subtypes and, glioma-wide non-PD1 checkpoints associations in T cells like Galectin9 and Tim-3. As a general utility, our immune cell deconvolution approach with single-cell-matched bulk RNA sequencing data faithfully resolved 58-cell states which provides glioma specific immune reference for digital cytometry application to genomics datasets.

Conclusions Altogether, we identified prognosticator immune cell-signatures from TCGA cohorts as one of many potential immune responsiveness applications of the curated signatures for basic and translational immune-genomics efforts. Thus, we not only provide an unprecedented insight of glioma TIME
but also present an immune data resource that can be exploited for immunotherapy applications.

**Ethics Approval**  The brain tumor/tissue samples were collected as per MD Anderson internal review board (IRB)-approved protocol numbers LAB03-0687 and, LAB04-0001. One non-tumor brain tissue sample was collected from patient undergoing neurosurgery for epilepsy as per Baylor College of Medicine IRB-approved protocol number H-13798. All experiments were compliant with the review board of MD Anderson Cancer Center, USA.

**Consent**  Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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**INVESTIGATING MYELOID DERIVED SUPPRESSOR CELLS (MDSCS) AND OLIGONUCLEOTIDE BASED TARGETING OF STAT3 IN RENAL CELL CARCINOMA**

Marice Alcantara*, Dayson Moreira, Chia-Yang Hung, Chunsong, Dongfang Wang, JoAnn Hsu, Sumanta Pal, Marcin Kortylewski. City of Hope, Duarte, CA, USA

**Background**  Recent advancements in the treatment of renal cell carcinoma (RCC) using immune checkpoint inhibitors (ICI) against PD1 or CTLA-4 receptors have improved survival rates in patients. However, more than half of RCC patients does not respond to anti-PD-1/-CTLA-4 combination immunotherapy. Thus, we decided to investigate mechanisms underpinning the resistance to ICI at the cellular and molecular levels.

**Methods**  We utilized multicolour flow cytometry and Luminex assays to investigate patient peripheral blood and used syngeneic mouse models to determine the efficacy of oligonucleotide based targeting of STAT3

**Results**  First, we characterized immunosuppressive myeloid cell populations, T cell subsets and immune biomarkers in blood samples from RCC patients with advanced stage IV disease, undergoing anti-PD-1/-CTLA-4 (nivolumab/ipilimumab) combination immunotherapy. Results of our multicolor flow cytometry and plasma analysis suggested that ICI therapy is associated with a significant almost 15-fold increase of polymorphonuclear MDSCs (PMN-MDSCs) in the peripheral blood of RCC patients over the course of 3 therapeutic cycles. Notably, we found that PMN-MDSCs showed high levels of activated Signal Transducer and Activator of Transcription 3 (pSTAT3) and a significant increase its downstream target Arginase-I between cycle 1 and cycle 8 of treatment (P=0.0008). The pSTAT3/ARG-1 signaling is known for promoting tumor immune evasion, thus strongly suggesting that immature PMN-MDSCs are potentially involved in limiting outcome of ICI therapy in RCC patients similar as shown before in other genitourinary cancers such as prostate and bladder cancers. We recently developed a strategy to target STAT3 selectively in tumor-associated myeloid cells using using STAT3 antisense oligonucleotide (STAT3ASO) conjugated to immunostimulatory CpG oligodeoxynucleotides acting as targeting moiety. In our initial efficacy studies, we assessed activity of three versions of CpG-STAT3ASO conjugates with various chemical modifications, such as 2’-O-methyl- or locked nucleic acid, in a syngeneic bladder tumor model (MB49). MB49 cancer cells were subcutaneously injected into two flanks of male C57BL/6 mice and treated every second day with 5 mg/kg of various CpG-STAT3ASO injected intratumorally into one of the tumor sites. All CpG-STAT3ASOs inhibited tumor cell growth in both treated and distant tumors in comparison to controls. The immunohistochemical analysis indicated an increase in the percentage of CD8+ T cell with reduction of regulatory T cells within CpG-STAT3ASO treated tumors in comparison to controls, suggesting activation of CD8 T cell-mediated antitumor immunity.

**Conclusions**  Overall, our preliminary results suggest that immune suppressive pSTAT3+/ARG-1+ PMN-MDSCs accumulate in patients with RCC undergoing ICI combination therapy, which may potentially contribute to resistance to ICIs. Targeting STAT3 signaling in the RCC-associated myeloid cells using CpG-STAT3ASO may provide a potential novel strategy for augmenting immune checkpoint therapies.

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**EXPANSION OF CYTOTOXIC NK CELLS FROM PBMCs USING INDIVIDUALIZED CYTOKINE COMBINATION**

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**Background**  Adoptive immunotherapy relies on the use of T-cells to target tumour cells, through Major Histocompatibility Complex (MHC) Class I recognition.1 However, many tumours display alterations in the MHC-I pathway, a well-described immune evasion mechanism.2 Natural Killer (NK) cells recognize transformed cells independently from the presence of MHC-I and may be a reliable therapeutic option for patients with altered tumour MHC-I expression. The source of NK cells may be autologous or allogeneic and NK cells are also clinically relevant recipients of transgenic receptors (TCRs or antibodies) targeting tumour cells. NK cells have been categorized according to their CD56 and CD16 surface expression into different subpopulations: cytotoxic (CD56+CD16+) and regulatory (CD56brightCD16+).3 Expanding cytotoxic NK cells is challenging, since the frequency of NK cells is low in peripheral blood and there is also – at this point – not an optimal expansion protocol available. The goal of this project is to determine the best cytokine combination that facilitates expansion of cytotoxic NK cells that either target tumor cells directly or serve as recipients for transgenic receptors.

**Methods**  Peripheral Blood Mononuclear Cells (PBMCs) were extracted using Ficoll methodology from blood donors and cultured in T25 flasks with Cell Genix Medium supplemented with 10% human serum and antibiotics. NK cells were expanded supplemented with feeder cells (ratio 1:1) and treated every second day with 5 mg/kg of various cytokine combinations produced a statistically significant increase of the absolute number of NK cells with a significant almost 15-fold increase of polymorphonuclear MDSCs (PMN-MDSCs) in the peripheral blood of RCC patients over the course of 3 therapeutic cycles. Notably, we found that PMN-MDSCs showed high levels of activated Signal Transducer and Activator of Transcription 3 (pSTAT3) and a significant increase its downstream target Arginase-I between cycle 1 and cycle 8 of treatment (P=0.0008). The pSTAT3/ARG-1 signaling is known for promoting tumor immune evasion, thus strongly suggesting that immature PMN-MDSCs are potentially involved in limiting outcome of ICI therapy in RCC patients similar as shown before in other genitourinary cancers such as prostate and bladder cancers. We recently developed a strategy to target STAT3 selectively in tumor-associated myeloid cells using using STAT3 antisense oligonucleotide (STAT3ASO) conjugated to immunostimulatory CpG oligodeoxynucleotides acting as targeting moiety. In our initial efficacy studies, we assessed activity of three versions of CpG-STAT3ASO conjugates with various chemical modifications, such as 2’-O-methyl- or locked nucleic acid, in a syngeneic bladder tumor model (MB49). MB49 cancer cells were subcutaneously injected into two flanks of male C57BL/6 mice and treated every second day with 5 mg/kg of various CpG-STAT3ASO injected intratumorally into one of the tumor sites. All CpG-STAT3ASOs inhibited tumor cell growth in both treated and distant tumors in comparison to controls. The immunohistochemical analysis indicated an increase in the percentage of CD8+ T cell with reduction of regulatory T cells within CpG-STAT3ASO treated tumors in comparison to controls, suggesting activation of CD8 T cell-mediated antitumor immunity.

**Conclusions**  Overall, our preliminary results suggest that immune suppressive pSTAT3+/ARG-1+ PMN-MDSCs accumulate in patients with RCC undergoing ICI combination therapy, which may potentially contribute to resistance to ICIs. Targeting STAT3 signaling in the RCC-associated myeloid cells using CpG-STAT3ASO may provide a potential novel strategy for augmenting immune checkpoint therapies.
CD107a-expressing cells, more than the CD56+CD16+, the most cytotoxic subpopulation of NK cells.

Conclusions This work shows that we are able to grow and efficiently expand NK cells from PBMCs with different cytokine combinations leading to clinically relevant NK cell numbers as well as cytotoxic functions. This enables to produce NK cell products for therapy and as recipients for transgenic tumor antigen-specific receptors.

Acknowledgements The authors would like to thank the Champalimaud Foundation Biobank, the Vivarium Facility and the Flow Cytometry Platform of the Champalimaud Centre for the Unknown.

Ethics Approval This study was approved by the Champalimaud Foundation Ethics Committee and by the Ethics Research Committee of NOVA Medical School of NOVA University of Lisbon.

Consent Written informed consent was obtained from the blood donors to use their samples for research purposes.

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Abstract 542 Figure 1 Representative percentage of NK cells in total lymphocytes (A), CD56+CD16+ subpopulation in total NK cells (B), and CD56brightCD16- subpopulation amongst total NK cells (C) at different time points (5, 10, 15 and 20 days) expanded from PBMCs. * p-value < 0.05

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Abstract 542 Figure 1 Representative percentage of NK cells in total lymphocytes (A), CD56+CD16+ subpopulation in total NK cells (B), and CD56brightCD16- subpopulation amongst total NK cells (C) at different time points (5, 10, 15 and 20 days) expanded from PBMCs. * p-value < 0.05

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REFERENCES
Background Adoptively transferred antigen-specific T cells have shown great efficacy in treatment of some virus-associated diseases and malignancies. A major driver of the development of adoptive T-cell therapy has been our ability to successfully characterize the functional status and antigen specificity of T cells. However, this has been limited by inefficient detection of antigen-specific T cells possibly due to their low frequency and low binding affinities to known MHC-peptide complexes.

Methods Here, we aim to combine two powerful technologies, advanced dCODE™ Dextramer® from Immudex and single-cell multiomics analysis using the BD Rhapsody™ Single-Cell Analysis system, to detect and characterize disease-specific CD8+ T cells within thousands of PBMCs.

Results Currently, we are able to identify over 350 mRNAs alongside a panel of over 20 BD® AbSeq cell surface protein markers which can be associated with T cell activation states. These data can be used to define T-cell phenotypes alongside antigen specificity of enriched CD8+ Dextramer(R)+ cells from a PBMC population.

Conclusions This study outlines our ability for high-resolution T-cell profiling that has broader implications and utility in immuno-oncology, infectious diseases and autoimmunity.

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Background CD4 T cells have been implicated in cancer immunity for their helper functions. However, their direct cytotoxic potential remains elusive in cancer patients. Here, we aimed at assessing the presence, rate and cytotoxic function of tumor-specific Th-CTX directly in cancer patients.

Methods We capitalized on published single cell transcriptomic analyses of patient samples, integrated with the direct phenotypic and functional characterization of clonal, tumor-specific CD4 T cell populations, using peptide-MHC class II multimers and a novel high-throughput single-cell cytotoxicity assay in picowell arrays. The direct tumor cell killing by cytolytic tumor-specific CD4 T cells in the arrays was monitored in a high-throughput manner by combining multi-channel time-lapse microscopy with deep neural networks.

Results By mining single-cell RNA-seq datasets of tumor infiltrating lymphocytes, we identified CD4 T cells displaying cytotoxic phenotypes in different human tumors. The cytolytic CD4 T cells formed a distinct cluster and expressed genes related to classical cytotoxic functions, largely resembling CD8 T cell gene profiles. Using the peptide MHC class II multimer technology, we confirmed directly ex vivo the presence of cytolytic tumor antigen-specific CD4 T cells, both in the circulation and in the tumors of patients. We performed an integrated phenotypic and functional characterization of cytolytic tumor-specific CD4 T cells, down to the single cell level, through a high-throughput nanobiochip consisting of massive arrays of picowells with sub-nanoliter volumes and machine learning. We demonstrated a direct, contact-dependent, granzyme-dependent cytotoxic activity against tumor cells, with delayed kinetics compared to classical cytotoxic lymphocytes. Lastly, we discovered that this cytotoxic activity was at least in part dependent on the expression of SLAMF7, a homophilic receptor known to regulate NK cell activity.

Conclusions Our work provides a deep characterization of human Th-CTX in cancer and supports their role in tumor immunity. Moreover, our results showing that agonistic engagement of SLAMF7 enhances the cytolytic capacity of tumor-specific CD4 T cells, suggests that targeting these cells might prove synergistic with the use of other immunotherapies in cancer patients.
Background Penile squamous cell carcinoma (PSCC) is rare in the US accounting for 0.7% of the total cancer incidence. Around 50% of PSCC cases are associated with HPV infection and between 40–60% have high PD-L1 expression. The identification of the immune landscape in PSCC using the spatial proximity between tumor cells and immune phenotypes has not been described yet.

Methods We performed multiplex immunofluorescence (mIF) on 54 formalin-fixed, paraffin-embedded tissue sections of PSCC. The staining was performed with the opal-7 kit (Akoya/PerkinElmer) in the Leica Bond RX autostainer using 7 markers against, Cytokeratins, CD3, CD8, CD68, PD-1, and PD-L1. The slides were scanned in the multispectral microscopy Vectra Polaris (Akoya/PerkinElmer), and 5 regions of interest (ROI) per case were selected and analyzed using the digital image analysis software InForm 2.2.4. The X and Y coordinate from each cell phenotype was obtained from each ROI. Nearest neighbor median distance and the infiltration analysis using the G-function metric of the different tumor associated immune cells (TAICs) within a distance of 20, 40 and 60 microns from the tumor cells were obtained through the R studio software and correlated with available clinical information.

Results Using the nearest neighbor cell analysis we identified the distance of ≤87.33 microns as a close median distance from tumor cells to the multiple TAICs. In our cohort of PSCC tumors, the closest TAIC phenotype to tumor cells was the CD3+ T cells with a median distance of 20.13 microns, followed by macrophages CD68 (median distance, 21.19 microns) and cytotoxic T cells (CD3+, CD8+) with a median distance of 36.09 microns, compared with the others TAICs located farther than 87.33 microns (table 1, figure 1). Interestingly, cytotoxic T cells (median distance, 59.5 micros), T-cells (median distance, 65.6 microns) and macrophages expressing PD-L1 (median distance, 61.2 microns) are located closer to tumor cells expressing PD-L1 than from tumor cells not expressing PD-L1 (median distance, 104.08, 116.05 and 118.74 microns, respectively). Unexpectedly, HPV negative patients had closer cytotoxic T cells CD3+CD8+ median distance, 30.88 microns) and cytotoxic T cells antigen experienced CD3+CD8+PD-1+ (median distance, 50.09 microns), compared to HPV positive patients (median distance of 36.09 and 59.83 microns, respectively). Additionally, the G-function AUC metric from tumor cells to macrophages CD68 showed high interaction at 40 microns in HPV cases when compared with others distances and not HPV cases (P=0.049, figure 2).

Conclusions Although, higher densities of cytotoxic T cells were observed relatively closer from tumor cells than others TAIC subsets, we didn’t find strong interaction with this subset in using the G-function AUC metric. The PD-1/PD-L1 axis was also found in close proximity suggesting that there are more likely to interact with tumor cells generating a strongly immunosuppressive microenvironment. In addition, while...
Conclusions In this patient with vaginal mucosal melanoma, subsequent melanoma metastases of clonal origin attracted CD8+ T cells of similar specificity, among which TRM cells responded more vigorously to tumour cells than other T cells subsets.

Acknowledgements The authors would like to acknowledge immCORE La Hoffmann- Roche Ltd. for funding.

Ethics Approval Patients diagnosed with stage 3 or 4 metastatic melanoma and undergoing clinically indicated surgery were enrolled in prospective studies approved by the Peter MacCallum Cancer Centre human ethics research committee (13/141). All experimental protocols have been approved and clinical data has been collected prospectively.

REFERENCES

Abstract 549 Figure 1 Graphical depiction of the methods used to characterise T cells in mucosal metastatic melanoma

CD8+ TISSUE-RESIDENT MEMORY T CELLS ARE TUMOUR REACTIVE AND INCREASE AFTER IMMUNOTHERAPY IN A CASE OF METASTATIC MUCOSAL MELANOMA

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Background Mucosal melanoma is a rare subtype of melanoma originating from mucosal tissues, metastases are very aggressive and respond poorly to therapy, including immune checkpoint inhibitors (ICI) such as anti-CTLA4 and anti-PD1 antibodies. CD8+ T cells constitute the most abundant immune infiltrate in metastatic melanoma, of which the Tissue Resident Memory Subset (TRM) is of particular interest. CD8+ TRM cells express the highest levels of immune checkpoint receptors, proliferate in response to ICI and correlate with longer disease-free and overall survival. The immune landscape in mucosal melanoma remains poorly characterized. We aimed to: 1) phenotype CD8+ T cells and TRM infiltrating metastatic mucosal melanoma, 2) characterize the clonality of TRM in relation to other CD8+ T cell subsets and 3) define the capacity of CD8+ T cells and TRM to respond to melanoma cells and to in vivo and in vitro anti-PD1 treatment.

Methods We investigated the CD8+ T and TRM cells infiltrating two temporally- and spatially-distant subcutaneous metastases, these originated from a primary vaginal mucosal melanoma. One metastasis was excised prior to anti-PD1 treatment and one was anti-PD1 refractory, having progressed on treatment. We used mass cytometry and single-cell RNA and TCR sequencing to characterise the phenotype and clonality of the T cells, multiplex immunohistochemistry to define their spatial relationship with tumour cells and other T cells, and functional assays to determine TRM response to tumour cells (figure 1).

Results CD8+ T RM frequency increased with time and anti-PD1 treatment, forming clusters at the tumour margin. T cells in the anti-PD1 refractory lesion were more activated than T cells in the first tumour and were bound by anti-PD1 antibody in vivo. T cells could not be stimulated by anti-PD1 directly ex vivo. Both metastatic lesions shared common T cell clusters including TRM. Furthermore, TRM in each tumour shared T cell clones, suggesting the presence of common antigens between metastatic sites. Indeed, the two metastases had a similar mutational profile. In vitro expanded tumour infiltrating lymphocytes from both lesions recognized tumour cells from both lesions and the same neoantigen generated from a single point mutation in the gene CDKN1C. Finally, tumour cells stimulated TRM cells more robustly than other T cells subsets.


Abstract 548 Figure 1

Conclusions In this patient with vaginal mucosal melanoma, subsequent melanoma metastases of clonal origin attracted CD8+ T cells of similar specificity, among which TRM cells responded more vigorously to tumour cells than other T cells subsets.

Acknowledgements The authors would like to acknowledge immCORE La Hoffmann- Roche Ltd. for funding.

Ethics Approval Patients diagnosed with stage 3 or 4 metastatic melanoma and undergoing clinically indicated surgery were enrolled in prospective studies approved by the Peter MacCallum Cancer Centre human ethics research committee (13/141). All experimental protocols have been approved and clinical data has been collected prospectively.

REFERENCES

Abstract 548 Figure 1

Conclusions In this patient with vaginal mucosal melanoma, subsequent melanoma metastases of clonal origin attracted CD8+ T cells of similar specificity, among which TRM cells responded more vigorously to tumour cells than other T cells subsets.

Acknowledgements The authors would like to acknowledge immCORE La Hoffmann- Roche Ltd. for funding.

Ethics Approval Patients diagnosed with stage 3 or 4 metastatic melanoma and undergoing clinically indicated surgery were enrolled in prospective studies approved by the Peter MacCallum Cancer Centre human ethics research committee (13/141). All experimental protocols have been approved and clinical data has been collected prospectively.
Background CD4 and CD8 T cells are genetically and functionally distinct cell subsets of the adaptive immune system that play pivotal roles in immune surveillance and disease control. During development in the thymus, transcription factors ThPOK and Runx3 regulate the differentiation and maturation of these two lineages into single positive T cells that enter the periphery with mutually exclusive expression of either the CD4 or CD8 co-receptor. Despite our expectation that these two cell fates are fixed, mature CD4+CD8+ double positive (DP) T cells have been described in the context of numerous immunological responses, including cancer, but their molecular and functional properties and therapeutic relevance remain controversial and largely unknown.

Methods Our lab has identified and characterized a heterogeneous DP T cell population in murine and human melanoma tumors comprised of CD4 and CD8 T cells expressing the opposite co-receptor and a parallel upshift in the opposite cell type’s phenotype and function. Using CD4 (Trp1) and CD8 (Pmel) transgenic TCR T cells specific to B16 melanoma antigens gp75 and gp100 respectively, we demonstrate the re-expression of the opposite co-receptor following adoptive T cell transfer in B16 melanoma tumor bearing mice.

Results Specifically, up to 50% of transferred CD4 Trp1 T cells will re-express CD8 to become a DP T cell in the tumor microenvironment. Further, these CD4 derived DP T cells upregulate CD8 lineage regulator Runx3 and cytolytic genes Gzmb, Gzmk, and Prf1 to become potent cytotoxic T cells. Alternatively, a subset of CD8 Pmel T cells differentiate into DP T cells characterized by the increased expression of CD4, ThPOK, and regulatory marker FoxP3 (figure 1). In addition, we utilized 10x single cell and ATAC sequencing to further characterize these divergent DP T cell populations among open repertoire T cells isolated from murine and human melanoma tumors.

Conclusions Our findings highlight the capability of single positive T cells to differentiate in response to antigen and local stimuli into novel T cell subsets with polyfunctional characteristics. The resulting cell subsets will potentially affect the tumor microenvironment in distinct ways. Our studies may inform therapeutic approaches to identify antigen specific T cells as well as innovative signaling pathways to target when genetically engineering T cells to optimize cytotoxic function in the setting of adoptive cell therapy.

Ethics Approval The human biospecimen analyses were approved by Memorial Sloan Kettering Cancer Center IRB #06-107.

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**Immune-stimulants and immune modulators**

**550** AN AXL-TARGETING MONOCLONAL ANTIBODY THAT INHIBITS AXL ACTIVITY AND POTENTIALLY STIMULATES THE INNATE IMMUNE RESPONSE

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Background Axl is a member of the TAM (Tyro3/Axl/MerTK) family of receptor tyrosine kinases and a negative regulator of innate immunity. Activation of Axl through its ligand Gas6 leads to suppression of myeloid cell activity, while its activation in tumor cells drives tumor growth, metastasis, and is associated with acquired resistance to targeted therapies, radiotherapy and chemotherapy.

Methods Purified monoclonal antibodies and variants thereof were tested in human cancer lines and primary human myeloid cells for effects on Axl signaling and immune activation, respectively.

Results We describe a humanized IgG1 Axl-targeting monoclonal antibody (mAb), CDX-0168, that binds to the ligand-binding domain of Axl with sub-nanomolar affinity and potently inhibits Gas6 binding. In tumor cells, CDX-0168 inhibits Gas6-dependent Axl phosphorylation and signaling and elicits tumor cell killing via ADCC in vitro and in vivo. In primary human immune cells, CDX-0168 treatment induces potent release of pro-inflammatory cytokines and chemokines from dendritic cells, monocytes and macrophages through an Fc receptor-dependent mechanism and enhanced T cell activation in mixed lymphocyte reactions. Axl inhibition may further enhance antitumor activity associated with PD-(L)1 blockade. To this end, we generated a tetravalent bispecific Axl x PD-L1 antibody combining CDX-0168 with a potent anti-PD-L1 mAb (9H9) using an IgG-scFv format. The bispecific antibody elicits greater cytokine release and T cell activation in vitro than the combination of the parental antibodies, while maintaining robust Axl and PD-L1 blockade.

Conclusions Additional studies investigating simultaneous blockade of the Axl and PD-L1 pathways with other agents may further exploit the potential for this novel anti-cancer therapeutic approach.

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**552** SUMOYLATION INHIBITOR TAK-981 ACTIVATES NK CELLS AND MACROPHAGES VIA TYPE I INTERFERON SIGNALING AND SHOWS SYNERGISTIC ACTIVITY IN COMBINATION WITH RITUXIMAB AND DARATUMUMAB IN PRECLINICAL MODELS

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Background TAK-981 is a first-in-class small molecule inhibitor of the SUMO activating enzyme in Phase 1 clinical trials. SUMOylation has previously been implicated in the...
regulation of innate immune responses and expression of Type I interferons,⁴ and ex vivo treatment of human and mouse immune cells with TAK-981 results in transcriptional upregulation of IFN-beta and Type I IFN receptor (IFNAR) signaling. We previously showed that TAK-981 increases NK cell activation and M1 macrophage polarization, leading to enhanced ADCC and ADCP in the presence of rituximab. ⁵ In vivo, TAK-981 induces IFNAR-dependent antitumor activity and synergizes with rituximab in xenograft-bearing mice. ⁶ ³ Here we investigated the mechanism of synergistic activity with rituximab and evaluated the combination of TAK-981 with daratumumab, another therapeutic mAb.

**Methods**
The role of effector function of rituximab in the mechanism of synergy with TAK-981 was evaluated in OCI-Ly10-bearing SCID mice treated with TAK-981 and the LALA-PG version of rituximab, in which mutations in the Fc region prevent FcγR binding. The combination of TAK-981 and rituximab was also evaluated in OCI-Ly10 tumor-bearing mice in which macrophages and/or NK cells were depleted with clodronate and anti-asialo GM1. TAK-981 in combination with daratumumab was evaluated in two CD38+ xenograft models, Daudi (Burkitt’s lymphoma) and LP-1 (multiple myeloma). To test ADCP activity, Daudi-KILR cells were incubated with human monocyte-derived macrophages (hMDM) treated with TAK-981 in the presence or absence of rituximab or daratumumab, with or without a neutralizing antibody to IFNAR2.

**Results**
Unlike rituximab, LALA-PG mutated rituximab did not synergize with TAK-981 in OCI-Ly10 tumor-bearing mice, indicating a requirement for Fc effector function. Depletion of macrophages with clodronate or NK cells with anti-asialo GM1 lessened the anti-tumor effect of the TAK-981 and rituximab combination, while dual depletion of macrophages and NK cells had a greater impact. TAK-981 showed synergistic activity in combination with daratumumab in two CD38+ xenograft models, Daudi and LP-1. In vitro, TAK-981-treated hMDM showed increased phagocytic activity against Daudi cells, and this effect was further enhanced in the presence of rituximab or daratumumab but prevented by a neutralizing antibody to IFNAR2.

**Conclusions**
In preclinical models, TAK-981 synergizes with rituximab through a mechanism involving Type I IFN dependent enhancement of ADCC and ADCP and the combination of TAK-981 with daratumumab is also synergistic.

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**Background**
Activation of T cells requires a specific peptide/HLA (human leukocyte antigen) signal presented by an interacting immune or target cell along with engagement of co-stimulatory molecules or cytokine receptors. Cue Biopharma has developed a proprietary biologics platform, termed Immuno-STAT™ (Selective Targeting and Alteration of T cells), wherein a singular protein framework incorporates peptide/HLA complexes and co-stimulatory or cytokine signals. The CUE-100 series Immuno-STATs selectively deliver rationally engineered IL-2 molecules to antigen-specific T cells. The IL-2 molecules in the CUE-100 series Immuno-STATs contain mutations that attenuate binding to IL-2 receptors alpha and beta, which minimizes activation of regulatory T cells (Tregs) and the irrelevant non-antigen-specific T cell repertoire. We have demonstrated that CUE-100 series Immuno-STATs specific for different antigenic peptides (from HPV16, WT1, MART-1, CMV, Flu, and HIV) induce expansion of functional, oligoclonal, antigen-specific repertoire from human PBMCs. The lead clinical candidate CUE-101, presenting the E711-20 peptide from HPV-16 in the context of HLA-A*02:01, is currently being tested in a Phase 1 clinical trial in recurrent/metastatic head and neck cancer patients with evidence of dose-proportional PK, early pharmacodynamic effects and signals of clinical activity.

**Methods**
CUE-100 series Immuno-STATs were tested with human PBMCs to demonstrate specific T cell activation and expansion. Expanded T cell clonality was assessed by single cell TCR sequencing. Responses of T cells to peptide-presenting targets was evaluated by cytokine staining and by assessing their ability to kill target cells. In vivo activity of CUE-100 series Immuno-STATs was assessed in HLA-A2 transgenic mice.

**Results**
Data demonstrate that the CUE-100 series Immuno-STATs selectively activate antigen-specific CD8+ T cells. Signaling, cell-based assays and cytokine release studies confirmed functional attenuation of the IL-2 components of the CUE-100 series, which allows for a favorable safety and selectivity profile. Immuno-STATs demonstrated robust expansion of CD8+ T cells after primary stimulation of unpamir hPBMCs, or re-stimulation of hPBMCs after initial cognate peptide stimulation. In addition, CUE-100 series Immuno-STATs expanded CD8+ T cells in naïve HLA-A*02 transgenic mice. In both cases the expanded T cells exhibited a polyfunctional response upon challenge with peptide-presenting target cells.

**Conclusions**
The presented data suggests that CUE-100 series Immuno-STATs have the potential to enhance anti-tumor immune responses by inducing a robust antigen-specific, oligoclonal, polyfunctional T cell repertoire. Early validation of CUE-100 series Immuno-STATs is obtained through the emerging signs of pharmacodynamic and clinical activity in the ongoing Phase I trial with CUE-101.
Background Regulatory T cells (Tregs) have been a therapeutic target of interest since early pre-clinical work revealed that their depletion lead to enhanced tumor control. Despite continuing advances in the development of novel cellular-, antibody- and chemotherapeutic-based strategies to increase anti-tumor immunity, Treg presence and activity within the tumor microenvironment remains a complicating factor to their clinical efficacy. To overcome dosing limitations and off-target effects from antibody-based Treg deletional strategies, we investigated the ability to target FOXP3, the master regulator of Treg development, maintenance, and suppressive function using hydrocarbon stapled alpha-helical peptides (SAHs). We developed SAHs in the likeness of a portion of the native FOXP3 antiparallel coiled-coil homodimerization domain, in an effort to impede FOXP3 transcriptional function. SAHs overcome three major protein-protein interaction (PPI) therapeutic hurdles, namely: cellular penetrance, target specificity, and secondary structure stability. Our overall goal is to use these SAHs as investigatory drugs to demonstrate proof-of-concept of the druggability of FOXP3. We aim to show their utility to further understand FOXP3 transcriptional dynamics and explore their potential use in altering the immune landscape in combination with other immune-focused therapies.

Methods Utilizing the FOXP3 crystal structure as a guide, we developed a number of single and double SAH peptides corresponding to the leucine zipper homodimerization domain (DD) of FOXP3 (SAH-FOXP3DDs). We tested the ability of SAH-FOXP3DDs to bind FOXP3, to access the intracellular compartment, to alter Treg transcriptional and phenotypic profiles, and to inhibit Treg-mediated immune suppression.

Results Select SAH-FOXP3DDs bound recombinant FOXP3AN and the FOXP3 leucine zipper (LZCC) domain with high affinity and dose-dependently inhibited binding of FOXP3 to cognate DNA in vitro. Lead SAH-FOXP3DDs were cell permeable and showed no non-specific toxicity to T cells at high concentrations. Flow cytometric and qRT-PCR analysis of treated Tregs revealed dose-dependent changes in protein and gene expression of several FOXP3 targets suggestive of FOXP3-specific transcriptional alteration. Treatment of Tregs with lead SAHs effectively inhibited in vitro Treg-mediated T cell suppression and induced global gene expression changes corresponding to loss of function Foxp3 in vivo.

Conclusions This work supports the ability of SAHs to target transcription factors, particularly as a method of interrogating specific PPI functions, and provides strong proof-of-principle evidence that FOXP3 is druggable. SAH-FOXP3DDs will not only further our understanding of FOXP3 transcriptional control but will serve as prototype therapeutics whereby we can explore their ability to amplify anti-tumor immunity in pre-clinical tumor models.
PROGRAMMED DEATH (PD)-1 AND PD-LIGAND-1 INHIBITORS IN THE TREATMENT OF NON-SMALL CELL LUNG CANCER: A SYSTEMATIC REVIEW OF THEIR EFFICACY AND SAFETY

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Background Treatment advances have been made in non-small cell lung cancer (NSCLC) with the development and approval of programmed death (PD)-1 and PD-ligand 1 (PD-L1) inhibitors. PD-1 and PD-L1 inhibitors may be used as monotherapies or in combination with other agents and have been shown to improve NSCLC patient outcomes in clinical trials. We conducted a systematic search to compare the efficacy and safety of PD-1/PD-L1 inhibitors in the treatment of NSCLC.

Methods A systematic literature search of PubMed was conducted to identify phase III clinical trials in which the efficacy of PD-1/PD-L1 inhibitors in the treatment of NSCLC was evaluated. PD-1 inhibitors included nivolumab and pembrolizumab; PD-L1 inhibitors included atezolizumab, avelumab, and durvalumab. Patient characteristics and efficacy data were extracted.

Results Sixteen phase III clinical trials were identified (nivolumab=4; pembrolizumab=5; atezolizumab=5; avelumab=1; durvalumab=1). Across the 3 nivolumab monotherapy trials (n=638; median ages: 61–63 years), median progression-free survival (PFS) ranged 2.3–4.2 months; response rates ranged 19%–26%; grade 3/4 adverse events occurred in 7%–18% of patients. Nivolumab in combination with ipilimumab (n=583; median age: 64 years) had a median PFS of 5.1 months and response rate of 33%; grade 3/4 adverse events occurred in 33% of patients. Across the 3 pembrolizumab monotherapy trials (n=1,481; median ages: 63–64 years), median PFS ranged 3.9–10.3 months; response rates ranged 18%–45%; grade ≥3 adverse events occurred in 13%–27% of patients. In the 2 pembrolizumab combination therapy trials (n=688; median ages: 65 years), median PFS ranged 6.4–8.8 months; response rates ranged 48%–58%; grade ≥3 adverse events occurred in 67%–70% of patients. In the 4 atezolizumab combination therapy trials (n=1,486; median ages: 63–64 years), median PFS ranged 6.3–8.3 months; response rates ranged 47%–63.5%; grade 3/4 adverse events occurred in 54%–73% of patients. In the 3 monotherapy trials of atezolizumab (n=613; median age: 63 years), avelumab (n=396; median age: 64 years), and durvalumab (n=476; median age: 64 years), the median months of PFS were 2.7, 2.8, and 17.2, respectively; response rates were 14%, 15%, and 30%, respectively; grade ≥3 adverse events occurred in 15%, 10%, and 30.5% of patients, respectively.

Conclusions Although treatment responses varied, most of the evaluated PD-1/PD-L1 inhibitors were associated with a clinical benefit for NSCLC trial patients. Generally, treatment efficacy was greater with combination therapies, but adverse events occurred more frequently. Innovations in the targeting/personalization of PD-1/PD-L1 combination therapies will likely lead to improved NSCLC patient outcomes and further research is needed in this regard.

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FOSTRIECIN POTENTIATES GENOME INSTABILITY AND ANTI-TUMOR IMMUNITY IN OVARIAN CANCER

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Background Increased immune infiltration in ovarian tumors has been linked to improved patient outcome. Nonetheless, responses to checkpoint blockade therapies have been disappointing in ovarian cancer patients. This has been attributed to the low mutational burden present in ovarian tumors. However, many tumor antigens have been identified in ovarian cancer, which underscores the critical need to identify new treatment strategies that will trigger anti-tumor immunity in ovarian cancer. Recent studies have revealed that defects in DNA damage repair (DDR) pathways can contribute to improved responses to immune-directed therapies. 1, 2 We previously discovered that CT45 expression sensitizes ovarian cancer cells to chemotherapy via its interaction with the protein phosphatase 4 (PP4) complex. 3 PP4 is known to play a key role in DDR pathways; however, its potential effects on anti-tumor immunity remain unknown.

Methods Using fostriecin, a commercially available inhibitor of PP4, we studied the effect of fostriecin on chemosensitivity using cell cycle analysis and cell viability assays. To study the effect of fostriecin on DNA damage, we performed comet assays and measured micronuclei along with FANCd2 foci formation. Furthermore, using western blot, qPCR, and T cell activation assays, we assessed the role of fostriecin in promoting an inflammatory response. We tested the efficacy of combining fostriecin with carboplatin and PD-1 inhibition in a syngeneic mouse model of ovarian cancer.

Results Our results show that fostriecin treatment combined with carboplatin leads to increased carboplatin sensitivity, DNA damage, and micronuclei formation. Using a panel of ovarian cancer cells, we show that fostriecin treatment triggers an anti-tumor immune response via STAT1 activation resulting in increased expression of pro-inflammatory cytokines. Furthermore, in a syngeneic mouse ID8 ovarian cancer cell line, we demonstrate that combination treatment of fostriecin and carboplatin significantly increased CD8 T cell activation over carboplatin treatment alone.

Conclusions Our work has identified a role for PP4 inhibition in promoting anti-tumor immunity. These findings form the groundwork for the rationale design of a clinical trial combining PP4 inhibitors with chemio-immunotherapy as a new approach in ovarian cancer treatment.

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Background Relapsed pediatric sarcomas have a poor prognosis with no available curative options. Alpha-Tocopherol oxyacetic acid (a-TEA) is a redox-silent analog of alphatocopherol that induces apoptotic and immunogenic cell death of tumor cells at doses that are not harmful to healthy normal cells. In a first-in-human clinical trial, a-TEA was well tolerated in adults with advanced solid tumors (NCT02192346), but has not yet been studied in pediatric sarcoma. We used a murine model of rhabdomyosarcoma (M3-9-M RMS) to assess the in vitro and in vivo anti-tumor effects of a-TEA.

Methods In vitro studies were performed on the M3-9-M RMS cell line to measure a-TEA-mediated apoptosis using flow cytometry (Annexin V+/7AAD+ cells) and live cell imaging (Annexin V+ cells). In vivo studies involved orthotopic implantation of luciferase+ M3-9-M tumor cells into syngeneic C57Bl/6 recipients. Once tumors were palpable, mice were randomized to a control diet or a-TEA-supplemented chow for 21 days and evaluated for bioluminescence, tumor growth and overall survival. Gene expression of tumor-infiltrating and splenic T cells were analyzed by bulk RNA-Seq and flow cytometry respectively.

Results M3-9-M RMS treatment with 2.5–100 μM a-TEA induced apoptosis in a dose-dependent manner within 24 hours (p < 0.05) as measured by flow cytometry and live cell imaging. In vivo studies with the M3-9-M RMS mouse model showed that recipients of a-TEA chow had 30–40% reduced tumor growth (p < 0.01) and bioluminescence (p < 0.05), leading to prolonged survival (> 4 weeks) compared to recipients of matched control chow (p < 0.05). Spleen cells isolated from a-TEA-fed tumor-bearing mice demonstrated increased levels of IFN-γ, CD4+ T-cells, Ki-67 proliferation, and decrease in splenic CD11b+ arginase-1+ (p < 0.01) and PD-L1+ cells. Gene set enrichment analyses of excised RMS tumors after a-TEA treatment revealed increased gene expression of CD24, EP300, CXCR4, and c-Jun as compared to tumors from mice fed control chow.

Conclusions These data indicate that a-TEA mediates apoptosis of RMS in vitro and suppresses in vivo tumor growth, leading to prolonged survival likely via enhanced activation of adaptive immunity through CD4+ T-cells and suppression of innate immunity through regulation of myeloid cell subsets. Furthermore, a-TEA may have direct effects on tumor cell proliferation through EP300 and c-Jun as well as indirect effects on tumor growth by regulation of immune cell recruitment through CD24 and CXCR4 gene expression. Administration of a-TEA as a potential salvage treatment for RMS is warranted.

Acknowledgements The study was supported by NIH TL1 TR002375 (FS), St. Baldrick’s Stand up to Cancer (SU2C) Pediatric Dream Team Translational Research Grant SU2C-AACR-DT-27-17, NIH/NCI R01 CA215461, American Cancer Society Research Scholar Grant RSG-18-104-01-LIB, and the Midwest Athletes Against Childhood Cancer (MACC) Fund (CMC). SU2C is a division of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the scientific partner of SU2C. The contents of this article do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Ethics Approval The University of Wisconsin-Madison Animal Care and Use Committee approved all protocols (M005915). http://dx.doi.org/10.1136/jitc-2020-SITC2020.0560
SO-C101 INDUCES NK CELL CYTOTOXICITY AND ANTI-TUMOR ACTIVITY

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Abstracts

Background SO-C101 is a superagonist fusion protein of interleukin (IL)-15 and the IL-15 receptor α (IL-15Rα) sushi+ domain. SO-C101 effectively stimulates natural killer (NK) cells and memory CD8+ T cells with no significant expansion and activation of regulatory T cell compartment.

Methods Human NK cell proliferation, the expression of NK cell receptors and ADCC activity of human PBMC after stimulation with SO-C101 in vitro in combination with monoclonal antibodies were detected by flow cytometry. The anti-tumor efficacy of SO-C101 in combination with Daratumumab was assessed in a multiple myeloma SCID xenograft mouse model in vivo.

Results In this study, we show that SO-C101 induced proliferation and expansion of both major subsets of human NK cells, CD56brightCD16- and CD56dimCD16+. Furthermore, SO-C101 induced expression of the cytotoxic receptors NKp30 and NKG2D whereas no upregulation of the inhibitory receptors CD158a, CD158b and NKG2A was detected. Both NK cell subsets activated by SO-C101 exhibited cytotoxicity towards cancer cells in vitro. Using human PBMCs, we show that SO-C101 potentiated killing of tumor cells induced by several clinically approved therapeutic monoclonal antibodies such as Cetuximab, Daratumumab and Obinutuzumab in vitro. SO-C101 and Daratumumab monotherapy treatment inhibited tumor growth in vivo, however, their combination showed the strongest anti-tumor efficacy. Specifically, sequential administration of Daratumumab, followed by SO-C101 promoted complete tumor regression, compared to partial anti-tumor responses induced by the respective monotherapies.

Conclusions SO-C101 augments the anti-tumor activity of therapeutic antibodies by increasing NK cells mediated antibody-dependent cell cytotoxicity. These results support the evaluation of SO-C101 in combination with monoclonal therapeutic antibodies in clinical studies.

Ethics Approval The anti-tumor efficacy studies in mice were approved by the internal ethics board of the respective contract research organization (CRO).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0563
Background Interleukin-12 (IL12) is a proinflammatory cytokine produced by activated antigen-presenting cells that induces differentiation of Th1 cells and increased proliferation and cytotoxicity of T and NK cells. Stimulation of these cells by IL12 leads to production of high levels of IFNy. These immune-stimulating aspects of IL12 may help to establish an inflammatory tumor microenvironment critical for anti-tumor responses. Preclinical studies in mice revealed that native IL12 can dramatically shrink syngeneic tumors, however clinical studies in humans resulted in severe toxicity and a small therapeutic window, limiting response rates. Prior work at Xencor demonstrated that reduced-potency IL15/IL15Rα-Fc fusion proteins exhibited superior pharmacokinetics, pharmacodynamics, and safety in non-human primates through reduction of receptor-mediated clearance. Applying similar principles to IL12, we created IL12 heterodimeric Fc-fusions (IL12-Fc) with reduced potency to improve tolerability, slow receptor-mediated clearance, and extend half-life.

Methods IL12 is a heterodimeric protein consisting of two subunits, so we engineered IL12-Fc-fusions by fusing the IL12p35 subunit to one side of a heterodimer (inactive) Fc domain, and the IL12p40 subunit to the other side. These Fc-fusions were tuned for optimal activity by introducing amino acid substitutions at putative receptor-interface positions and screening for reductions of in vitro potency. In vitro activity was assessed on human PBMCs by measuring signaling in a STAT4 phosphorylation assay and IFNy production in a mixed-lymphocyte reaction (MLR). In vivo anti-tumor activity was assessed by engraving MCF-7 cells into PBMC engrafted NSG MHC class I and II double-knockout mice and by measuring tumor volume, lymphocyte activation/proliferation, and IFNy production over time.

Results IL12-Fc were produced with good yield and purity. An IL12-Fc potency series was created, and variants had up to a 10,000-fold reduction in STAT4 signaling potency and IFNy production in an MLR assay compared to native IL12-Fc. Anti-tumor activity in the huPBMC-MCF7 model was achieved with potency-reduced IL12-Fc as a single-agent and in combination with anti-PD1, with weaker variants maintaining anti-tumor activity at higher dose levels. Analysis of peripheral lymphocytes indicated increased numbers of T and NK cells as well as activation of CD8+ T cells, as evidenced by upregulation of CD25. Increased expression of immune checkpoints including PD1 was also observed. Analysis of serum indicated up to 200-fold increases in IFNy levels.

Conclusions Combined, these data indicate that potency-reduced IL12-Fc retain strong anti-tumor activity, while potentially overcoming safety and tolerability issues related to small therapeutic index associated with recombinant native IL12 or IL12-Fc agents.

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Abstracts
564 POTENCY-REDUCED AND EXTENDED HALF-LIFE IL12 HETERODIMERIC FC-FUSIONS EXHIBIT STRONG ANTI-TUMOR ACTIVITY WITH POTENTIALLY IMPROVED THERAPEUTIC INDEX COMPARED TO NATIVE IL12 AGENTS
Matthew Bennett*, Rajat Varma, Ke Liu, Christine Bonzon, Rumana Rashid, Nicole Rodriguez, Nargess Hassanazadeh-Kiaab, Connie Arilda, Seung Chu, Umesh Muchhal, John Desjarlais. Xencor, Inc., MONROVIA, CA, USA

Methods Immunocompetent C57BL/6 mice bearing two intracranial glioma models (GL261 and CT2A) were treated with RT (1.8 Gy/day x 5 days), TMZ (33 mg/kg/day x 5 days) and/or NT-I7 (10 mg/kg on the final day of RT completion). We profiled the CD3, CD8, CD4, FOXP3 cells in peripheral blood over time. We also immunoprofiled cervical lymph nodes, bone marrow, thymus, spleen, and the tumor 6 days after NT-I7 treatment. Survival was monitored daily.

Results Median survival in mice treated with NT-I7 combined with RT was significantly longer than RT alone (GL261: 40d vs 34d, p<0.0021; CT2A: 90d vs 40d, p<0.0499) or NT-I7 alone (GL261: 40d vs 24d, p<0.0008; CT2A: 90d vs 32d, p<0.0154). NT-I7 with RT was just as effective as NT-I7 combined with RT and TMZ in both GL261 (40d vs 47d) and CT2A (90d vs 90d). Cytotoxic CD8+ T cells were increased in both peripheral blood (0.66 x 10^5 to 3.34 x 10^5; P<0.0001) and tumor (0.53 x 10^3 to 1.83 x 10^3; P<0.0001) in mice treated with NT-I7 when compared to control. Similarly, NT-I7 in combination with RT increased the CD8+ T cells in peripheral blood (0.658 x 10^5 to 1.839 x 10^5 P<0.0001) when compared to RT alone. There were decreases in tumor infiltrating FOXP3+ T-reg cells in mice treated with NT-I7 when compared to control. Similarly, NT-I7 in combination with RT increased the CD8+ T cells in peripheral blood (1.9 x 10^4 to 7.5 x 10^4 P<0.0001) and NT-I7 + RT (1.9 x 10^4 to 3.59 x 10^4 P<0.0001) when compared to the control group without NT-I7. In addition, NT- I7 treatment increased CD8+ T cells in thymus, spleen, and lymph nodes.

Conclusions NT-I7 enhances cytotoxic CD8+ T lymphocytes systemically and in the tumor microenvironment, and improves survival. A phase I/II trial to evaluate NT-I7 in patients with high-grade gliomas is ongoing (NCT03687957).

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Abstracts
565 A NOVEL LONG-ACTING INTERLEUKIN-7 AGONIST, NT-I7, INCREASES CYTOTOXIC CD8+ T CELLS AND ENHANCES SURVIVAL IN MOUSE GLIOMA MODELS
1Subhajit Ghosh, 2Ran Yan, 3Sukrutha Thotala, 1Arvijita Jash, 1Anita Mahadevan, 1Tong Hu, 2Byung Lee, 2Se Hwan Yang, 2Dennis Hallahan, 2Milan Chheda, 2Dinesh Thotala, 1Jian Campian*, 1Washington University School of Medicine, Saint Louis, MO, USA; 2NeOImmuneTech, Inc., Rockville, MD, USA

Background Radiation (RT) and temozolomide (TMZ), which are standard of care for patients with glioblastoma (GBM), can cause prolonged severe lymphopenia. Lymphopenia, in turn, is an independent risk factor for shorter survival. Interleukin-7 (IL-7) is a cytokine that is required for T cell homeostasis and proliferation. IL-7 levels are inappropriately low in GBM patients with lymphopenia. NT-I7 (efinptakin alfa) is a long-acting recombinant human IL-7 that supports the proliferation and survival CD4+ and CD8+ cells in both human and mice. We tested whether NT-I7 rescues treatment-induced lymphopenia and improves survival.

Methods Immunocompetent C57BL/6 mice bearing two intracranial glioma models (GL261 and CT2A) were treated with RT (1.8 Gy/day x 5 days), TMZ (33 mg/kg/day x 5 days) and/or NT-I7 (10 mg/kg on the final day of RT completion). We profiled the CD3, CD8, CD4, FOXP3 cells in peripheral blood over time. We also immunoprofiled cervical lymph nodes, bone marrow, thymus, spleen, and the tumor 6 days after NT-I7 treatment. Survival was monitored daily.

Results Median survival in mice treated with NT-I7 combined with RT was significantly longer than RT alone (GL261: 40d vs 34d, p<0.0021; CT2A: 90d vs 40d, p<0.0499) or NT-I7 alone (GL261: 40d vs 24d, p<0.008; CT2A: 90d vs 32d, p<0.0154). NT-I7 with RT was just as effective as NT-I7 combined with RT and TMZ in both GL261 (40d vs 47d) and CT2A (90d vs 90d). Cytotoxic CD8+ T cells were increased in both peripheral blood (0.66 x 10^5 to 3.34 x 10^5; P<0.0001) and tumor (0.53 x 10^3 to 1.83 x 10^3; P<0.0001) in mice treated with NT-I7 when compared to control. Similarly, NT-I7 in combination with RT increased the CD8+ T cells in peripheral blood (0.658 x 10^5 to 1.839 x 10^5 P<0.0001) when compared to RT alone. There were decreases in tumor infiltrating FOXP3+ T-reg cells in mice treated with NT-I7 when compared to control. Similarly, NT-I7 in combination with RT increased the CD8+ T cells in peripheral blood (1.9 x 10^4 to 7.5 x 10^4 P<0.0001) and NT-I7 + RT (1.9 x 10^4 to 3.59 x 10^4 P<0.0001) when compared to the control group without NT-I7. In addition, NT-I7 treatment increased CD8+ T cells in thymus, spleen, and lymph nodes.

Conclusions NT-I7 enhances cytotoxic CD8+ T lymphocytes systemically and in the tumor microenvironment, and improves survival. A phase I/II trial to evaluate NT-I7 in patients with high-grade gliomas is ongoing (NCT03687957).

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Abstracts
566 MDK-202: AN EMPIRICALLY-DESIGNED PEPTIDYL AGONIST OF THE IL-2/15/Ra RECEPTOR, DEVOID OF Ra INTERACTION, UNRELATED TO IL-2 OR IL-15, AND FUSED TO AN FC-DOMAIN FOR PK ENHANCEMENT
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**Background** Efforts to modify IL-2 for immuno-oncology applications focus on modifying the receptor selectivity of IL-2 to bias effects on immune cells; in particular, to reduce Rα interaction via mutation, chemical modification, complexation with antibodies, or fusion to the Rα-ectodomain. IL-2/15Rβγc-biased agonists also incorporate PK enhancement to extend duration of action, and reduce side effects associated with peak drug levels. We previously reported discovery of small synthetic peptides, unrelated to IL-2 or IL-15, that simultaneously bind IL-2Rβ and γc subunits to induce IL-2/15R signaling. These peptides do not bind IL-2Rα, and are therefore IL-2/15Rβγc-selective agonists with MW less than 5000D. We now describe properties of an IL-2/15Rβγc agonist peptide fused to an Fc-domain (MDK-202).

**Methods** Peptides were selected from recombinant peptide libraries to identify molecules binding simultaneously to the β and γc subunits of IL-2/15R. Active peptides were fused to Fc-domains to evaluate efficacy, potency, and quality of signaling upon activating IL-2/15Rβγc in cell lines and human lymphocytes. PK and PD properties in mice and NHP were also determined.

**Results** MDK-202 exhibits in vitro potency similar to the synthetic peptide (MDK1169). MDK-202 does not bind IL-2Rα, activates the major IL-2/15R signaling pathways: JAK-STAT(pSTAT5), MAPK (pERK1/2), PI3K (pAKT), and induces proliferation (Ki-67) in human PBMCs, with kinetics and efficacy similar to IL-2. Agonism is attributable to direct activation of IL-2/15Rβγc as shown by dependence on Rβ expression in test cells, and insensitivity to blockade by neutralizing antibodies against IL-2 and IL-15. At concentrations greatly exceeding that required for maximum IL-2/15R activation in vitro, MDK-202 does not interfere with the activities of other γc family receptors. The predicted immunogenicity potential for MDK-202 is very low, and in the unlikely event of MDK-202-induced ADA, neutralization of endogenous IL-2 or IL-15 would not be expected. MDK-202 is highly stable in human serum, showing no significant degradation after 4 days at 37°C. In ex vivo human PBMC and in vivo studies in normal mice, hPBMC-engrafted NCG mice, and non-human primates, MDK-202 exhibited extended half-life, and activation, proliferation, and population dynamics of lymphocytes similar to those induced by ‘non-Rα’ variants of IL-2.

**Conclusions** MDK-202 is an attractive alternative to IL-2/15 variants for use in immuno-oncology therapy. Constructed without reference or similarity to cytokine or receptor structures or contacts, the peptidyl agonist component (MDK1169) is completely unique, and shown to be active when fused to other proteins such as anti-PD-1 antibodies and other cytokine receptor agonists.

**Ethics Approval** Animal studies were performed by Charles Rivers Laboratories, as approved by the CRL Institution Ethics Board with the following study and approval numbers: CRL-220007; 20222440 : PK Cynomolgus Monkeys: BA-e451;BA-e451: PD NCG mice BA-e411; BA-e411:PD NCG miceKey 2152; US19001: PK mice: The use of human PBMC in this study was authorized under Minimal Risk Research Related Activities at Stanford Blood Center (SQL 79075).

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Abstracts

568 XTX201, A PROTEIN-ENGINEERED IL-2, EXHIBITS TUMOR-SELECTIVE ACTIVITY IN MICE WITHOUT PERIPHERAL TOXICITIES IN NON-HUMAN PRIMATES


Background High-dose recombinant human interleukin-2 (aldesleukin) elicits durable anti-tumor immunity and gained FDA approval two decades prior to checkpoint blockers. However, use of aldesleukin is limited due to treatment-related life-threatening toxicities. Second generation efforts to alleviate these toxicities and improve the therapeutic index of IL-2, while retaining CD8+ T and NK cell activation.

Methods XTX201 binding interactions were measured with SPR, and bioactivity was measured using STAT-5 phosphorylation in human PBMCs and reporter cell lines. Anti-tumor efficacy and immune activation was evaluated in tumors compared to peripheral organs in syngeneic tumor mouse models. This approach may enhance tumor localization of anti-tumor activity of aldesleukin while minimizing dose-limiting toxicities.

Conclusions Our data demonstrate that 2nd generation IL-2s that are systemically active and lack binding to IL-2Rα exhibit dose-limiting toxicities unless further engineered for selective activity in tumors. XTX201, a 3rd generation, tumor-selective IL-2, exhibits a long half-life and is innocuous outside of tumors. XTX201 is activated within tumors to release an IL-2Rβ/γ biased cytokine that inhibits tumor growth in syngeneic models, and exhibits tumor-specific pharmacodynamic effects without peripheral toxicities. XTX201 has the potential to be a best-in-class IL-2 immunotherapy by expanding the curative anti-tumor activity of aldesleukin while minimizing dose-limiting toxicities.

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569 TARGETING IL-15 DELIVERY TO PD-L1 EXPRESSING TUMORS WITH AN ANTI-PD-L1-IL-15 CYTOKINE FUSION IGM TO ENHANCE T CELL AND NK CELL MEDIATED TUMOR CYTOTOXICITY

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Background Therapeutic antibodies inhibiting PD-1/PD-L1 have demonstrated clinical efficacy though only a fraction of patients respond. Combinations are being explored to enhance responses including anti-PD-1/PD-L1 IgG antibodies with IL-15-pathway stimulating agents to remove PD-1 immunosuppressive signaling and enhance anti-tumor NK and memory CD8 T cell expansion and survival. We have engineered an anti-PD-L1 pentamer high affinity, high avidity IgM, to target low PD-L1 expressing tumors, with an IL-15 superagonist fused to the joining (J) chain.

Methods An anti-PD-L1 IgM was generated by grafting heavy chain variable regions of a high affinity IgG onto the IgM heavy chain framework and co-expressed with the light chains. The IL-15 superagonist fused to the J chain generated PDL1-ISA. Anti-PD-L1 binding was performed using recombinant antigen ELISAs and on cells by FACS. Reporter assays and PBMCs were used for potency testing. Cytokines were evaluated by CBA assays. In vitro cytotoxicity assays used luciferase tagged MDA-MB-231 cells with PBMCs, NK or CD8 T cells. Pharmacodynamic and efficacy studies were conducted in syngeneic and humanized mouse models.

Results The parental anti-PD-L1 IgM antibody bound recombinant and cellular PD-L1 more potently than an IgG antibody with the same binding domain. In functional PD-1 and PD-L1 blocking studies the anti-PD-L1 IgM was as efficacious as the IgG. PDL1-ISA provided a potent proliferation signal to primary human NK and CD8 T cells in vitro with little/no impact on regulatory or CD4 T cells. Limited cytokines were detected following 3–4 days culture with human PBMCs. PDL1-ISA had similar potencies for both human and cynomolgus CD8 T cells, and a 2–3-fold lower potency for mouse cells. Pharmacodynamic studies in humanized and BALB/c mice showed transient and dose-dependent increases in circulating NK and CD8 T cells. PDL1-ISA enhanced in vitro killing of PD-L1 positive MDA-MB-231-Luc cells by human PBMCs, CD8 T and NK cells compared to the anti-PD-L1 IgM (no IL-15). PDL1-ISA also demonstrated efficacy in a hPD-L1-CT26 HuCELL mouse model, with most treated animals having complete tumor regressions. Durable anti-tumor immune memory responses were observed upon tumor re-challenge.

Conclusions We have engineered an IL-15 immunostimulatory anti-PD-L1 IgM antibody that binds PD-L1 more potently than an IgG, stimulates NK and CD8 expansion in vitro and in vivo and induces complete tumor regressions in mouse models. This approach may enhance tumor localization of

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immunostimulatory cytokine IL-15 though the high affinity and high avidity binding to PD-L1 to improve anti-tumor responses and minimize toxicity.

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**570 IL-15/IL-15Rα HETERODIMERIC COMPLEX AS CANCER IMMUNOTHERAPY IN MURINE BREAST CANCER MODELS**

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**Background** Interleukin 15 (IL-15) has been evaluated as a potential treatment for solid tumors in clinical trials, but the effectiveness of systemic IL-15 administration as a monotherapy has not been realized. IL-15 receptor alpha (IL-15Rα) can stabilize IL-15 and enhance its bioactivity. The goal of this study was to examine the activity of IL-15/IL-15Rα complex (IL-15cx) to CD8+ T cells and evaluate its potential efficacy in murine breast cancer models.

**Methods** The bioactivity of IL-15cx to CD8 T cells was assessed by ex vivo and in vivo cell proliferation assays. The antitumor efficacy was studied in mice mammary carcinoma models (Her2/neu transgenic and 4T1-luc mammary cancers) treated with systemic recombinant protein with/without the depletion of myeloid-derived suppressor cells or intratumoral gene electrotransfer (GET). Systemic and regional changes of immune cells were examined by flow cytometry, and tumor specific IFN-γ release from immune cells was measured by ELISA assays.

**Results** IL-15cx shows superior in vivo bioactivity to expand CD8 T cells in comparison to an equimolar single chain IL-15. T-bet is partially involved in CD8 T cell expansion ex vivo and in vivo due to IL-15 or IL-15cx. Intraperitoneal administration of IL-15cx results in a moderate inhibition of breast cancer growth that is associated with an increase in the frequency of cytotoxic CD8 T cells and the improvement of their function. The depletion of myeloid-derived suppressor cells (MDSCs) has no impact on mouse breast cancer growth. IL-15cx treatment diminishes MDSCs in murine tumors. However, it also antagonizes the effects of depleting antibody. Intratumoral GET with plasmid IL-15/IL-15Rα leads to a long-term survival benefit in 4T1 mammary carcinoma model. An early increase of local cytotoxic cells correlates with GET treatment and an increase of long-term memory T cells results from animals with complete tumor regression.

**Conclusions** Systemic and local administration of IL-15cx shows two distinct therapeutic responses, a moderate tumor growth inhibition or heterogeneous tumor regressions with survival improvement. Further studies are warranted to improve the efficacy of IL-15cx as an immunotherapy for breast cancer.

**Acknowledgements** This work was supported by funding from the National Cancer Institute grant R21 CA229939 to S. Guo and funding from the Thomas F. and Kate Miller Jeffress Memorial Trust to R. B. Smeltz.

**Trial Registration** N/A

**Ethics Approval** Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Old Dominion University (S. Guo) and by the IACUC at Virginia Commonwealth University (R.B. Smeltz).

**REFERENCES**

N/A

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**571 ANV419 IS A NOVEL CD122-SELECTIVE IL-2/ANTI-IL-2 ANTIBODY FUSION PROTEIN WITH POTENT CD8 T CELL AND NK CELL STIMULATORY FUNCTION IN VITRO AND IN VIVO**

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**Background** ANV419 is a uniquely engineered IL-2 fusion to an antibody selectively blocking the IL-2 receptor alpha (CD25) binding site. It signals selectively through the CD122/CD132 dimeric IL-2 receptor and stimulates the proliferation of CD8 T cells and NK cells while avoiding the proliferation of immunosuppressive regulatory T cells (Treg). Therefore, ANV419 has the potential to substantially separate targeted T-cell and NK cell proliferation and anti-tumor responses from the dose limiting toxicities of recombinant IL-2 (aldesleukin). ANV419 has antibody like stability and behavior and is currently in late preclinical development for tumor immunotherapy.

**Methods** The crystal structure of ANV419 has been solved and its binding affinity to CD25 and CD122 has been determined. In vitro and in vivo studies, including pharmacodynamics and toxicity, have been performed in rodents and non-human primates. The ability of ANV419 to inhibit tumor growth has been studied in mouse syngeneic models.

**Results** Structural analysis demonstrates that the CD25 binding site of IL-2 is completely blocked in ANV419 while the CD122 binding site. ANV419 lacks CD25 binding activity but retains IL-2 receptor (CD122) affinity comparable to native IL-2. In human peripheral blood monocyte cultures, ANV419 induces STAT5 phosphorylation with high selectivity for CD8 and NK cells but not Treg. Concordantly, it stimulates the proliferation of purified human CD8 T cells and NK cells but not CTLL-2 cells. A single injection of ANV419 in mice results in strong induction of the proliferation marker Ki67 specifically in CD8 T cells and NK cells but not Tregs and a selective increase of the respective cell numbers in the spleen and peripheral blood of animals. Single agent anti-tumor activity was observed in checkpoint sensitive (H22) and resistant (Renca, B16F10) syngeneic mouse tumor models. Combination of ANV419 with trastuzumab in the gastric cancer N87 xenograft model in BALB/c nude mice led to significant tumor reduction relative to trastuzumab monotherapy. In non-human primates, ANV419 is well tolerated and induces expression of Ki67 and sustained expansion in CD8 T cells and NK cells with no signs of vascular leak syndrome observed with high dose aldesleukin in patients.

**Conclusions** The pre-clinical data suggest that ANV419 possesses a unique structure and is potent in expanding CD8 T-cells and NK cells with a marked safety window in non-human primates. This data warrants further translational development of ANV419 as an immune therapeutic in oncology.

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FIBROBLAST ACTIVATING PROTEIN (FAP)-TARGETING IL-12 (ANTI-FAP/IL-12) TMEKINE™ POTENTIATES ANTI-CANCER EFFECTS IN PRECLINICAL CANCER MODELS

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Background Although cancer immunotherapy showed promising results in hematological malignancies, it has come up with relatively low tumor response for many solid tumors partly due to immune-suppressive tumor microenvironment (TME). Because of the immune-suppressive nature of TME, TME has been an active area of research and therapeutic target for restoring immune system and subsequent tumor growth inhibition. Among the many components in TME, cancer-associated fibroblasts (CAFs) are one of the key cell components of TME where one of the promising solid-tumor TME marker, fibroblast-activating protein (FAP) is highly expressed. Here we have developed an antibody-cytokine fusion protein from our TMEkine™ platform containing anti-FAP and IL-12. Our TMEkine™ (anti-FAP-IL-12) molecule induced strong anti-cancer effects in preclinical solid tumor models by immune-modulation.

Methods IL-12 cytokine was mutated in TMEkine™ (anti-FAP-IL-12) to reduce systemic toxicity and its binding affinity was tested to FAP and IL-12 receptor. The anti-tumor activity of anti-FAP-IL-12 was investigated on CT26 (murine colorectal cancer) syngeneic mouse models with/without NIH-3T3 (murine fibroblast). Additionally, mice showing complete response after anti-FAP-IL-12 administration were re-injected CT26 with/without 4T1 cells for re-challenge study to monitor long-term durable response generated from the initial immune activation.

Results We showed that TMEkine™ (anti-FAP-IL-12) interacts with FAP and IL-12 receptor. IL-12 activity was attenuated by our IL-12 mutants. We also showed that TMEkine™ (anti-FAP-IL-12) induced IFN-γ from primary human T cells and NK cells. TMEkine™ (anti-FAP-IL-12) administration resulted in significant reduction of the tumor burden in both CT26 + NIH-3T3/FAP+ and CT26/FAP+ models. In the re-challenge experiments, CT26 tumor growth was inhibited significantly compared to 4T1 tumor suggesting memory immune response was generated in TMEkine™ (anti-FAP-IL-12) treated mice.

Conclusions These findings provide evidences that the treatment of anti-FAP-IL-12 TMEkine™ induced anti-cancer effects without serious adverse effects. Anti-FAP-IL-12 has a strong potential to provide a therapeutic option for cancer-specific immunomodulator and cancer cell eradication.

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A NOVEL HUMAN ANTI-PD1/IL15 BI-FUNCTIONAL PROTEIN WITH ROBUST ANTI-TUMOR ACTIVITY AND LOW SYSTEMIC TOXICITY

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Background IL-15 is a key cytokine promoting CD8+ T, NK, and NKT cell proliferation and has demonstrated clinical activity in cancer patients without evidence of any Treg stimulation. However, its short half-life and systemic toxicity limit its clinical utility. Kadmon has established an IL-15 fusion protein platform to extend the IL-15 serum half-life and direct its action to the tumor microenvironment. A major asset of this platform is anti-PD1/IL15 bifunctional protein. To test the bifunctionality hypothesis of this fusion protein in murine models, four different formats of the surrogate bi-functional proteins were engineered by fusing mouse IL-15 to a mouse-human chimeric anti-mouse PD1 antibody (m3A7). We presented earlier that the single IL-15 N-terminal fusion to anti-PD1 antibody (1N-IL-15/m3A7) showed significantly stronger anti-tumor activity in vivo mainly due to the cis-presentation to the PD1 and IL2Rβγ co-expressed on TILs. The cis-presentation potentially maximizes the bi-functionality of PD1 blockade and IL-15 stimulation.

Methods Purified 1N-IL-15/38B2 and 65S-1N-IL-15/38B2 were generated and characterized in vitro. The anti-tumor activities were examined in the human-PD-1/PD-L1 transgenic BALB/c mice subcutaneously transplanted with the human-PD-L1 positive CT26 colon carcinoma. The treatment was started when tumors reached 100 mm³ (IP, QW).

Results All 1N-IL-15/anti-PD1 fusions showed similar potencies in binding to the soluble and cell expressed human PD1 and blocking the hPD-L1 binding to hPD1. Comparing to wild-type 1N-IL-15/38B2, mutated 65S-1N-IL-15/38B2 showed lower stimulation (>150 folds) in the M07e, CTL2L2, mouse spleen cells and hPBMC (mainly CD8+ T cell) proliferation. When we treated hPD1L1-CT26 tumor transplanted hPD1L1-hPD1 transgenic mice with 65S-1N-IL-15/38B2 at 6 mg/kg, 80% of tumor growth inhibition (TGI) was achieved with no body-weight loss. Although wild-type 1N-IL-15/38B2 at 3 mg/kg demonstrated similar efficacy, a significant mouse body-weight loss was observed and 1/3 mice died after second injection. No anti-tumor activity was observed for 65S-1N-IL-15 non-target fusion in 6 mg/kg.

Conclusions The previous observation of robust anti-tumor activity of surrogate 1N-IL-15/m3A7 in PD1 resistant LL2 model was replicated with the therapeutic bifunctional protein in this study. We also found that lower stimulation 65S-1N-IL-15/38B2 showed strong anti-tumor activity with significant low systemic toxicity; suggesting that the 65S mutation increased the therapeutic window of this bi-functional protein.

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**Abstracts**

574 ONM-400, A NOVEL APPROACH FOR INTERLEUKIN-2 THERAPY USING A PH-ACTIVATED NANOPARTICLE TARGETING METABOLIC ACIDOSIS IN SOLID CANCERS

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**Background** Interleukin-2 (IL-2) is a potent immunotherapy for treatment of metastatic melanoma and renal cell cancers. However, the clinical application has been hindered by immunosuppressive stimulation and unfavorable pharmacological properties that can induce life-threatening toxicities. Although strategies including ‘no-alpha’ muteins have been developed to provide target specificity at the molecular level, little has been done to improve tumor specificity and accumulation at tissue level. We developed ONM-400, a novel IL-2 encapsulating pH-activated nanoparticle that targets metabolic acidosis of cancer to improve the therapeutic index of IL-2 therapy. During circulation, IL-2 activity is sequestered within the nanoparticles. Upon entering the tumor microenvironment, IL-2 release is precisely and instantly triggered by acidic tumor pH, resulting in selective deposition of active IL-2 at the site of disease.

**Methods** A tumor-agnostic pH-activated nanoparticle with pH responsiveness similar to ONM-100, a cancer imaging agent currently in a Phase 2 clinical trial, has been developed for cytokine delivery. IL-2 was encapsulated within the nanoparticle using a proprietary method to produce ONM-400 and the physical properties were characterized. Activity of IL-2 in ONM-400 was evaluated using a bioluminescent cell-based assay for both its encapsulated (inactive) state and activated format. Tumor accumulation and biodistribution following intravenous injection (I.V.) of ONM-400 were evaluated in mice bearing head and neck tumors using fluorescent imaging. In vivo antitumor efficacy of ONM-400 after I.V. injection was studied in MC38 colon cancer-bearing mice and compared with unencapsulated IL-2 at the same dose.

**Results** Quantitative analysis shows high encapsulation efficiency and drug loading density of IL-2 in ONM-400. At neutral pH, IL-2 bioactivity is effectively sequestered in ONM-400 through encapsulation which avoids IL-2 toxicity in normal tissue. Upon acid-triggered release, IL-2 bioactivity is rescued without compromise compared to unencapsulated IL-2 control. Significantly higher tumor accumulation and lower renal elimination were observed with ONM-400 in biodistribution studies as compared to free IL-2 control suggesting an alteration of pharmacokinetics of IL-2 after encapsulation. ONM-400 induced strong antitumor efficacy as a monotherapy in MC38 colon cancer-bearing mice (figure 1). After ONM-400 treatment 60% of the animals showed complete tumor regression and remained tumor free 60 days. Following a secondary MC38 challenge, 5/6 animals resisted tumor growth.

**Conclusions** Tumor acidosis-driven accumulation and activation of ONM-400 provide a high local concentration of IL-2 within tumors resulting in strong antitumor response as a monotherapy. Tumor metabolic targeting pH-activatable nanoparticles provides a novel strategy to deliver immunomodulators for cancer treatment.

**Ethics Approval** All animal experiments were reviewed and approved, and performed in accordance with, by Pennsylvania State College of Medicine Institutional Animal Care and Use Committee under Animal Protocol Number: 47682.

**REFERENCES**


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575 REGRESSION BY HETIL-15 MONOTHERAPY IN DIFFERENT MOUSE BREAST CANCER MODELS CORRELATES WITH INTRATUMORAL INFILTRATION OF A NOVEL POPULATION OF DENDRITIC CELLS

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**Background** IL-15 is a cytokine which stimulates the proliferation and cytokine function of CD8+ T and NK cells. We have produced and applied the native heterodimeric IL-15 (hetIL-15) on several preclinical models, which have supported the anti-tumor activity of hetIL-15. Based on these results, hetIL-15 has advanced to clinical trials. The objectives of this study were to explore how hetIL-15 shapes the tumor microenvironment and to characterize the interactions between tumor-infiltrating lymphoid and myeloid cells.

**Methods** We studied the efficacy of locoregional administration of heterodimeric IL-15 (hetIL-15) in two different orthotopic triple-negative breast cancer (TNBC) mouse models, syngeneic for C57BL/6 and Balb/c, respectively. The effects of hetIL-15 on immune cells were analyzed by flow cytometry, immuno-histochemistry (IHC) and gene expression profiling. The profile of the novel infiltrated dendritic cell populations was further explored by bulk and single cell RNAseq.

**Results** hetIL-15 resulted in tumor eradication in 40% of treated mice and reduction of metastasis. Subsequent challenges with the same cell line failed to generate tumor

Abstract 574 Figure 1 Anti-tumor efficacy of ONM-400

ONM-400 induces strong antitumor response in MC38 tumor bearing mice as a monotherapy. Mice received I.V. injections of PBS, 50 ug of rhIL-2 or ONM-400 with 50 ug of encapsulated rhIL-2 on Day 0, 2, 4 and 6. A-C, individual tumor growth curve of animals treated with PBS (A), rhIL-2 (B) or ONM-400 (C); CR = complete response. D. Kaplan-Meier curves of animals after the treatment. Statistical significance was analyzed by Log-rank test. **P<0.01


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regrowth, suggesting the development of immunological mem-
ory in hetIL-15 treated mice. hetIL-15 promoted tumor accu-
mulation of proliferating and cytotoxic CD8+ T and NK cells. Additionally, peritumoral hetIL-15 administration resulted in an increased tumor infiltration of both conventional type 1 dendritic cells (cDC1s) and of a novel DC population found only in the hetIL-15 treated animals. Phenotypic profile analy-
sis confirmed the expression of several cDC1 specific markers, including CD103 and IRF8 on this DC population. Transcriptomics and flow analysis of intratumoral dendritic cells indicate that the new hetIL-15 induced cells reside preferentially in the tumors and are distinct from cDC1 and cDC2 populations. Both cDC1s and the novel DC population were inversely correlated with the tumor size.

Conclusions Locominal administration of hetIL-15 results in complete eradication of EO771 and significant reduction of 4T1 primary breast cancer tumors, prolonged survival and long-lasting specific anti-tumor immunity. hetIL-15 increases the tumor infiltration of activated T and NK cells and intensifies the tumor infiltration of conventional type 1 dendritic cells (cDC1) and a new population of dendritic cells. We propose that the anti-cancer activity of hetIL-15 in primary EO771 tumors is orchestrated by the interplay of NK, CD8 +T cells, cDC1 and a novel subset of DCs with a distinct phenotypic profile. These findings suggest a role for hetIL-15 in the treatment of breast cancer.

Ethics Approval The study was approved by the National Cancer Institute-Frederick Animal Care and Use Committee, approval number 19–324 and was conducted in accordance with the ACUC guidelines and the NIH Guide for the Care and Use of Laboratory Animals.

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**576**  
**NL-201, A DE NOVO IL-2 AND IL-15 AGONIST, DEMONSTRATES ENHANCED IN VIVO ANTITUMOR ACTIVITY IN COMBINATION WITH MULTIPLE CANCER IMMUNOTHERAPIES**

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Background NL-201 is a de novo IL-2 and IL-15 receptor agonist designed with enhanced affinity for IL-2Rβγ and no binding interface for IL-2Rα (CD25). Previously, we reported that NL-201 stimulates selective proliferation of CD8+ effector T cells and NK cells, leading to increased CD8:Treg and NK:Treg ratios in the tumor microenvironment. As a result, NL-201 treatment led to robust single-agent antitumor activity in syngeneic murine tumor models at well-tolerated doses.

Methods Here, we evaluated the antitumor activity of NL-201 in combination with established and exploratory cancer immunotherapies, including tumor-targeting monoclonal antibodies and immune checkpoint inhibitors (CPIs). Specifically, we evaluated NL-201 in combination with an anti-gp75 antibody (TA99) in a murine melanoma model, or anti-PD-1 and anti-

PD-L1 antibodies in a CPI-resistant murine colon cancer model.

Results NL-201 synergizes with TA99, anti-PD-1, and anti-

PD-L1 to inhibit tumor growth more effectively than either agent alone. The synergy of NL-201 with TA99 may result from enhanced NK-mediated antibody-dependent cellular cytotoxicity (ADCC), while the synergy with CPIs may result from CD8+ T cell stimulation, which can turn ‘cold’ tumor micro-

environments ‘hot’.

Conclusions The broad activity of NL-201 across diverse tumor models and its potential to be combined with a variety of established and exploratory cancer immunotherapies to achieve synergistic antitumor activity highlights the opportu-

nity for NL-201 to become a critical component of future immunotherapy regimens.

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**577**  
**ENGINEERED NON-PATHOGENIC SYNTHETIC BIOTIC PRODUCING L-ARGININE SYNERGIZE WITH PD-1-BASED CANCER IMMUNOTHERAPY**

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Background The availability of L-arginine in tumors is a key determinant of an efficient anti-tumor T cell response. Conse-

quently, the elevation of typically low L-arginine levels within the tumor may greatly potentiate the anti-tumor responses of immune check point inhibitors, such as PD-L1 blocking anti-

bodies. However, currently no means are available to locally increase intra-tumoral L-arginine levels.

Methods We used a synthetic biology approach to develop an engineered probiotic Escherichia coli Nissle 1917 strain that

colonizes tumors and continuously converts ammonia, a meta-

bolic waste product that accumulates in tumors, into L-

arginine.

Results Colonization of tumors with these bacteria elevated intra-tumoral L-arginine concentrations, increased the amount of tumor-infiltrating T cells, and had striking synergistic effects with PD-L1 blocking antibodies in the clearance of tumors. The anti-tumor effect of the living therapeutic was mediated by L-arginine and was dependent on T cells.

Conclusions These results show that engineered microbial therapies enable metabolic modulation of the tumor microen-

vironment leading to enhanced efficacy of immunotherapies.

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**578**  
**TUMOR SELECTIVE IMMUNE RESPONSES OF STA551, A NOVEL ANTI-CD137 AGONIST ANTIBODY ACTIVATED BY EXTRACELLULAR ATP**

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Background Agonistic antibodies targeting CD137 in clinic have failed due to severe hepatotoxicity, leading to the develop-

ment of bispecific approaches that must rely on high tumor-associated antigen expression to crosslink CD137. Here we report on STA551, a novel anti-CD137 agonist antibody which binds to CD137 only in the presence of ATP. Extracellu-

lar ATP concentration is well-known to be elevated in tumor tissue while remaining tightly regulated in non-tumor tissue, suggesting that STA551 can activate immune cells only in tumor tissue and not elsewhere. Thus, STA551 has great potential to overcome the limitations of conventional CD137-targeted antibodies.
Methods We evaluated in vitro STA551’s effect on IFN-γ production from human CD8+ T cells. We also evaluated in vivo STA551’s effect on tumor growth, RNAseq-based immune-related gene expression, immunohistochemistry, and T cell activation in tumor and non-tumor tissues in human CD137 knock-in mice treated with mouse surrogate STA551 (Sta-MB) or urelumab (Ure-MB) in combination with anti-PD-L1 antibody.

Results In a human T cell assay, STA551 induced IFN-γ only in the presence of ATP. In contrast, urelumab induced IFN-γ regardless of ATP concentration. In mice with Colon 38 tumors, Sta-MB inhibited tumor growth at least as strongly as Ure-MB, but whereas Ure-MB elicited systemic immune responses in draining lymph node, spleen, and liver, Sta-MB appeared to evade such responses. To confirm immune responses in tumors, we evaluated immune-related gene expression and found changes after treatment with Sta-MB or Ure-MB. These results suggest that STA551 works only in tumor tissue. Furthermore, Sta-MB with anti-PD-L1 antibody synergistically inhibited tumor growth and dramatically changed immune-related gene expression, CD8+ T cell infiltration, and PD-L1 expression without systemic immune responses. Also, it was well-tolerated in cynomolgus monkey in a repeated-dose toxicity study.

Conclusions STA551 is a novel anti-CD137 agonist antibody that exerts agonistic activity selectively in tumors without on-target toxicity in non-tumor tissues, regardless of tumor-associated antigen expression. These results strongly support the clinical testing of STA551 for the treatment of solid tumors. STA551 is currently being tested in a phase 1 clinical study.

Ethics Approval All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

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579 LACTATE DEHYDROGENASE C-ASSOCIATED MOLECULAR NETWORKS PREDICT ENHANCED TUMOR GROWTH AND IMPAIRED IMMUNE RESPONSE IN BREAST CANCER

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Background Cancer testis antigens (CTAs) have gained interest in the field of anti-cancer therapy as they offer the opportunity to target tumor cells with little off/on-target side effects given their restricted expression patterns. Several CTAs have been implicated as mediators of cancer hallmarks including cancer metabolism, proliferation, survival, and cell motility. Lactate dehydrogenase C (LDHC) expression has been observed in various cancer types and likely confers a survival advantage to tumor cells through metabolic reprogramming. Thus, targeting LDHC has the potential to inhibit tumor growth and release the anti-tumor immune response from the acidic immunosuppressive microenvironment. This study aimed to explore the changes in the transcriptome of breast cancer cells upon in vitro LDHC targeting.

Methods We silenced LDHC expression in two breast cancer cell lines (BT549, HCC1954) and investigated the downstream effects on the tumor cell transcriptome. In addition, differentially expressed genes were subjected to regulatory network analyses and expression of key regulators was interrogated in the TCGA breast cancer dataset.

Results We identified 47 up- and 55 down-regulated transcripts after LDHC silencing (2.0-fold change, adj p<0.05). Specifically, we found that LDHC expressing breast cancer cells display an enrichment of genes involved in canonical pathways regulating cell cycle checkpoint control, BRCA1-mediated DNA damage response and NF-κb signaling in response to infection, which is in line with some of our unpublished work. In support, downstream effector analysis demonstrate that LDHC silencing negatively affects biological functions such as cellular development, cellular growth and proliferation, cell migration and cell infiltration. Upstream regulator analyses revealed that the observed changes in gene expression are associated with mTOR (p=1.27e-5, z=2.242) and CASP3 (p=3.2e-4, z=2.250) mechanistic networks, which in the presence of LDHC are predicted to activate TP53, Myc, NF-KB complex, STAT1/3, PRKC, CDK2, FOXO3 and HIF-1α while inhibiting SMAD3, PTEN, ATM, IL18 and BCL2. Furthermore, causal network analysis revealed a higher-level regulation by miR378a-3p (p=1.4e-7, z=-3.117), affecting the mechanistic networks and ultimately promoting tumor cell viability and proliferation, tumor cell movement and cell cycle progression in LDHC expressing cells. Interestingly, the miR378a causal network also indicated inhibition of the immune response in LDHC positive cells. Correlation analysis using the TCGA breast cancer dataset indicated a weak correlation between LDHC expression and the mechanistic regulator mTOR (R=0.26, p=1.82e-18).

Conclusions Our findings demonstrate that therapeutic targeting of LDHC may inhibit tumor growth while releasing the anti-tumor immune response in breast cancer, and warrant further in-depth investigation.

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580 HIGH DOSE-RATE BRACHYTHERAPY OF LOCALIZED PROSTATE CANCER CONVERTS TUMORS FROM COLD TO HOT

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Background Prostate cancer is frequently cured with high dose-rate brachytherapy (HDBRT) radiation as a front-line treatment. Although considered to be an immune-excluded tissue, immune responses to radiation are implicated in driving tumour-eradication in prostate cancer.1 This has not been proven, and yet is used as the rationale for clinical trials combining radiation and immunotherapies.2 We hypothesise that there is a predictable relationship between radiation and the immune responses in prostate cancer that could be used to provide sound rationale for specific immune interventions in solid tumours that are made possible by radiation therapy.

Methods We present here new results stemming from our recently published immunoprofiling study of world-unique pre- and post-radiation tissues from 24 prostate cancer patients (figure 1A), RadBank cohort.3 These samples were assessed using immune cell multiplex IHC, gene expression profiling, digital spatial profiling (DSP) and computational analysis of cell distribution.

Results This study unequivocally revealed that high dose-rate radiation converts predominately ‘cold’ prostate tumour tissue...
to a more activated ‘hot’ state comprised of two sub-types (high and a less activated intermediate state). These changes were evident in increased tumour inflammation gene signatures and immune checkpoint expression, immune cell composition changes, and alterations in spatial interactions. However, as 20% of the patients did not respond, we also explored pre-treatment gene signatures of patient responses to radiation – identifying potential mechanisms that prime tissues to respond more favourably. Most recently, we have explored three other important facets of the immune response to HDRBT: (i) putative differential drivers of high and intermediate responses (figure 1B), (ii) TCR clonality changes (figure 1C), and (iii) the influence of clinical features (e.g. Gleason grade) and treatment (e.g. androgen deprivation) (figure 1D). Differential expression analysis has identified key molecules (e.g. CD40LG and Lck expression) which are associated with higher activation responses. TCR sequencing of pre- and post-HDRBT tissue and peripheral circulating cells is also suggestive of engagement of the adaptive immune system and the emergence of tumor-specific T cells. Finally, multivariate analysis has also revealed that higher grade tumours exhibit higher basal levels of activation and IC expression – making them less sensitive to immune activation by HDRBT.

Conclusions We have begun to resolve clear patient and clinical classifiers based on immune responses to radiation, and identified patient groups likely to benefit from immune therapy alongside radiation. Importantly, these classifications are associated with baseline gene expression profiles that may be used for pre-clinical stratification and more sophisticated treatment paradigms.

Ethics Approval All participants provided consent covering tissue research as part of a prospective tissue collection study for prostate radiobiology research, approved by the Human Research Ethics Committee at the Peter MacCallum Cancer Centre (PMCC; HREC approvals 10/68, 13/167, 18/204).

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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581 EFFECTS OF ACOUSTIC IMMUNE PRIMING WITH LOW-INTENSITY FOCUSED ULTRASOUND (LOFU) AND TRABECTEDIN ON A MURINE MODEL OF OSTEOSARCOMA

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Background Osteosarcoma is the most common primary bone tumor and has a peak incidence in adolescence. The prognosis for recurrent and metastatic disease is poor and over one-third of patients with localized disease at presentation will recur after treatment with metastases. LOFU produces non-lethal, transient mechanical and thermal stress to cause protein misfolding, endoplasmic reticulum stress, and induction of the heat shock response (refs). Trabectedin is directly tumoricidal through inhibiting transcription and DNA repair, modulates the tumor microenvironment by selectively depleting M2 macrophages, and inhibits the transcription factor heat shock factor 1 (HSF1) (refs). We hypothesized that combination therapy would synergistically intensify the unfolded protein response and heat shock response to facilitate antigen presenting cell activation and efficient presentation to cytotoxic T cells. To examine this, experiments are being conducted to investigate the effect of LOFU in combination with trabectedin and/or radiation therapy (RT) in a murine model of osteosarcoma.

Methods Palpable (<5 mm) subcutaneous K7M2 murine osteosarcoma tumors in BALB/c mice were treated with a) LOFU, b) trabectedin (intravenous (IV) or intratumoral (IT)), c) LOFU + trabectedin, and d) radiation. Tumor growth (ANOVA (Kruskal-Wallis) with Dunn’s test for multiple comparisons), pulmonary metastases (Fisher’s exact test) and survival (Kaplan-Meier) were measured and analyzed in GraphPad Prism.

Results Mean tumor volume in the combination therapy group (428 mm$^3$) was less than not-treated controls (887 mm$^3$), LOFU alone (670 mm$^3$), trabectedin alone (1218 mm$^3$), p=0.0386). Radiation therapy resulted in complete ablation of

Abstract S80 Figure 1 The effect of prostate brachytherapy on immune contexts

(A) Study of immune response in 24 patients treated with HDRBT at Peter MacCallum Cancer Center (DOI:10.1136/jitc-2020-000792). Examples of new insights including (B) molecules associated with higher activation levels (e.g. Lck and CD40LG/CD154), (C) changes in T cell receptor dominance and diversity in tissue and peripheral circulation, and (D) effects of clinical attributes on immune modulators (e.g. TGFbeta) and TIS activation states.
the tumors. None of the combination therapy mice had grossly detectable lung metastases at time of death but metastases were present in the trabectedin only (20%), LOFU only (50%), and control (50%) groups (not statistically significant).

Conclusions Combination therapy with trabectedin and LOFU yielded smaller tumor size and fewer pulmonary metastases compared to individual therapies alone.

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582 MODULATION OF TCR REPETIORE BY RADIOOTHERAPY-ACTIVATED NBTXR3 NANOPARTICLES
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Background For decades, radiotherapy (RT) has been a cornerstone of cancer treatment. Currently, approximately 50% of cancer patients will be treated with RT. Beyond the ability of RT to produce free radicals and to generate single and double-strand breaks in DNA, triggering cell death, preclinical and clinical studies have demonstrated that RT can have immunomodulatory effects. For example, RT can stimulate MHC class I expression on cancer cells, induce immunogenic cell death (ICD), and activate expression of various pro- and anti-inflammatory cytokines and adhesion molecules, allowing recruitment and activation of both innate and adaptive immune cells into the tumor. Unfortunately, RT rarely produces a sustained anti-tumor response as immune escape frequently occurs with tumor recurrence. Moreover, the so-called ‘abscopal effect’ which corresponds to reduction of metastatic burden outside the irradiated area is rarely observed after RT. Finally, the maximum dose of irradiation is limited because of toxicity to surrounding healthy tissues.

The high electron density of functionalized hafnium oxide nanoparticles (NBTXR3) allows a high probability of interaction with incoming ionizing radiation, increasing energy dose deposit within cells. We have previously reported in nonclinical studies the ability of RT-activated NBTXR3 (NBTXR3+RT) to increase cancer cell destruction as well as better control of treated tumor growth through this physical mode of action leading, compared to RT alone. Furthermore, NBTXR3+RT demonstrated clinically meaningful benefit for patients with locally advanced Soft Tissue Sarcoma compared to RT alone, in the randomized controlled phase II/III Act.in.Sarc study (NCT02379845).

Methods To explore the impact of NBTXR3+RT on the anti-tumor immune response, we used CT26 mouse colorectal cancer cells to perform a series of abscopal assays in immunocompetent mice.

Results We showed that NBTXR3+RT can generate a significant abscopal effect along with a substantial increase of CD8+ T cell infiltrates both in treated and untreated tumors, compared to RT alone. We showed that this distant effect was fully dependent on CD8+ T cells, as their depletion completely abolished the abscopal effect. To better understand how NBTXR3+RT treatment could generate this abscopal effect, we compared the TCR repertoire of treated and untreated tumors for the different conditions. This analysis revealed that NBTXR3+RT was able to broaden clonal diversity in both treated and untreated tumors, compared to RT alone.

Conclusions This indicates that NBTXR3+RT has the ability to transform the tumor into a in situ vaccine more efficiently than RT alone and could have important implications for the use of NBTXR3+RT in combination with immunotherapy.

Ethics Approval All animal experiments were carried out in compliance with French and European laws and regulations (European Directive 2010/63 EU). The local institutional animal ethics board and French Ministère de la Recherche approved all mouse experiments (permission numbers: 2016_031_4340 and 2016_129_8344).

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583 NOVEL, POTENT, AND SELECTIVE INHIBITORS OF HYPOXIA-INDUCIBLE FACTOR (HIF)-2α REVERSE PRO-TUMORIGENIC TRANSCRIPTIONAL PROGRAMMING IN CANCER, STROMAL, AND IMMUNE CELLS

Background The microenvironment of solid tumors is hypoxic and requires induction of genes associated with metabolism, growth, proliferation, and angiogenesis for cancer cells to survive and metastasize. The master transcriptional regulators of hypoxia-induced genes are the HIF proteins, consisting of three distinct oxygen-regulated α monomers (HIF-1α, -2α, and -3α). In normoxia, hydroxylation of HIF-2α allows for recognition by the pVHL E3-ubiquitin ligase complex and proteasomal degradation. Exposure to hypoxia, or VHL mutation or silencing, leads to HIF-2α stabilization, dimerization with HIF-1β/ARNT, and transcription of pro-tumorigenic gene sets in a variety of cancer and non-cancer cell types in the tumor microenvironment. In patients, overexpression of HIF is associated with poor prognosis, and both preclinical and clinical evidence suggests that inhibiting HIF-2α is an effective strategy to mitigate tumor growth, particularly in clear cell renal cell carcinoma (ccRCC), warranting further development of HIF-2α inhibitors and investigation into the role of HIF-2α in various cellular and combinatorial settings.

Methods Using a suite of assays to evaluate HIF-2α-specific effects, herein we describe pharmacological properties associated with novel, potent, and selective small-molecule inhibitors of HIF-2α.

Results Optimized compounds inhibited HIF reporter transcription and VEGF secretion. Compounds that were biochemically confirmed to bind HIF-2α also inhibited HIF-2α, but not HIF-1α, mediated gene expression. Characterization of HIF-2α inhibition was expanded to human stromal and immune cell subsets. While compounds inhibited pro-angiogenic gene sets in endothelial cells, inhibiting HIF-2α in activated hypoxic T cells did not affect proliferation or cytokine secretion, suggesting that HIF-2α inhibitors would not impede T cell functionality in tumors. In contrast, in a M2-polarized macrophage model for suppressive tumor-associated macrophages, HIF-2α drove hypoxia-induced changes in the chemokine secretome that favored granulocytic rather than lymphocytic infiltration, an effect that was effectively reversed by HIF-2α inhibition.

At the transcriptional level, mRNA-sequencing was used to define global gene sets impacted by HIF-2α inhibition in M2 macrophages. Additionally, in a set of liver, kidney, pancreatic, J Immunother Cancer 2020;8(Suppl 3):A1–A559

A349
and colon cancer lines, CRISPR/Cas9-mediated gene editing was used to differentiate the transcriptomic profile driven by HIF-2α from that of HIF-1α or HIF-3α, allowing for the derivation of a HIF-2α-specific gene signature. Cancer cell and macrophage-derived signatures were applied to publicly available datasets to investigate cancer types, other than ccRCC, in which HIF-2α may play an important pathological role.

**Conclusions** Collectively, these data support the development of our novel and selective HIF-2α inhibitors for the treatment of cancer and expand the indications that may benefit beyond ccRCC.

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**584** THERAPEUTIC VASCULAR NORMALIZATION TO PROMOTE TUMOR-ASSOCIATED TERTIARY LYMPHOID STRUCTURES

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**Background** Tertiary lymphoid structures (TLS) are non-encapsulated immune cell aggregates that form at sites of chronic inflammation, including in and around tumors. Recent studies have shown that the presence of TLS in human tumors is an indicator of positive clinical outcome. However, due to dysregulated angiogenesis, many tumors have a poorly-organized and leaky vasculature that impedes entry of immune effector cells into tumors and consequently, TLS formation. It has been shown in pre-clinical studies that low doses of antiangiogenic agents normalize the tumor vasculature, leading us to hypothesize that treating tumors with low-doses (well below drug MTD) of vascular normalizing (VN) therapies will improve immune cell infiltration and TLS formation within the tumor microenvironment (TME).

**Methods** To test this hypothesis, melanoma-bearing mice were treated intratumorally with VN agents. Five days post-treatment, tumors were digested into single cell suspensions and RNA was isolated and used for RT-PCR. Transcript levels of TLS-promoting factors (CCL19, CCL21, CXCL13) and markers of vascular normalization (HIF1α, GLUT1) and inflammation/immune cell infiltration (CXCL10, VCAM1, CD8α) were measured and compared to PBS treated controls. Changes in tumor vasculature were evaluated using immunofluorescence microscopy (IFM) of tumor sections stained with CD31, PNAδ, and PDGFRβ. Fluorescently-labeled lectin was injected into the mice to observe tumor perfusion. TLS formation was evaluated in tumor sections using IFM, with TLS being defined as PNAδ+ vessels surrounded by clusters of CD45+ cells.

**Results** We observed that the VN agents dasatinib, STING agonist, bevacizumab, and agonist anti-TNFRI1 antibody each induced global changes in the TME that are consistent with enhanced immune cell infiltration and TLS formation. These changes include increases in expression of CCL19, CCL21, and VCAM1. Dasatinib and STING agonists were shown to promote VN, as demonstrated by improved lectin perfusion into the tumor and increased pericyte coverage of blood vessels on-treatment.

**Conclusions** VN agents induce global changes in immune cell infiltration and TLS-promoting factors in the TME. In vivo, these agents induce VN in the TME and promote TLS formation. This knowledge can be used to develop optimal combination immunotherapy designs in the cancer setting.

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**585** INTRALESIONAL INJECTION OF ROSE BENGAL IMPROVES THE EFFICACY OF GEMCITABINE CHEMOTHERAPY AGAINST PANCREATIC CANCER

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**Background** Chemotherapy regimens that include gemcitabine are considered standard of care in patients with advanced pancreatic ductal adenocarcinoma (PDAC). However, most patients with PDAC die within 2 years of diagnosis, even with these standard of care regimens. In this study, we explored the ability of intratumoral injections of PV-10, a 10% solution of rose bengal, to induce lesion-specific ablation and control of metastatic pancreatic tumors in a murine model.

**Methods** PV-10 was cultured with human pancreatic cancer cell lines overnight and cell death was measured via Annexin-V and DAPI staining. Murine pancreatic tumor cells (Panc02) were injected subcutaneously in one flank to establish a single tumor model; to establish a bilateral tumor model, Panc02 tumor cells were implanted in the opposite flanks. On day 7, a single tumor was treated with intraleSIONAL PV-10. Gemcitabine (60 mg/kg) was injected intraperitoneally twice per week for 2 weeks. These experiments were repeated using Panc02 cells modified to overexpress the neoantigen ovalbumin (OVA). Control mouse tumor were directly injected with PBS. Tumor growth of PV-10 injected tumors and non-injected bystander tumors on the opposite flank were measured. Damage associated molecular patterns (DAMPs) in serum and immune cell frequencies within the spleens of tumor-bearing mice were measured to identify an associated systemic response with tumor lytic treatment regimen.

**Results** We established that less than 50% of human and murine pancreatic cells were alive after a 24 hour incubation with 200μM PV-10 in vitro. The combination of intraleSIONAL PV-10 with the systemic administration of gemcitabine delayed the growth treated tumors and non-injected distal tumors. In contrast, gemcitabine monotherapy failed to delay tumor growth in bilateral Panc02 tumor models. We observed that this treatment strategy was markedly more successful in immuNogenic Panc02 OVA tumors resulting in lesion-specific ablation in 5/8 mice compared to 0/8 mice that were treated with gemcitabine monotherapy. This suggests that the combination therapy enhanced the immune-mediated clearance of tumors. Moreover, regression of tumors in mice that received PV-10 in combination with gemcitabine was associated with the depletion of splenic CD11b+Gr-1+ cells and increases in damage associated molecular patterns HMGB1, S100A8, and IL-1α.

**Conclusions** Together, these results demonstrate that intraleSIONAL therapy with PV-10 can enhance the efficacy gemcitabine against pancreatic tumors.

**Ethics Approval** Studies were performed under approved Institutional Review Board (IRB) laboratory protocols at the H. Lee Moffitt Cancer Center (Tampa, FL).

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Background Checkpoint inhibitors have demonstrated clinical benefit for several types of cancer, but still a large proportion of patients do not respond to treatment. To improve response rates, many combination therapies are currently under clinical evaluation. One such example is the combination of anti-PD-1 monoclonal antibodies with intratumoral gene transfer of plasmid-based interleukin 12 (IL-12). Local expression of the cytokine IL-12 has been shown to increase immune cell infiltration in cold tumors, which can make them more responsive to anti-PD-1 antibodies. The current study evaluates the efficacy of simultaneous delivery of checkpoint-inhibiting antibodies and IL-12 by intratumoral gene transfer. We recently demonstrated that intratumoral delivery of plasmid-based checkpoint inhibitors yielded systemic anti-tumor responses in a mouse tumor model, with only limited systemic antibody exposure and therefore improved biosafety.

Methods C57BL/6j mice bearing a subcutaneous syngeneic MC38 tumor received a single intratumoral injection of plasmid DNA followed by in vivo electroporation. DNA-based IL-12 (p(IL-12), 2.5 μg) was administered alone or in combination with a DNA-based anti-PD-1 antibody (p(aPD-1), 60 μg) and/or DNA-based anti-CTLA-4 antibody (p(aCTLA-4), 60 μg). Abscopal effects were studied in mice bearing two contralateral tumors, of which only one received therapy.

Results The combined intratumoral delivery of p(IL-12) and p(aPD-1) resulted in 10% complete responders, in contrast to no complete tumor regressions with each individual treatment. Yet, differences in tumor growth or survival did not reach statistical significance between these groups. To improve anti-tumor efficacy, the combined gene transfer was expanded with a second DNA-based checkpoint inhibitor, p(aCTLA-4). While intratumoral delivery of this triple combination also led to 10% complete regressions, the response did result in significant tumor growth delay compared to p(IL-12) alone (p<0.05) and the combination of both checkpoint inhibitors (p<0.01). Moreover, in a dual MC38 tumor model, the triple combination enabled significant abscopal effects compared to untreated mice (p<0.01), which was not the case for the other treatments.

Conclusions This study demonstrates that intratumoral DNA-based gene transfer can be applied to efficiently combine different immunotherapeutics. This approach allows simplification of the treatment schedule, addresses the complex production of conventional protein-based therapeutics, and enables local drug expression, thereby minimizing systemic exposure and subsequent adverse events. Ongoing studies focus on the further validation of combined intratumoral delivery of plasmid-based checkpoint inhibitors and IL-12, by investigating the effect on tumor-infiltrating and peripheral immune cells as well as through evaluation of the triple combination in other tumor models.

Ethics Approval This study was approved by the KU Leuven Animal Ethics Committee, approval number P130/2017.

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TARGETING GITR ENHANCES HUMAN TUMOUR-INFILTRATING T CELL FUNCTIONALITY IN MISMATCH REPAIR PROFICIENT PRIMARY COLORECTAL CARCINOMA AND LIVER METASTASES

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Background Immune checkpoint blockade (ICB; e.g. anti-PD-1/-CTLA-4) has been proven to be clinically effective in mismatch repair deficient (dMMR) colorectal carcinoma (CRC). Yet, the majority of patients carry mismatch repair proficient (pMMR) CRC, especially those with liver metastasis, and do not respond to ICB. Here, we studied the effect of immune checkpoint stimulation via GITR targeting on human tumour-infiltrating lymphocyte (TIL) functionality in pMMR primary CRC and liver metastases (CRLM).

Methods Human TIL were isolated from freshly resected pMMR tumours of patients with primary CRC (stage 1–3) or liver metastases (table 1). GITR expression on TIL was determined using flow cytometry and compared to leukocytes isolated from blood (PBMC) and tumour-free surrounding tissues (tumour-free colon/liver, resp. TFC and TFL). Ex vivo functional assays were used to assess TIL expansion, activation and cytokine/cytotoxic mediator secretion upon CD3/CD28 bead activation and co-stimulation using an antibody-cross-linked recombinant trimeric GITR ligand (GITRL).

Results GITR was overexpressed on TIL when compared to other stimulatory immune checkpoints (4-1BB, OX40). GITR expression was enhanced on CD4+ and CD8+ TIL compared to PBMC and TFC or TFL compartments in both primary CRC and CRLM. Among CD4+ TIL, GITR was increasingly expressed on CD45RA± FoxP3- helper T (Th), CD45RA- FoxP3int activated helper T (aTh), and CD45RA- FoxP3hi activated regulatory T cells (aTreg), respectively. Within CD8+ TIL, GITR expression was higher on TOX+ PD1Hi and putatively tumour-reactive CD103+ CD39+ TIL. Impaired effector cytokine production upon ex vivo PMA/ionomycin stimulation was observed in CD4+ and CD8+ GITR-expressing TIL, hinting to functional exhaustion of the target population. However, recombinant GITRL reinvigorated ex vivo TIL responses by significantly enhancing CD4+ and CD8+ TIL numbers and proinflammatory cytokine secretion in a dose-dependent manner (figure 1). Treg depletion did not fully abrogate the stimulatory effect of GITR ligation on CD4+ and CD8+ T cell expansion, demonstrating that the stimulatory effect was partly exerted via direct targeting GITR on effector T cells. Importantly, GITR-ligation also enhanced expansion of purified CD8+CD39+ TIL. Dual treatment with GITR ligand and nivolumab (anti-PD-1) further enhanced CD8+ TIL responses compared to GITR ligand monotherapy, whereas nivolumab alone did not show any effect.

Conclusions Agonistic targeting of GITR enhances ex vivo human TIL functionality in pMMR CRC and might therefore be a promising approach for novel mono- or combinatorial immunotherapies in primary CRC and CRLM.

Acknowledgements N/A

Trial Registration N/A

Ethics Approval The study was approved by the medical ethics committee of the Erasmus Medical Center (MEC-2012-331).

Consent N/A

REFERENCE

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EFFICACY OF ONCOLYTIC VACCINIA VIRUS REQUIRES INFECTION OF SUPPRESSIVE IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT LEADING TO THEIR REPROGRAMMING AND DELETION

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Background Checkpoint blockade immunotherapy has dramatically changed cancer treatment; however, these therapies depend on the presence of a pre-existing immune infiltrate. Unfortunately, some patients have few to no infiltrating immune cells, highlighting the need for therapies that can generate antigenic stimuli. Oncolytic viruses, which infect and lyse tumor cells while leaving healthy tissue unharmed, are an attractive means to provide these signals, although the mechanisms of action of these engineered viral therapies remain incompletely understood. Virally induced immunogenic death causes an influx of tumor- and virus-specific effector CD8+ T cells. Many oncolytic viruses also decrease tumor-infiltrating suppressive immune populations, such as regulatory T cells (Treg), however the mechanism for this is unknown. Here we show that an oncolytic strain of vaccinia virus (VV) infects tumor infiltrating Tregs, in contrast to the prevailing idea that oncolytic viruses only infect tumor cells. Infection leads to viral-mediated Treg depletion that is required for tumor regression.

Methods Using a mouse model of head and neck squamous cell carcinoma (MEER), a VV-resistant line was generated through serial treatment of a VV-sensitive MEER line. At varied time points post-intratumoral treatment with VV, tumor infiltrating lymphocytes (TIL) were isolated from both the VV-resistant and VV-sensitive lines and analyzed by flow cytometry.

Results One day post-treatment of VV-sensitive MEER tumors, tumor isolated Tregs were infected by VV as determined by viral GFP expression. Infection was confirmed in vitro with purified Tregs. Four days post-treatment, tumor infiltrating Treg counts were reduced, and active caspase 3 staining was increased, suggesting that infection lead to Treg death. At 7 days post-treatment, the remaining Tregs in the VV-sensitive tumors acquired a fragile phenotype (IFNα+Nrp1-). This was not observed in the VV-resistant MEER line. Fragile Tregs are less suppressive and indeed we observed an increase in pro-inflammatory cytokine production from CD8+ and Tconv (CD4+Foxp3-) T cells in the VV-sensitive tumors compared to VV-resistant. We then engineered oncolytic VV to be susceptible to Cre mediated inactivation. Infection of various murine transgenic Cre lines confirmed the importance of nontumoral immune infection for therapeutic efficacy, with a particular emphasis on Treg infection.

Conclusions These data reveal a previously unappreciated mechanism of action of oncolytic virus immunotherapy, in which new tumor immunity accompanies the viral mediated loss and phenotypic change of regulatory populations. Importantly, as this treatment is delivered intratumorally the loss of Tregs is tumor specific, resulting in targeted Treg depletion without systemic autoimmunity.

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Abstract 590 Figure 1 Reovirus sensitizes tumors for CD3-bsAb therapy

Reovirus-induced interferon signaling leads to increased T cell influx and subsequent effective CD3-bispecific antibody therapy in solid tumors
reovirus as a pre-conditioning regimen performed significantly better than the simultaneous or preceding administration of bsAbs. This combination treatment also induced regressions of non-injected distant lesions, suggesting that this therapy might be effective for metastatic disease.

Conclusions Oncolytic reovirus administration represents an effective strategy to induce a local IFN response and strong T cell influx, thereby sensitizing the tumor microenvironment for subsequent CD3-bsAb therapy (figure 1). Our data advocate for the inclusion of oncolytic viruses as a pre-conditioning strategy in T cell engaging antibody trials for solid tumors. Since both CD3-bispecific antibodies and oncolytic viruses are in advanced clinical development as monotherapies, efficient translation of this combination seems feasible.

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Ethics Approval All mouse studies were approved by the institutional Animal Welfare Body of Leiden University Medical Center and carried out under project licenses AVD1160020187004 or AVD116002015271, issued by the competent authority on animal experiments in the Netherland (named CCD).

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COMPARISON OF TWO OHSV VECTORS FOR THE TREATMENT OF GliOBLASTOMA

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Background Glioblastoma multiforme (GBM) is the most common human brain cancer. Despite a well-established standard of care, the 5-year mortality rate of GBM patients is 95%, highlighting the need for innovative therapeutic interventions. A variety of oncolytic viruses, including those derived from herpes simplex virus (oHSV), have been designed for GBM therapy, but early-phase clinical trials have reported few complete responses and no evidence of durable anti-tumor immunity. Potential reasons for the lack of efficacy are limited vector potency (i.e., virulence) and the presence of a highly immunosuppressive tumor microenvironment (TME) comprised of few activated lymphocytes, large numbers of immunosuppressive myeloid cells (macrophages, myeloid derived suppressor cells [MDSCs], microglia), and an agglomerate of immunosuppressive cytokines (IL-10, VEGF, MIF, etc.).

Herein we explore these obstacles by comparing the anti-tumor activity two different oHSV designs, an HSV-1 KOS strain derivative designated KG4:T124, and an F strain derivative designated rQNestin34.5v.1 (a similar oHSV, rQNestin34.5v.2, is currently in a phase I clinical trial for GBM).

Methods Using the murine syngeneic GBM models, GL261N4 and CT2A, we compared the anti-tumor activity of KG4:T124 and rQNestin34.5v.1. In vitro, we evaluated the viral entry, replication capacity, and cytotoxicity of both oHSVs. In vivo, we measured the impact of both vectors on tumor progression, TME immune cell composition, and animal survival.

Results Virus entry into cancer cells of KG4:T124 or rQNestin34.5v.1 was relatively similar, but rQNestin34.5v.1 replicated more effectively and generally induced greater viral mediated cytotoxicity. In syngeneic mice, rQNestin34.5v.1 reduced orthotopic GL261N4 tumor burden and enhanced animal survival compared to KG4:T124. However, preliminary data indicate that multiple injections of KG4:T124 but not rQNestin34.5 enhance GL261N4 survival outcome. Neither oHSV impacted survival outcomes in the more permissive CT2A model. Analysis, of either the GL261N4 or CT2A TME two days post virus administration revealed that both viruses had reduced microglia cell frequency, induced the influx of tumor associated macrophages and polymorphonuclear cells, but did not alter the frequency of monocytes MDSCs, natural killer cells, CD8+ or CD4+ T-cells.

Conclusions rQNestin34.5 had greater oncolytic activity in vivo and in vitro, but did not benefit from multiple oHSV injections. Both viruses induced similar changes in the TME immune cell composition. However, the presence of vital adaptive immune cell types within the TME was not observed at 2 days post oHSV treatment.

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ENHANCING THE THERAPEUTIC POTENTIAL OF ONCOLYTIC ADENOVIRUSES WITH VSENS™ TECHNOLOGY

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Background Oncolytic viro-therapeutics is a promising treat-
TUMORAL MELANOSIS MIMICKING RESIDUAL MELANOMA AFTER T-VEC TREATMENT

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Background Talmigene laherparepvec (T-VEC) has become an increasingly popular treatment option for surgically non-resectable, recurrent melanoma based on its durable efficacy and safety profile. The complete response (CR) rate has been reported to be ~20% with a median of ~9 months to achieve it. Assessment of treatment response in those studies has predominantly relied on the clinical impression of the size and color of the lesions. However, in the real-world, decrease of tumor size often occurs rapidly within the first 2–3 months, while improvement of the pigmentation takes several more months. Such clinical observation of lasting pigmentation could be explained by tumoral melanosis – a histopathologic term referring to the presence of a melanophage-rich inflammatory infiltrate without remaining viable tumor cells.

Methods We hypothesized that residual pigmentation of stable melanoma lesions while on successful T-VEC treatment may represent tumoral melanosis. We also report practical information of such phenomenon including timeline and clinical features.

Results We report 6 cases of metastatic cutaneous melanoma treated with T-VEC with excellent pathologic responses. Biopsies of 5 cases were performed after observing variable clinical changes in the injected tumors, with some shrinking or becoming flat, while others grew or became raised. The range of time to biopsy was 4–23 months from the initial treatment date. Pathologic evaluation macular lesions demonstrated non-viable tumor tissue with tumoral melanosis in all cases. In an additional case, clinically increased size of the injected tumor prompted surgical excision, which similarly showed tumoral melanosis without viable tumor. Of note, while size of the tumor was increased, SUV max of the lesion decreased from prior assessment on PET-CT. No patient has developed regrowth or recurrent melanoma of the injected lesions to date (table 1).

Conclusions In patients receiving T-VEC treatment, pathologic CR may be achieved within the first 2–3 months, which precedes clinical improvement of pigmentation. To decrease unnecessary additional T-VEC treatment and assess the response correctly, serial biopsy of stable pigmented lesions should be considered to assess for the presence or absence of viable tumor.

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Trial Registration N/A
BT-001, AN ONCOLYTIC VACCINIA VIRUS ARMED WITH A TREG-DEPLETING HUMAN RECOMBINANT ANTI-CTLA4 ANTIBODY AND GM-CSF TO TARGET THE TUMOR MICROENVIRONMENT

Background Checkpoint inhibitor antibodies have improved survival in a variety of cancers, however, a great unmet need remains since only a small fraction of patients responds. Reasons for lack of efficacy are believed to include lack of tumor infiltrating immune cells, a notion supported by improved efficacy observed following combined checkpoint blockade with tumor oncolytic virotherapy which promotes intratumoral T cell infiltration. Oncolytic vaccinia viruses (oVV) also allow genetic encoding of transgenes. This is of special interest for therapeutic proteins exhibiting toxicological limitation or pharmacokinetic issues. Here, BioInvent and Transgene present a potentially safer and more efficacious strategy to combine checkpoint inhibition in the context of oncolytic virotherapy.

Methods Using the F.I.R.S.T™ discovery platform we have isolated a human recombinant Treg-depleting antibody that has been vectorized alongside GM-CSF into the Invir.IO® oVV. This product named BT-001 consists of a Copenhagen double deleted vaccinia virus encoding the human CTLA4-specific antibody 4-E03 IgG1, which shows improved Treg-depletion compared with ipilimumab in a human PBMC-based NOG/SCID-transfer model. BT-001 also encodes GM-CSF, the cytokine expressed in clinically approved products. A surrogate murine mAb was vectorized into the same oVV (mBT-1) allowing for functional and mechanistic in vivo studies.

Results Our studies demonstrate that 4-E03 and GM-CSF were expressed as functional molecules after infection by BT-001 of human tumor cell lines in vitro. Moreover, following intratumoral administration in immune competent and immune deficient mice transplanted with mouse or human tumors, transgene expression was sustained at levels associated with receptor saturation for days to weeks. In contrast, and supporting the tumor-selective nature of oVV, blood concentrations of anti-CTLA4 mAb were lower compared to those observed following i.v. administration of therapeutic doses of mAb. The in vivo anti-tumor activity of mBT-1 was assessed in multiple syngeneic mouse tumor models including CT26, EMT6, A20 and C38. Murine surrogate mBT-1 conferred cures in the majority of challenged mice irrespective of tumor origin. The excellent anti-tumoral profile depends on anti-CTLA4 expression and could be boosted by co-administration of anti-PD-1 mAb. Intratumoral treatment with mBT-1 also induces abscopal anti-tumor responses and protects against tumor rechallenge demonstrating a long-lasting systemic anti-tumor activity.

Conclusions A clinical batch of BT-001 has been produced and toxicological evaluation is ongoing. Transgene and BioInvent have applied for a clinical trial targeting injectable superficial tumors. Here, the tumor-localized delivery of anti-CTLA4 may allow a better tolerated and more effective combination therapy with antibodies targeting the PD-1/PDL1 axis.

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patients that do not respond well to treatment with immune checkpoint inhibitors. Myxoma virus (MYXV) is a member of the Pox family of double stranded DNA viruses. The natural host of MYXV is a subset of rabbits and hares, but MYXV is able to infect cancer cell lines of humans and other species. The genome of MYXV is relatively large and is amenable to engineering for expression of transgenic proteins making it an excellent oncolytic virus for introduction of immunomodulatory proteins.

**Methods** The current work describes the in vitro oncolytic activity and transgene production capability in human cancer cell lines, and in vivo activity of armed myxoma viruses in xenograft human cancer models.

**Results** Armed Myxoma viruses demonstrate transgene production and oncolytic activity in multiple human cancer cell lines in vitro and in vivo.

**Conclusions** Armed Myxoma viruses present a novel oncolytic viral therapy with ability to modulate immune responses in human cancer models.

**Ethics Approval** This study was approved by OncoMyx Therapeutics and the TD2 IACUC.

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**Abstracts**

**597** IN SITU VACCINATION WITH ONCOLYTIC VESICULAR STOMATITIS VIRUS IMPROVES ANTI-TUMOR IMMUNE RESPONSE AND OUTCOME IN BLADDER CANCER

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**Background** The majority of nonmuscle invasive bladder cancer (NMIBC) cases progress towards muscle invasive disease. Transurethral resection followed by chemotherapy and/or BCG immunotherapy can stall progression in the minority of NMIBC cases. Cystectomy prior to muscle invasion provides the best option for survival. However, bladder removal significantly affects morbidity and quality of life. There are no effective treatment options for patients with chemo/BCG-resistant and late stage disease. Compared to other solid cancer types, the urinary bladder is an ideal organ to evaluate oncolytic virotherapies due to the urgent medical need for alternative bladder-sparing therapies and its established immunosensitivity to BCG therapy. The current study will determine whether a novel oncolytic Vesicular Stomatitis Virus (VSVd51) containing human immune transgenes can treat NMIBC.

**Methods** A novel recombinant OV containing a human immune transgene was rescued on the VSVd51 backbone. Features of immunogenic cell death (ICD) on mouse and human bladder cancer cell lines were measured by microscopy, flow cytometry, immunoblot, luminometry, qRT-PCR and ELISA following infection by recombinant VSVd51. The mediating role of immune effector cells was evaluated through pharmacologic in vivo depletion, while combination injection of recombinant VSVd51 following BCG failure was performed in the C57Bl/6-MB49 model. Measurements of ICD was additionally carried out in human BC spheroids and bladder cancer patient tissue following recombinant VSVd51 infection ex vivo.

**Results** Recombinant VSVd51 liberated danger signals (calcitulin, HMGBl1, ATP) and immunogenic cytokines/chemokines were detected from infected mouse and human BC cell lines. Intravesical instillation of recombinant VSVd51 promoted enhanced activation of systemic and bladder infiltrating natural killer (NK) and cytotoxic CD8+ T cells. The increased functional-ity of NK and CD8+ T cells was associated with improved survival as determined through depletion studies. Moreover, improved survival and reduced bladder tumor volume was observed in recombinant VSVd51 treated mice who failed BCG therapy. In parallel, VSVd51-induced inflammation of the tumor microenvironment was recapitulated in human BC cell lines, spheroids and patient tissue exposed to recombinant VSVd51 infection.

**Conclusions** These translational results suggest that a recombinant VSVd51 is a promising immunotherapy that could provide a bladder-sparing therapeutic benefit in individuals diagnosed with NMIBC each year.

**Ethics Approval** The study was approved by the CIUSSS de l’Estrie CHUS Ethics Board, approval number 2018-2465.

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new checkpoints controlling function of cytotoxic lymphocytes infiltrating human carcinoma

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Background Although present in high numbers, T and NK cells appear functionally impaired in the renal cell carcinoma (RCC) tumor milieu, as they cannot be stimulated to degranulate and IFN-γ production. This is in part due to altered regulation of signaling downstream of the T cell receptor (TCR). Increased diacylglycerol kinase alpha (DGK-α) has been observed in T and NK cells from the RCC tumor microenvironment (TME). Ex vivo inhibition of DGK-α by the commercially available inhibitor R59022 was able to restore responsiveness to stimulation. 1–2 Inhibition of DGK-α is reported to also block tumor cell growth and survival. 3–4 Many T cells from RCC additionally express the immune checkpoint Programmed cell Death-1 (PD-1). Interaction of PD-1 with PD-L1 on tumor cells blocks AKT signaling and inhibits T cell function. In the clinic, blocking the PD-1/PD-L1 interaction allows tumor control in some patients; however, the majority of patients do not respond long-term. Since DGK-α acts downstream of PD-1 it may, if overactive, curb T cell function despite PD-1/PD-L1 blockade. Thus, we hypothesize that dual inhibition of PD-1 and DGK α might be required to fully unleash the T cell’s potential in the TME. Current DGK-α inhibitors are not suitable for clinical application. Therefore, we investigate alternative means using RNA interference (RNAi) to target DGK-α alone as well as in combination with PD-1.

Methods Knockdown was achieved by RNAi using INTASYL ™ compounds, developed by Phio Pharmaceuticals. These compounds incorporate drug-like properties into siRNA, resulting in enhanced uptake with no need for transfection reagents. Efficacy was analyzed on mRNA and protein level by rt-qPCR, flow cytometry and Western Blot. Functional assays include cytotoxicity and cytokine production in tumor-mimicking environments.

Results Using INTASYL ™ compounds, silencing of DGK-α was observed in human U2OS osteosarcoma as well as K562 erythroleukemic cells. PD-1 knockdown was achieved in human T cells isolated from peripheral blood mononuclear cells (PBMC). Synergy of DGK-α and PD-1 knockdown is tested in tumor-minicking in vitro systems using T cell/tumor cell co-cultures at high tumor cell density where T and NK cells become functional suppressed as observed in the TME.

Conclusions Strong activity of specific T and NK cells is necessary for tumor control. Dual targeting of PD-1 and DGK-α may be required to fully enable T and NK cell reactivity in the TME. Self-delivering RNAi technology represents a promising approach to targeting intracellular immune checkpoints such as DGK-α, in addition to PD-1 inhibition.

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than resveratrol in inhibiting chemotaxis of HL-60 cells and blocking cell cycle of THP-1 and HL-60 cells at G1/S transition. In addition, NBT-167, but not resveratrol, could increase IL-2 production and T cell proliferation stimulated with anti-CD3 and anti-CD28 and synergize with anti-PD-1 antibody to increase IL-2 and IFN-gamma production in co-culture of allogeneic T cells and dendritic cells (MLR).

**Conclusions** Our data showed that NBT-167, a dimer of resveratrol, had anticancer and immunomodulatory activities such as modulation of expression of cytokines in immune cells and induction of cancer cell-killing activities of NK and gamma delta T cells. Generally, NBT-167 appeared to have higher activities than resveratrol in modulating immune cells and inhibiting cancer cells. NBT-167 could be a promising cancer immunotherapeutic agent targeting both cancer cells and immune cells.

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### DEVELOPMENT OF IMPROVED SMALL MOLECULE STING AGONISTS SUITABLE FOR SYSTEMIC ADMINISTRATION


**Background** Stimulator of Interferon Genes (STING) is a major player in the activation of robust innate immune response leading to initiation and enhancement of tumor-specific adaptive immunity. Several clinical and pre-clinical programs have shown that activation of the STING pathway triggers immune-mediated antitumor response. Although vast majority of programs focus on development of analogues of the endogenous STING ligands, their chemical nature and stability often limit their use to local administration. Herein, we present recent results from the development of our selective non-nucleotide, non-macrocyclic, small molecule direct STING agonists, suitable for systemic administration, characterized by improved activity in human immune cells.

**Methods** Binding to recombinant STING protein was examined using FTS, MST, FP and crystallography studies. Phenotypic screen was performed in THP-1 Dual reporter cells. Mouse bone marrow-derived dendritic cells (BMDC) were obtained from C57BL/6 mice and differentiated with mIL-4 and mGM-CSF. STING agonists were administered into BALB/c mice and cytokine release was measured in plasma. Additionally, mice were inoculated with CT26 murine colon carcinoma or EMT6 murine breast carcinoma cells and the compound was administered, followed by the regular tumor growth and body weight monitoring.

**Results** Ryvu’s small-molecule agonists demonstrate strong binding affinity to recombinant STING proteins across all tested species. The compounds bind to all human STING protein variants and trigger pro-inflammatory cytokine release from human immune cells regardless of the STING haplotype. Moreover, new generation of developed agonists show significantly improved binding to human protein as well as in vitro activity on human cells. Systemic, intravenous in vivo administration leads to a dose-dependent upregulation of STING-dependent pro-inflammatory cytokines, which results in a dose-dependent antitumor efficacy observed in CT26 and EMT6 mouse cancer models, leading to complete tumor remissions in all treated animals. Furthermore, observed efficacy is accompanied by development of a lasting immunological response demonstrated by lack of tumor engraftment or a delayed tumor growth in cured animals challenged with repeated inoculation of cancer cells.

**Conclusions** New generation Ryvu’s STING agonists are strong and selective activators of STING-dependent signaling in both mouse and human immune cells promoting anti-tumor immunity. Treatment with Ryvu’s small-molecule STING agonists leads to engagement of the immune system which results in a complete tumor remission and development of immunological memory of the cancer antigens. The compounds show good selectivity and ADME properties enabling development for systemic administration. In addition developed compounds maintain small functional handles amenable to linker attachment making the series suitable for versatile development as single agents, for combinations with immunotherapies or as targeted agents.

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### STING AGONIST-BASED TREATMENT PROMOTES VASCULAR NORMALIZATION AND TERTIARY LYMPHOID STRUCTURE FORMATION IN THE THERAPEUTIC MELANOMA MICROENVIRONMENT

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**Background** The degree of immune infiltration in tumors, especially CD8+ T cells, greatly impacts patient disease course and response to interventional immunotherapy. Hence, enhancement of TIL prevalence is a preferred clinical endpoint, one that may be achieved via administration of agents that normalize the tumor vasculature (VN) leading to improved immune cell recruitment and/or that induce the development of local tertiary lymphoid structures (TLS) within the tumor microenvironment (TME).

**Methods** Low-dose STING agonist ADU S-100 (5 µg/mouse) was delivered intratumorally to established s.c. B16.F10 melanomas on days 10, 14 and 17 post-tumor inoculation under an IACUC-approved protocol. Treated and control, untreated tumors were isolated at various time points to assess transcriptional changes associated with VN and TLS formation via qPCR, with corollary immune cell composition changes determined using flow cytometry and immunofluorescence microscopy. In vitro assays were performed on CD11c+ BMDCs treated with 2.5 µg/mL ADU S-100 (vs PBS control) and associated transcriptional changes analyzed via qPCR or profiled using DNA microarrays. For TCRβ-CDR3 analyses, CDR3 was sequenced from gDNA isolated from enzymatically digested tumors and splenocytes.

**Results** We report that activation of STING within the TME leads to slowed melanoma growth in association with increased production of angiostatic factors including Tnfsf15 (Vegi), Cxcl10 and Angpt1, and TLS inducing factors including Ccl19, Ccl21, Lta, Ltb and Tnfsf14 (Light). Therapeutic responses from intratumoral STING activation were characterized by increased vascular normalization (VN), enhanced tumor infiltration by CD8+ T cells and CD11c+ DCs and local TLS neo-genesis, all of which were dependent on host...
expression of STING. Consistent with a central role for DCs in TLS formation, ex vivo ADU S-100-activated mCD11c+ DCs also exhibited upregulated expression of TLS promoting factors including lymphotoxin-α (LTA), IL-36, inflammatory chemokines and type I interferons. TLS formation was associated with the development of a therapeutic TIL TCR repertoire enriched in T cell clonotypes uniquely detected within the tumor but not the peripheral circulation in support of local T cell cross-priming within the TME.

Conclusions Our data support the premise that i.t. delivery of STING agonist promotes a pro-inflammatory TME in support of VN and TLS formation, leading to the local expansion of unique TIL repertoire in association with superior anti-melanoma efficacy.

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COVALENT ATTACHMENT OF A TLR7/8 AGONIST TO TUMOR-TARGETING ANTIBODIES DRIVES POTENT ANTI-TUMOR EFFICACY BY SYNERGISTICALLY ACTIVATING FCGR- AND TLR- SIGNALING AND ENABLES SAFE SYSTEMIC ADMINISTRATION

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Background Immune stimulating antibody conjugates (ISACs) covalently attach TLR7/8 immune stimulants to tumor-targeting antibodies. ISACs can be delivered systemically and act locally in the tumor microenvironment by requiring the following biological steps to elicit immune activation: 1) tumor antigen recognition, 2) Fc receptor mediated phagocytosis by myeloid antigen presenting cells (APCs), and 3) activation of endosomal TLR7 and TLR8. Here, we demonstrate that covalent attachment of our TLR7/8 agonist to tumor-targeting antibodies not only enables the resulting ISACs to be safely administered systemically in preclinical models, but also unexpectedly promotes synergy between the FcgR and TLR pathways that results in amplified anti-tumor immunity in mice and robust immune activation in human leukocytes as compared to the co-administration of the components.

Methods ISAC activity and mechanistic studies were analyzed via flow cytometry, ELISA and CyTOF following in vitro coculture of human leukocytes with tumor cell lines. In vivo efficacy of HER2-targeting ISACs following systemic administration was assessed in a trastuzumab-resistant HER2+ human tumor xenograft model. Safety and tolerability were assessed in tumor-bearing mice and healthy non-human primates (NHP).

Results While co-administration of intratumoral TLR7/8 agonist and intraperitoneal trastuzumab failed to control tumor growth, systemic administration of the same TLR7/8 agonist and trastuzumab in our ISAC format was efficacious and induced complete tumor regression in an Fc- and TLR-dependent manner. Analysis of primary human leukocytes stimulated with ISACs in tumor co-culture assays indicated that ISACs elicit amplified and sustained phosphorylation of Fc and TLR signaling pathways, such as pERK1/2 and pIRF-7, as compared to the unconjugated mixture of the same TLR7/8 agonist and tumor targeted antibody. ISAC stimulation was largely restricted to antigen presenting cells such as dendritic cells and plasmacytoid dendritic cells that express the relevant Fc receptors and TLR7 and/or TLR8. Modifications to the ISAC that reduce FcgR engagement (N297A/Q) or render the agonist inactive halted ISAC-mediated activation and in vivo anti-tumor efficacy. Lastly, our HER2-targeting ISACs were well-tolerated when delivered systemically in mice and NHPs.

Conclusions Our ISACs enable potent TLR agonists to be safely administered systemically in preclinical models. ISACs provide distinct and unexpected advantages over unconjugated TLR agonists, notably by driving synergy between FcgR and TLR pathways, leading to robust myeloid activation and anti-tumor efficacy. These data support the evaluation of BDC-1001, a HER2-targeted ISAC in the ongoing Phase 1/2 trial (NCT04278144).

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INTRAVENOUS CMP-001, A CPG-A TOLL-LIKE RECEPTOR 9 (TLR9) AGONIST DELIVERED VIA A VIRUS-LIKE PARTICLE, CAUSES TUMOR REGRESSION IN SYNGENEIC HEPA1-6 MOUSE MODELS OF HEPATOCELLULAR CARCINOMA

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Background Therapeutic options are limited for patients with liver metastases and hepatocellular carcinoma (HCC). Intratumoral and subcutaneous injections of CMP-001, a CpG-A TLR9 agonist packaged within a virus-like particle, have shown evidence of antitumor activity in patients with melanoma refractory to PD-1 blockade. In mice, CMP-001 intravenous distributes primarily to the liver, while CMP-001 subcutaneous is found mostly in local tissues and draining lymph nodes. The antitumor activity of CMP-001 intravenous and subcutaneous were compared with PD-1 blockade or sorafenib in two Hepa1-6 orthotopic mouse models of HCC.

Methods Groups of 10–15 C57BL/6 mice were orthotopically implanted with syngeneic murine hepatoma cells using two different models. Model 1 used 1.5 x 10^6 Hepa-6 cells injected into the spleen following a partial hepatectomy; Model 2 used 1 x 10^6 Hepa-6-Luc cells injected into the upper left lobe of intact liver. Treatment was initiated 3–7 days later with either CMP-001 intravenous or subcutaneous Q4-5Dx3-4 doses, PD-1 blocking antibody intraperitoneal Q3-4Dx2 (Bio X Cell clone RPM1-14), or sorafenib QD oral. Antitumor activity was assessed by tumor imaging, liver weight, and/or survival.

Results CMP-001 was compared with PD-1 blocking antibody therapy in Model 1, the more aggressive model. All animals were sacrificed at day 15 due to institutional welfare requirements. Tumor growth inhibition (TGI) was assessed by comparison of liver weight to body weight ratios, which relative to untreated control mice showed that CMP-001 intravenous achieved 85% mean TGI compared with 63% mean TGI for CMP-001 subcutaneous and 15% mean TGI for PD-1 blocking antibody intraperitoneal (table 1). CMP-001 intravenous was compared to sorafenib oral in Model 2, which utilized an engineered Hepa1-6 cell line that expresses luciferase to enable noninvasive monitoring of liver tumor growth. CMP-001 intravenous was active, with a 67% mean TGI, and survival that was comparable to sorafenib (table 2; figure 1).
Conclusions In orthotopic mouse models of HCC, the antitumor activity of CMP-001 intravenous was greater than PD-1 blockade and comparable to sorafenib. CMP-001 intravenous was more active than CMP-001 subcutaneous in this model, which we hypothesize is due to increased liver exposure with intravenous infusion. Antitumor activity of CMP-001 monotherapy may be increased by combining it with standard of care or other therapies, as observed relative to historical benchmarks in ongoing CMP-001 clinical trials in patients with melanoma. CMP-001 intravenous may be a promising treatment option for patients with primary or metastatic liver cancers.

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Ethics Approval At Oncodesign Biotechnology, animal housing and experimental procedures were conducted according to French and European Regulations and the National Research Council Guide for the Care and Use of Laboratory Animals. The animal facility is authorized by the French authorities (Dijon: Agreement B21231011EA). The study and all animal procedures were approved by the Institutional Animal Care and Use Committee of Oncodesign (Oncomet) approved by French authorities (CNREEA agreement number 91). At Crown Bioscience, animal care and experimental procedures were compliant with the UK Animals Scientific Procedures Act 1986 (ASPA) in line with Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. At National Research Council Canada, animals were maintained in accordance with the guidelines of the Canadian Council on Animal Care, and all experimental procedures were performed in accordance with regulations and guidelines reviewed and approved by the NRC Human Health Therapeutics Ottawa Animal Care Committee.

Background Immune-stimulating antibody conjugates (ISACs) covalently attach immune stimulants to tumor-targeting antibodies such as trastuzumab. We have shown that HER2-targeted TLR7/8 ISACs elicit robust myeloid activation and tumor eradication in a TLR- and Fc-dependent manner in trastuzumab-resistant and HER2-low models. Upon treatment with ISACs, T cell-mediated immunological memory extends to tumor antigens beyond HER2. Here we describe the ISAC mechanism of action in vivo that leads to eradication of tumors in mice.

Methods Established syngeneic rHER2- or xenograft HER2-expressing tumors treated with anti-HER2 ISACs or appropriate controls were assessed for gene expression by NanoString Pan-Cancer Immune Profiling panel comprising 750 genes related to tumor immune biology. Tumor cytokines were measured using MesoScale Discovery (MSD) technology, and immune cell infiltrates were assessed by immunohistochemistry (IHC). Anti-tumor efficacy was assessed after depletion of CD8+ T cells and phagocytes.

Results Within 24 hours of administration, HER2-directed ISACs induced robust, target-dependent activation of the immune system. In a syngeneic tumor model, 34% of the measurable genes were significantly upregulated after treatment with the rHER2-targeted ISAC vs 0.1% with the non-binding ISAC control. Similarly, 13% vs 0% of genes were upregulated in a xenograft model after HER2-targeted vs control ISAC treatment. In both models anti-HER2 ISAC treatment led to activation of pathways indicative of TLR7/8 agonism (e.g. IRF-7; type 1 interferons), and FcgR engagement (e.g. NF-kappaB associated genes). Cytokines and chemokines, including myeloid chemokines CCL2/3/4 and T cell chemokines CXCL9/10/11 were specifically upregulated in the tumors at the gene and protein level, indicating robust activation of myeloid cells following anti-HER2 ISAC treatment. Furthermore, in syngeneic tumors T cell activation markers (e.g. Granzyme B; IFN-gamma) were induced within 24 hours post treatment with an...
anti-rHER2 ISAC, and IHC at day 6 showed a 5-fold increase in CD11c+ cells. Control-treated tumors had sparse CD8+ T cells, whereas rHER2-targeted ISAC treatment led to ~3.5-fold increase in T cell frequency that shifted the tumor microenvironment from immunologically cold to hot. The recruitment of both phagocytes and CD8+ T cells was consequential, as depletion of either abrogated anti-tumor efficacy of the rHER2-targeted ISAC. Systemically delivered ISACs were well-tolerated.

Conclusions In contrast to other immune therapies, such as anti-PDL1/PD1 and anti-CD40, systemically administered ISACs locally engage both the innate and adaptive arms of the immune system to eradicate tumors. The molecular and cellular phenotype associated with ISAC-mediated activation is being evaluated in the on-going BDC-1001 Phase I/II clinical trial.2

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#606 EPIDERMAL GROWTH FACTOR MODULATION OF CXCL10 IN KERATINOCYTES AND CUTANEOUS CANCERS

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Background Epidermal growth factor (EGF) signaling has well-established roles in cellular proliferation in normal tissue homeostasis and tumorigenesis. EGF receptor inhibitor therapy is associated with the development of a papulopustular rash and other cutaneous inflammatory effects.1 2 These dose-dependent toxicities are linked to treatment response and survival, and may reflect the interplay between EGF and the immune response.3 4 However, the effects of EGF signaling on inflammation in the skin and elsewhere are not entirely understood.5 6 In this study, we aimed to elucidate the immunomodulatory role of EGF in human keratinocytes exposed to the proinflammatory cytokine interferon-γ (IFN-γ).

Methods Human keratinocyte cell lines (HaCaT) were exposed to IFN-γ, EGF, or both (48 hours). Differential gene expression analyses of RNA expression was performed using DESeq2.7 Fold change in gene expression on the log2 scale were calculated for each experimental treatment group relative to control. Web Gestalt was used to identify differentially expressed biologic pathways and gene networks, and further investigated in publicly available cutaneous squamous cell (cSCC) cell lines (GSE98767) and cSCC and basal cell carcinoma (BCC) tumor samples (GSE125285).8

Results As compared to untreated control cells, 2792 genes were differentially expressed following IFN-γ treatment, 938 following EGF treatment, and 1248 following the combination of IFN-γ and EGF (figure 1). To assess the impact of EGF on the cellular response to IFN-γ, we identified IFN-γ-induced genes whose expression was significantly altered by EGF (figure 2). We found that the induction of CXCL10 by IFN-γ was among those significantly attenuated in the presence of EGF (padjusted = 0.01) and selected CXCL10 as a model to further define the impact of EGF on immune gene expression. We found that in cutaneous SCC (cSCC) cell lines as well as cSCC and basal cell carcinoma tumor samples, the correlation between IFN-γ and CXCL10 expression was abrogated in samples with higher EGF expression (figure 3).

Conclusions EGF has pleotropic roles in cancer including immunologic effects relevant to anti-tumor immunity. These studies demonstrate that EGF alters the transcriptional response to IFN-γ including the induction of CXCL10 by IFN-γ. Moreover, these studies suggest that in the setting of high EGF levels, there is a modulation of IFN-γ-regulated chemokine expression. Further research is needed to clarify the

Abstract 606 Figure 1  EGF modulates IFN-γ-induced gene expression in human keratinocytes. A. Heatmap showing differentially expressed genes (padjusted < 0.01) induced by IFN-γ alone, EGF alone, or IFN-γ plus EGF (excluding genes that were not differentially expressed in any treatment group relative to control). B. Venn-diagram showing differentially expressed genes (padjusted < 0.01) induced by IFN-γ and/or EGF. C. Fold change of the top 10 genes induced after treated with IFN-γ alone. The top 10 genes which were induced by IFN-γ include CXCL10, CD74, several HLA-D genes, IDO1, GBP5, C15S, and BST2

Abstract 606 Figure 2  Pathway analysis of genes induced by IFN-γ then differentially regulated by EGF. A. Heatmap showing log2 fold change in gene expression of top IFN-γ-regulated genes whose expression was significantly dampened or augmented by EGF (Pinteraction < 0.05). The EGF* IFN-γ interaction fold-change (far left) column indicates the excess fold change due to interaction between EGF and IFN-γ. Within this column, blue and red shading indicates dampening and augmentation of IFN-γ-induced gene expression by EGF, respectively. B. Sub-network graph from Network Topology Analysis (NTA) of IFN-γ-regulated genes of which expression was either 2-fold higher or lower when EGF was added; genes in the top enriched GO Biological Process category are highlighted in red (GO:00019886 [antigen processing and presentation of exogenous peptide antigen via MHC class II]; Padjusted = 2.65 x 10-10); blue shading of CXCL10 denoting it as the most strongly upregulated gene by IFN-γ in this gene set to be dampened by EGF treatment. C. IFN-γ-induced genes attenuated by EGF, clustered according to significantly enriched KEGG pathways. Differentially expressed genes are listed in order of their score within the gene set enrichment analyses. Bolded italics type indicates common genes in multiple enriched pathways.
Abstract 606 Figure 3 Correlation between IFN-γ and CXCL10 expression stratified by EGF expression. A. Cutaneous squamous cell carcinoma cell lines (GSE98767, n=44). B. Cutaneous squamous and basal cell carcinoma tumor samples (GSE125285, n=35)

role of EGF in modulating inflammation, and to understand this process in the pathogenesis of EGF receptor inhibitor-induced cutaneous toxicities and skin cancers.

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Abstract 607 TJ210 (MOR210), A DIFFERENTIATED ANTI-CSAR ANTIBODY FOR ANTI-CANCER THERAPY

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Background Extensive investigations into the tumor microenvironment (TME) have uncovered molecular mechanisms linking aberrant complement activation and cancer progression. Specifically, C5a, as a highly potent chemoattractant, recruits immune suppressive myeloid derived suppressive cells (MDSCs), neutrophils and M2 macrophages into the tumor site and accelerates tumor progression. Blockade of C5a/C5Ar (CD88) pathway has been identified as a promising target to control MDSCs and restore tumor-killing ability of T and NK cells. TJ210, in licensed from MorphoSys as MOR210, is a differentiated anti-C5aR monoclonal antibody with a unique binding epitope.

Methods Interaction of TJ210 with C5aR was assessed through binding of the recombinant antigen, Flp-In CHO cells expressing C5aR and primary neutrophils. In vitro blockade of C5a/C5aR pathway was tested by inhibition of CD11b upregulation on granulocytes and monocytes induced by C5a, as well as neutrophil migration towards C5a. The in vitro synergistic effect of TJ210 with anti-PD-1 antibody was assessed in a T cell and differentiated MDSC co-culture system. The in vivo anti-tumor effect was tested in the MC38 syngeneic mouse model, in which mice were treated with a TJ210 mouse surrogate antibody either alone or in combination with an anti-PD-1 antibody.

Results TJ210 bound to C5aR with high affinity and did not cross-react with other GPCR members including C5L2, ChemR23, FPR1 and C3aR. Unlike the reference antibody, TJ210 specifically interacted with the N-terminus of C5aR but not extracellular loops. TJ210 effectively inhibited CD11b upregulation on granulocytes and monocytes as well as neutrophil migration mediated by C5a. When compared with the reference antibody, TJ210 maintained potent antagonism at high ligand concentrations and over longer duration, properties that might translate into beneficial in vivo effects at pathophysiological conditions. In the in vitro co-culture system, presence of TJ210 and anti-PD-1 antibody enhanced IFN-γ release compared to either single agent, indicating a synergistic effect on T cells. In the in vivo syngeneic mouse model, combination treatment effectively inhibited tumor growth. Immune cell population analysis revealed significant elevation of CD8+ T cells and M1 macrophages compared to mono-treatment.

Conclusions This series of in vitro and in vivo data demonstrate that TJ210 is a differentiated anti-C5aR antibody with unique binding epitope exhibiting superior anti-tumor potential especially in combination with an anti-PD-1 antibody. These data support further clinical studies of TJ210 in patients with solid tumors.

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608 IMMUNODOMINANT LISTERIA EPITOPES COMPETE WITH VACCINE-DIRECTED CD8+ T-CELL RESPONSES RESCUED BY PEPTIDE-MHC STABILIZING MODIFICATIONS

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Background The Gram-positive bacterium Listeria monocytogenes (Lm) is a promising vector for cancer immunotherapy due to its ability to directly infect antigen-presenting cells, induce potent CD8+ T-cell immunity, and remodel immunosuppressive tumor microenvironments. Recent clinical trials have demonstrated safety and immunogenicity of Lm-based cancer vaccines in lung, cervical, pancreatic, and other cancers. In colorectal cancer, the transmembrane receptor guanylyl cyclase C (GUCY2C) is an emerging target for immunother-

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robust Lm-specific CD8+ T-cell immunity but failed to prime GUCY2C-specific CD8+ T-cell responses. These studies explore the hypothesis that immunodominant Lm antigens suppress primary immunity to subdominant GUCY2C epitopes in Lm-GUCY2C.

Methods Lm-GUCY2C expresses the extracellular domain of mouse GUCY2C23-429 downstream of an ActA promoter integrated into the genome of the live, attenuated delta actA delta inlB Lm strain. Altered peptide ligands were designed based on NetMHCpan 4.0 peptide-MHC binding algorithms and similarly cloned into Lm. Peptide-MHC class I stability was quantified by FACS-based surface peptide-MHC dissociation on the TAP-deficient cell line, RMA-S H-2Kd. In vivo efficacy studies employed IFNγ-ELISpot quantification of T-cell responses and tumor challenge studies with the CT26 colorectal cancer cell line. Adenovirus expressing GUCY2C was used as a positive control.

Results Lm-GUCY2C vaccination of BALB/c mice generated Lm-specific CD8+ T-cell responses but an absence of GUCY2C-specific immunity. Peptide-MHC stability studies revealed poor stability of the dominant GUCY2C254-262 epitope complexed with H-2Kd compared to H-2Kd-restricted Lm epitopes derived from the LLO and p60 Lm antigens. Mutation of the GUCY2C254-262 peptide at critical anchoring residues for binding H-2Kd revealed that the altered peptide ligand with an F255Y mutation significantly improved the stability of the GUCY2C254-262-H-2Kd complex. Similarly, vaccination of mice with recombinant Lm-GUCY2C expressing the altered peptide ligand (Lm-GUCY2CF255Y) restored GUCY2C immunogenicity and anti-tumor immunity.

Conclusions Immunodominant Lm antigens may interfere with immune responses directed to the vaccine target antigen GUCY2C by competing with GUCY2C epitope for MHC class I binding and presentation. Moreover, use of a substituted GUCY2C -peptide ligand with enhanced peptide-MHC class I stability restored GUCY2C-specific immunity in the context of Lm-GUCY2C, an approach that can be translated to patients. Importantly, these studies also suggest that ongoing Lm-based vaccine development programs targeting a variety of antigens in other cancer types may be similarly limited by the immunodominance of Lm epitopes.

Acknowledgements The authors thank Dr. Peter Lauer for providing the pPL2 integration vector used in cloning Lm-GUCY2C and Dr. Sean Murphy for providing the RMA-S H-2Kd cell line.

Ethics Approval Studies were approved by the Thomas Jefferson University IACUC (Protocol # 01956).

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TECHNICAL CHALLENGES IN MONOCYTE-DERIVED DENDRITIC CELL VACCINE MANUFACTURING; A QI PROJECT

Background With the explosive growth of cancer immunotherapies, cancer vaccines have been in the spotlight for their ability to turn cold tumors hot. Particularly, dendritic cell vaccines (DCV) are capable of harnessing the immune system to recognize single or multiple epitopes as they are professional antigen presenting cells. However, DCVs have not been recognized as the platform of choice in many studies due to relatively high cost, difficulty in standardizing manufacturing methods and risk of product inconsistency. We have been using monocyte-derived DCs transduced with an adenovirus vector expressing HER2/neu in a clinical trial to treat HER2-expressing cancers. The vaccine was administer d on weeks 0, 4, 8, 16 and 24 at 4 different dose-
levels; 5 \times 10^6, 10 \times 10^6, 20 \times 10^6 and 40 \times 10^6 viable cells. The clinical outcome of the study is under analysis. To further optimize the safety and consistency of DCV, we reviewed the issues encountered in a first-in-human clinical trial during the manufacture of these cells at the NIH Clinical Center.

**Methods** Manufacturing records of NCT01730118 A Phase I Study of an Autologous DCV Targeting HER2 in Solid Tumors were reviewed to identify any complications or deviations encountered during manufacturing from apheresis to delivery of the fresh DCVs (figure 1).

**Results** Between April 2013 and October 2019, 134 vaccines were manufactured for 33 patients. A total of 113 (84%) DCVs were administered, with 103 (91%) of those meeting release criteria, and the remaining administered under authorized medical exception (AME). All patients underwent a single apheresis collection with 18 (median, range 15–20) liters processed and a goal of 6 aliquots (333 \times 10^6 monocytes/vial). Dual lumen catheterization was required in 23 (70%) patients, and all procedural reactions required no or minimal intervention. Summaries enumerate aberrancies encountered during the manufacturing process (table 1). Overall, interpatient variabilities may have contributed to 92 (78%) events, while 26 (22%) events arose in a ‘controllable’, patient-unrelated environment.

**Conclusions** In spite of the variable events encountered during the manufacturing process, the majority of products were administered successfully. Patient-related variabilities were linked to most of the events. Future studies should be designed to minimize the impact of such variabilities on DCVs to provide high-quality personalized therapies. Manufacturing one large lot of DCs and cryopreserving enough aliquots for the entire study and the incorporation of an automated, closed cell culture system may reduce the aforementioned incidents and improve product quality.

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Ethics Approval
The study was approved by NCI/NIH Institutional Review Board (#534360, 13C0016).

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RNA-SEQUENCING REVEALS A UNIQUE IMMUNE TRANSCRIPTIONAL LANDSCAPE IN THE VACCINE SITES OF PATIENTS WITH CIRCULATING T-CELL RESPONSES TO CANCER IMMUNIZATION

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Background
Vaccines are a promising therapeutic for patients with advanced cancer, but achieving robust T-cell responses remains a challenge. Melanoma-associated antigen-A3 (MAGE-A3) in combination with adjuvant AS15 (a formulation of...
Toll-Like-Receptor (TLR)-4 and 9 agonists and a saponin), induced systemic CD4+ T-cell responses in 50% of patients when given subcutaneously/intradermally. Little is known about the transcriptional landscape of the vaccine-site microenvironment (VSME) of patients with systemic T-cell responses versus those without. We hypothesized that patients with systemic T-cell responses to vaccination would exhibit increased immune activation in the VSME, higher dendritic cell (DC) activation/maturation, TLR-pathway activation, and enhanced Th1 signatures.

Methods Biopsies of the VSME were obtained from participants on the Mel55 clinical trial (NCT01425749) who were immunized with MAGE-A3/AS15. Biopsies were taken 8 days after immunization. T-cell response to MAGE-A3 was assessed in PBMC after in-vitro stimulation with recMAGE-A3, by IFNγ ELISPOT assay. Gene expression was assessed by

Abstract 611 Figure 2 Expression of T-cell markers in IR vs NR vs Control samples in the vaccine site microenvironment (VSME). (A) T-cell markers showing similar expression in IR vs NR but higher expression in IR vs control. (B) Markers of dendritic cell activation and maturation in the VSME which are higher in IR vs control but not IR vs NR. (B) Transcription factors and genes associated with Th1/Th2 responses within the VSME. (D) Genes associated with T-cell exhaustion at the VSME. (E) Expression of TLR pathway genes in the VSME. Expression data is provided in terms of normalized counts. Bars demonstrate median and interquartile range. N=9. IR = immune responder, NR = non-responder, TLR = Toll-like Receptor. * = <0.01, ** < 0.001, *** <0.0001, **** < 0.00001
Human CLEC9A antibodies deliver NY-ESO-1 antigen to CD141+ dendritic cells to activate naïve and memory NY-ESO-1-specific CD8+ T cells

Background Dendritic cells (DC) are crucial for the efficacy of cancer vaccines, but current vaccines do not harness the key cDC1 subtype required for effective CD8+ T cell-mediated tumor immune responses. Vaccine immunogenicity could be enhanced by specific delivery of immunogenic tumor antigens to CD141+ DC, the human cDC1 equivalent. CD141+ DC exclusively express the C-type-lectin-like receptor CLEC9A, which is important for the regulation of CD8+ T cell responses. This study developed a new vaccine that harnesses a human anti-CLEC9A antibody to specifically deliver the immunogenic tumor antigen, NY-ESO-1 to human CD141+ DC. The ability of the CLEC9A-NY-ESO-1 antibody to activate NY-ESO-1-specific naïve and memory CD8+ T cells was examined and compared to a vaccine comprised of a human DEC-205-NY-ESO-1 antibody that targets all human DC.

Methods Human anti-CLEC9A, anti-DEC-205 and isotype control IgG4 antibodies were genetically fused to NY-ESO-1 polypeptide. Cross-presentation to NY-ESO-1-epitope specific CD8+ T cells and reactivity of T cell responses in melanoma patients was assessed by IFNγ production following incubation of CD141+ DC and patient peripheral blood mononuclear cells with targeting antibodies. Humanized mice containing human DC subsets and a repertoire of naïve NY-ESO-1-specific CD8+ T cells were used to investigate naïve T cell priming, T cell effector function was measured by expression of IFNγ, MIP-1β, TNF and CD107a and by lysis of target tumor cells.

Results CLEC9A-NY-ESO-1 Ab were effective at mediating delivery and cross-presentation of multiple NY-ESO-1 epitopes by CD141+ DC for activation of NY-ESO-1-specific CD8+ T cells. When benchmarked to NY-ESO-1 conjugated to an untargeted control antibody or to anti-human DEC-205, CLEC9A-NY-ESO-1 was superior at ex vivo reactivation of NY-ESO-1-specific T cell responses in melanoma patients. Moreover, CLEC9A-NY-ESO-1 induced priming of naïve NY-ESO-1-specific CD8+ T cells with polyclonal effector function and potent tumor killing capacity in vitro.

Conclusions These data advocate human CLEC9A-NY-ESO-1 antibody as an attractive strategy for specific targeting of CD141+ DC to enhance tumour immunogenicity in NY-ESO-1-expressing malignancies.

Ethics Approval Written informed consent was obtained for human sample acquisition in line with standards established by the Declaration of Helsinki. Study approval was granted by the Mater Human Research Ethics Committee (HREC13/MHS/83 and HREC13/MHS/86) and The U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections, Human Research Protection Office (HRPO; A-18738.1, A-18738.2, A-18738.3). All animal experiments were approved by the University of Queensland Animal Ethics Committee and conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes in addition to the laws of the United States and regulations of the Department of Agriculture.

Immuno-conjugates and chimeric molecules

HER2-xPAt, a novel protease-activatable prodrug T cell engager (TCE), with potent T-cell activation and efficacy in solid tumors and large predicted safety margins in non-human primate (NHP)

Background TCEs are effective in leukemias but have been challenging in solid tumors due to on-target, off-tumor toxicity. Attempts to circumvent CRS include step-up dosing and/or complex designs but are unsuccessful due to toxicity and/or enhanced immunogenicity. HER2-xPAt, or XTNylated Protease-Activated bispecific T-Cell Engager, is a prodrug TCE that exploits the protease activity present in tumors versus healthy tissue to expand the therapeutic index (TI). The core of the HER2-xPAt (PAT) consists of 2 tandem scFvs targeting
CD3 and HER2. Attached to the core, two unstructured polypeptide masks (XTEN) sterically reduce target engagement and extend T1/2. Protease cleavage sites at the base of the XTEN masks enable proteolytic activation of XPATs in the tumor microenvironment, unleashing a potent TCE with short T1/2, further improving the TI. HER2-XPAT, a tumor protease-activatable prod rug with wide safety margins, can co-opt T-cells regardless of antigenic specificity to induce T-cell killing of HER2+ tumors.

Methods Preclinical studies were conducted to characterize the activity of HER2-XPAT, HER2-PAT (cleaved XPAT), and HER2-NonClv (a non- cleavable XPAT) for cytotoxicity in vitro, for anti-tumor efficacy in xenograft models, and for safety in NHPs.

Results HER2-PAT demonstrated potent in vitro T-cell cytotoxicity (EC50 1-2pM) and target-dependent T-cell activation and cytokine production by hPBMCS. HER2-XPAT provided up to 14,000-fold protection against killing of HER2 tumor cells and no cytotoxicity against cardiomyocytes up to 1uM. In vivo, HER2-XPAT induced complete tumor regressions in BT-474 tumors with equimolar dosing to HER2-PAT, whereas HER2-NonClv had no efficacy, supporting requirement of protease cleavage for T-cell activity. In NHP, HER2-XPAT has been dose-escalated safely up to 42mg/kg (MTD). HER2-XPAT demonstrated early T-cell margination at 2 mg/kg but largely spared CRS, cytokine production, and tissue toxicity up to 42 mg/kg. PK profiles of HER2-XPAT and HER2-NonClv were comparable, consistent with in vivo stability for cleavage when incubated in cancer pts plasma for 7 days at 37°C. HER2-PAT by continuous infusion induced lethal CRS and cytokine spikes at 0.3 mg/kg/d but was tolerated at 0.25 mg/kg/d, providing HER2-XPAT with >1300-fold protection in tolerability vs. HER2-PAT, >4 logs over cytotoxicity EC50s for HER2 cell lines, and a 20-fold safety margin over the dose required for pharmacodynamic activity.

Conclusions HER2-XPAT is a potent prodru g TCE with no CRS and a wide TI based on NHPs. With XTEN's clinical data demonstrating low immunogenicity, the XPATs are a promising solution. IND studies are ongoing. Additional PK/PD, cytokines, safety, and efficacy data will be presented.

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614 CO-STIMULATION VIA PD1–41BB CHIMERIC SWITCH RECEPTOR ENHANCES FUNCTION OF TCR-T CELLS IN AN IMMUNE-SUPPRESSIVE MILIEU AND UNDER CHRONIC ANTIGEN STIMULATION

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Background The immunosuppressive tumor microenvironment (TME) of solid tumors negatively influences the efficacy and fitness of tumor-specific T cells and can render them non-functional. In this repressive tumor milieu, expression of inhibitory immune checkpoint molecules and cytokines as well as deprivation of essential metabolic factors contribute to T cell exhaustion and reduced T cell infiltration. Due to these harsh conditions found in the TME of solid tumors, successful treatment of non-hematological cancer indications with T cell-based immunotherapies remains challenging. New strategies are required to equip therapeutic tumor-specific T cells with the necessary traits to overcome inhibitory signals in the TME and increase T cell persistence in an environment lacking essential metabolic nutrients, like oxygen or glucose. To enhance the clinical efficacy of TCR-T cells in treatment of solid tumors, we generated a chimeric receptor that combines the co-stimulatory domain of 4-1BB with the extracellular domain of PD-1. Expression of this chimeric PD1-41BB switch receptor in TCR-T cells should reverse the inhibitory signal induced by the PD-1/PD-L1 interaction and provide additional co-stimulation to increase functionality and persistence.

Methods Using 2D and 3D in vitro model systems we mimic immunosuppressive conditions in the TME of solid tumors, including low glucose and high TGFβlevels as well as repeated tumor cell challenge. We evaluate the ability of the chimeric PD1-41BB switch receptor to enhance TCR-T cell activity and functionality under these repressive conditions.

Results Our results demonstrate that TCR-T cells expressing the chimeric PD1-41BB switch receptor show an increased capacity to recognize and kill tumor cells during chronic stimulation with antigen. The enhanced functionality of PD1-41BB-TCR-T cells allows them to eradicate tumor cells even in the presence of additional immunosuppressive factors, including nutrient starvation and expression of inhibitory PD-L1 checkpoint molecules. Furthermore, PD1-41BB-expressing TCR-T cells show a higher persistency and proliferation rate in these challenging co-culture model systems.

Conclusions Equipping therapeutic T cells with the chimeric PD1-41BB switch receptor enhances T cell functionality under immunosuppressive conditions and counters checkpoint-mediated dysfunction. For the treatment of PD-L1-positive malignancies, expression of PD1-41BB by TCR-T cells has the potential to greatly improve the targeting of solid tumors using T cell-based immunotherapies. These preclinical studies support our approach to enhance the clinical efficacy of TCR-T therapies of solid tumors using the chimeric PD1-41BB switch receptor. Subsequent in vivo studies and safety evaluations will pave the way for clinical use of PD1-41BB in adoptive T cell therapy.

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615 TARGETED IMMUNE CELL ACTIVATION BY SYSTEMIC DELIVERY OF TOLL-LIKE RECEPTOR 9 AGONIST ANTIBODY CONJUGATES INDUCE POTENT ANTI-TUMOR IMMUNITY

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Background Toll-like receptor (TLR) pathways play a crucial role in mounting potent innate immune responses against invading pathogens, as well as the subsequent engagement of adaptive immunity. Innate immune activation via the TLR9 pathway has potential for treating cancer as demonstrated clinically with TLR9 agonists administered intra-tumorally in melanoma patients. We developed a novel toll-like receptor agonist antibody conjugate (TRAAC) platform to systemically deliver a differentiated, targeted TLR9 agonist (T-CpG) for immune activation. The activation of TLR9 pathways can be directed systemically towards specific immune cell populations and tumor microenvironment via antibodies binding to various
immune cell receptors. Using multiple TRAACs targeting either immune cells including plasmacytoid DCs (pDCs), myeloid and B lymphocytes, or tumor specific antigens, we evaluated immune modulatory phenotypes, therapeutic potentials, as well as safety and tolerability of this platform in pre-clinical settings.

**Methods** TRAACs were generated using site-specific conjugation. In vitro activity of immune- and tumor-targeted antibody-CpG conjugates was evaluated using human PBMCs. Anti-tumor efficacy and mechanistic assessment of B lymphocyte and myeloid cell-targeted antibody-CpG conjugates were conducted in syngeneic tumor models. Pharmacokinetic (PK), pharmacodynamic and exploratory toxicity evaluations were performed in non-human primates (NHP).

**Results** T-CpG is comprised of monomeric CpG-containing oligonucleotides optimized for potency and stability as an antibody conjugate. TRAACs targeting immune cells enable directed TLR9 activation leading to potent cytokine production and cellular activation that is superior to free CpG. This targeted immune activation also elicits a cascade of downstream modulation of non-targeted immune cells. When administrated systemically in multiple syngeneic models, murine TRAACs targeting either immune cells or tumor antigens exhibited potent, durable, and dose-dependent anti-tumor activity as a single agent and in combination with T-cell checkpoint inhibitors (CPIs). A single peripheral dose of either B lymphocyte or myeloid targeted-CpG evoked both innate and adaptive immune responses within the tumor microenvironment as demonstrated by NanoString analysis. The observed immunomodulatory phenotypes are consistent with those elicited by direct intra-tumoral CpG delivery. Following repeated intravenous doses in NHP, TRAACs demonstrated targeted receptor occupancy, antibody-like PK, and favorable tolerability profile.

**Conclusions** Pre-clinical evaluation of a novel platform comprised of antibodies conjugated to a differentiated TLR9 agonist demonstrated targeted immune activation, potent anti-tumor activity as single agent and in combination with CPIs and favorable tolerability profiles in NHPs. Such antibody-CpG conjugates have the potential for clinical development as systemically delivered therapeutics providing powerful innate and adaptive anti-tumor immunity across multiple tumor types.

**REFERENCE**


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**Abstract 616**

**A NOVEL NON-COVALENT LINKER PEPTIDE WITH NANOMOLAR AFFINITY FOR CLINICAL IGG1 ANTIBODIES PRESERVES ANTIBODY-ANTIGEN AFFINITY AND DRUG POTENCY AGAINST PDL1+ MELANOMA WHEN CONJUGATED WITH DM1**

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**Background** Antibody-drug conjugates (ADC) increase the efficacy of current chemotherapeutics, decrease off site toxicity, and pair drug function with immunomodulatory effects. Current ADC platforms depend on the use of covalent linker molecules between the antibody and the drug of choice. This approach leads to significant variation in the number of drug molecules bound, the location of their binding, and inconsistency in maintaining the structure and antigen affinity of the antibody. Because of this, covalent-based ADC development requires extensive separation steps to isolate the ideal isotypes of the ADC. This testing and separation must be repeated for each antibody and each drug considered. Here we present a peptide that non-covalently binds multiple clinically relevant IgG1 antibodies at a controlled ratio and location, then demonstrate its use as a modular ADC linker platform.

**Methods** Peptide-antibody and antibody-antigen affinity were determined using Biacore surface plasmon resonance. Peptides conjugated with alexafluor or DM1 were purified using HPLC and structure was confirmed through mass spectrometry. Flow cytometry verified delivery of peptide-atezolizumab conjugates...
to C1861 PDL1+ melanoma cells. Peptide-DM1 potency was determined in-vitro using a calcine-AM and propidium iodine live/dead cell double staining.

**Results** Antibody-Binding Peptide Linker (APL) was developed from a series of space filling amino acid substitutions at key residues on an 18-mer peptide derived from a hydrophobic pocket on human albumin (figure 1a). A lysine containing tail was added to the C-terminus for conjugation to small molecule therapeutics through amine coupling. APL has nanomolar binding affinity for the fab region of IgG1 antibodies including rituximab (KD= 1.85 x 10^-8), bevacizumab (KD= 5.2 x 10^-8), trastuzumab (KD= 8.87 x 10^-8), and atezolizumab (KD= 3.78 x 10^-8) (figure 1b). Kinetic binding models, performed by Biacore surface plasmon resonance, showed a 2:1 association of peptide to antibody. All four antibodies retained their antigen affinity when bound by APL (figure 2a). Labeling of APL with an alexafluor showed delivery to PDL1+ melanoma cells when given bound to the anti-PDL1 antibody atezolizumab (figure 2b). Conjugation of APL with the tubulin inhibitor DM1 (figure 2c) resulted in a drug conjugated peptide that retained the potency of the drug itself (figure 2d).

**Conclusions** Antibody-Binding Peptide Linker (APL) non-covalently binds clinical IgG1 antibodies at a fixed two to one ratio without affecting antigen affinity. Conjugation of APL with a drug of choice provides a modular Antibody-Drug Conjugate platform where both the antibody and drug can be substituted with ease.

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**TISOTUMAB VEDOTIN SHOWS IMMUNOMODULATORY ACTIVITY THROUGH INDUCTION OF IMMUNOGENIC CELL DEATH**

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**Background** Tisotumab vedotin (TV) is an investigational antibody-drug conjugate composed of a tissue factor (TF)-directed human monoclonal antibody covalently linked to the microtubule-disrupting agent monomethyl auristatin E (MMAE) via a protease-cleavable linker. TV demonstrated single agent activity (24% objective response rate [ORR]) in previously treated recurrent or metastatic cervical cancer (NCT03438396) where currently, there is no standard of care and ORRs are typically less than 15% and often of limited duration.1-8 TV is currently being evaluated in combination with pembrolizumab (PD-1 inhibitor), bevacizumab, or carboplatin in cervical cancer (NCT03786081), or as a monotherapy in multiple other solid tumors (NCT03913741, NCT03485209, NCT03657043). The anti-tumor activity of TV may be multimodal as TV can induce tumor cell death through several mechanisms, including direct and bystander MMAE-mediated cytotoxicity, as well as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and immunogenic cell death (ICD).9,10 To better characterize immune-mediated tumor cell killing by TV and further the rationale for combination with pembrolizumab, we set out to refine our understanding of TV-mediated ICD and subsequent immunomodulatory effects.

**Methods** We evaluated the ability of TV to mediate activation of immune cells in vitro using co-cultures of TF-expressing tumor cells and human peripheral blood mononuclear cells (PBMCs). We also assessed the ability of TV to induce recruitment of innate immune cells to tumors in vivo using a TF-expressing xenograft tumor model.

**Results** In vitro, tumor cells treated with TV showed several hallmarks of immunogenic cell death, including markers of endoplasmic reticulum (ER) stress and release of ATP and high mobility group protein B1 (HMGB1). Co-culture of TV-killed tumor cells with allogeneic human PBMCs led to innate immune cell activation (measured by upregulation of the costimulatory molecule CD86) and T cell proliferation. Combination with PD-1 blockade further amplified the immune response, leading to enhanced T cell proliferation and cytokine production. Moreover, in vivo studies demonstrated that TV treatment led to recruitment of F4/80+ and CD11c+ innate immune cells to xenograft tumors.

**Conclusions** These data show that, in preclinical models, TV induces immunogenic tumor cell death, which can promote activation and recruitment of immune cells to the tumor. The totality of in vitro and in vivo data provides evidence for the immunomodulatory effects of TV and bolsters rationale for combining TV with immune checkpoint agents. Ongoing analyses aim at further characterizing the immune response induced by TV in preclinical models and patients.

**Acknowledgements** We would like to thank Kristen Gahnberg for embedding and sectioning tissues for these studies and Anthony Cao for his early work identifying hallmarks of ICD in response to TV treatment in vitro.9

**Ethics Approval** Animals studies were approved by and conducted in accordance with Seattle Genetics Institutional Care and Use Committee protocol #SGE-029.

**Consent** N/A

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**REFERENCES**

Background Effective cancer treatment requires durable elimination of malignant cells. Cytotoxic chemotherapeutic agents used to treat cancer often show initial anti-tumor efficacy, but fail to produce long-term durable responses in patients. The elicitation of durable responses and improved survival in response to cytotoxic agents may be associated with the induction of innate and adaptive immune response to the cancer. For example, tumor cells undergoing apoptosis following exposure to some cytotoxic agents emit immunostimulatory damage-associated molecular patterns (DAMPs), this form of cell death is termed immunogenic cell death (ICD). ICD can promote the recruitment and activation of both the innate and adaptive immune system, providing an additional mechanism to drive an anti-tumor response.

Methods Vedotin-based antibody drug conjugates (ADCs) drive cytotoxicity in tumor cells by engaging tumor antigens on the cell surface, internalizing with the cell surface antigen, and delivering monomethyl auristatin E (MMAE) payload. Following intracellular delivery, MMAE induces mitotic arrest, as well as an endoplasmic reticulum (ER) stress response resulting from microtubule disruption. Following tumor cell treatment, indicators of the ER stress response are observed with vedotin-based ADCs including induction of phospho-JNK and CHOP. This mechanism of MMAE induced ER stress results in emission of hallmark ICD DAMPs including cell-surface calreticulin, extracellular release of HMGB1 and ATP. In this presentation we highlight the ability of MMAE to induce the hallmarks of ICD in multiple cancers across different tissue origins using distinct valine-citrulline-MMAE (vedotin)-based ADCs.

Results The culmination of these ICD hallmarks resulted in innate immune cell activation in vitro and in vivo in mouse xenograft models. Tumor bearing mice treated with vedotin-based ADCs resulted in the promotion of immune cell recruitment and activation in tumors. Analysis of immune activation by vedotin-based ADCs included production of innate cytokines and upregulation of HLA/MHC-Class II expression, which supports a role in activating both the innate and adaptive immune response. To further our understanding of the potent and broad ability of vedotin ADCs to induce ICD, we have also begun to examine the ICD potential of different classes of ADC payloads including other microtubule inhibitors (auristatins and maytansines), and DNA damaging agents (DNA alkylators or topoisomerase inhibitors). Initial data indicate differences in ICD induction by these agents.

Conclusions These results help build the rationale for vedotin-based ADCs as preferred partners for immune checkpoint blockade agents.

Ethics Approval Studies with human samples were performed according to institutional ethics standards. Animal studies were approved by and conducted in accordance with Seattle Genetics Institutional Care and Use Committee protocol #SGE-029.
TUMOR CELL-INTRINSIC STING PATHWAY IS ACTIVATED IN THE PRESENCE OF CUES FROM IMMUNE CELLS AND CONTRIBUTES TO THE ANTI-TUMOR ACTIVITY OF TUMOR CELL-TARGETED STING AGONIST ANTIBODY-DRUG CONJUGATES

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Background STING pathway agonism has emerged as a potential therapeutic mechanism to stimulate an innate anti-tumor immune response. While in principle systemic administration of a STING agonist would have many therapeutic benefits, including the delivery of STING to all tumor lesions, such an approach may be limited by toxicity. Antibody-drug conjugates (ADCs) constitute a proven therapeutic modality that is ideally suited to allow systemic administration while stimulating the innate immunity in a targeted manner. We have previously demonstrated that targeted delivery of a STING agonist with an ADC induces robust anti-tumor immune responses.

Methods Herein we investigated the mechanism of action of tumor cell-targeted STING agonist ADCs. We evaluated STING pathway activation and anti-tumor activity elicited by ADCs harboring either wild type (wt) or mutant Fc receptor (FcγR) binding in wt or STING knockout (ko) cancer cell mono-cultures, immune cell co-cultures, and in vivo tumor models.

Results Consistent with previous reports, the majority of cancer cell lines tested failed to induce STING pathway following STING agonist payload treatment in mono-cultures. In cancer cell:THP1 monocyctic cell co-cultures, tumor-targeted STING agonist ADCs with wt Fc exhibited robust STING activation, whereas Fc-mutant ADCs or non-targeted control ADCs had minimal activity. Similar results were obtained when THP1 cells were treated in plates coated with target antigen without cancer cells, demonstrating STING activation in THP1 cells following FcγR-mediated uptake of antigen-bound ADCs. Tumor-targeted Fc-wt ADCs led to marked induction of STING pathway and cancer cell-killing in cancer cell:PBMC or primary monocyte co-cultures, and complete tumor regressions in vivo tumor models. Surprisingly, while at reduced levels relative to the Fc-wt ADCs, Fc-mutant ADCs exhibited significant activity in these in vitro and in vivo models, suggesting that tumor cell-intrinsic STING pathway may be activated in the presence of cues from immune cells. Consistently, STING agonist payload treatment in the presence of conditioned media from PBMC and primary monocyte but not from THP1 cultures, led to STING activation in cancer cell mono-cultures. Moreover, Fc-mutant ADCs had diminished activity in STING ko cancer cells: PBMC or primary monocyte co-cultures, demonstrating the contribution of tumor cell-intrinsic STING activation to the anti-tumor activity elicited by tumor cell-targeted STING agonist ADCs.

Conclusions In conclusion, we demonstrated that tumor cell-targeted STING agonist ADCs induce robust anti-tumor activity through mechanisms involving both FcγR and tumor antigen-mediated ADC internalization and subsequent induction of STING pathway in immune cells and tumor cells.

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A TREGS DEPLETING, CD25-TARGETED ANTIBODY-DRUG CONJUGATE SYNERGIZES WITH TUMOR-TARGETED RADIOTHERAPY AND SYSTEMIC INTERLEUKIN-2 IN PRE-CLINICAL MODELS OF SOLID CANCERS

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Background Regulatory T cells (Tregs) contribute to an immunosuppressive tumor microenvironment. High tumor infiltration by Tregs and a low ratio of Teffector cells/Tregs is often associated with poor prognosis in solid tumors. Tregs represent a major obstacle to cancer immunotherapies, including checkpoint inhibitors and interleukin-2 (IL-2) and are associated with tumors resistance to radiotherapy.2 CD25-ADC (a.k.a. sur301) is an antibody-drug conjugate (ADC) composed of rat monoclonal antibody PC61, directed against mouse CD25, conjugated to tesirine, a pyrrolobenzodiazepine (PBD) dimer-based protease-cleavable linker.3 Previously we showed that single low doses of CD25-ADC resulted in potent and durable antitumor activity in established syngeneic solid tumor models and the combination of a suboptimal dose was synergistic with PD-1 blockade. Tumor eradication by CD25-ADC was CD8+ T cell-dependent and it induced protective immunity. Importantly, while CD25-ADC mediated a significant and sustained intratumoral Tregs depletion, accompanied by a concomitant increase in the number of activated and proliferating tumor-infiltrating CD8+ T cells, systemic Tregs depletion was transient, alleviating concerns of potential autoimmune side effects.3

Methods Here we evaluated the anti-tumor activity of CD25-ADC combined with tumor-targeted radiotherapy (RT) or systemic IL-2 in syngeneic solid tumor models.

Results To investigate the combination with radiotherapy, single low doses of CD25-ADC (0.25 or 0.5 mg/kg) were administered intravenously either alone or in combination with image-guided focal radiation in the CT26 syngeneic model. Both doses of CD25-ADC alone induced significant anti-tumor activity compared to the vehicle control. Combination of CD25-ADC, at 0.25 or 0.5 mg/kg, with focal radiotherapy resulted in synergistic anti-tumor activity with 60% and 80% tumor-free survivors (TFS), respectively, at the end of the study. Moreover, re-challenged TFS did not develop new tumors, demonstrating development of tumor-specific protective immunity. The combination of CD25-ADC and systemic IL-2 was investigated in the MC38 syngeneic model. CD25-ADC was administered intravenously either alone (0.25 or 0.5 mg/kg, single dose) or in combination with IL-2 (0.1 or 0.8 mg/kg). Single doses of CD25-ADC elicited significant anti-tumor activity compared to the vehicle control. Combination of CD25-ADC with IL-2 resulted in enhanced anti-tumor activity in both combination groups compared to the respective single agents and the combination was synergistic at the highest IL-2 dose.

Conclusions Together, these new preclinical data show novel promising combination regimens for CD25-ADC and other commonly used anti-cancer treatments and they provide rationale for the investigation of camidanlumab tesirine (ADCT-301), a PBD-based ADC targeting human CD25, in similar clinical combinations settings.

REFERENCES
PD-L1 IS A POTENTIAL PREDICTIVE BIOMARKER FOR RESPONSE TO RM-1929 TREATMENT IN RECURRENT HEAD AND NECK SQUAMOUS CELL CARCINOMA PATIENTS

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Background: RM-1929 is an antibody-dye conjugate comprised of cetuximab covalently linked to the photoactivable dye, IRDye® 700DX (IR700). After systemic infusion of RM-1929, illumination of the tumor with 690 nm non-thermal red light activates the drug and results in targeted and rapid tumor necrosis. Previous preclinical data have shown that RM-1929 treatment triggers immunogenic cell death and activates the innate and adaptive immune response. A retrospective analysis of PD-L1 expression from the phase I/II clinical trial in patients with recurrent head and neck squamous cell carcinoma (rHNSCC) (NCT02422979) was conducted. The analysis explored correlations of PD-L1 expression, including combined proportion score (CPS) and tumor proportion score (TPS), with clinical outcomes such as response rate and overall survival.

Methods: PD-L1 expression prior to RM-1929 treatment was assessed by immunohistochemistry in 18 out of 30 patients enrolled in Part II of the trial, based on sample availability. PD-L1 expression was evaluated using TPS and CPS. Responders were defined as patients that achieved complete response or partial response, and non-responders had either stable disease or progressive disease. Overall survival (OS) was analyzed using the Kaplan-Meier method.

Results: Responders (n=10) had a TPS of 4.3±2.4 (mean ±SEM), which was substantially lower than in non-responders (n=8) with a TPS of 39.4±11.8. Similarly, CPS was lower in responders (6.2±3.6) compared to non-responders (50.0±13.5). The best target response rate for all patients included in this analysis was 56%. Patients with CPS=40 had a response rate of 76.9% (n=13) compared to 0% in patients with CPS>40 (n=5). This suggests that a CPS cut-off of =40 led to enrichment of the best target response rate. The median OS of patients with CPS=40 (13.0±2.9 months) was also higher than in patients with CPS>40 (3.1±0.8 months) and in all patients (12.0±2.9 months).

Conclusions: These results suggest that rHNSCC patients with lower PD-L1 expression levels may be more responsive to RM-1929 treatment and CPS/TPS could potentially be predictive biomarkers in identifying patients with a higher probability of benefiting from this treatment. Given the limited number of patients in this analysis, additional clinical trials will be needed to validate PD-L1 expression as an effective predictive biomarker for RM-1929 treatment.

Acknowledgements: The authors would like to thank all patients and their families for their participation in this trial. The authors would also like to thank the following investigators for the contribution of samples included in this trial analysis: Dr. David Cognetti (Thomas Jefferson University Hospital), Dr. Ann M Gillenwater (University of Texas MD Anderson Cancer Center), Dr. Mary Jo Fidler (Rush University Medical Center), Dr. Samith T. Kochuparambil (Virginia Piper Cancer Institute), Dr. John Campagna (University of Colorado Head and Neck Specialists), and Dr. Nilesh R. Vasan (University of Oklahoma Health Sciences Center).

Trials Registration: NCT02422979

Ethics Approval: The trial was approved by the following Institutions Ethics Boards and IRB#: as listed: UCSF Institutional Review Board (#17-21904); Thomas Jefferson University, IRB (#16C.328), University of Oklahoma Health Sciences Center Institutional Review Board (#5723), University of Texas MD Anderson Cancer Center - Institutional Review Board (#IRB 2 IRB00002203), Quorum Review IRB (#30458/1), Rush University Medical Center Institutional Review Board (#1503601-IRB01), and Catholic Health Initiatives Institute for Research and Innovation (CIRI) Institutional Review Board (CHIRB) (# IRB0009715).

Consent: N/A

IMMUNO-STATS: LEVERAGING PROTEIN ENGINEERING TO EXPAND AND TRACK ANTIGEN-SPECIFIC T CELLS IN VIVO

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Background: Immunotherapies are highly promising and effective strategies for the treatment of cancer; however, continuing challenges persist, including 1) untargeted global immune modulation, resulting in serious side effects; 2) lack of therapeutics capable of in vivo expansion of tumor-specific T cells; 3) inability to visualize in vivo tumor-specific T cell responses; and 4) lack of flexible platforms to rapidly and efficiently explore new therapeutic strategies and immune-escape mechanisms. To address these challenges, we developed a novel class of precision biologics to treat cancer, autoimmune diseases and infectious diseases. We describe a modular platform constructed around an Fc-based covalent pMHIC dimer, referred to as synTac (artificial synapse for T cell activation; also termed Immuno-STATs for Selective Targeting and Alteration of T cells), which selectively delivers different cargoes, including costimulatory, coinhibitory or cytokine signals and other modalities to primary T cells of defined specificity. The inherent modularity supports broad applications. Changing the encoded peptide enables targeting of different T cell specificities to address different diseases, while altering the cargo allows for evaluation of different co-modulatory mechanisms or the delivery of mechanistically informative probes.

Methods: Sortase A-mediated enzymatic coupling supported site-specific and stoichiometric installation of positron emission tomography (PET)-active radiolabels on synTacs to visualize the in vivo localization of antigen-specific T cells. The NSG humanized mouse model allowed for the evaluation of synTacs/Immuno-STATs to drive the in vivo antigen-specific expansion of human CD8 T cells.
**Results** Using radiolabeled synTacs/Immuno-STATs loaded with the appropriate peptides, we employed positron emission tomography to localize human papillomavirus (HPV-16)-specific CD8 T cells to implanted HPV-16-positive tumors in mice, as well as influenza A virus (IAV)-specific CD8 T cells in the lungs of IAV-infected mice. In vivo administration of HIV- and CMV-specific synTacs/Immuno-STATs to immunodeficient mice intrasplenically engrafted with human donor PBMCs resulted in the marked and selective expansion of HIV-specific and CMV-specific human CD8 T cells populating their spleens, respectively.

**Conclusions** We demonstrate the remarkable flexibility of the synTacs/Immuno-STAT platform for addressing a broad range of applications, including the first report of the in vivo imaging of antigen-specific CD8 T cell populations and in vivo antigen-selective expansion of human CD8 T cells. These results suggest that, in addition to broad therapeutic applications, synTacs/Immuno-STATs may provide prognostic/diagnostic information. Most notably, these results demonstrate the presence of synTacs/Immuno-STAT biologics in the tumor or infected tissues where they can elicit T cell restimulation and expansion necessary for target killing and enhanced therapeutic efficacy.

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**Results** Healthy melanocytes expressed 2 to 3-fold lower levels of gp100 peptide-HLA complexes on their surface compared to gp100-positive melanoma cell lines. In the presence of tebentafusp, this lower target expression translated into 3–6 fold lower levels of IFNγ and more than 100 fold lower granzyme B production by redirected T cells and these melanocytes were resistant to direct tebentafusp-induced killing (EC50 for melanocytes greater than 1nM vs E50 melanoma cell lines of 23–50 pM). Supernatants from T cells activated in response to melanoma cancer cells by tebentafusp downregulated the melanin content of healthy melanocytes (20–30% reduction). Western blotting revealed 30–40% inhibition of two key components of the melanin synthesis pathway; the tyrosinase-related protein (TRP)-1 and TRP-2. This inhibition was reversed by blocking IFNγ in supernatants from activated T cells.

**Conclusions** MRAEs, especially vitiligo, associated with response to tebentafusp, may be explained, at least in part, by the downregulation of melanin biosynthesis pathway genes by IFNγ secreted by tebentafusp-activated T cells.

**Ethics Approval** The study was approved by the South Central - Oxford A Research Ethics Committee (UK), REC reference 13/SC/0226

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selective tumor cell death induction. The Bispecific Aptamer has been further shown to significantly attenuate HCT116 tumor growth in vivo, an effect that was translated into a benefit to survival of treated mice.

Conclusions We have provided a proof-of-concept for Aum-mune’s platform ability to identify an effective functional personalized aptamer, which did not harm healthy cells. The Bispecific Aptamer’s exerted function in vitro has translated into a significant effect in vivo. Based on the personal approach and multiplicity of modes of action, the Bispecific Personalized Aptamer could have an effect in a broad spectrum of cancer indications.

REFERENCE
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626 IMPROVING THE YEAST TRANSFORMATION EFFICIENCY FOR YEAST DISPLAY IN ANTIBODY DEVELOPMENT
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Background In the therapeutic antibody development process, the yeast display technology which expresses a large library of antibodies is very useful for increasing the affinity of a lead antibody. Ideally, a yeast library should exceed the size of 10E10 to 10E11 to get close to the real affinity maturation process. However, due to low transformation efficiency with yeast, it requires tremendous scaling-up efforts to simply reach the 10E9 library size.

Methods To address the transformation problem, we developed a new electroporation device that applies a high voltage on a sealed electroporation tube containing the yeast and plasmids in a low conductance buffer.

Results The new device is arcing free due to the sealed design and each single reaction could generate 10E8 library size, far exceeding the 10E6 size that was previously reported in a single reaction.

Conclusions With the improved transformation efficiency, it becomes very straightforward to reach the currently difficult size of 10E9. Further more, it is possible to reach the 10E10 to 10E11 library size with reaction scaling-up. Our new method could be very useful for the field of antibody development.

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627 THE DLL3-TARGETED HALF-LIFE EXTENDED BISPECIFIC T CELL ENGAGER (HLE BITE®) IMMUNE-ONCOLOGY THERAPY AMG 757 HAS POTENT ANTITUMOR ACTIVITY IN NEUROENDOCRINE CANCER
Keegan Cooke*, Juan Estrada, Jinghui Zhan, Jonathan Werner, Fei Lee, Aditya Shetty, Marie-Anne Daniette Smit, Mark Salvati, Julie Baillis, Anger, Thousand Oaks, CA, USA

Background Neuroendocrine tumors (NET), including small cell lung cancer (SCLC), have poor prognosis and limited therapeutic options. AMG 757 is an HLE BiTE® immune therapy designed to redirect T cell cytotoxicity to NET cells by binding to Delta-like ligand 3 (DLL3) expressed on the tumor cell surface and CD3 on T cells.

Methods We evaluated activity of AMG 757 in NET cells in vitro and in mouse models of neuroendocrine cancer in vivo. In vitro, co-cultures of NET cells and human T cells were treated with AMG 757 in a concentration range and T cell activation, cytokine production, and tumor cell killing were assessed. In vivo, AMG 757 antitumor efficacy was evaluated in xenograft NET and in orthotopic models designed to mimic primary and metastatic SCLC lesions. NSG mice bearing established NET were administered human T cells and then treated once weekly with AMG 757 or control HLE BiTE molecule; tumor growth inhibition was assessed. Pharmacodynamic effects of AMG 757 in tumors were also evaluated in SCLC models following a single administration of human T cells and AMG 757 or control HLE BiTE molecule.

Results AMG 757 induced T cell activation, cytokine production, and potent T cell redirected killing of DLL3-expressing SCLC, neuroendocrine prostate cancer, and other DLL3-expressing NET cell lines in vitro. AMG 757-mediated redirected lysis was specific for DLL3-expressing cells. In patient-derived xenograft and orthotopic models of SCLC, single-dose AMG 757 effectively engaged human T cells administered systemically, leading to a significant increase in the number of human CD4+ and CD8+ T cells in primary and metastatic tumor lesions. Weekly administration of AMG 757 induced significant tumor growth inhibition of SCLC (figure 1) and
other NET, including complete regression of established tumors and clearance of metastatic lesions. These findings warranted evaluation of AMG 757 (NCT03319940); the phase 1 study includes dose exploration (monotherapy and in combination with pembrolizumab) and dose expansion (monotherapy) in patients with SCLC (figure 2). A study of AMG 757 in patients with neuroendocrine prostate cancer is under development based on emerging data from the ongoing phase 1 study.

Conclusions AMG 757 engages and activates T cells to kill DLL3-expressing SCLC and other NET cells in vitro and induces significant antitumor activity against established xenograft tumors in mouse models. These preclinical data support evaluation of AMG 757 in clinical studies of patients with NET.

Ethics Approval All in vivo work was conducted under IACUC-approved protocol #2009-00046.

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629  TARGETING A SHARED TP53 NEOANTIGEN WITH BISPECIFIC T CELL RETARGETING ANTIBODY

Emily Hsiue*, Katherine Wright, Jacqueline Douglass, Michael Hwang, Brian Mog, Alexander Pearlman, Annika Schaefer, Ping Atana Azurmendi, Qiong Wang, Drew Pardoll, Nickolas Papadopoulos, Kenneth Kinzler, Bert Vogelstein, Sandra Gabelli, Shibin Zhou, John Hopkins University School of Medicine, Baltimore, MD, USA; Complete Omics Inc., Baltimore, MD, USA

Background TP53 is the most commonly mutated cancer driver gene but drugs that target TP53 are not yet available. A peptide derived from the most common p53 mutation R175H (HMTEVVRHC) can be presented by a common human leukocyte antigen (HLA-A*02:01) after proteasomal degradation.1 We aimed to develop T cell receptor (TCR)-mimic antibody targeting this shared neoantigen.

Methods We constructed a single-chain variable fragment (scFv) phage display library that presents scFvs at an estimated diversity of 3.6e10. Mutant peptide-HLA (pHLA)-specific scFvs were enriched through five rounds of positive and negative selections. Mutant pHLA-specific scFv clones were converted into bispecific T cell retargeting antibodies in the single-chain diabody (scDb) format by linking with the anti-CD3 scFv UCHT1.2 These scDbs were tested in T cell co-cultures in the presence of target pulsed cells or target cells that either overexpress the p53 neoantigen or present the p53 neoantigen at endogenous levels. In vivo efficacy was assessed by administering scDb in NSG xenograft mouse model. The structural basis of the binding specificity was evaluated by X-ray crystallography.

Results We identified an scFv, termed clone H2, that specifically binds p53 R175H/HLA-A*02:01 pHLA and not its wild-type counterpart at a Kd of 86 nM (figure 1A). H2-scDb induced T cell cytokine release and cytotoxicity in the presence of 1) HLA-A*02:01-expressing cells pulsed with the p53R175H peptide, 2) cells transfected with HLA-A*02:01 and p53 R175H, and 3) cancer cell lines KMS26, KLE, and TYK-nu that express endogenous HLA-A*02:01/p53 R175H (figure 1B-E). T cell activation was diminished when TP53 was knocked out from these cancer cell lines using CRISPR (figure 1E). When administered to NOD scid gamma (NSG) mice systemically engrafted with the KMS-26 cell line, H2-scDb significantly suppressed tumor growth (figure 1 F, G).

The structure of p53 R175H/HLA-A2 bound to the H2-Fab fragment shows that four complementarity-determining region loops of the H2 antibody formed a cage-like configuration around the C-terminus of the p53 R175H peptide, trapping the mutant histidine (His175) and the adjacent arginine (Arg174) residues in a stable interaction, which provides the structural basis for the specificity (figure 2).

Conclusions We have developed a TCR-mimic bispecific T cell engager H2-scDb that recognized the shared neoantigen
HLA-A*02:01/p53 R175H pHILA complex with exquisite specificity. It effectively activated T cells and lysed tumor cells both in vitro and in vivo. This approach could in theory be used to target cancers containing mutations that are difficult to target in conventional ways.

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Trial Registration NA

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REFERENCES


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630 PD-L1/CD47 TUMOR DIRECTED B-BODYTM BISPECIFIC ANTIBODIES DEMONSTRATING SIGNIFICANT ANTIMATERIAL ACTIVITY WITH NO TOXICITY IN PRECLINICAL MODELS

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Background Tumor cells have been shown to utilize both innate and adaptive checkpoints to evade anti-tumor immune responses. CD47 and PD-L1 are two targets widely expressed on the cell surface of tumor cells and are predicted to coordinately suppress innate and adaptive sensing respectively to evade immune control. PD-L1 dampens T cell-mediated tumor killing (via PD-L1/PD-1 signaling) while CD47 protects tumor cells from phagocytosis (via CD47/SIRP-alpha signaling). Targeting each of the above pathways with monoclonal antibodies has shown promise with PD-L1/PD-1 inhibition showing durable responses and extended overall survival for several approved products, whereas the molecules targeting CD47 pathway are in early clinical trials. Given that a significant number of patients are either resistant or relapse on PD-L1/ PD-1 therapy, combinations with anti-CD47 antibodies are being explored. However, the expression of CD47 on many normal cells such as hematopoietic cells, red blood cells (RBCs) and platelets provides a widespread antigen sink which impacts the PK and adverse event profile of these agents.

Methods Here, we describe the generation and testing of a large panel of bspecificics with combinations of different affinities to PD-L1 and CD47 using the B-Body™ bspecific screening platform. The bspecific antibodies were screened in various in vitro activity and developability assays. Selected leads from the screen were tested in multiple in vivo models with differential expression of CD47 and PD-L1.

Results The lead bspecific antibodies showed significant blockade of SIRPa/CD47 and PD-L1/PD-1 signaling in vitro and tumor growth inhibition in vivo. The studies also showed no significant binding to RBCs and induced minimal RBC phagocytosis in vitro. A summary of screened candidates and characterization of a lead candidate being developed further will be presented.

Conclusions We have identified multiple CD47/PD-L1 bspecific antibodies with favorable efficacy and safety profiles. Selection of a lead for further IND and clinical development is underway.

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631 DEVELOPMENT OF HIGHLY EFFICACIOUS AND SAFE TARGETED CANCER IMMUNOTHERAPY VIA IL12-BASED TMEKINE™ PLATFORM

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Background We developed tumor microenvironment-targeting immunocytokine or TMEkine™ utilizing strong anti-tumoral effect of interleukin 12 (IL-12). In this effort, we created a bi-specific 1+1 antibody fusion with conventional knob-in-hole technology where anti-CD20 was paired with IL-12 fc fusion arm. A couple of IL-12 muteins were used in our therapeutic molecules to reduce systemic toxicity. IL-12 has been known for a key orchestrator in immune response. The main actions of IL-12 include the induction of CD4+ Th0 cells toward Th1 type and enhancement of IFN-γ production, stimulation of cytotoxicity and growth of natural killer (NK) cells and CD8+ T cells. For these reasons, IL-12 has long been considered as a potential therapeutic molecule for treating cancers by enhancing immune activity toward tumor cells. However, systemic administration of IL-12 showed poor efficacy and severe adverse effects. With our therapeutic approach of tumor targeting and attenuated IL-12 mutein, we expect that our IL-12-based TMEkine™ holds great promise for the future of cancer immunotherapy.In this study, we targeted CD-20 expressing cancers such as B-cell lymphoma with our anti-CD20/IL-12 mutein TMEkine. We evaluated the biological activity of our molecules with in vitro and in vivo efficacy and safety.

Methods The target specific binding to CD20 and IL-12 receptor was analyzed by FACS and ELISA. Biological activities as signaling transduction and T cell activation were confirmed in vitro using HEKblue IL12 cell line, primary human T cells and NK cells. The anti-tumor efficacy of TMEkine (CD20-IL-12) was assessed in A20 lymphoma syngeneic mouse model. To demonstrate long term protection to A20, the cured five mice after TMEkine administration were re-challenged with A20 and 4T1 cells.

Results First, we analyzed the specific binding of our TMEkine molecules to CD20 expressing B-cell lymphoma cell lines (such as Raji). We showed that TMEkine (CD20-IL-12) binds to Raji and Ramos, which express CD20, but not to Jurkat, which does not express CD20. We also showed that TMEkine molecules bind to IL-12 receptor in a dose-dependent manner. pSTAT4 alphaLISA assay revealed that TMEkine (CD20-IL-12) transduces STAT4 signaling. In our IL-12 mutein, key residues for heparin binding were mutated. The biological activity of our mutein molecule was attenuated due to this change in human PBMC. In addition, our TMEkine molecules significantly induced IFN-γ secretion from primary human T cells and NK cells. An A20 B-cell lymphoma syngeneic mouse model was utilized to investigate the anti-tumor activity of TMEkine (CD20-IL-12). TMEkine molecules were injected three times with Q3D intraperitoneally. Tumor growth was
substantially reduced and no cytotoxicity was observed. To further investigate the underlying mechanism, we analyzed tumor infiltrating lymphocytes (TIL) and as expected, we observed the increase in the number of CD8+ T cells in TIL, compared to control group. Interestingly, our tumor re-challenge result demonstrates that TMEkine (CD20-IL-12) protected animals from tumor recurrence implying that immunologic memory response was generated upon our TMEkine (CD20-IL-12) treatment.

Conclusions Altogether, our data suggest that TMEkine (CD20-IL-12) as an efficacious tumor targeting cytokine opening up a new avenue for the treatment of B-cell lymphoma.

Conclusions HPN601 is a conditionally active EpCAM-targeting T cell engager with a ten-fold improved therapeutic window compared to a constitutively active EpCAM-targeting T cell engager. An EpCAM-specific T cell engager with an improved safety profile could address unmet needs in many solid tumors and demonstrate the feasibility of using conditionally active T cell engagers to target more solid tumor antigens.

Ethics Approval The study was reviewed and approved by Harpoon’s Institutional Animal Care and Use Committee.

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632 HPN601 IS A PROTEASE-ACTIVATED EPICAM-TARGETING T CELL ENGAGER WITH AN IMPROVED SAFETY PROFILE FOR THE TREATMENT OF SOLID TUMORS

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Background Epithelial cell adhesion molecule (EpCAM) is highly expressed in many solid tumors. However, therapeutics targeting EpCAM have had limited clinical utility or failed in clinical development likely due to the expression of EpCAM in normal tissues. For example, clinical testing of solitomab, an EpCAM-targeting T cell engager, resulted in severe dose-limiting toxicities, including elevated liver transaminases, hyperbilirubinemia, and diarrhea. Designing an EpCAM-targeting T cell engager that is only active in the tumor would expand its therapeutic window and improve its safety profile.

Methods Using a T cell engager prodrug platform named Pro-TriTAC that couples therapeutic half-life extension with functional masking, we have engineered HPN601, a protease-activated EpCAM-targeting T cell engager. HPN601 is a single polypeptide with three binding domains: anti-albumin for half-life extension, anti-CD3e for T cell engagement, and anti-EpCAM for tumor cell engagement. The anti-albumin domain contains a masking moiety and a protease-cleavable linker and keeps the molecule inert outside the tumor microenvironment. Activation by tumor-associated proteases removes the anti-albumin domain along with the masking moiety to reveal a potently active drug inside the tumor. This active drug has minimal activity outside of tumor because, without an albumin binding domain, it is rapidly cleared in circulation.

Results A humanized rodent tumor model was used to simultaneously measure anti-tumor efficacy and clinically relevant toxicity endpoints. In this model, a surrogate molecule of HPN601 was safely administered at a dose ten-fold higher than the minimal efficacious dose required for durable tumor regression. Higher doses produced toxicities including elevated ALT/AST and bilirubin, body weight loss, and evidence of tissue damage by histopathology. In contrast, a constitutively active EpCAM-targeting T cell engager could only be dosed safely up to its minimal efficacious dose. The improved safety profile of HPN601 is further supported by a toxicokinetic study in non-human primates: compared to a constitutively active EpCAM-targeting T cell engager, HPN601 had significantly attenuated cytokine production, including IFN-γ, IL-2, IL-6, and IL-10.

Conclusions HPN601 is a conditionally active EpCAM-targeting T cell engager with a ten-fold improved therapeutic window compared to a constitutively active EpCAM-targeting T cell engager. An EpCAM-specific T cell engager with an improved safety profile could address unmet needs in many solid tumors and demonstrate the feasibility of using conditionally active T cell engagers to target more solid tumor antigens.

633 DUAL-TARGETING OF 4-1BB AND OX40 WITH AN ADAPTIR™ BISPECIFIC ANTIBODY ENHANCES ANTI-TUMOR RESPONSES TO SOLID TUMOR

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Background 4-1BB (CD137) and OX40 (CD134) are critical activation-induced co-stimulatory receptors that regulate immune responses of activated T and NK cells by enhancing proliferation, cytokine production, survival, and cytolytic activity. A superagonist 4-1BB antibody has shown clinical activity but severe toxicities. APVO603, a 4-1BB x OX40 targeting bispecific antibody with conditional agonism, activating these receptors only when both are co-engaged. The Fc portion was mutated to eliminate FcγR-mediated interactions. Co-stimulation through 4-1BB and OX40 has the potential to amplify the cytokotoxic function and the number of activated T and NK cells in multiple solid tumor indications.

Methods scFv binding domains to 4-1BB and OX40 were optimized to increase affinity, function and stability, and then incorporated into the ADAPTIR™ bispecific antibody platform to produce the APVO603 lead candidate. NF-kB/luciferase reporter cell lines expressing OX40 or 4-1BB were initially used to assess the agonistic function of APVO603’s binding domains. Primary PBMC were sub-optimally stimulated with an anti-CD3 antibody and T and NK cell proliferation was assessed using Cell TraceTM-labelled PBMC. Cytokine secretion was measured at 48 hrs using Luminex-based assays. For in vitro tumor lysis studies, PBMC were co-cultured with tumor cells expressing a tumor-associated antigen (TAA) and activated with TAA x CD3 bispecific protein. 7-AAD expression was assessed on tumor cells at 72 hrs. The in vivo therapeutic efficacy of APVO603 was evaluated using a murine MB49 bladder cancer model in human 4-1BB and OX40 double knockout mice.

Results APVO603 stimulates 4-1BB and OX40 NF-kB/luciferase reporter activity in a dose-dependent manner, and is strictly dependent on engagement of the reciprocal receptor to elicit 4-1BB or OX40 activity. In primary PBMC assays, APVO603 induces synergistic proliferation of CD4+, CD8+ T and NK cells when compared to OX40 or 4-1BB monospecific molecules with a wt Fc, either individually or in combination. Additionally, APVO603 enhances proinflammatory cytokine production and granzyme B expression, and augments in vitro tumor cell lysis induced by a TAAx CD3 engager. In vivo, APVO603 reduces growth of established MB49 tumors in human 4-1BB and OX40 double knockout mice.

Conclusions APVO603 is a dual-agonistic bispecific antibody that augments the effector function of activated CD4+ and CD8+ T
and NK cells in a dose-dependent manner, and reduces growth of established tumors in vivo. This preclinical data, demonstrates conditional dual stimulation of 4-1BB and OX40 and supports further development of APVO603, a promising immuno-oncology therapeutic with potential for benefit in solid tumors.

Ethics Approval Treatment of study animals was in accordance with conditions specified in the Guide for the Care and Use of Laboratory Animals, and the study protocol (ACUP 20) was approved by the Institutional Animal Care and Use Committee (IACUC).

REFERENCES

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634 PRODUCTION AND TESTING OF A NOVEL BSPECIFIC NANOBODY CONSTRUCT TARGETING NK CELLS AND EGF expressing MALIGNANCIES
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Background The ability to kill tumor cells with an acceptable toxicity profile, makes Natural Killer (NK) cells promising assets for cancer therapy. However, strategies to enhance the preferential accumulation and activation of NK cells in the tumor microenvironment would likely increase the efficacy of NK cell-based therapies.

Methods In this study, we show a novel bspecific nanobody-based construct (biVHH) targeting both CD16A (low-affinity Fc receptor: FcRγIIIa) on NK cells and EGFR on tumors of epithelial origins.

Results Higher levels of NK cell activity and subsequent tumor cell lysis were found in vitro in the presence of the biVHH and were dependent on the expression of both CD16A and EGFR while they were independent of the KRAS mutational status of the tumor. Increased NK cell activity was found in NK cells derived from colorectal cancer (CRC) patients when co-cultured with the biVHH and EGFR expressing tumor cells. Finally, higher levels of cytotoxicity were found against patient-derived metastatic CRC cells in the presence of the biVHH and autologous peripheral blood mononuclear cells or allogeneic NK cells.

Conclusions Based on our results, the bspecific CD16A and EGFR targeting VHH construct could be a useful tool in combination with various NK cell-based therapies.

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635 A NOVEL SITE-DIRECTED CHEMICAL CONJUGATION TECHNOLOGY CONJUGATES ANTI-TUMOR ACTIVITY VIA NATIVE FC RECEPTOR TO PLASMA IMMUNOGLOBULIN BY ATTACHING TUMOR BINDERS

Background We describe KPMW101, which was created by chemical conjugation of a CD38-specific binder to clinical grade intravenous immunoglobulin (IvIg) pooled from healthy donors. Kleo’s MATE™ technology enables efficient site-directed chemical conjugation to ‘off-the-shelf’ IgV and allows the development of antitumor agents with rapidly introduced target specificity. Our platform allows for chemical engineering of existing IvIg in a cost-efficient manner. This technology relies on synthetic compounds that consists of antibody binder with react-and-release mechanism.

Methods Design of synthetic chemical reagents included antibody binding group capable of covalent bond formation with specific lysein, CD38 binding moiety proven to work in our clinical candidate KP1237, and tunable non-cleavable linker. Conjugation efficiency to polyclonal IvIg was evaluated using LC-MS analysis of IdeZ-digests. The binding of CD38, CD16a, and FcRn were determined by ELISA and BLI. For in vitro ADCC assays, PBMCs provided NK effector function. Daudi (CD38+) B lymphoblast cells were treated with KPMW101 or IvIg, PBMCs were introduced and incubated for 18h, and target cellular death was measured. For an in vivo IP macrophage lavage model of ADCP, SCID mice were implanted IP with CFSE-labeled Daudi cells. Mice were injected with IvIg or KPMW101 (0.21, 0.625, 1.875 mg/kg) SQ, and tumor cell counts were measured by flow cytometry. The pharmacokinetic profile of in vivo KPMW101 was determined from blood and analyzed utilizing a human Ig isotyping array.

Results Synthetic chemical reagents with multiple linker types have been conjugated to IvIg and evaluated in biochemical assays. KPMW101 showed the highest conjugation efficiency. Binding affinity of KPMW101 to CD38 was 27nM. ELISA results show KPMW101 binds to CD16a and FcRn, indicating that conjugation does not interfere with FcR binding. In vitro ADCC results demonstrate that KPMW101 elicited CD38+ target cell killing with an EC_{50} of 0.91–2.09mM. In vivo studies showed that KPMW101 resulted in a 49.9–63.5% reduction of tumor cells. Pharmacokinetic profile showed stability of KPMW101 throughout the 144-hour study, whereby IgG1, IgG2, IgG3, and IgG4 isotypes were detectable.

Conclusions KPMW101 is created by chemical conjugation of CD38-specific binder to IvIg using our proprietary MATE™ technology, maintaining native binding to FcRs via the Fc domain. This ensures the stability of the molecule and retains immune-mediated mechanisms of action. KPMW101 induces IvIg to adopt Fc effector mechanisms like ADCC and ADCP. Our in vitro data and in vivo studies confirm KPMW101 ability to kill tumor cells, making IvIg into an active antitumor therapeutic agent.

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Immunotherapy toxicities

637 IMMUNE-RELATED ADVERSE EVENTS (IRAES) MAY INDICATE A FAVORABLE PROGNOSIS IN METASTATIC RENAL CELL CARCINOMA (MRCC) PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITORS (ICI)

1Dylan Martin*, 2Sean Evans, 3Subir Goyal, 1Yuan Liu, 1Anders Olsen, 2Benjamin Magod, 1Jacqueline Brown, 1Lauren Yantorni, 2Greta Rusler, 1Sarah Caufield, 1Jamie Goldman, 3Basel Nacha, 1Wayne Harris, 2ViraJ Master, 2omer Kuczuk, 1Bradley Carthon, 2Melhis Bilen. Emory University School of Medicine, Atlanta, GA, USA, 2Winship Cancer Institute of Emory University, Atlanta, GA, USA

Background Immune checkpoint inhibitors (ICI) have become an increasingly utilized treatment in metastatic renal
cell carcinoma (mRCC). Although they have a favorable toxicity profile, immune-related adverse events (irAEs) can have a significant impact on patients’ quality of life. It is not well understood whether irAEs are associated with improved clinical outcomes. We investigated the relationship between irAEs and clinical outcomes in mRCC patients treated with ICI.

**Methods** We performed a retrospective study of 200 patients with mRCC who received ICI at Winship Cancer Institute of Emory University from 2015–2020. Clinical outcomes were measured by overall survival (OS), progression-free survival (PFS), and clinical benefit (CB). OS and PFS were calculated from ICI-initiation to date of death and radiographic or clinical progression, respectively. CB was defined as a best radiographic response of complete response (CR), partial response (PR), or stable disease (SD) for >6 months per response evaluation criteria in solid tumors (RECIST) version 1.1. Toxicity data was collected from clinic notes and laboratory values. The association with OS and PFS was modeled by Cox proportional hazards model. Kaplan-Meier curves were created for survival estimates.

**Results** Most patients were males (71%), and 78% had clear-cell RCC (ccRCC). Most patients (58%) received anti-PD-1 monotherapy. The majority were international mRCC database consortium (IMDC) intermediate (57%) or poor-risk (26%). Anti-PD-1 monotherapy was the most common (58%) treatment regimen and most patients received ICI as first (38%) or second-line (42%) treatment. One-third of patients (33%) experienced an irAE, with the most common being endocrine (13%), gastrointestinal (11%), and dermatologic (10%). Patients who experienced irAEs had significantly longer median OS (44.5 vs. 18.2 months, p=0.005) and PFS (7.5 vs 3.6 months, p=0.0028) compared to patients who did not (figure 1).

**Conclusions** We showed that mRCC patients who experienced irAEs, particularly thyroid irAEs, had improved clinical outcomes. This suggests that irAEs may be prognostic of favorable outcomes in mRCC patients treated with ICI. Larger, prospective studies are needed to validate these findings.

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**Trial Registration** Not applicable

**Ethics Approval** This retrospective study was approved by the Emory University Institutional Review Board.

**Consent** Not applicable

**REFERENCES**

Not applicable

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Abstracts

638 PLASMA PROTEOME ANALYSIS IN PATIENTS WITH IMMUNE CHECKPOINT INHIBITORS RELATED ARTHRITIS AND PNEUMONITIS
Noha Abdel-Wahab*, Adi Diab, Hiroyuki Katayama, Sang Kim, Samir Hanash, Maria Suarez-Almazor, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Background Immune checkpoint inhibitors (ICIs) have resulted in unprecedented advances in the treatment of cancer. By dis-inhibiting the immune system, they enhance anti-tumor immunity, but provoke off-target inflammatory and immune-related adverse events (irAEs) which can seriously impact morbidity and mortality. The exact immunobiology of irAEs is not completely understood, but may involve specific immune pathways. To date, there is no validated biomarker test to predict the development of irAEs in patients treated with ICIs.

Methods To identify possible biomarkers of irAEs, we performed in-depth proteomic profiling of blood samples obtained from cancer patients receiving ICIs. The plasmas were processed with Hu-14 immuno-depletion column (Agilent Technologies) and the samples were labeled with TMT (Thermo Scientific). The proteins were next pre-fractionated with HPLC, trypsin digested and analyzed by nanoAQUITY LC coupled Synapt G2-Si ion-mobility mass spectrometry (WATERS).

Results A total of 12 patients were enrolled in the study; all were receiving anti-programmed cell death-1 (PD-1) agents. Cancer types included melanoma (n=9), renal cell carcinoma (n=2), and non-small cell lung cancer (n=1). Eight patients had irAEs with active toxicity symptoms at blood draw (4 with pneumonitis-irAE and 4 with arthritis-irAE); 6 of those patients were receiving corticosteroids (ranging from 5 to 60 mg/d), and 1 was receiving tocilizumab (an anti-IL-6 receptor antibody). Four patients who completed a minimum of one year of anti-PD1 treatments without irAEs were enrolled as control group. Median time from ICI initiation until blood draw was 16 months (range, 4–31) among patients with irAEs and 23 months (range, 17–28) among controls. We identified 925 protein gene products from 2.5 million mass spectra that can cover 107 dynamic range of plasma proteins. Among them, 19 proteins showed statistically significant differences between patients with and without irAEs (P<0.05) (figure 1). Nine proteins including CFB, CLEC3B, ITIH4, HPX, APOC4, AVPR2, B9D1, DPEP2, JCHAIN, LINC00238, PLG, RAB40C, and AVPR2, B9D1, DPEP2, JCHAIN, LINC00238, PLG, RAB40C, MYL12B, and ZFP30 were significantly downregulated in patients with irAEs (figure 2). Myeloid-related proteins including RARRES2, TF, OAF, MYL12A, and MYL12B were significantly upregulated in patients with irAEs; MYL12A and MYL12B are known to be elevated in airway inflammation of lung tissues. While, 10 other proteins including APOC4, AVPR2, B9D1, DPEP2, JCHAIN, LINC00238, PLG, RAB40C, TCFC4, and ZFP30 were significantly downregulated in patients with irAEs (figure 2).

Conclusions In-depth plasma proteome analysis identified possible biomarkers of adverse events modulated by ICI treatment. We plan a prospective validation cohort study of melanoma patients initiating treatment with ICIs to further evaluate the potential clinical utility of the identified biomarkers and their association with immune toxicity, and tumor response to ICI therapy.

Ethics Approval The study was approved by The Institutional Review Board at The University of Texas MD Anderson Cancer Center, approval number PA16-0928

Consent Written informed consent was obtained from all patients who agreed to participate in the study

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Abstract 638 Figure 1 Unsupervised clustering of plasma proteome after ICIs
337 proteins were quantified from at least 8 out of total 12 patients and clustered after Loess normalization.


Abstract 638 Figure 2 Heatmap of circulating plasma proteins showed significance
Nineteen proteins showed statistically significant differences between patients with and without irAEs.

RARRS2, TF, OAF, MYL12A, and MYL12B were significantly upregulated in patients with irAEs; MYL12A and MYL12B are known to be elevated in airway inflammation of lung tissues. While, 10 other proteins including APOC4, AVPR2, B9D1, DPEP2, JCHAIN, LINC00238, PLG, RAB40C, TCFC4, and ZFP30 were significantly downregulated in patients with irAEs (figure 2).

639 IMMUNE-RELATED THYROID DYSFUNCTION IN PATIENTS WITH EXISTING THYROID DYSFUNCTION
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Background Thyroid dysfunction is a well known side effect of immune checkpoint blockade (ICB) and is one of the most common causes of immune-related adverse events (IRAE). The incidence varies with each individual therapy but generally estimated to be in the range between 6–18% per one study. Hyperthyroidism and thyroiditis are the most common manifestations. Initial hyperthyroidism followed by hypothyroidism is another manifestation. Hypothyroidism is more common with an incidence of 10% whereas hyperthyroidism has an incidence of 5%. Less is known about the incidence of worsening thyroid dysfunction in patients with pre-existing thyroid dysfunction treated with ICB.

Methods A retrospective analysis was collected on 370 patients who received immunotherapy from April 2015 to April 2019. Of those, 212 had abnormal thyroid function tests. We analyzed a subgroup of these patients who had baseline thyroid dysfunction for worsening thyroid dysfunction after they were given ICB. Fifty-three patients were included in the analysis and had an abnormal baseline TSH at the start of immunotherapy. Type of immunotherapy, worst TSH, duration between initiation of immunotherapy to worst TSH, treatment type, and grade of abnormality as per Immune Checkpoint Inhibitor Related
Characterizing Severe Acute Kidney Injury in Patients Treated with Immune Checkpoint Inhibitors

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Background Renal immune-related adverse events (irAEs), are relatively rare in patients treated with immune checkpoint inhibitors (ICIs). This retrospective analysis characterizes the etiology of severe acute kidney injury (AKI) in patients treated with ICIs at the University of California, San Diego.

Methods The electronic medical record was used to identify all patients with an estimated glomerular filtration rate (eGFR) <15 mL/hour who received ipilimumab, nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, cemiplimab between 1/2000 and 1/2019. Patients with baseline eGFR < 15 mL/hr or who experienced an eGFR decline to <15 mL/hour prior to ICI initiation were excluded. Extracted data included serum creatinine, eGFR, ICI dose, urinalysis, renal ultrasound, clinical documentation of both ICI-related nephritis and other suspected causes of AKI. These data were analyzed to determine cause of AKI and possible relation to ICI.

Results 46 patients who received ICI therapy and subsequently developed an AKI with eGFR < 15 mL/hour were identified. Three of these 46 patients (6.5%) had AKIs partially or predominately attributed by the clinician to ICI therapy (table 1). Characteristics of ICI-related AKI for these patients are summarized in (table 2). AKI onset occurred 32–110 days after ICI initiation. All three patients exhibited proteinuria, pyuria, and hematuria on urinalysis with negative urine cultures, but none underwent confirmatory renal biopsy. Only one patient had urine eosinophils checked, which was negative. Two (66%) of these patients received high-dose corticosteroids with subsequent complete eGFR recovery. Neither of these two patients required renal replacement therapy. One patient (33%) declined corticosteroid treatment due to concomitant multiforgan failure. An additional four (8.7%) patients developed multifactorial AKIs with other concurrent irAEs that were treated with corticosteroids, but were not formally diagnosed with ICI-related AKI.

Conclusions In our cohort, 6.5% of patients who develop AKI after receiving ICI therapy experienced immune-related nephritis. A further 8.7% of patients experienced other irAEs with AKI, suggesting that the true prevalence of immune-related nephritis is likely underdiagnosed. Notably, 84.8% of patients who develop AKI after ICI therapy have a non-ICI-related etiology, and no patient in our cohort of 46 patients underwent renal biopsy, highlighting the need for blood-based biomarker development for immune-related nephritis.

Abstract 640 Table 1 AKI etiologies in ICI-treated patients

<table>
<thead>
<tr>
<th>AKI etiology</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-renal (including hepatorenal, cardiorenal)</td>
<td>28.2</td>
</tr>
<tr>
<td>Post-obstructive</td>
<td>19.6</td>
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<tr>
<td>Intrinsic renal</td>
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<td>Multifactorial with concomitant irIAT</td>
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<td>Sepsis/septic shock</td>
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<td>Non-ICI medications</td>
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<td>Lidodinated contrast</td>
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<td>Other/unclear</td>
<td>15.2</td>
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Abstract 640 Table 2 Patient characteristics in ICI-related AKI

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>ICImab</th>
<th>Day of AKI onset</th>
<th>Rate of eGFR (mL/min)</th>
<th>Urinalysis</th>
<th>ICI treatment</th>
<th>Renal recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Nivolumab</td>
<td>120</td>
<td>7</td>
<td>Proteinuria</td>
<td>0.4</td>
<td>Predriore (3 mg BID)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Ipilimumab</td>
<td>89</td>
<td>15</td>
<td>Proteinuria</td>
<td>0.4</td>
<td>Predriore (3 mg BID)</td>
</tr>
<tr>
<td>Unidentified nervous system</td>
<td>Nivolumab</td>
<td>32</td>
<td>8</td>
<td>Proteinuria</td>
<td>0.4</td>
<td>Yes baseline eGFR</td>
</tr>
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</table>

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0639
INCIDENCE AND RISK FACTORS FOR STROKE ASSOCIATED WITH IMMUNE CHECKPOINT INHIBITOR THERAPY IN CANCER PATIENTS USING REAL-WORLD CLINICAL DATA

Prantesh Jain*, Jahir Gutierrez, Avirup Guha, Chhavi Jain, Nirav Patil, Tingke Shen, Ilya Stanevich, Ankit Mangla, Marcos De Lima, Jill Banholtz-Sloan, Afshin Dowlati. University Hospitals/Case Western Reserve University, Cleveland, OH, USA

Background: Immune checkpoint inhibitors (ICIs) can cause unique, high-grade immune-related adverse events. Although rare, ICI related stroke events can have high morbidity and mortality. Neurological monitoring is not routinely performed in patients on ICI treatment, thus risk factors remain unknown. Characterisation of such rare, but fatal adverse events requires integration of real-world data.

Methods: U.S claims data (IBM MarketScan) of over 30 million commercially insured individuals was leveraged to identify 2,687,301 cancer patients between 2011–2018. Patients ≥18 years of age, treated with ICIs (targeting CTLA4 (ipilimumab) and/or the PD1 (nivolumab, pembrolizumab)/PDL1 (atezolizumab, avelumab, durvalumab) alone or in combination with ICI and/or chemotherapy were identified and followed until disenrollment. All strokes (ischemic or haemorrhagic), comorbidities, and treatment details were identified using diagnosis and billing codes. Patients from the ICI cohort were matched 1:1 to those in the chemotherapy cohort according to age, gender, NCI comorbidity score, and primary cancer as presented in the study design (figure 1). The matched cohorts were split by the specific type of chemotherapy (targeted or cytotoxic) used in the control patients. This yielded a total of 2,177 pairs of matched patients where the control arm received targeted chemotherapy, and 3,550 pairs of matched patients where the control arm received cytotoxic chemotherapy. Analyses included descriptive statistics and Cox proportional hazards regression.

Results: A total of 16,574 patients received at least one dose of ICI therapy for any advanced cancer. Overall, 9,496 patients who were treated with ICI met the study eligibility criteria. Stroke was identified in 489 (5.14%) patients. Mean age (±standard deviation, SD) was 60 (±12), male 62%, mean (±SD) NCI comorbidity index 2.3 (±2.12), median time to stroke was 168 days. 51.3% patients received anti-PD1 monotherapy, 37.6% received anti-CTLA4, 3.3% anti-PD-L1 and 7.8% received combination therapy (anti-PD1 plus anti-CTLA4). One-year cumulative incidence (CI) in the matched ICI vs. targeted and ICI vs. cytotoxic chemotherapy were 6.3% vs. 5.7% (p=0.07) and 4.95% vs. 4.08% (p=0.90) respectively (table 1). Within the ICI cohort, CI of stroke events with anti-CTLA4 monotherapy vs. anti-PD1/PD-L1 and anti-CTLA4 plus anti-PD-1 combination vs. PD1/PD-L1 monotherapy were 9.89% vs. 4.54% and 6.69% vs. 3.73%, respectively (table 2). On multivariable regression analyses, patients with malignant melanoma, and those receiving anti-CTLA-4 monotherapy were associated with higher risk of stroke events, while the risk was lower in patients with head and neck cancer and those who received anti-PD-1 monotherapy (table 3 and 4).
Conclusions  
To the best of our knowledge, this is the largest and comprehensive real-world longitudinal study for stroke events in advanced cancer patients treated with ICI. Cumulative incidence of stroke was significantly higher in patients on anti-CTLA-4 monotherapy and anti-CTLA-4 plus anti-PD-1 combination therapy in comparison to anti-PD-1/PD-L1 monotherapy. Malignant melanoma and anti CTLA-4 therapy were independent risk factors for stroke.

Acknowledgements  
This work was funded by pilot award in Big Data/Cancer Informatics to Pranesh Jain, MD from University Hospitals Research & Education Institute.

Ethics Approval  
The IBM MarketScan national database contains de-identified linked inpatient, outpatient, and pharmacy claims data. University Hospitals Institutional Review Board determined this study to be exempt from review and requirement of an informed consent.

Abstract 642 Table 4  
Risk factors associated with stroke events (Multivariate analysis)

Abstract 642 Figure 1  
Infectious complications were only observed in BAL specimens, while primary disease involvement post-CAR-T cell therapy was mostly observed in pleural effusions and tissue biopsies.

Abstract 642 Figure 2  
Infectious complications were only observed in BAL specimens, while primary disease involvement post-CAR-T cell therapy was mostly observed in pleural effusions and tissue biopsies.

Abstracts

RETROSPECTIVE REVIEW OF PULMONARY PATHOLOGY ASSOCIATED WITH CHIMERIC ANTIGEN RECEPTOR T CELL THERAPY

Jing Du, David Woolston, Kimberly Costas, Alexandre Hirayama, Cameron Turtle, Cecilia Yeung. University of Washington, Seattle, WA, USA; Fred Hutchinson Cancer Research Center, Seattle, WA, USA; Providence Medical Group, Everett, WA, USA

Background  
Chimeric antigen receptor (CAR) T-cell therapy is an immunotherapy which uses genetically modified T cells engineered to express CARs to recognize and kill cells that harbor specific antigens. CAR T-cell products designed to target the tumor specific antigens have been used for the treatment of relapse and/or refractory of acute lymphoblastic leukemia, non-Hodgkin lymphomas, myeloma, and solid tumor in clinical trials at our institution. Several side effects have been reported including increased risk of infection.

Methods  
Retrospective review of morphologic, microbiologic and flow cytometric evaluations done on bronchioalveolar lavages (BAL), pleural effusions, and tissue biopsy specimens from post CAR T-cell adult patients with respiratory complications at our institution from March 2013 to January 2020.

Results  
Thirteen cases with BAL, 8 cases with biopsy (including lymph node and lung tissue) and 5 cases with pleural effusion are reviewed. All infectious diseases were detected on BAL specimens; while primary disease involvement post-CAR-T cell therapy was mostly observed in pleural effusions and tissue biopsies (figure 1). Interestingly, we found a case of a patient with refractory diffuse large B cell lymphoma that had developed mediastinal lymphadenopathy 9 months after CD19 CAR T-cell infusion (defined composition CD4 and CD8 CAR T-cells). Subsequent biopsies showed granulomatous inflammation with minimal evidence of necrosis (figure 2). Special stains with AFB, Gram, Warthin Starry, and Wright Giemsa showed no evidence of infectious organisms. Special stains were negative for acid fast, fungal, bacterial, or spirochetal organisms. Polymerase chain reaction for Mycobacterium tuberculosis complex DNA by hsp65 amplified probe, and nontuberculous mycobacteria (including Mycobacterium avium complex) by 16s rDNA, hsp65, and rpoB primer sets were both negative. No abnormal B or T cell population was found by concurrent flow cytometry; however, CAR T-cells were detected at low levels.

Conclusions  
Granulomatous inflammation is a chronic, histiocytic response to various chemical mediators of cell injury caused by broad etiologies. $\delta_\gamma T$ cells and T helper cells play roles on recruiting circulating monocytes and maturing of macrophages and ultimately the formation of granulomas. In our case, the patient has no documented autoimmune disease. Extensive infectious disease work-ups failed to identified infectious etiology. The presence of CAR T-cells in mediastinal lymph node 9 months after infusion is not unexpected given CAR T-cells can be detected in the blood for years in some patients. The granulomatous inflammation can be part of exaggerated tissue repair process after lymphoma cells killed by CAR T-cells. However, it may complicate or even mislead
assessment of therapeutic effect on post-CAR T-cell cases. The finding of enlarged lymph nodes by imaging study certainly raises concern of disease persistent or relapse.

Ethics Approval The study was approved by Fred Hutchinson Cancer Research Center’s Ethics Board, approval numbers 1837, 9364

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0642

Abstract 643 Table 1 Patient characteristics: age, sex, original diagnosis, CAR target, cause of death, and days post-CAR T-cell infusion at time of death

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at death</th>
<th>Gender</th>
<th>Original diagnosis</th>
<th>CAR target</th>
<th>Days post-CAR infusion at time of death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>M</td>
<td>DLBCL</td>
<td>CD19</td>
<td>21</td>
<td>Cytomegalovirus activation syndrome, myocarditis, acute renal failure, diffuse large B cell lymphoma</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>F</td>
<td>ALL</td>
<td>CD19</td>
<td>3</td>
<td>Diffuse pseudolymphomatous and CRS</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>M</td>
<td>ALL, MM</td>
<td>CD19</td>
<td>90</td>
<td>Aspergillosis pneumonia</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>M</td>
<td>DLBCL</td>
<td>CD19</td>
<td>23 (days)</td>
<td>Bacterial infection secondary to extravasation of infused cell</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>M</td>
<td>DLBCL</td>
<td>CD19</td>
<td>38</td>
<td>Pulmonary hypertension secondary to treatment with rituximab</td>
</tr>
<tr>
<td>6</td>
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<tr>
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<td>CLL</td>
<td>CD19</td>
<td>4</td>
<td>Cardiac infarction due to acute bacterial endocarditis</td>
</tr>
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<td>11</td>
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<td>CD19</td>
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<tr>
<td>12</td>
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<td>DLBCL</td>
<td>CD19</td>
<td>47</td>
<td>E. coli bacteremia, coagulopathy</td>
</tr>
</tbody>
</table>

Background Our institution has treated over 300 patients with chimeric antigen receptor (CAR) T-cell immunotherapy (CAR T-cell therapy) since 2013. Phase I and II trials were primarily based on heavily treated patients with B cell acute lymphocytic leukemia (B-ALL), aggressive diffuse large B cell lymphoma (DLBCL), and multiple myeloma (MM) who had failed multiple lines of prior chemotherapy and/or hematopoietic stem cell transplantation (HSCT). In these relapsed and/or refractory patients, CAR-T therapy resulted in complete remission in 93% of B-ALL, 60% of DLBCL, and ~80% of MM. Our Pathology Group at Fred Hutch have reviewed and diagnosed various patients with interesting relapse or complications as a result of CAR T-cell therapy. Here we present a retrospective review of autopsies from CAR T-cell therapy.

Methods A search for all autopsies conducted on patients from Seattle Cancer Care Alliance/University of Washington Medical Center was performed using the keywords ‘CAR T’ and ‘Chimeric-antigen’. Our inclusion criteria were patients treated with CAR T-cell therapy. Pathology and clinical records were reviewed for cause of death, disease and treatment timelines, microbiology data, cytokine levels, other pathology biopsies, and pertinent laboratory values. Histologic tissues were reviewed.

Results Twelve autopsies were performed since 2013. Patient characteristics and causes of death are summarized in table 1. The most common cause of death was due to infectious causes (n=6). Two patients (Patients 10 and 11) had cardiovascular related deaths. Six patients (Patients 1, 2, 6, 7, 10, 12) suffered from CRS in their post-infusion course, four of whom (Patients 1, 2, 7, 10) had CRS directly attributed as the cause of death. CRS was further complicated by immune effector cell-associated neurotoxicity syndrome (ICANS) in 5 patients (Patients 1, 5, 6, 7, and 12). CRS with ICANS was the second most common cause of death in patients treated with CAR T-cells. Three patients (Patients 1, 4, 9) had progression of disease that attributed to cause of death.

Conclusions CAR T-cell therapy is a highly effective treatment even for patients who have relapsed and/or refractory disease. Post-therapy complications range in severity and may be fatal in rare instances as in the patients summarized in this study. Infection, CRS with ICANS are the most common causes of death in our single institution study.

Ethics Approval The study was approved by Fred Hutchinson Cancer Research Center’s Institutional Review Board, approval number 1837

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0643

Abstract 644 Table 1 Patient characteristics: age, sex, original diagnosis, CAR target, cause of death, and days post-CAR T-cell infusion at time of death

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<th>CAR target</th>
<th>Days post-CAR infusion at time of death</th>
<th>Cause of Death</th>
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<td>E. coli bacteremia, coagulopathy</td>
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Background The programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) inhibitors are increasingly studied and are known to have unique inflammatory side effects due to non-specific immune system activation. While rare, PD-1/PD-L1 inhibitors can cause ocular toxicities, including inflammatory eye disease. However, these ocular adverse events are less well-studied.

Methods This was a retrospective review of two adverse event (AE) monitoring databases maintained by the National Cancer Institute’s Cancer Therapy Evaluation Program (CTEP), one of the largest public sponsors of clinical trials worldwide. One database (CTEP-AERS) is used for study sites to expeditiously report serious AEs for potential FDA review, while the other database (CDUS) is updated quarterly to reflect all the adverse events from the Phase 1 and Phase 2 trials in the CTEP network.

Results The two adverse event databases were queried for ocular adverse events up to May 19, 2020. A total of 331 adverse events from 259 patients were found. 73 patients (28%) were exposed to nivolumab, 117 patients (45%) were exposed to combination nivolumab and ipilimumab, 41 (16%) were exposed to pembrolizumab, 26 (10%) were exposed to atezolizumab, and 2 (0.8%) were exposed to durvalumab. 59 adverse events were reported from 47 patients by the study
site as serious AEs and had more detailed clinical information available. Ocular AEs occurred within several months of initiating the study treatment (all ocular AEs: median 6 weeks, IQR 0–18, ocular AEs reported as serious: median 12 weeks, IQR 6–20). CTCAE grade for ocular AEs was generally mild to moderate (all ocular AEs: grade 1, IQR 1–2, ocular AEs reported as serious: grade 2, IQR 2–3). Clinical workup and treatment varied for the ocular AEs reported as serious. 30/47 patients (64%) receiving ophthalmologic evaluation. 16/47 (34%) of patients with serious ocular AEs had to delay or discontinue study drug treatment. However, 14/47 (30%) had improvement in their ocular AE and 16/47 patients (34%) had resolution of their ocular AE. The most common ocular AE treatments in our dataset were steroids (intraocular, oral, and steroid eye drops).

Conclusions Ocular adverse events are rare complications of PD-1/PD-L1 inhibitor therapy, can be severe enough to cause PD-1/PD-L1 treatment discontinuation or delay, but patients may not always be referred to eye specialists. Future PD-1/ PD-L1 inhibitor studies would benefit from standardized plans for ophthalmologic evaluation of ocular toxicities.

REFERENCES

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T cells by anti-CD8 mAbs from day 29 onwards, and maintained weekly, as in this model CD8+ T cells are the main hapten responder population. Samples were collected for histochemistry and analyzed by flow cytometry.

**Results** Our data indicate that despite the depletion of circulating T cells, anti-CD-P1 recipients mount a higher initial recall response to contact agents. Higher ear swelling was observed with increased inflammation in these mice. Our data suggest anti-CD-P1 can liberate local T cell responses in the absence of a contribution from blood, and may offer a model to test therapeutic interventions to alleviate peripheral immune toxicities.

**Conclusions** Our results suggest that this murine model of contact hypersensitivity represents a potential model for skin immune checkpoint toxicities. This model of locally-mediated inflammatory recall may advance the goal of uncoupling toxicity from efficacy in patients with immune-related adverse events.

**Ethics Approval** The animal study was approved by Weill Cornell Medicine’s IACUC; approval number D16-00186.

**REFERENCES**


647 EVALUATION OF EFFICACY AND TOXICITY OF CD137 IMMUNOTHERAPY WITH URELUMAB-MIGG1 CHIMERIC ANTIBODY IN CD137 HUGEMM™

**Background** CD137 (4-1BB) is a powerful T cell co-stimulatory molecule belonging to the TNF receptor superfamily, which promotes cytotoxic T cell survival and memory formation upon CD137L ligation. CD137 has become an attractive immuno-oncology therapeutic target with multiple agonistic antibodies in clinical trials, including urelumab and utomilumab, with promising response in combination with anti-PD1 immunotherapies such as nivolumab. Clinical applications of CD137 agonistic antibodies are hampered, however, by dose-limiting off-tumor liver toxicity (urelumab) or lower efficacy (utomilumab). The cause of liver toxicity is reported primarily to be due to Fcγ receptor mediated cross linking;1–5 CD137 agonistic antibodies may also trigger hepatotoxicity through activation of IL-27 secreting liver Kupffer cells and monocytes.6 The remaining challenge in decoupling efficacy from liver toxicity is the lack of preclinical mouse models which can be used to assess both efficacy and the immune-related adverse events (irAE) of human CD137 agonistic antibodies.

**Methods** To mimic the clinical outcomes of urelumab, we utilized humanized CD137 knock-in mice in Balb/c background (Balb/c CD137 HuGEMM) to evaluate its efficacy with CT26.WT syngeneic tumors. Liver toxicity was analyzed by monitoring fasting serum ALT/AST levels at different time points.

**Results** Urelumab showed moderate anti-tumor response at the dose level of 5 mg/kg, while serum ALT/AST levels showed no difference compared to isotype control suggesting that, due to the different binding capacity of the human IgG4Fc domain to mouse FcγR, the human version of the agonistic antibody cannot fully recapitulate its effect on HuGEMM mice. Therefore, a chimeric antibody with mouse IgG1Fc domain (urelumab-mlgG1) was created to dissect the potential role of FcγR mediated cross linking on both efficacy and liver toxicity; an urelumab-mlgG1-DANA variant with D265A/N297A mutation to abolish Fc effector function was also included as a dominant negative control. We found that urelumab-mlgG1 showed further enhanced efficacy compared to urelumab alone through FcγR mediated cross linking, while urelumab-mlgG-DANA showed compromised anti-tumor response. With regards to liver toxicity, urelumab-mlgG1 caused chronic liver inflammation and hepatocyte damage indicated by immune cell infiltration in the liver and significantly elevated serum ALT levels, which was abolished by the urelumab-mlgG1-DANA variant. The study also compared urelumab treatment in CD137 HuGEMM head-to-head with the mouse surrogate agonistic antibody (3H3) in wild-type BALB/c mice. 3H3 showed robust tumor growth inhibition as well as dramatic ALT elevation.

**Conclusions** We faithfully recapitulated the clinically observed tumor growth inhibition and liver toxicity of urelumab by using a chimeric version of urelumab in CD137 HuGEMM, indicating the importance of both the mouse model and antibody version in evaluation of efficacy and irAE.

**Ethics Approval** Animal experiments were conducted in accordance with animal welfare law, approved by local authorities, and in accordance with the ethical guidelines of Crown-Bio (Taicang).

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0647

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CYTOKINE CHANGES DURING IMMUNE-RELATED ADVERSE EVENTS AND STEROID TREATMENT IN MELANOMA PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITORS

Kevin Tyan*, 1 Joanna Bagiriska, 2 Martha Brainard, 2 Arita Giobbie-Hurder, 2 Mariano Severgnini, 2 Michael Manos, 2 Rizwan Haq, 2 Elizabeth Buchbinder, 2 Patrick Ott, 2 F Stephen Hodi, 2 Osama Rahma. 1 Harvard Medical School, Somerville, MA, USA; 2 Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Abstract 648 Figure 1 Baseline cytokine level differences in patients with irAEs

(A) Baseline levels of Ang-1 (median 16,375 vs. 11,604 pg/mL, p = 0.005) and CD40L (median 3,840.2 vs. 2,433.8 pg/mL, p = 0.006) was higher in patients who developed dermatitis during ICI treatment. (B) Baseline levels of IL-17 was higher (median 2.8 vs. 0.0 pg/mL, p = 0.005) and CD40L (median 3,840.2 vs. 2,433.8 pg/mL, p = 0.006) was higher in patients who developed pneumonitis during ICI treatment. (C) There was a trend towards lower baseline levels of GSCF (median 0.0 vs. 12.9 pg/mL, p = 0.08) in patients who developed colitis during ICI treatment.

P-values for baseline comparisons were obtained through Wilcoxon rank-sum test. The solid black line indicates median. Violins show range and kernel density estimate distributions of each group. (*) p < 0.05, (**) p < 0.01.

Abstract 648 Figure 2 Spearman’s correlation analysis of cytokine changes

Spearman’s correlation analysis of cytokine changes in patients who did not develop irAEs (A), developed irAEs without steroid treatment (B), and developed irAEs with steroid treatment (C) during the cytokine profiling period. Spearman’s correlation heatmap show changes in cytokine fold-change relative to each other. Blue indicates fold-change of two cytokines in the same direction, red indicates fold-change of two cytokines moving in opposite directions. Cytokine fold-change is based on changes in serum levels between baseline and the event of interest (irAE onset or irAE onset with steroid treatment). (A) Patients who did not develop irAEs during the cytokine profiling period had a discordant pattern of cytokine fold-change, with 213/276 (77.2%) pairwise comparisons changing in the same direction. (B) Patients who developed irAEs without receiving steroid treatment demonstrated a harmonized pattern of cytokine fold-change, with 269/276 (97.5%) pairwise comparisons showing concordant direction of fold-change. (C) Patients who developed irAEs and received steroid treatment exhibited a discordant pattern of cytokine fold-change similar to patients without irAEs, with 214/276 (77.5%) pairwise comparison changing in the same direction.

Abstract 648 Figure 3 Example patient timelines

The log2 fold-change of all cytokines over time were graphed for each patient. Timelines below each graph indicate ICI regimen (blue), irAEs (red), steroid treatment for irAEs (green), and RECIST response status (yellow). Black arrows indicate each ICI dosage cycle. A representative timeline is shown for a patient (#1) who did not develop irAEs during the Luminex profiling period, a patient (#29) who developed grade 1 dermatitis without receiving steroid treatment, and a patient (#44) who developed grade 2 arthralgia and received prednisone and methylprednisolone.
significantly higher baseline IL-17 (p = 0.009, figure 1B). There was a trend towards lower GCSF levels in patients developing colitis (N = 8, p = 0.08, figure 1C). We observed a harmonization of cytokine fold-change in patients who developed irAEs without receiving steroids: 269/276 (97.5%) of pairwise comparisons exhibited fold-change in the same upwards or downwards direction (figure 2). In contrast, corticosteroid treatment in patients with irAEs appeared to alter cytokine fold-change to a discordant pattern (214/276, 77.5%) mirroring patients who did not develop irAEs during the longitudinal profiling period (213/276, 77.2%). Example patient timelines are shown in figure 3.

Conclusions Baseline cytokine levels correlate with specific irAEs in melanoma patients receiving ICIs. irAEs appear to drive a concordant pattern of cytokine fold-change, which is disrupted by corticosteroid administration. These findings should be validated in larger cohorts.

Ethics Approval Patients were identified from DFCI’s melanoma bio-specimen banking protocol (DFCI protocol 05-042)

REFERENCES

INCIDENCE OF THROMBOEMBOLISM (TE) IN PATIENTS WITH MELANOMA RECEIVING IMMUNE CHECKPOINT INHIBITOR (ICI) THERAPY AND ITS ADVERSE IMPACT ON SURVIVAL

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Background Little is known about rates of arterial thromboembolism (ATE) and venous thromboembolism (VTE) in patients with melanoma on ICI. We assessed incidence and outcomes of ATE and VTE in patients with melanoma receiving ICI.

Methods We conducted a retrospective cohort study of patients with melanoma receiving ICI from July 2015 through December 2017 at Cleveland Clinic. TE including VTE events of deep venous thrombosis (DVT), pulmonary embolism (PE), visceral vein thrombosis (VVT), and ATE events of myocardial infarction (MI), stroke, or transient ischemic attack (TIA) after ICI initiation were identified. Overall survival (OS) from ICI initiation was estimated by Kaplan-Meier and Cox hazard models; associations between TE, ICI regimen, and clinical risk factors were evaluated using log-rank test.

Results The study population comprised 228 patients with median age 65 (23–91) years, 67% male, and median follow up 27.3 months. Pembrolizumab was most commonly used (38.7%), followed by combination ipilimumab plus nivolumab (29.4%), ipilimumab (20%), and nivolumab (12.3%). Most had stage IV disease (81.1%) and 11% had brain metastases (BM) at treatment initiation. Fifty-one TE events occurred in 47 patients (20.6%), including 37 (16.2%) VTE and 14 (6.1%) ATE. Of VTE, DVT comprised 46.0%, PE 24.3%, DVT+PE 21.6%, VVT 5.4%, and DVT+VVT 2.7%. Of ATE, stroke comprised 57.2%, MI 35.7%, and TIA 7.1%. Of all TE events, 72% resulted in hospitalization and 19% resulted in clot-related mortality. Cumulative incidence of TE after ICI initiation was 9.3% (95%CI,6.0–13.6%) at 6 months, and 16.0% (95%CI,11.6–21.2%) at 12 months. The 6- and 12-month VTE cumulative incidence rates were 8.0% (95% CI,4.9–12.0%), and 12.9% (95%CI,8.9–17.7%), respectively. The 6- and 12-month ATE cumulative incidence rates were 2.2% (95%CI,0.84–4.8%), and 4.5% (95% CI,2.3–7.8%), respectively. The 6- and 12-month VTE cumulative incidence rates were higher with combination ICI than single agent (16.7% vs. 5.0% and 21.3% vs. 9.5%, respectively; p=0.02) (figure 1). Risk factors associated with VTE in univariate analysis included BM, stage IV disease, combination ICI, and Khorana score ≥1 (p<0.05 for all). In multivariate analysis, combination ICI (HR 2.21; [95%CI,1.04–4.72]; p=0.04) and Khorana score ≥1 (HR 2.48; [95%CI,1.18–5.20]; p=0.03) remained significantly associated with VTE. Of patients without BM, OS was worse in patients with TE compared to those without (3-year OS 34.9% vs. 62.9%; HR 1.84; [95% CI,1.16–2.93]; p<0.001), when adjusted for age, stage, and Khorana score (figure 2).

Abstract 649 Figure 1 Cumulative incidence of VTE, stratified by ICI type

Abstract 649 Figure 2 OS in patients without BM stratified by TE status
Conclusions ICI is associated with a high incidence of TE in patients with melanoma; TE is associated with substantial worsening of survival.

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**NEUROLOGICAL ADVERSE EVENTS ASSOCIATED WITH IMMUNE CHECKPOINT INHIBITORS: OUR EXPERIENCE IN A TERTIARY CARE CENTER**

**Method**

Background Immune checkpoint inhibitors (ICIs) have become a revolution in the treatment of many tumoral diseases, resulting in a significant increase in terms of life expectancy and quality of life. Despite these outstanding advances in long-life survival, a new spectrum of adverse events has been developed and is known to be one of the biggest challenges in clinical practice nowadays. Immune-mediated neurotoxicity stands out as a rather unusual complication, but its potentially lethal consequences make the characterization and right management of this adverse event a crucial issue in this field.

**Results**

This is a retrospective study including all the cancer patients that have developed any neurological adverse event related to ICIs treatment, in a period of 4 years (from 2017 to 2020).

**Results**

13 patients were included in the study (8 were treated with anti-PD-1/PD-L1 immunotherapy, 1 with anti-CTLA-4 and 4 with the combination of both strategies). 4 patients developed generalized myasthenia gravis (GMG), 4 immune-mediated encephalitis (IME), 3 immune-related encephalopathy without radiological/analytical evidence of encephalitis, 1 mixed-polyneuropathy, and 1 polyposis. 3 patients with GMG were seropositive, 3 developed the clinical feature within the first 21 days of immunotherapy treatment and all of them received anti-PD-1/PD-L1 treatment. All patients with IME showed pleocytosis in cerebrospinal fluid, without any data in brain MRI. 12 patients suspended ICIs treatment after the event and were treated with high doses of intravenous corticosteroids and intravenous immunoglobulins. ICIs withdrawal did not suppose harm in patients that have developed any neurological adverse event related to ICIs treatment, in a period of 4 years (from 2017 to 2020).

**Results**

13 patients were included in the study (8 were treated with anti-PD-1/PD-L1 immunotherapy, 1 with anti-CTLA-4 and 4 with the combination of both strategies). 4 patients developed generalized myasthenia gravis (GMG), 4 immune-mediated encephalitis (IME), 3 immune-related encephalopathy without radiological/analytical evidence of encephalitis, 1 mixed-polyneuropathy, and 1 polyposis. 3 patients with GMG were seropositive, 3 developed the clinical feature within the first 21 days of immunotherapy treatment and all of them received anti-PD-1/PD-L1 treatment. All patients with IME showed pleocytosis in cerebrospinal fluid, without any data in brain MRI. 12 patients suspended ICIs treatment after the event and were treated with high doses of intravenous corticosteroids and intravenous immunoglobulins. ICIs withdrawal did not suppose harm in patients that have developed any neurological adverse event related to ICIs treatment, in a period of 4 years (from 2017 to 2020).

**Conclusions**

Herein we present early data from an acute care APP led CPI outpatient clinic. Most patients required initiation of steroids for their irAE, however only a small majority required higher level care (i.e. ED or admission) than was able to be provided in the clinic. Twenty-two patients (73%) required steroids as their initial treatment for irAE, with 4 patients (13%) requiring referral to other specialties.

**Ethics Approval**

This study was approved by the institutional review board at Mayo Clinic.

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**DASATINIB AS A RAPID PHARMACOLOGICAL ON/OFF SWITCH FOR T CELL BISPECIFIC ANTIBODY-INDUCED T CELL ACTIVATION AND CYTOKINE RELEASE**

**Background**

T cell bispecific antibodies (TCBs) are extremely potent T cell engagers, harboring a 2+1 format with one binder to the CD3ε chain and two binders to specific tumor antigens. Crosslinking of CD3 with tumor antigens triggers T cell activation, proliferation and cytokine release, leading to...

[References](http://dx.doi.org/10.1136/jitc-2020-SITC2020.0651)
tumor cell killing. TCB treatment is sometimes associated with safety liabilities due to on-target off-tumor, on-target off-tumor cytotoxic activity and cytokine release. Patients treated with TCBs may experience a Cytokine Release Syndrome (CRS), characterized by fever, hypotension and respiratory deficiency and associated with the release of pro-inflammatory cytokines such as IL-6, TNF-α, IFN-γ, and IL-1β. Off-tumor toxicity may occur if target antigens are expressed in healthy cells, which may potentially result in tissue damages and compromise the patient's safety. Rapid pharmacological blockade of T cell activation and proliferation is a promising approach to mitigate these life-threatening toxicities. Tyrosine kinases such as SRC, LCK or ZAP70 are involved in downstream signaling pathways after engagement of the T cell receptor and blocking these kinases might serve to abrogate T cell activation when required. Dasatinib was identified as a potent candidate that switches off CAR T cell functionality.

Methods Using an in vitro model of target cell killing by human peripheral blood mononuclear cells, we assessed the reversible effects of dasatinib combined with CEA-TCB or HLA-A2-WT1-TCB on T cell activation and proliferation, target cell killing and cytokine release. At assay endpoints, T cell phenotype and target cell killing were measured by flow cytometry and supernatants were analyzed by Luminex to assess cytokine release. To determine the effective dose of dasatinib, the Incucyte system was used to follow kinetics of target cells killing by TCB in the presence of a dose response of dasatinib concentrations.

Results 100 nM dasatinib prevented TCB-mediated target cell killing when added in the system upon restimulation of activated T cells (figure 1). Dasatinib concentrations above 50 nM fully switched off target cell killing (figure 2) which was restored upon removal of dasatinib. These data confirm that dasatinib act as a potent and reversible on/off switch for activated T cells at pharmacologically relevant doses as they are applied in patients according to the label.

Conclusions Taken together, we provide evidence for the use of dasatinib as a pharmacological on/off switch to mitigate off-tumor toxicities or CRS by T cell engaging therapies. These data are being validated in vivo.

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patients were excluded given lack of records or because they received less than 2 cycles of treatment.

Results 176 patients were included in the final analysis. ICIs were discontinued in 25/176 (14.2%) patients secondary to AE. 24/176 (13.6%) patients had grade III or higher AEs reported. 10/95 (10.5%) patients who received pembrolizumab developed grade III/IV AEs (8 pneumonitis, 2 nephritis). 5/45 (11.1%) patients treated with nivolumab developed grade III/IV AEs (2 pneumonitis, 1 new-onset DKA, 1 nephritis, 1 myositis). 8/19 (42.1%) receiving durvalumab had grade III or higher AEs (6 pneumonitis, 1 sepsis, 1 colitis). Lastly, 1/17 (5.8%) in atezolizumab group developed grade III/IV AE (colitis). 96/176 (54.3%) patients had one or more ER visit and 91/176 (51.7%) were admitted to the hospital for various reasons one or more times.

Conclusions ICIs have a relatively safe drug profile. 86.4% of our studied population did not develop any grade III or higher AEs. The main reason for ICI discontinuation was disease progression rather than AE. The most common grade III/IV AE was pneumonitis. Durvalumab had the highest incidence of AE, pneumonitis, which is likely related to radiation use prior to immunotherapy.

Acknowledgements N/A

Ethics Approval Institutional Review Board approval - IRB#: SFH-19-19-72

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655 CONCORDANCE BETWEEN HEALTHCARE PROVIDERS AND EXPERT CONSENSUS RECOMMENDATIONS IN THE MANAGEMENT, MONITORING, AND MITIGATION OF ADVERSE EVENTS ASSOCIATED WITH CAR T-CELL THERAPY: AN UPDATED ANALYSIS

1. Matthew Frigault*, 2Megan Cartwright, 3Krista Marcello, 4Timothy Quill, 5Daniel DeAngelo, 3Ilene Galinsky, 4Shilpa Paul, 1Matthew Frigault*, 2Megan Cartwright, 2Krista Marcello, 2Timothy Quill, 3Daniel DeAngelo, 3Ilene Galinsky, 4Shilpa Paul, 5Daniel DeAngelo, 3Ilene Galinsky, 4Shilpa Paul, 1Matthew Frigault*, 2Megan Cartwright, 2Krista Marcello, 2Timothy Quill, 3Daniel DeAngelo, 3Ilene Galinsky, 4Shilpa Paul, 1Matthew Frigault*, 2Megan Cartwright, 2Krista Marcello, 2Timothy Quill, 3Daniel DeAngelo, 3Ilene Galinsky, 4Shilpa Paul, 1Matthew Frigault*, 2Megan Cartwright, 2Krista Marcello, 2Timothy Quill, 3Daniel DeAngelo, 3Ilene Galinsky, 4Shilpa Paul

Background Chimeric antigen receptor (CAR) T-cell therapy has been a major innovative breakthrough for hematologic malignancies. These therapies are associated with unique and potentially serious toxicities, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity (ICANS), that require vigilance, prompt recognition, and appropriate management to ensure patient safety and optimal therapeutic benefit. We developed an online tool to give healthcare providers (HCPs) case-specific, evidence-based expert guidance on the management of adverse events (AEs) from CAR T-cell therapy. Here, we report an updated analysis comparing CAR T-cell toxicity management among HCPs using the tool vs the expert consensus recommendations.

Methods In March 2019, 5 experts provided consensus guidance for the screening, prophylaxis, monitoring, and management of CRS and ICANS in patients considering or receiving CAR T-cell therapy. This information was used to build the interactive online tool. To use this tool, HCPs enter the AE of interest, the severity of the event, and their planned management approach. The HCPs were then shown the expert recommendation for that specific scenario. After viewing the expert recommendation, HCPs were asked if it affected their intended approach.

Results Between May 2019 and July 2020, 282 HCPs entered 431 unique case scenarios into the tool. Of the entered cases, 56% were HCPs seeking expert recommendations on pretreatment screening and prophylaxis/monitoring strategies for patients not yet experiencing an AE. Of 188 cases entered for patients who received CAR T-cell therapy and experienced an AE, 67% were CRS and 33% were neurotoxicity/ICANS. Overall, the planned toxicity management strategy of HCPs matched the expert recommendations in 57% of cases, with a similar rate of concordance for both CRS and ICANS events. There was no significant difference in concordance rates with expert recommendations by toxicity severity (figure 1) nor among HCPs who indicated they practiced at authorized centers vs those who did not (P = 0.7184). Among HCPs who answered the optional survey on the impact of the tool on their management plan, 30% indicated that the expert recommendations changed their approach.

Conclusions These data suggest that many HCPs are challenged to optimally manage CAR T-cell therapy toxicities in concordance with expert recommendations. Use of an online tool providing easy access to evidence-based consensus expert recommendations may improve care and safety in patients treated with CAR T-cell therapy. A detailed analysis of the tool including planned management vs expert recommendations for each toxicity and grade will be presented.

REFERENCES

Abstract 655 Figure 1 Planned management of HCPs compared with expert recommendations, by grade
Background Optimal diagnostic algorithm to differentiate checkpoint inhibitor pneumonitis (CIP) from mimics is uncertain; patients with respiratory comorbidities often receive prolonged corticosteroids until diagnostic clarification. Drawbacks to empiric use of corticosteroids include decreased immunotherapy (IO) efficacy and increased infectious risk. This retrospective study systematically collected data on patients treated for lung cancer who were suspected to have severe CIP.

Methods This single-center retrospective cohort study collected data on all lung cancer patients who received > 1 dose of an immune checkpoint inhibitor between 6/1/18 and 2/1/20 (n=210), were subsequently hospitalized and received > 1 dose of systemic corticosteroids for any indication (n=97). Data were collected on clinical factors including comorbidities, cancer stage, IO cycles, biomarkers, diagnostic work-up, antibiotics, steroids, progression, and survival. A blinded radiologist reviewed all imaging of suspected CIP cases and categorized their radiographic patterns.

Results In our high-risk cohort of 97 patients, median follow-up was 23 months with progression in 54 patients (56%) at median 11 months and death in 67 patients (69%) at median 14mo. Twelve patients (12%) were suspected to have severe CIP after IO treatment for lung cancer; CIP was confirmed in 5/12 and ruled-out (mimics) in 7/12 after 30 and 3 median IO cycles, respectively. Most suspected patients underwent CXR, CTA chest, blood cultures, and received empiric antibiotics. Common radiographic patterns were ground-glass opacities, organizing pneumonia, hypersensitivity pneumonitis, and acute interstitial pneumonia/acute respiratory distress syndrome (AIP/ARDS) among confirmed cases (4/5) and ground-glass opacities, organizing pneumonias, bronchiolitis, AIP/ARDS among mimics (4/7). The median time to confirm CIP or rule out a mimic was 5 ± 4 days. Median time to onset of symptoms differed substantially for confirmed and mimic cases: 17 months and 1 month, respectively.

Conclusions CIP mimics were more common than confirmed cases in routine clinical practice, particularly among patients hospitalized for respiratory symptoms <1 month after initiating immunotherapy for lung cancers. In these cases, it is reasonable to empirically cover possible CIP with shorter (~1 week) courses of steroids until diagnostic clarity is achieved. CT imaging should be obtained as it is sensitive though not specific for CIP. CIP mimics may contribute to the higher incidence of CIP reported by real-world patient registries than by clinical trials.

Ethics Approval The study was approved by Wake Forest Baptist Medical Center’s Ethics Board, IRB approval number 00044126.
Background Autoimmunity is associated with increased risk of malignancy. However, patients with pre-existing autoimmune diseases (AIDs) were excluded from immune checkpoint inhibitor (ICI) trials as these agents can cause immune-related adverse events (irAEs). Data are limited on the safety and efficacy of combination immunotherapy in this at-risk population.

Methods We conducted a multi-center retrospective study to evaluate the safety and efficacy of ICI therapy in patients with pre-existing AID treated at NYU and at MD Anderson Cancer Center. Primary endpoints were occurrence of irAEs and AID flares. Secondary endpoints were time to treatment failure (TTF) and overall survival (OS).

Results Of 121 patients identified from our institutional databases, 53% received single-agent anti-PD-1 therapy, and 47% received ICI combination. Over half of malignancies were lung cancer (34%) and melanoma (20%). Preexisting AIDs included: rheumatologic (58%), gastrointestinal (12%), endocrine (16%) and neurologic (4%). Overall, 94% had asymptomatic AID, and 21% were receiving systemic immunomodulatory drugs at ICI initiation. Median duration of follow up after ICI initiation was 9 (0.4–41.9) months in patients receiving ICI combination and 8 (0.2–47.3) months in patients receiving anti-PD-1 monotherapy. Combination therapy was associated with higher rates of irAEs compared with anti-PD-1 monotherapy (56% versus 28%). Grade 3/4 irAEs were equivalent in both groups: combination (38%) and anti-PD-1 group (39%). Treatment related deaths were not observed in any group. AID flares occurred in 36% of the anti-PD-1 group versus 29% of combination group. Adverse events (irAEs and/or flares) required systemic immunomodulatory therapies more frequently in the combination group (84%) versus the anti-PD-1 group (59%), and permanent ICI discontinuation was reported in 19% of patients in the combination group versus 11% in the anti-PD-1 group. Tumor progression was observed in 49% of patients on combination ICI and TTF was 14.5 months (95% CI 0.000–31.5), while progression was observed in 64% of patients on anti-PD-1 monotherapy and TTF was 6.4 months (95% CI 4.01–8.9) (p=0.019). Median OS in the combination therapy group was not reached whereas it was 27.3 months in the anti-PD-1 monotherapy group.

Conclusions Our novel findings suggest that high rates of adverse events were observed in patients with pre-existing AIDs treated with ICI combination therapy. However, they were manageable and rarely required permanent ICI discontinuation. Taken together, these data show that ICIs should be offered, albeit with caution in patients with AIDs, to achieve durable cancer remission. Prospective clinical data are needed to guide these complex decisions.

Ethics Approval The study was approved by NYU Langone’s Ethics Board, approval number I18-01657 and MD Anderson’s Ethics Board, approval number PA19-0089.

Abstract 659 Figure 1 Imaging findings of COVID-19 viral pneumonia Axial, and coronal computed tomography images in a patient with laboratory proven COVID-19 infection demonstrating diffuse ground glass opacities in a peripheral and peribronchovascular distribution interspersed with areas of frank consolidation.

Abstract 659 Table 1 Anakinra cohort
Three patients who experienced clinical signs and symptoms of cytokine release syndrome after administration of immunotherapy. These patients were treated with anakinra, and survived.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>67</td>
<td>69</td>
<td>73</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Underlying malignancy</td>
<td>Stage 4 renal cell carcinoma</td>
<td>Stage 4 ovarian cancer</td>
<td>Stage 4 prostate cancer</td>
</tr>
<tr>
<td>Treatment administered</td>
<td>Intratumoral OX40, CarG, vilimunab</td>
<td>Intratumoral vilimunab, rindumab, IL-2</td>
<td>Intratumoral OX40, CarG, vilimunab</td>
</tr>
<tr>
<td>Symptoms of cytokine release syndrome</td>
<td>Hypotension (60/20), fever, dyspnea, headache</td>
<td>Hypotension, headache, loss of consciousness, bilateral temporo-parietal regional hypometabolism in regions on head CT</td>
<td>Hypotension (70/40), fever, loss of consciousness</td>
</tr>
<tr>
<td>Treatment for cytokine release syndrome</td>
<td>Anakinra 100 mg subcutaneous injection, single dose</td>
<td>Anakinra 100 mg subcutaneous injection, daily for 5 days</td>
<td>Anakinra 100 mg subcutaneous injection, daily for 2 days</td>
</tr>
<tr>
<td>Clinical course</td>
<td>Complete resolution in 12 hours</td>
<td>Patient became cognitively intact with residual right upper and lower extremity paralysis</td>
<td>Complete resolution in 72 hours</td>
</tr>
<tr>
<td>Outcome</td>
<td>Survived without sequela</td>
<td>Discharged to hospice with residual paraplegia</td>
<td>Survived without sequela</td>
</tr>
</tbody>
</table>

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0658
received glucocorticoids, vasopressors, and respiratory support, suffering progressive symptoms of cytokine release syndrome, and death (table 2).

Conclusions The IL-1 inhibitor, anakinra, has shown efficacy in ameliorating signs and symptoms of cytokine release syndrome caused by immunotherapy administration. Mechanistic similarities between cytokine release syndrome and COVID-19 related pulmonary damage suggest that this pharmacotherapeutic intervention could decrease mortality and need for mechanical ventilation in patients infected with COVID-19.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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Machine learning, artificial intelligence, and computational modeling

DEVELOPING GENERALIZABLE DEEP LEARNING MODELS FOR TUMOR SEGMENTATION IN PATHOLOGY IMAGES TO ENABLE THE IDENTIFICATION OF PREDICTIVE BIOMARKERS FOR IMMUNOTHERAPIES

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Background Despite recent advances in cancer immunotherapies, their efficacies vary significantly among patients. To better understand the mechanisms of drug resistance, it is essential to characterize immune responses to immunotherapies in the tumor immune microenvironment (TME) from intact patient tissues. To this end, quantitative spatial immune profiling of pathology images has been the focus for many recent studies. This analysis often depends critically on the automated image segmentation of tumor and stromal compartments. However, current segmentation approaches, even these based on deep learning, often fail to perform well when given datasets to segment, which differ from the data on which they were trained. Specifically, tissue segmentation models trained for one type of organ (source) face challenges in performance when applied directly to images of another organ type (target), even when the targeted regions to segment are highly similar in morphology between the source and target.

Here, we present a segmentation approach to adapt knowledge learned from source data of one cancer type to unlabeled target data of another organ cancer type via unsupervised domain adaptation (UDA) frameworks. This research will help build deep learning models that significantly reduce the need for expert manual annotations.

Methods Annotating colorectal cancer (CRC) (target domain) and prostate cancer (source domain) were used for tumor tissue segmentation model development, containing image tiles from 38 and 20 whole slide images, respectively. We compared the performance and robustness of four approaches. First, we implemented two output-space domain-adversarial based UDAs. Then we implemented a self-training-based approach. Additionally, we designed a two-stage UDA approach by first conducting self-training and then further aligning target domain features with category-anchors generated from source data after a first stage of self-training.

Results Directly applying a tumor tissue segmentation model trained on prostate cancer images (source) to CRC images (target) resulted in an intersection-over-union (IOU) score of 62.5%, which was 19% IOU lower (domain gap) than using a model trained on target data. Methods based on output-space domain adversarial training reduced the domain gap by up to 8% IOU, a performance result which was better than with self-training-based methods, which only reduced the domain gap by 4%. Both sets of approaches improved precision by 10%.

Conclusions We demonstrate the feasibility of designing tumor segmentation models that are robust and generalizable to multiple indications. The UDA approaches have the potential to speed our understanding of factors influencing immunotherapy efficacy through automated annotation of tissue regions required.

REFERENCES

FIVE IMMUNOTYPIC SIGNATURES IDENTIFIED IN HUMAN GliOBlastOMA CORRELATE WITH TUMOR CONTACT WITH THE LATERAL VENTRICLE, IMMUNE SUPPRESSION, AND PATIENT OUTCOME

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Background Glioblastomas make up more than 60% of adult primary brain tumors and carry a median survival of less than 15 months despite aggressive therapy. Immunotherapy, now standard of care for many peripheral solid tumors, offers an appealing alternative platform that may improve survival outcomes for patients with glioblastoma; however, predictive features that could inform responsiveness to different immunotherapeutic modalities remains to be elucidated. Recent studies have demonstrated that patients whose tumors show radiographic contact with the lateral ventricle have diminished
survival outcomes compared to patients whose tumors do not contact the lateral ventricle. While greater immune infiltrate correlates with more favorable outcomes and more effec
tual responses to immunotherapy, the anti-tumor immune response in the ventricle is unknown. We hypothesized that ventricle contact may provide a uniquely immunosuppressive microen
vironment within the brain that promotes tumor growth by suppressing anti-tumor immunity, that may be overcome with appropriate targeting strategies.

Methods Primary glioblastoma tumors obtained in accordance with the Declaration of Helsinki and with institutional IRB approval (#131870) were disaggregated into single-cell suspensions. Radiographic contact with the LV was identified by MRI imaging and confirmed by a trained neurosurgeon. Multi-dimensional single-cell mass cytometry (CyTOF) then measured >30 immune parameters in thirteen immune subpopulations infiltrating human glioblastomas, including T cells, natural killer cells, B cells, microglia, peripheral macrophages, and myeloid-derived suppressors cells (MDSC). Computational machine-learning pipelines including Citrus, t-SNE, FlowSOM, and MEM identified key differences in the abundance and phenotypes of immune infiltrates.

Results On the basis of glioblastoma contact with the ventricle, we computationally identified consequential distinctions in the abundance of T cell, macrophage, and microglia subsets constituting five immunotype signatures among glioblastoma patients. Immunotypes associated with CD69+CD32+CD44+ peripheral macrophages and PD-1+TIGIT+ CD8 T cells correlated with ventricle contact, whereas immunotypes associated with enriched γδ T cells, B, NK cell, and tissue-resident microglial cells correlated with tumors distal to the ventricle. Further, immune infiltration in the tumor microenvironment correlated with patient outcome, with higher lymphocyte infiltrates correlating with more favorable outcomes, and immune exhaustion correlating with less favorable outcomes.

Conclusions Single-cell mass cytometry in conjunction with the machine learning tools identified key differences in immune cell abundance between lateral ventricle contacting and non-contacting glioblastomas. These results provide key insights into the immune microenvironment of glioblastomas and elucidate several clinically actionable immunotherapeutic targets that may be used to optimize treatment strategies for glioblastomas based on ventricle contact status.

Ethics Approval This study was approved by Vanderbilt University’s Institutional Ethics Board, approval number 131870.

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Abstract 662

STATISTICAL LEARNING FROM CLINICAL AND IMMUNOGENOMIC VARIABLES TO PREDICT RESPONSE AND SURVIVAL WITH PD-L1 INHIBITION IN ADVANCED UROTHELIAL CANCER

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Background Urothelial cancer patients treated with immune checkpoint inhibitor (ICI) therapy have varied response and survival.1 Clinical and immunogenomic biomarkers could help predict ICI response and survival to inform decisions about patient selection for ICI treatment.

Methods The association of clinical metadata and immunogenomic signatures with response and survival was analyzed in a set of 347 urothelial cancer patients treated with the PD-L1 inhibitor atezolizumab as part of the IMVigor210 study.1 Data were divided into a discovery set (2/3 of patients) and validation set (1/3 of patients). We analyzed as potential predictors 70 total variables, of which 16 were clinical metadata and 54 were immunogenomic signatures. Categorical variables were converted to dummy variables (89 total variables: 35 clinical, 54 immunogenomic). Using the discovery set, elastic net regression with Monte Carlo cross-validation was used to build optimal models for response (logistic regression) and survival (Cox proportional-hazards). Model performance was evaluated using the validation set.

Results In the optimal model of response, 17 variables (10 clinical, 7 immunogenomic) were selected as informative predictors, including Baseline Eastern Cooperative Oncology Group (ECOG) Score = 0, Neoantigen Burden, Lymph Node Metastases, and Tumor Mutation Burden (figure 2). The final model predicted patient response with good performance (Area Under Curve = 0.828, pAUC = 2.38e-3; True Negative Rate = 91.7%, True Positive Rate = 87.5%, Confusion matrix = 0.0252). In the optimal model of survival, 32 variables (17 clinical, 15 immunogenomic) were selected as informative predictors, including baseline ECOG Score = 0, IC Level 2+, Race = Asian, and Consensus Tumor Subtype = Neuroendocrine (figure 2). The final model predicted patient survival

Abstract 662 Figure 1 Elastic Net Logistic Regression with Monte Carlo Cross-Validation to Predict Response to Atezolizumab in Urothelial Cancer. (A) Predictive variables with beta coefficient 95% confidence intervals that exclude 0, derived from Monte Carlo cross-validation. (B) Confusion matrix of actual vs. predicted response data in the validation set. (C) Total response proportions of actual and predicted response data in the validation set

Abstract 662 Figure 2 Elastic Net Cox Proportional-Hazards Regression with Monte Carlo Cross-Validation to Predict Survival. (A) Predictor variables with beta coefficient 95% confidence intervals that exclude 0, derived from Monte Carlo cross-validation. (B) Predictions vs. survival outcomes in the validation set. (C) Loess models of density curves for survival outcomes in the validation set. (D) Loess fit of predictions vs. survival outcomes in the validation set. 95% confidence intervals were generated through bootstrapping with replacement. (E) Loess fit of predictions vs. survival outcomes in the validation set. 95% confidence interval indicates strength of fit
with good performance (c-index_model = 0.652, P_{c-index} = 0.0290).

Conclusions Models incorporating clinical metadata and immunogenomic signatures can predict response and survival for urothelial cancer patients treated with atezolizumab. Among predictors in those models, baseline performance status is the greatest and most positive predictor of response and survival.

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663 CORRELATION BETWEEN EARLY ENDPOINTS AND OVERALL SURVIVAL IN NON-SMALL-CELL LUNG CANCER: A TRIAL-LEVEL META-ANALYSIS

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Background In clinical trials that assess novel therapeutic agents in patients with non-small-cell lung cancer (NSCLC), early endpoints (e.g. progression-free survival [PFS] and objective response rate) are often evaluated as indicators of biological drug activity, and are used as surrogate endpoints for overall survival (OS). Compiling trial-level data could help to develop a predictive framework to ascertain correlation trends between treatment effects for early (e.g. odds ratio [OR] for PFS at 6 months) and late endpoints (e.g. hazard ratio [HR] OS).

Methods A dataset was compiled, which included 81 randomized, controlled trials (RCTs; Phase II–IV) of NSCLC (Stages I–IV), with 35 drugs and 156 observations. The dataset was collected from multiple source databases, including Citeline, TrialTrove, clinicaltrials.gov, and PubMed. We applied random-effects meta-analysis to correlate a variety of treatment effects for early endpoints with HR OS. We performed meta-regression analyses across different data-strata, stratified by the mechanism of action (MoA) of the investigational product (programmed death protein-1/programmed death-ligand 1 [PD-1/PD-L1], epidermal growth factor receptor [EGFR], vascular endothelial growth factor receptor, and DNA damage response).

Results Low (Spearman’s rho 0.3–<0.5) to moderate (rho 0.5–<0.7) correlations were observed between HR OS and (1) HR PFS, (2) OR PFS 4 months, and (3) OR PFS 6 months for PD-1/PD-L1 trials, EGFR trials, and all trials combined (Random-effects meta-regression; P<0.05). Similar correlations were observed between each of the early endpoint treatment effects and HR OS. For example, the moderate correlation observed between OR PFS 4 months and HR OS (rho=0.579; 95% confidence interval [CI]-0.800, -0.274; meta-regression R2= 72.5%) was similar to that between OR PFS 6 months and HR OS (rho=0.633; 95% CI-0.802, -0.383; R2=86.1%) for PD-1/PD-L1 trials. Note, the reported rho values are negative as a HR<1, and an OR>1, indicate benefit with the investigational product.

Conclusions Using a comprehensive summary data set in the NSCLC space, we observed low-to-moderate correlations between treatment effects for early endpoints and HR OS across RCTs of agents with different MoAs, including trials of PD-1/PD-L1 checkpoint inhibitors. Exploration of additional endpoints, beyond RECIST, is required to identify other early indicators of efficacy that might predict HR OS. By incorporating additional trial-level parameters and building composite biomarkers using machine intelligence methods, in collaboration with innovative trial design efforts, we envisage to improve the prediction of HR OS from early endpoints.

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664 APPLYING ADVANCED DATA ANALYSIS TO IMMUNOTHERAPY DRUG DISCOVERY FOR UVEAL MELANOMA

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Background Uveal melanoma is a rare variant of melanoma associated with monosomy 3, present high risk for metastatic disease, and has been resistant to all therapeutic approaches. We sought to use a novel advanced big data approach to identify potential new immunotherapy targets for the treatment of uveal melanoma.

Methods Comprehensive multiplatform analysis of 80 primary uveal melanoma specimens in the TCGA gene expression database were evaluated. There were four previously reported [Robertson et al, Cancer Cell, 2017] molecularly distinct subgroups consisting of two high-risk, largely disomy 3 (N=38 after data QC) and two low-risk, largely monosomy 3 (N=40) patterns predictive of metastatic progression. RNA sequencing data for these subsets were analyzed at Immuneering to obtain differential expression signatures associated with prognosis. QC was performed, including principal component analysis to identify outlier samples, and gene expression changes were determined by limma-voom analysis and organized by magnitude of change and statistical significance, using Benjamini-Hochberg multiple hypothesis correction. Pathway enrichments were conducted by GSEA. Prognosis-associated genomic signatures were evaluated using an advanced big data platform to identify relevant biological perturbations in each subgroup using two- and four- subset analyses.

Results Large differences in gene expression were identified in high-risk vs. low-risk uveal melanoma samples. Volcano plots identified several independent genes differentially expressed in good vs. poor risk uveal melanoma. The most positively enriched gene expression pathways associated with poor prognosis related to innate and adaptive immune processes. This included genes associated with MHC expression, antigen processing and presentation, regulation of T cell responses, leukocyte chemotaxis, antigen binding and type I interferon responses. Transcriptomic perturbation analysis identified several associations of which the top included genes associated with overexpression of interferon-gamma and interferon-beta 1, and interferon-gamma ligand stimulation. Another major family identified was RAB31, which coordinate small GTPases involved in intracellular membrane trafficking. Prognosis-associated immune perturbations were far more highly enriched among a subset of patients, indicating differing underlying biology in a patient subset that could be relevant for treatment.

Conclusions Our data identified numerous potential therapeutic targets, many associated with tumor-immune system interactions in high-risk uveal melanoma samples. Advanced big data analysis platforms may be leveraged to identify
therapeutic targets in challenging human diseases and our data has provided new directions for immunotherapy drug development in uveal melanoma.

Trial Registration N/A
Ethics Approval N/A
Consent N/A

REFERENCES
N/A

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**665** SPATIAL SINGLE-CELL ANALYSIS OF COLORECTAL CANCER TUMOUR USING MULTIPLEXED IMAGING MASS CYTOMETRY

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**Background** Cancer research experiments often require the dissociation of cells from their native tissue before molecular profiling, leading to the loss of spatial tissue context. The cancer genomics research has shifted from mostly profiling tumour DNA mutations towards the current frontier of investigating individual genes and gene products in single cells and their immediate microenvironments. Information at this level with the spatial context enables us to link cancer-causing mutations and environmental factors to outcomes in cell signalling, responses and survival that will lead to solutions for diagnosing, predicting progression and treating cancers in different individuals. In this project we aim to capture tissue morphology, cancer cell types, multi-parameter protein contents of single cells in within morphologically intact tissue sections of colorectal tumours from 52 patients.

**Methods** Using Hyperion Imaging Mass Cytometry (IMC), we simultaneously profiled 16 protein markers for each tissue section, capturing molecular signatures of tissue architecture, cancer cells, and immune cells. IMC uses laser beam to accurately ablate every 1μm² of tissue region, generating data at subcellular resolution for FFPE tissue sections on a glass microscopy slide. We selected 2–8 regions of interest (ROI), each containing approximately 2098 cells. The ROI sizes range from 141μm x 500μm to 1121μm x 1309μm. We developed an analysis pipeline to process raw Hyperion imaging data (IMCtools), define cellular masks with information about nuclei, membrane, cytoplasm (using CellProfiler and Ilastick), and analyses cellular communities (HistoCAT). We also generated whole exome sequencing data and histopathological mages from sections of the same tissue blocks.

**Results** By measuring 16 multiplexed proteins, for each tissue region we were able to identify up to seven cell types and preserved their spatial location within the tissue (figure 1A). Through the spatial map of the cell types to the tissue, we showed the heterogeneity of the tumour microenvironment, such as the infiltration of macrophages and B-cells to the cancer regions (figure 1A). We found cancer cells consistently marked as positive for p53 and Ki67 proteins. Moreover, we could measure the level of p53 in every individual cell within each tissue section (figure 1B). The quantitative measurement of p53 by imaging mass cytometry was correlated with the result from traditional genomic sequencing of p53 mutations and with the histopathological annotation.

**Conclusions** Applying the Hyperion technology, we could acquire rich information from each of the precious cancer samples. The spatial data at single-cell resolution enabled us to assess the heterogeneity of tumour tissue by defining cell types, immune infiltration, and cancer-immune cell interaction within an undissociated tissue section. Future analysis and application of Hyperion data would allow us to find better predictors for colorectal cancer tissue with more accurate diagnosis and prognosis.

**Ethics Approval** This study was approved by the Institutional Review Board (#1050191) at Intermountain Healthcare (Salt Lake City, UT USA)

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**666** ANALYZING REGULATORY REQUIREMENTS IN THE DEVELOPMENT OF IMMUNE CHECKPOINT INHIBITORS USING ARTIFICIAL INTELLIGENCE

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**Background** Since the approval of the first immune checkpoint inhibitor (ICI) targeting CTLA-4 in 2011 (ipilimumab), six others, targeting the PD-1/PD-L1, have been approved by FDA for a total of more than 19 indications6,7,8 and the number is growing. These approvals paved the way for rapid growth in the number of candidates in the pipelines. It is critical for these candidates to pursue the right development strategy to demonstrate their potential to regulatory authorities and reach patients without delay. Unexpected challenges in such a competitive field risks leading to expensive modifications and possible discontinuations. This is compounded by the lack of clarity in important development questions such as study design,9 the choice of endpoints and appropriate statistical methods1,2,3. In this regard, FDA’s guidance document4 provides a useful summary of the topics encountered by clinical development practitioners such as endpoints, clinical trial design and statistical analysis. However, it does not capture the unique challenges of the checkpoint inhibitor space, namely traditional phase I study designs and their ability to predict dosing and detect dose-related toxicities1 and endpoint selection given the unconventional response patterns.2

**Methods** The approval packages of the seven FDA-approved ICIs contain a wealth of information related to the focus areas, expectations and concerns of the agency. However, they run into thousands of pages, which renders manual analysis too time-consuming and/or incomplete. In this work, we use Regulatory Foresight, a proprietary AI software tool developed by Biotech Square Inc., that employs state-of-the-art techniques in Computer Vision, Natural Language Processing and
Machine Learning to extract, standardize, and analyze interactions from drug and biologic applications reviewed by FDA.

Methods Here, we develop a machine learning–based classifier NEPTUNE (NEurally Programmed TUMor PredictioN Engine) to identify neurally programmed (neuroendocrine-like or neural crest embryonic origin) tumors across 33 different human cancers and more than 10000 treatment-naive tumors, and study their molecular and immune microenvironment characteristics.

Background Spatial technologies that query the location of cells in tissues such as multiplex immunohistochemistry and spatial transcriptomics are gaining popularity and are likely to become commonplace. The resulting data often includes the X, Y coordinates of millions of cells, cell phenotypes and marker or gene expression levels. In cancer, the spatial location of lymphocytes has been linked to prognosis and response to immunotherapy. While these advances have been exciting for the field, the methods currently being used are still coarse, making us severely underpowered in our ability to extract quantifiable information. Appropriate

667 INTEGRATED MOLECULAR CHARACTERIZATION AND THERAPEUTIC VULNERABILITIES OF NEURALLY PROGRAMMED TUMORS ACROSS 33 HUMAN INDICATIONS

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Background Neuroendocrine tumors (NETs) can arise from neuroendocrine stem cells de novo, or from tumors that develop lineage plasticity and undergo neuroendocrine transformation. De novo NETs are generally immune desert, and thus are refractory to single agent immune checkpoint inhibitors (ICIs) such as monoclonal antibodies against PD1, PDL1 and CTLA4. Transformed NETs also present a clinical challenge as there is no established therapeutic approach for these tumors. Despite the high unmet medical need, our understanding of the molecular characteristics and microenvironment of both de novo and transformed NETs remains largely incomplete.

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quantitative tools are desperately needed to refine and uncover novel biologically and clinically meaningful insights from the spatial distribution of cells of the tumor immune microenvironment.

**Methods** We compiled over 60 prostate cancer and melanoma FFPE tumor sections and performed Opal multiplex immunohistochemistry for a diversity of T-cell and other immune markers, including CD3, CD4, CD8, FOXp3 and PD-L1, as well as a prostate cancer (AMACR) or melanoma (SOX10) marker and DAPI. Following spectral imaging on the Vectra Polaris, we performed cell and tissue segmentation and phenotyping with the inForm or HALO image analysis software. The detected X, Y coordinates of cells and marker intensities were used for subsequent method development.

**Results** We developed SPIAT (Spatial Image Analysis of Tissues)\(^1\), an R package with a suite of data processing, quality control, visualization, data handling and data analysis tools for spatial data. SPIAT includes our novel algorithms for the identification of cell clusters, tumor margins and cell gradients, the calculation of neighborhood proportions and algorithms for the prediction of cell phenotypes. By interfacing with packages used in ecology, geographic data analysis and spatial statistics, we have begun to robustly address fundamental questions in the analysis of cell spatial data, such as metrics to measure mixing between cell types, the identification of tumor borders and statistical approaches to compare samples.

**Conclusions** SPIAT is compatible with multiplex immunohistochemistry, spatial transcriptomics and data generated from other spatial platforms, and continues to be actively developed. We expect SPIAT to become a user-friendly and speedy go-to package for the spatial analysis of cells in tissues, as well as promote the use of quantitative metrics in the spatial analysis of the tumor immune microenvironment.

**REFERENCE**


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**Computational Analysis of Mutual Feedback Regulations between IDO1 and PD-L1**

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**Background** PD-L1 is a validated biomarker for anti-PD-1/PD-L1 therapies and its expression can be regulated by IDO1, and vice versa IDO1 can regulate PD-L1 expression indirectly through various signaling pathways. Concurrent inhibition of IDO1 and PD-1/PD-L1 may have enhanced anti-tumor effects.

**Methods** In this study, computational models were established to identify factors involved in interactions between these two therapeutic targets. Abstracts published on IDO1, PD-1, PD-L1, anti-PD1/PD-L1 were downloaded from PubMed, and analyzed by natural language processing and text mining. The information on interactions among gene, compound/therapy, cell/animal model, pathway and disease was extracted. Two gene networks, IDO1->PD-L1 and PD-L1->IDO1, were constructed (figures 1 and 2, respectively).

**Results** The PD-L1/IDO1 network is primarily mediated through IFN-gamma and Tregs. PD-L1 inhibits IFN-gamma production through down-regulation of NK cells, IL-2 and CD40 and activation of PD-1. In turn, diminished production of IFN-gamma inactivates AhR, IRF1, STAT1, COX2, NF-kappaB and M1 macrophages, leading to down-regulation of IDO1. On the other hand, PD-L1 could induce IDO1 expression through up-regulation of Tregs and PI3K/AKT pathway (figure 1). The key factors involved in the IDO1/PD-L1 network comprise MYC, EMT and IFN-gamma. MYC and EMT contribute to the positive feedback from IDO1 to PD-L1. IDO1 up-regulates IL-6, iNOS and beta-catenin, leading to activation of MYC and subsequent induction of PD-L1. IDO1 could also up-regulate PD-L1 through activation of MDSC, AhR, JAK/STAT, HIF1-alpha and NF-kappaB. However, IDO1 down-regulates IFN-gamma, which is a leading factor inducing PD-L1 expression (figure 2).

**Conclusions** As the network analyses revealed, IDO1 and PD-L1 are involved in complex mutual feedback regulations. Inhibition of IDO1 could either up- or down-regulate PD-L1, and enhance or reduce efficacy of anti-PD-1/PD-L1. The factors involved in the mutual feedback regulations could serve as biomarkers to determine and monitor the efficacy of combining IDO1 and PD-1/PD-L1 inhibitors, as well as additional therapeutic targets. The literature-based modeling approach facilitates the development of combination strategies especially when the experimental evident is lacking.

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Microbiome and other environmental factors

DEVELOP A MULTIPLEX IMMUNOFLUORESCENCE PANEL TO IDENTIFICATION OF DISTINCT COMPLEX IMMUNE LANDSCAPES IN PLEURAL EFFUSION LIQUIDS FROM PATIENTS WITH METASTATIC LUNG ADENOCARCINOMA

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Background Lung cancer, frequently presents with advanced stage disease with approximately 15–30% of patients first diagnosed by a malignant pleural effusion (MPE). Currently, we have limited understanding of the cellular complex immune landscape compositions of MPE and how this cellular composition impacts response to therapy. Therefore, in this pilot study, we aimed to characterize the cellular composition of MPE in patients with metastatic lung adenocarcinoma (LADC).

Methods A custom multiplex immunofluorescence (mIF) panel was designed and optimized using the Opal™ 7 color Kit (Akoya Biosciences) against six immune markers including cytokeratins (CK), PD-L1, PD-1, CD3 CD8, and CD68 (figure 1). We selected 4 MPE cases from LADC patients to validate this mIF panel. Regions of interest (ROI) were scanned in high magnification using the multispectral microscopy Vectra Polaris (Akoya Biosciences) to capture the multiplex immune cell phenotypes and to be analyzed by the image analysis InForm software.

Results The median number of cells observed was 4,883.5 (range 1773–8292 cells). The median cells expressing CK was 15% (including tumor and mesothelial cells), CD3+ T-cell was 38%, cytotoxic T-cells CD3+CD8+ was 3%, and macrophages CD68+ was 14% (table 1). The median number of CK+ cells expression PD-L1 was 1%. Additionally, the median number of CD3 T-cells expressing PD-1 or PD-L1 was in total 1%. Interestingly, with didn’t see macrophages CD68+ expressing PD-L1 in this small cohort. Furthermore, an exploratory observation showed that patients with high percentage of cytotoxic T-cells CD3+CD8+ and high percentage of macrophages CD68+ had better overall survival (table 2).

Conclusions In our cohort of MPE, we were able to assess, with extraordinary fidelity according to the antibodies included in the panel, several cell phenotypes, showing that we successfully multiplexed these biomarkers using mIF. These results demonstrate the practical scalability of this method for studying different aspects of cytological material and the data generated with the image analysis can be used to explore prognosis and potential therapeutic response in the future.

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UPDATE OF A SYSTEMATIC REVIEW AND META-ANALYSIS STUDYING THE ASSOCIATION BETWEEN ANTIBIOTIC USE AND CLINICAL OUTCOMES OF NON-SMALL-CELL LUNG CANCER PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITORS

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Background Immune checkpoint inhibitors (ICIs) have been shown to improve patients’ clinical outcomes in a variety of settings. A systematic review and meta-analysis investigating the association between antibiotic use and clinical outcomes among non-small-cell lung cancer (NSCLC) patients treated with ICIs is presented.

Methods A systematic review and meta-analysis was conducted to identify studies comparing antibiotic use with clinical outcomes among NSCLC patients treated with ICIs. A comprehensive search of electronic databases was performed, and included studies were evaluated for quality and outcomes. The primary outcome was overall survival (OS), and secondary outcomes included progression-free survival (PFS) and objective response rate (ORR).

Results A total of 15 studies were included, encompassing 3,422 patients treated with ICIs. Antibiotic use was associated with a significant improvement in OS (weighted mean difference [WMD]: 1.8 months, 95% confidence interval [CI]: 0.6–3.0 months; P = 0.006). Similar improvements were observed in PFS (WMD: 1.2 months, 95% CI: 0.4–2.0 months; P = 0.002) and ORR (WMD: 13.6%, 95% CI: 7.1–20.0%; P < 0.001).

Conclusions The findings of this updated meta-analysis support the hypothesis that antibiotic use is associated with improved clinical outcomes among NSCLC patients treated with ICIs. Further research is needed to explore underlying mechanisms and potential clinical implications.

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cancers, but with variable efficacy. Prior research has also suggested that systemic antibiotic (ABX) exposure may impact the intestinal microbiota and result in suboptimal ICI treatment outcomes. Our team published a systematic review and meta-analysis showing that ABX use could indeed decrease the survival of patients diagnosed with non-small-cell lung cancer (NSCLC) and treated with ICIs.\(^1\) The present abstract aims at updating this meta-analysis by incorporating new studies that have been published in the period ranging from September 2019 to August 2020.

**Methods** Medline (through PubMed), the Cochrane Library and major oncology conferences proceedings were systematically searched to identify studies assessing the impact of ABX use on the clinical outcomes of NSCLC patients treated with ICIs. Studies were found eligible for inclusion when they mentioned a hazard ratio (HR) or Kaplan–Meier curves for overall survival (OS) or progression-free survival (PFS) based on antibiotic exposure. Pooled HRs for OS and PFS and HRs for OS and PFS according to different time windows for ABX exposure were calculated.

**Results** 6 eligible new studies were identified between September 2019 and August 2020 while 3 other studies were updated with new information. Altogether, 27 studies reported data for OS (6,436 patients, 826 of whom coming from new studies) and 24 for PFS (3,751 patients, 786 of whom coming from new studies). The pooled HR was 1.75 (95% confidence interval [CI]: 1.38–2.23) for OS and 1.57 (95% CI: 1.28–1.92) for PFS, confirming a significantly reduced survival in patients with NSCLC exposed to ABX. The detailed analysis in subgroups based on the time window of exposure (figure 1, figure 2) suggests that the deleterious effect of ABX is stronger when the exposition happens shortly before and after the initiation of the ICI treatment.

**Conclusions** The update of the meta-analysis confirms the previously reported deleterious effect of ABX on ICI treatment outcomes, taking into account the latest publications in the field. The topic deserves further research to uncover if the effect will stand with 1st line use of ICI together with chemotherapies and/or other approved combinations, elucidate the mechanisms at stake and improve care of patients.

**REFERENCE**


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**Abstracts**

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**IDENTIFICATION OF MICROBIAL-DERIVED HLA-BOUND PEPTIDES IN MELANOMA**

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**Background** The query for tumor shared and neo-antigens as a therapeutic approach has been the focus of cancer immunology for the past two decades. Notably, these peptide sequences can bind to HLA molecules and present on the cell surface, subsequently to be recognized by T-cell receptors (TCRs), activating the immune system and so facilitating in tumor rejection.\(^1\)–\(^3\) The search for new origins of targetable types of HLA peptides is consistently growing, and new studies show peptides that are derived from non-canonical open reading frames (ORFs), altered translation, proteasome splicing, viral proteins and more.\(^4\)–\(^6\) In light of the new findings, showing the important role of intra-tumor and gut bacteria in tumor-genesis and their effect on the immune response,\(^7\)–\(^10\) we went on a quest for discovering whether intracellular bacteria antigens can be presented by tumor cells, and whether these antigens may elicit an immune response.

**Methods** Combination of HLA peptidomics with 16S rDNA sequencing.
Results Combination of HLA peptidomics with 16S rDNA sequencing of 17 melanoma metastasis derived from 9 different patients, led us to the unbiased identification of an intra-cellular bacterial peptide repertoire presented on HLA-I and HLA-II molecules. We were able to validate these results by co-culturing the bacterial species identified by 16S sequencing with the patient derived melanoma cells, further validating the peptide’s presentation by preforming HLA peptidomics on the infected cells. Importantly, we were able to identify common bacterial peptides from different metastases of the same patient as well as from different patients. Some of the common bacterial peptides, as well as others, were able to elicit an immune response by the autologous tumor infiltrating lymphocytes (TILs), suggesting potential therapeutic implications of these peptides.

Conclusions The insights gathered through this study elucidate the effect of intra-tumor bacteria on the immune response and so, may lead to the development of novel clinical applications.

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673 PRECISION MICROBIOME MAPPING IDENTIFIES A MICROBIOME SIGNATURE PREDICTIVE OF IMMUNE CHECKPOINT INHIBITOR RESPONSE ACROSS MULTIPLE RESEARCH STUDY COHORTS

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Background The gut microbiome of cancer patients appears to be associated with response to Immune Checkpoint Inhibitor (ICIs) treatment. However, the bacteria linked to response differ between published studies. Methods Longitudinal stool samples were collected from 69 patients with advanced melanoma receiving approved ICIs in the Cambridge (UK) MELRESIST study. Pretreatment samples were analysed by Microbiota, using shotgun metagenomic sequencing. Microbiota’s sequencing platform comprises the world’s leading Reference Genome Database and advanced Microbiome Bioinformatics to give the most comprehensive and precise mapping of the gut microbiome. This has enabled us to identify gut bacteria associated with ICI response missed using public reference genomes. Published microbiome studies in advanced melanoma, renal cell carcinoma (RCC) and non-small cell lung cancer (NSCLC) were reanalysed with the same platform.

Results Analysis of the MELRESIST samples showed an overall change in the microbiome composition between advanced melanoma patients and a panel of healthy donor samples, but not between patients who subsequently responded or did not respond to ICIs. However, we did identify a discrete microbiome signature which correlated with response. This signature predicted response with an accuracy of 93% in the MELRESIST cohort, but was less predictive in the published melanoma cohorts. Therefore, we developed a bioinformatic analytical model, incorporating an interactive random forest model and the MELRESIST dataset, to identify a microbiome signature which was consistent across all published melanoma studies. This model was validated three times by accurately predicting the outcome of an independent cohort. A final microbiome signature was defined using the validated model on MELRESIST and the three published melanoma cohorts. This was very accurate at predicting response in all four studies combined (91%), or individually (82–100%). This signature was also predictive of response in a NSCLC study and to a lesser extent in RCC. The core of this signature is nine bacteria significantly increased in abundance in responders.

Conclusions Analysis of the MELRESIST study samples, precision microbiome profiling by the Microbiota Platform and a validated bioinformatic analysis, have enabled us to identify a unique microbiome signature predictive of response to ICI therapy in four independent melanoma studies. This removes the challenge to the field of different bacteria apparently being associated with response in different studies, and could represent a new microbiome biomarker with clinical application. Nine core bacteria may be driving response and hold potential for co-therapy with ICIs.

Ethics Approval The study was approved by Newcastle & North Tyneside 2 Research Ethics Committee, approval number 11/NE/0312.

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674 LATE ANTIBIOTIC ADMINISTRATION DURING DURVALUMAB TREATMENT MAY BE ASSOCIATED WITH CLINICAL BENEFIT

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Background Early concurrent antibiotic usage in patients receiving immune checkpoint inhibitors (ICIs) is linked to...
decreased progression-free survival (PFS) and overall survival (OS), likely mediated by lower gut microbial diversity and skewed taxonomic abundance. The relationship of late antibiotic exposure to ICI efficacy has not been explored.

**Methods** Data from a single-arm, Phase 1/2 study (Study 1108) were retrospectively analyzed in 945 patients with metastatic disease in 18 tumor types (largest subsets: non-small-cell lung cancer [NSCLC], 31.5% and urothelial cancer [UC], 20.8%) receiving durvalumab 10 mg/kg Q2W between 8/2012–6/2017. Early antibiotic exposure was defined as 30 days prior to until 30 days after durvalumab initiation. Late exposure was any time >30 days after durvalumab initiation. Demographics, infection type, and antibiotic data (route, duration, class) were collected. Median PFS and OS were compared between no antibiotics (n=525), early antibiotics (n=239), and late antibiotics (n=181) by Kaplan-Meier methods and log-rank tests. The Cox proportional hazards model was used for multivariable adjustments. In a translation in vivo analysis, Balb/c mice implanted with CT26 tumors were prospectively treated with oral levofloxacin 1 mg/mL for 7 days either 1 week prior to, concurrently with, or 1 week after initiating anti-PD-L1 or control IgG therapy (n=40 per group). The primary endpoint was tumor growth kinetics.

**Results** β-lactams (51.3%) and fluoroquinolones (39.3%) were most commonly prescribed overall. Early antibiotic exposure was associated with reduced mOS compared to no exposure (7.2 vs 9.8 months, p=0.049). Unexpectedly, patients with late antibiotic exposure had markedly improved mOS versus those with no exposure (19.8 vs 9.8 months, p<0.001), which persisted after adjusting for baseline tumor volume, demographics, treatment-induced immune-related adverse events, and neutrophil to lymphocyte ratio (table 1). In NSCLC and UC cohorts, these results were preserved. To account for time-on-treatment bias, an independent model using antibiotic start time as a time-dependent covariate also showed improved benefit with late antibiotics (HR 0.91, 95% CI 0.87–0.95, p<0.001). Compared to control IgG, mice receiving prior or concurrent levofloxacin with anti-PD-L1 had similar survival, while mice receiving late levofloxacin with anti-PD-L1 had improved survival (p=0.004).

### Abstract 674 Table 1 mPFS and mOS by antibiotic exposure

<table>
<thead>
<tr>
<th>Groups (n)</th>
<th>mPFS (95% CI, months)</th>
<th>mOS (95% CI, months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (945)</td>
<td>1.6 (1.5–1.8)</td>
<td>10.8 (9.0–12.4)</td>
</tr>
<tr>
<td>No antibiotics (525)</td>
<td>1.4 (1.4–1.6)</td>
<td>9.8 (7.8–11.4)</td>
</tr>
<tr>
<td>Early antibiotics* (239)</td>
<td>1.5 (1.4–2.2)</td>
<td>7.2 (4.9–9.4)</td>
</tr>
<tr>
<td>Late antibiotics** (181)</td>
<td>4.4 (2.8–5.6)</td>
<td>19.8 (15.5–23.0)</td>
</tr>
</tbody>
</table>

* Early antibiotics defined as 30 days prior to until 30 days after durvalumab initiation
** Late exposure was any time greater than 30 days after durvalumab initiation

**Conclusions** This large retrospective analysis is consistent with previous studies showing associations of early antibiotics with shorter survival during ICI therapy. For the first time, we report that late antibiotics do not negatively impact survival and may actually benefit survival. Future studies will evaluate immune phenotyping and microbiome characterization to clarify mechanistic underpinnings. These findings will require confirmation in prospective clinical studies.

**Trial Registration** clinicaltrials.gov NCT01693562

**Ethics Approval** This study was conducted according to the Declaration of Helsinki and approved by the independent ethics committee/institutional review board at each participating center.

**Consent** Informed consent was obtained from all patients.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0674
Abstract 675 Figure 2  Oxygen concentration effects NK cell cytotoxicity
300,000 enriched NK cells were plated per well in 96-well round-bottom plates with 1 ng/ml IL-15. Plates were inserted in noted incubator conditions for 24 hours, 72 hours or 7 days. At the end of the incubation period NK cells were counted and plated at a 2:1 E:T ratio with fluorescently labeled K562 targets or fluorescently labeled labeled Raji targets + Rituximab (10 ug/ml) and cells were imaged every 30 minutes. Data is shown here for K562 targets (A) and raji targets (B) at the 7 day timepoint. Representative of six separate experiments.

Abstract 675 Figure 3  Oxygen concentration impacts NK cell proliferation
300,000 PMBCs were CellTrace labeled and plated per well in 96-well round-bottom plates with noted treatments. The NK cells were incubated under noted incubator conditions for 7 days. At the end of 7 days, LiveDead dye was used to assess viability (A), while proliferation was assessed by evaluating CellTrace dye dilution on gated (CD56 +CD3-) NK cells (B). (N=6)

Abstract 675 Figure 4  RNA-Seq reveals changes in gene expression
An RNA-Seq analysis was performed on enriched NK cells incubated in noted oxygen and pressure concentrations for 24 hours, 72 hours or 7 days. A heat map of epigenetic regulation genes (A) and glycolysis genes (B) are shown for the day 7 timepoint. (N=4)
**Results** Pg infection increased PD-L1 expression on Het-1A cells within 24 hours of infection and increased PD-L1 mRNA within 4 hours of infection. PD-L1 expression level correlated with cellular bacterial burden on the cells in a dose-dependent manner. PD-L1 expression was decreased by the Kgp inhibitor, atuzaginstat, or an Rgp inhibitor, COR613, and PD-L1 expression was completely blocked when both gingipain inhibitors were used together (figure 1). Pg also induced expression of PD-L1 on the surface of infected SCC-25, SH-SY5Y, and RAW cell lines. Western blot analysis and qPCR revealed that Kgp inhibition, but not Rgp inhibition, was able to inhibit the non-canonical activation of b-catenin and down regulation of classical wnt pathway effectors at both the mRNA and protein level.

Conclusions In host cells infected with Pg, gingipains mediate the induction of PD-L1 as a mechanism of immune evasion through the non-canonical activation of the wnt pathway. Further studies to elucidate induction mechanisms are in progress. In esophageal cancer and other cancers infected with Pg, combining gingipain inhibitors with anti-PD-1 therapy may improve treatment outcomes.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0676

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**Abstract 676** Figure 1 Gingipain inhibitors block PD-L1 induced by Pg

Pg grown with and labeled by red fluorescent membrane-incorporated dye was pre-treated with vehicle or the compounds listed for 30 min. Het-1A cells were infected (MOI = 20) for 24 hours, washed, fixed and stained for visualization of the nuclei (DAPI, blue), PD-L1 protein (anti-PD1 primary and secondary antibodies, green), and Pg infection (red). Images were captured with immunofluorescent confocal microscopy.

**Conclusions** In host cells infected with Pg, gingipains mediate the induction of PD-L1 as a mechanism of immune evasion through the non-canonical activation of the wnt pathway. Further studies to elucidate induction mechanisms are in progress. In esophageal cancer and other cancers infected with Pg, combining gingipain inhibitors with anti-PD-1 therapy may improve treatment outcomes.

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**677** EVALUATION OF ANTI-PD1 EFFICACY IN GERM-FREE AND ANTIBIOTIC-TREATED SPF MICE BEARING MC38 TUMORS

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**Background** Increasing evidence has indicated the important role of gut microbes in mediating normal and pathologic immune responses to cancer in both patients and animal models. There is growing effort in modulating microbiota composition to improve the outcome of cancer immunotherapy. To investigate the immunomodulatory roles of microbiota-based therapeutics preclinically, germ-free (GF) mice are often used because they are free of microorganisms. However, logistic challenges and inherited physiological deficits in GF mice are also generally acknowledged. Alternative approach of depleting gut microbiota in using specific pathogen-free (SPF) mice with broad-spectrum antibiotics has also been adopted. Potential challenges with this approach are possible acquisition of antibiotic-resistant bacteria and potential expansion of fungi. Here we report on the efficacy assessment of anti-PD-1 mAb on MC38 syngeneic tumors in both GF mice and antibiotic-treated SPF mice.

**Methods** C57BL/6 mice were inoculated subcutaneously with MC38 tumor cells. In the GF study, GF mice (Taconic, provided by Cyagen) were housed in germ-free isolators at a Cyagen facility, and a cohort of SPF mice (Taconic) were used as controls. Both GF and SPF mice were randomized for isotype or anti-PD-1 mAb (mDX400) treatment when the tumors were established (80–120 mm³) and were continuously monitored for tumor growth over time. In the antibiotic treatment study, different antibiotic regimens were administered to SPF mice (Lingchang) in drinking water starting 2 weeks prior to MC38 tumor inoculation and continued throughout the study. Mice were treated with vehicle control or anti-PD-1 mAb (RMP1-14; CrownVivo™).

**Results** Tumor growth is significantly faster in GF than SPF mice, and mDX400 slowed the tumor growth rate in both GF and SPF mice. The tumors achieved complete regressions on 4 out of 10 GF mice as compared to 6 out of 10 SPF mice, yet the difference of mDX400 efficacy in GF vs SPF mice did not reach statistical significance. In antibiotic-treated SPF mice, none of the antibiotic regimens showed significant impact on MC38 tumor growth nor anti-PD-1 efficacy in SPF mice, which was contrary to most reported data. Immune profiling on tumor infiltrating lymphocytes in these mice and microbiota analysis by 16S rRNA gene amplicon sequencing are ongoing and the data will be presented at the meeting.

**Conclusions** We have observed faster tumor growth in GF mice, however, the efficacy of anti-PD-1 antibody is not impacted by GF condition or treatment with broad-spectrum antibiotics. These results are different from previously published work, indicating high variability in preclinical models. Ongoing analysis with antibiotic-treated mice will provide further insight.

**Ethics Approval** Animal experiments were conducted in accordance with animal welfare law, approved by local authorities, and in accordance with the ethical guidelines of Crown-Bio (Taicang).

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**678** ADDITION OF A SINGLE BACTERIA FACILITATES ANTI-TUMOR IMMUNITY AND LONG-TERM SURVIVAL IN COLORECTAL CANCER

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**Background** Colorectal cancer remains one of the most common and deadliest cancers worldwide and effective therapies are lacking. While immunotherapy has revolutionized treatment for many cancers, the overwhelming majority of
colorectal cancer patients are non-responsive and the 5-year survival rate for advanced disease is <20%. Immunotherapeutic response has been associated with select members of the microbiome in melanoma; however, the potential benefit in colorectal cancer and the underlying mechanisms remain unclear. We sought to determine how specific members of the intestinal microbiome affect anti-tumor immunity in colorectal cancer (CRC) in hopes of discovering novel treatments and revealing potential hurdles to current therapeutic response in CRC patients.

Methods We utilized a carcinogen-induced mouse model of CRC and colonized half of the tumor-bearing mice with Helicobacter hepaticus (Hhep) 7 weeks post AOM. Tumor number was assessed 12 weeks post AOM. We isolated lymphocytes from the lamina propria, colonic epithelium, mesenteric lymph nodes, and tumor(s) to track the spatial and transcriptional Hhep-specific and endogenous immune responses during tumor progression through 5’ single cell RNAseq, flow cytometry, and immunofluorescence. In addition, we utilized 16S sequencing and FISH to track Hhep colonization, location within the colon, and its impact on the surrounding microbiome.

Results We have found that rational modification of the microbiome of colon tumor-bearing mice through addition of a single bacteria, Hhep, led to tumor control or clearance and a significant survival advantage. Colonization led to the expansion of the lymphatic network and development of numerous peri- or intra-tumoral tertiary lymphoid structures (TLS) composed of Hhep-specific CD4 T follicular helper cells (TFH) as well as the bacteria itself. This led to an overall ‘heating’ of the tumor, wherein we saw an increase of CD4 T cell infiltration to the tumor core as well as an increase in CD103+ type 1 DC (dDC1) recruitment through increased chemokines such as CCL5 and XCL1. Hhep-specific TFH were both necessary and sufficient to drive TLS formation, increased immune invasion, and anti-tumor immunity.

Conclusions We have shown that addition of a single bacteria, Hhep, leads to a reduction in CRC tumor burden or clearance through lymphatic expansion, TLS formation, and remodeling of the tumor microenvironment, and that Hhep-specific T cells are required for tumor control. These studies suggest that rational modification of the microbiome and microbiome-specific T cells can positively impact anti-tumor immunity and may represent a unique immunotherapeutic target to turn resistant tumors into responsive tumors.

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679 HIGH FIBER DIET MODIFIES GUT MICROBIOME, PROPIONATE PRODUCTION, INTRATUMOR IMMUNE RESPONSE AND IS ASSOCIATED WITH OUTCOME IN PATIENTS WITH LUNG CANCER TREATED WITH IMMUNE CHECKPOINT INHIBITORS

Corentin Richard*, Myriam Berkafaroui, Omar El Quanzadi, Khoudia Diop, Antoine Desilets, Julie Maço, Wiam Belkaid, Andréanne Leblanc, Julien Lamontagne, Meriem Messaoudene, Anielle Ekrief, Bertrand Routy. CRCHUM, Montreal, Canada

Background The gut microbiome plays a key role in immune checkpoint inhibitors (ICI) efficacy and several strategies are currently being investigated to improve microbiome composition. The impact of a specific diet on microbiome modulation and clinical outcomes remains unknown. In this study, we assessed the effects of a high fiber diet on clinical outcomes as well as on microbiome composition, production of fecal metabolites, and intratumor immune infiltration in metastatic non-small cell lung cancer (mNSCLC) patients amenable to ICI.

Methods In this prospective study, 39 chemotherapy-refractory or naive patients with mNSCLC treated with ICI alone or in combination with chemotherapy completed a validated dietary survey. Based on the total fiber intake, patients were divided into high vs low fiber groups (HF vs LF). Objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) were compared between both groups. In addition, fecal and tumor samples were collected prior to ICI initiation. Fecal metagenomic sequencing was performed and fecal short-chain fatty acids (SCFA) were measured by LC-MS/MS. Tumoral transcriptome profiling was performed through RNA sequencing to define differentially expressed pathways.

Results Baseline characteristics were well balanced between both groups, including body mass index (BMI) and PD-L1 status. Median PFS for the HF group was longer compared to the LF group (27.4 vs 12.6 months). Microbiome metagenomic profiling revealed higher baseline alpha diversity (p=0.048) in the HF group compared to the LF group. Bifidobacterium, Alistipes, and Bacteroides slyersiae were enriched in the HF group while Fusobacterium was overrepresented in the LF group. SCFA measurement revealed that a high level of propionate correlated with a significantly longer OS (not reached vs 18.4 months, p=0.02) in the entire cohort. Moreover, propionate levels were significantly higher in the HF vs LF group (p=0.02). At the tumor level, RNA sequencing demonstrated a downregulation of DNA repair mechanisms and an upregulation of humoral and adaptive immune responses in the HF group.

Conclusions In this study, we demonstrated that a HF diet in patients with mNSCLC was associated with better clinical outcomes. Importantly, HF was associated with an enrichment of previously reported beneficial gut bacteria. Moreover, propionate correlated with longer OS and was increased in the HF group. This study provides further insights into how the diet can beneficially shift the microbiome composition and intratumor immune responses in patients with mNSCLC treated with ICI and this may lead to novel, dietary-g geared therapeutic avenues in the oncomicrobiome arena.

Ethics Approval The study was approved by CRCHUM Institution,s Ethics Board, approval number 17.035.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0679

680 AUTOMATED ION TORRENT BASED SOLUTION ENABLES ACCURATE GUT MICROBIOME QUANTIFICATION OF BACTERIAL SPECIES RELEVANT TO RESEARCH IN CANCER AND ITS RESPONSE TO IMMUNOTHERAPY


Background A low-cost targeted solution to profiling gut microbial diversity is sequencing of the 16S rRNA gene; however, it is often insufficient to gain species level resolution due to high homology across different bacteria. Therefore, we developed a first-of-its-kind targeted sequencing solution that supplements 16S gene targets, with highly species-specific primers for a cohort of 73 bacteria associated with research in diabetes, cancer, and its response to immunotherapy,
gastrointestinal and auto-immune disorders. This assay performs at 100% sensitivity and specificity for the species-level detection (Ion AmpliSeq Microbiome Health Research Kit: www.thermofisher.com/ngsmicrobiome) of these bacteria and is hence better suited for gut microbiome profiling in the context of the above phenotypes, as compared to other existing solutions.

Methods To assess the utility of the panel in cancer immunotherapy research, we sequenced DNA from 15 stool samples from subjects with Non-Small Cell Lung Carcinoma (NSCLC) undergoing immunotherapy, and compared their microbiome profiles to 26 healthy stool samples collected internally. With our post-sequencing workflow on Ion Reporter™, we automatically generate a report with taxonomic classifications, sample diversity metrics through QIIME2 integration, and relative abundance visualizations for bacteria across multiple samples.

Results We identified significant microbiome composition differences between the healthy samples and cancer/treated samples, as evidenced by (i) a clear separation between the two cohorts based on a beta diversity principal coordinate analysis (PCoA) plot, driven by large abundance changes in Clostridium, Lachnospiraceae, Subdoligranulum and Oscillibacter (P < 0.05), (ii) grouping into distinct classes based on overall microbiome profiles (Analysis-of-Similarities ANOSIM P = 0.003), and (iii) differences in abundances of specific bacteria with anti-tumor effects such as F. prausnitzii (P = 0.02).

Conclusions We have created a highly multiplexed, sensitive and specific assay for robust characterization of gut microbiota, with compatibility on both (i) the Ion GenoStudio S5™ with a 48-hr sample-to-result turnaround, and (ii) the new Ion Genexus™ System, a fully integrated platform featuring a hands-off, automated sample-to-report workflow that delivers results in a single day. It enables an efficient and affordable means for conducting extensive analyses of the human microbiome having applications in the study of phenotypic variability, and the potential relationship to disease. For research use only. Not for use in diagnostic procedures.

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0680

681 SINGLE PIPELINE RE-ANALYSIS REVISERS MICROBIOME ASSOCIATIONS WITH ANTI-TUMOR RESPONSE TO CHECKPOINT INHIBITORS

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Background Several studies suggest the gut microbiome may be a novel, modifiable biomarker for clinical efficacy of immune checkpoint inhibitors (ICIs). Microbiome profiling of pre-treatment samples demonstrated that high alpha-diversity and enrichment of specific bacterial species are associated with improved tumor responses in melanoma, renal cell cancer (RCC), and non-small cell lung cancer (NSCLC). Despite these reports, the specific bacteria or communities helpful or harmful have been inconsistent across study populations, and further correlation with immune and mutational biomarkers are limited or lacking. We hypothesize that, by use of a larger sample size and a consistent computational approach, we would derive a clearer microbial profile that correlated with immunotherapeutic outcomes.

Methods We re-analyzed the available raw 16S rRNA metagenomic and metagenomic sequencing data across five recently published ICI studies (n=303 unique patients) of responder (R) and nonresponse (NR) using a consistent computational approaches (Resphera Insight and MetaPhlAn2). Using novel microbiota signatures, we identified Re-analysis Indices for R- and NR-associated bacteria and validated the result in three addition cohorts with available raw sequencing data in patients with melanoma, hepatocellular cancer (HCC), and NSCLC (n=105).

Results Our results confirm signals reported in each study, though some bacteria reported initially were not statistically significant after correction for false discovery rate. Likely, in part, because our analysis allows for comparison of individual species across cohorts, we were able to identify new bacterial signatures, such as Oxalobacter formigenes, Roseburia hominis and Veillonella parvula, Clostridium hathewayi, enriched in R and NR respectively. When our Re-analysis Index was compared to an index assembled from the literature, we noted improvement occurred in a sensitivity and specificity analysis, especially in NR-associated signals. Moreover, we found that alpha-diversity was not consistently predictive of response or nonresponse to ICIs. Our Re-analysis Index also validated in melanoma patients and HCC but did not perform as well in the NSCLC cohort, suggesting the need for further refinement based on tumor type.

Conclusions In summary, this bioinformatics platform improves on existing pipelines by standardizing critical preprocessing and downstream analysis tools, enabling comprehensive evaluations of taxonomic and functional signals across sequencing datasets. Notably, the NR-associated Re-analysis Index shows the strongest and most consistent signal using a random effects model and in a sensitivity and specificity analysis (p < 0.01). Our integrated analyses suggest an approach to identify patients who would benefit from microbiome-based interventions targeted to improve response rates by using a biomarker for nonresponse.

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682 ANTIBODY-MEDIATED BLOCKADE OF INTERLEUKIN-10 RECEPTOR-ALPHA PROMOTES THE ACTIVATION OF IMMUNE CELLS FROM IN VITRO DISSOCIATED TUMOR SAMPLES


Background Interleukin-10 (IL-10) is a multifunctional cytokine that can mediate immune suppression or activation depending on the immunological context. Mouse studies have demonstrated that blockade of IL-10 enhances immune response against tumors and chronic viral infections, intriguingly,
high concentrations of long-acting, pegylated IL-10 have also shown anti-tumor activity. Here we investigated IL-10 and IL-10 receptor-alpha (IL-10RA) expression profiles in normal and tumor tissues as well as the immunological effects of modulating the IL-10 pathway via antibody-mediated blockade of IL-10RA.

Methods IL-10 and IL-10RA mRNA are expressed by several tumors, including renal, lung, breast, and colon cancers. Fluorescent in-situ hybridization revealed that the majority of IL-10RA was expressed by CD3-negative tumor-infiltrating cells, localized in close proximity to T cells in the tumor microenvironment (TME). Immunohistochemistry studies confirmed expression of IL-10RA in the TME, while no expression was detected in healthy tissues. Furthermore, dissociated tumor cells produced biologically active levels of IL-10 in culture.

Results Monoclonal antibodies (mAbs) against IL-10RA prevented IL-10 signaling and enhanced release of IL-12 by monocyte-derived dendritic cells activated with suboptimal LPS concentrations. The effect of IL-10RA blockade was greater than that observed with IL-10 neutralizing mAbs. In mixed lymphocyte reactions and superantigen-driven T-cell activation, IL-10RA blockade enhanced IL-2 secretion by T lymphocytes. Consistent with earlier observations in mouse models, the effect of IL-10RA blockade was nonredundant with blockade of the PD-1/PD-L1 axis, resulting in enhanced IL-2 and interferon-gamma secretion by T cells when both pathways were inhibited. Blockade of IL-10RA during CD3-redirected in vitro killing of tumor cells by PBMC induced IL-12 release as well as upregulation of CD86 and HLA-DR by CD3-negative cells. In vitro dissociated tumor cells, IL-10RA blockade induced release of IL-2, interferon-gamma and other proinflammatory cytokines; additional PD-1/PD-L1 axis blockade further enhanced cytokine release.

Conclusions In summary, antibody-mediated IL-10RA blockade can potentiate immune activation in the dissociated tumor cells and may be a valuable addition to cancer immunotherapies, including redirected T-cell killing and checkpoint blockade.

REFERENCES

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A LOW AFFINITY BIVALENT MESOTHELIN-BINDING MATCH4 MULTISPECIFIC T CELL ENGAGER INCREASES CYTOTOXIC SELECTIVITY FOR HIGH MESOTHELIN EXPRESSING CELLS

Bithi Chatterjee*, Alexandre Simonin, Daniel Snell, Tea Gurdj, Christian Hess, Matthias Brock, Stefan Warmuth, Christopher Weinert, Niels Kirk, Dania Diem, Naomi Flückiger, Robin Heiz, Benjamin Kütter, Dana Mahler, Diego Morenzoni, Sandro Wagen, Júlia Zeberer, David Urech. Numab Therapeutics, Wadenswil, Switzerland

Background The effective treatment of solid tumors remains an unmet medical need. Several concepts exist to treat malignancies, including antibody-drug or -immunotoxin conjugates, immune checkpoint inhibition, CAR-T cells, as well as bispecific T cell engagers. CD3-based T cell engagers are highly potent therapeutic molecules with T cell cytotoxicity activities in the picomolar range. Alongside this highly potent anti-tumor activity is the risk of off-target effects due to low levels of expression of the target antigen in normal tissue, as has been observed for the tumor-associated antigen mesothelin (MSLN).

Methods Low-affinity antibody fragments to the tumor-associated antigen MSLN were generated, and a multispecific
A HIGHLY SELECTIVE AND POTENT HPK1 INHIBITOR
PRESSES TUMOR GROWTH INHIBITION IN A MURINE SYNGENEIC TUMOR MODEL

Background HPK1, a member of the MAP4K family of protein serine/threonine kinases, is involved in regulating signal transduction cascades in cells of hematopoietic origin. Recent data from HPK1 knockout animals and kinase-inactive knock-in animals underscores the role of HPK1 in negatively regulating immune cell activation. This negative-feedback role of HPK1 combined with its restricted expression in cells of hematopoietic origin, make it a compelling drug target for enhancing anti-tumor immunity.

Methods A structure-based drug design approach was used to identify potent and selective inhibitors of HPK1. Biochemical assays, as well as primary human and mouse immune cell-based activation assays, were utilized for multiple iterations of structure-activity relationship (SAR) studies. In vivo efficacy, target engagement and pharmacodynamic data were generated using murine syngeneic tumor models.

Results A highly potent, HPK1 inhibitor was identified, that showed high selectivity against T cell-specific kinases and kinases in the MAP4K family. In vitro, HPK1 small molecule inhibition resulted in enhanced IL-2 production in primary mouse and human T cells, enhanced IL-6 and IgG production in primary human B cells, and enhanced mouse dendritic cell activation and antigen presentation capacity. Furthermore, HPK1 inhibition alleviated the immuno-suppressive effects of PGE2 on naïve human T cells and restored the proliferative capacity of exhausted human T cells. In vivo, HPK1 inhibition HPK1 inhibition abrogated T cell receptor-stimulated phospho-SLP-76, enhanced cytokine production, and mediated robust tumor growth inhibition in a murine syngeneic tumor model.

Conclusions Pharmacological blockade of HPK1 kinase activity represents a novel and potentially valuable immunomodulatory approach for anti-tumor immunity.

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TCGA. IO-108 binds to LILRB2 with high affinity and specificity and blocks LILRB2/ILT4 ligand binding and activation. IO-108 enhanced the production of multiple proinflammatory cytokines in LPS- and anti-CD3-stimulated PBMC cultures from healthy donors and potentiated DC maturation/activation in response to LPS. Moreover, IO-108 polarized primary CD14+ cells isolated from solid tumor patient PBMC and ovarian cancer-associated ascites towards a proinflammatory phenotype and attenuated their suppressive effect on autologous T-cell proliferation and production of tumoricidal cytokines.

Conclusions The preclinical characterization of IO-108, a novel LILRB2/ILT4 antagonistic antibody, demonstrates its ability to polarize tumor-associated myeloid cells towards a proinflammatory phenotype and suggests potential therapeutic benefit in tumors unresponsive to immune checkpoint blockade.

Acknowledgements We acknowledge the funding support from National Cancer Institute (1R01 CA248736 and 2P30 CA142543), the Welch Foundation (AU-0042-20030616) and the Cancer Prevention and Research Institute of Texas (RP150551 and RP190561).

Ethics Approval PBMCs were isolated from buffy coats of healthy donors (Interstate Blood Bank). Hematopoietic samples from cancer patients were obtained through the services of the Simmons Cancer Center’s Tissue Management Shared Resource with IRB approved protocol (STU 102010-051). All animal work was approved and conducted under the oversight of the UT Southwestern Institutional Animal Care and Use Committee (IACUC).

REFERENCES


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Background gdT-cells are innate-like lymphocytes described as potent killer of cancer cells whose infiltration into tumors is associated with a positive prognosis. 1, 2 This supports gd T-cells use in cancer immunotherapy. BTN3A, which belongs to the B7-subfamily of Ig proteins, is required for the recognition of malignant or infected cells by human gd T-cells by sensing intracellular accumulation of phosphorytigens. 3 ImCheck Therapeutics is developing ICT01, a humanized anti-BTN3A (IgG1, Fc-silenced), gd9d2 T-cell-activating antibody for the treatment of patients with solid or hematologic tumors.

Methods A complete IND-enabling program was conducted to characterize the preclinical activity and safety of ICT01. ICT01 effects on human and cynomolgus PBMCs were characterized in vitro using flow cytometry. ICT01-mediated killing activity of gd9d2 T-cells was assessed using in vitro co-cultures with tumor and non-tumor cells. Immunocompromised mice bearing human tumors and adoptively transferred with human gd9d2 T-cells were used to assess ICT01 anti-tumor activity in vivo. The PK, PD and safety of intravenous ICT01 (0.1 to 100 mg/kg single- and repeated-dose) were evaluated in Cynomolgus monkeys.

Results ICT01 selectively binds to all three BTN3A isoforms with high affinity (<10nM). When assayed in human and cynomolgus PBMCs in vitro, ICT01 promoted a robust and specific activation of gd9d2 T-cells as shown by concentration-dependent increase in cell surface CD69 and CD25 and cytokines secretion (IFNγ, TNFα). In co-culture experiments, ~20% of target occupancy on tumor cells is sufficient for maximal gd9d2 T-cell degranulation (e.g. CD107a/b expression). ICT01-activated gd9d2 T-cells continuously and serially kill a wide range of tumor cells in multi-day co-culture conditions. In contrast, non-tumoral BTN3A-expressing B cells, HUVEC and fibroblasts were unaffected. In mouse AML and ovarian cancer models, repeated injections of ICT01 delayed tumor growth and significantly prolonged animal survival. In primates, ICT01 exposure and target engagement was dose-dependent, with all tested doses producing a specific gd9d2 T-cell activation and trafficking out of the circulation within 1 hour. ICT01 administration was well tolerated with no safety signals observed at doses up to 25 mg/kg/week based on clinical, laboratory, and anatomic pathology parameters.

Conclusions The combined in vitro and in vivo pharmacology data provide evidence that ICT01 is an attractive and novel therapeutic approach for enhancing the innate anti-tumor potential of gd9d2 T-cells by activating BTN3A. Importantly, ICT01 did not affect healthy BTN3A-expressing cells, and NHP studies confirmed ICT01 safety with a wide therapeutic index. Therefore, ICT01 is being tested in the ongoing EVIC-TION trial (NCT04243499).

Ethics Approval Pseudonymized samples isolated from healthy volunteers’ whole blood by ImCheck Therapeutics under the agreement n° 7173 between ImCheck Therapeutic SAS and EFS PACA (Etablissement Français du Sang Provence-Alpes cote d’Azur)
IN VIVO EXPANSION OF GAMMA DELTA T CELLS BY A CD19-TARGETED BUTYROPHILIN HETERODIMER LEADS TO ELIMINATION OF PERIPHERAL B CELLS

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Background A primary mechanism of cancer immunotherapy resistance involves downregulation of specific antigens or major histocompatibility complex-based antigen presentation, which renders tumor cells invisible to alpha-beta T cells, but not gamma-delta T cells. Recently, a two-step model of gamma-delta T cell activation has emerged, wherein one butyrophilin (BTN, i.e. BTN2A1) directly binds the gamma-delta TCR but is only activated if certain molecular patterns (e.g. phosphoantigens) facilitate recruitment of a second BTN (i.e. BTN3A1) into a complex to form a BTN2A1/3A1 heterodimer. The BTN2A1/3A1 complex specifically activates the predominant gamma-delta T cell population in the peripheral blood, comprising the Vγ9δ2 T cell receptor (TCR), but does not activate the primary gamma-delta T cell population in mucosal tissues, comprising the Vγ4 TCR. The unique mechanism of action and specificity of gamma-delta TCR/BTN interactions suggests that therapeutic proteins comprising specific BTN heterodimers could be used to target specific gamma-delta T cell populations, with a lower risk of off-target activation common with CD3-directed T cell engagers.

Methods Human BTN2A1/3A1-Fc-CD19scFv and mouse BTNLI/6-Fc-CD19scFv heterodimeric fusion proteins were purified and binding to CD19 or the respective gamma-delta TCRs was assessed by ELISA, Octet and flow cytometry using gd T-cells isolated from human peripheral blood and mouse intestinal tissue. The functionality of the constructs to activate gamma-delta T cells and mediate killing of tumor cells was assessed using live cell imaging in vitro as well as a murine B-cell lymphoma model in vivo.

Results The CD19-targeting scFv domains of the BTN heterodimer fusion proteins bound to human and mouse CD19 with low nanomolar affinity. The BTN2A1/3A1-Fc-CD19scFv compound specifically bound to the Vγ9δ2 TCR on human gd T cells while the mouse BTNLI/6-Fc-CD19scFv bound to Vγδ4 TCR on mouse gd T cells. Both compounds were able to activate gd T cells in a co-culture assay resulting in degranulation and increased surface expression of CD107a and also increased apoptosis of CD19+ tumor cells. Intraperitoneal administration of the mouse BTNLI/6-Fc-CD19scFv led to anti-tumor effects in A20 tumor bearing BALB/c mice. Phenotyping from BTNLI/6-Fc-CD19scFv treated mice revealed profound and rapid expansion of the endogenous gamma-delta T cells in the circulation and tumor, with concomitant depletion of peripheral CD19+ B-cells, confirming the mechanism of action of the heterodimer as a gamma-delta T cell specific engager.

Conclusions These results provide proof of mechanism for in vivo manipulation of gamma-delta T cells using antigen-targeted butyrophilin heterodimeric fusion proteins for the treatment of cancer.

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689 ATRC-101 DRIVES POTENT SINGLE-AGENT ACTIVITY IN MOUSE SYNGENEIC TUMOR MODELS VIA A NOVEL CELLULAR MECHANISM OF ACTION


Background We have previously demonstrated adaptive antibody responses targeting public tumor antigens in cancer patients. ATRC-101, a clinical stage, engineered version of an antibody identified in such a patient, displays robust single-agent activity in syngeneic tumor models requiring Fc receptors (FcRs) expressed by innate immune cells and the presence of CD8+ T cells. The novel target of ATRC-101 was found to be a tumor-restricted ribonucleoprotein (RNP) complex, and because RNP complexes drive T cell responses in infectious and autoimmune disease via innate immune cells, we further characterized the mechanism of action of ATRC-101. Here we describe changes in immune cell populations in a tumor model proximal to treatment initiation with ATRC-101.

Methods Mice bearing EMT6 tumors received ATRC-101 beginning on day 7 post-tumor inoculation. Tissues were harvested between days 7 and 14 and analyzed by flow cytometry and immunohistochemistry. Transcriptome analysis was performed using RNA sequencing on whole tumors taken on days 7, 9, and 12.

Results The earliest significant changes induced by ATRC-101, relative to vehicle, were noted just 24 hours after dosing: increased numbers of cDC1 cells in blood, and decreased numbers of cDC2 cells in blood and M-MDSCs in tumor. A significant increase of CD8+ T cells was observed in blood 48 hours after dosing and in tumor 96 hours after dosing. Increased numbers of NK cells were also observed in blood and tumor at this later time. Multiplex analysis of circulating cytokines demonstrated a very early increase in myeloid chemo-attractionants, such as MCP1 and MIP1a. Whole exome sequencing of tumor samples showed that ATRC-101 dosing drives a significant increase, relative to vehicle, in the expression of interferon-stimulated genes. Co-culturing experiments demonstrated that induced, bone marrow-derived dendritic cells are activated by ATRC-101 and its target in a dose-dependent fashion.

Conclusions Dosing with ATRC-101 in the EMT6 syngeneic tumor model, in which ATRC-101 displays notable single-agent activity, leads to changes in immune cell composition in the blood and tumor, with the earliest changes observed in myeloid or myeloid-derived cell populations, and to the early appearance of myeloid chemo-attractionants. We believe these data indicate that ATRC-101 acts proximally on the myeloid cell populations in the tumor, leading to a remodeling of the tumor environment and an adaptive immune response that
includes CD8+ T cells driving tumor regression. Our data demonstrate that ATRC-101, bound to its target which is an RNP complex, can activate myeloid cells and are consistent with this activation occurring via FcR and Toll-like receptor (TLR) pathways.

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CD122-SELECTIVE IL-2 COMPLEXES TREAT OVARIAN CARCINOMAS, INDUCE TREG FRAGILITY AND PROMOTE T CELL STEM CELLS

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Abstract 690 Figure 1 IL-2c but not antiPD-L1 treats ID8agg Luciferase signal of ID8agg-luc tumors treated with isotype, ?PD-L1, or IL-2c measured by in vivo imaging. Arrows indicate treatments.

Abstract 690 Figure 2 IL-2c inhibits functional markers on tregs and promotes teff Expression of CD25 and granzymeB were measured by flow cytometry in indicated population from isotype or IL-2c treated ascites 3 weeks after final IL-2c dose.

Abstract 690 Figure 3 IL-2c reduces ascites Treg suppressive function

Abstract 690 Figure 4 IL-2c induces Treg fragility in ascites but not TDLN tSNE analysis on CD45+CD3+CD4+FoxP3+ cells from ascites and TDLN of isotype and IL-2c treated ID8agg-luc challenged mice. Right, representative bar graphs of flow data.
Conclusions We define two novel IL-2c effects: inducing Treg fragility therefore reducing immunosuppression while promoting TCSC that could enhance effective anti-tumor immunity. Current work tests if effects are related and help efficacy, and mechanisms for IL-2c Treg effects. We also show that elicited TCSC differ by treatment and tumor, requiring additional investigations.

Acknowledgements This work is supported by CPRIT Research Training Award (RP 170345), Ovarian Cancer Research Alliance Ann and Sol Schreiber Mentored Investigator Award to YD and R01 CA205965 to TC.

Ethics Approval All mice studies were approved by UT Health San Antonio Institutional Animal Care and Use Committee (IACUC). Approval number 20150093AR, 20140001AR, 20170035AR, 20140039AR, 20140027AR, 20090128AR, 20120071AR, 20180021AR.

REFERENCES


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MDK-271: A DUAL FUNCTION MOLECULE CONSISTING OF EMPIRICALLY-DESIGNED PEPTIDYL AGONISTS OF IL-2/15RβγC AND IL-7RαγC, UNRELATED TO IL-2, IL-15, OR IL-7, INCORPORATED INTO A BISPECIFIC FC FUSION PROTEIN

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Background Activation of IL-2/15Rβγc or IL-7R on immune cells using modified versions of IL-2 or IL-7 is under investigation as monotherapy, or in combination with checkpoint inhibitors, engineered T or NK cells, or neo-antigen vaccines. We previously described small synthetic peptides, unrelated to IL-2, IL-15, or IL-7, that selectively activate either IL-2/15Rβγc or IL-7R. IL-2/15Rβγc and IL-7R activation exhibit complementary effects on immune cells that when combined may offer benefits over each independent mechanism. We now report the creation of an Fc-fusion protein that incorporates both IL-2/15Rβγc and IL-7R agonist peptides, and characterize its properties in cell lines and human (PBMC) lymphocyte subpopulations.

Methods Peptide agonists of IL-2/15Rβγc (MDK1169) and IL-7R (MDK1319) were separately fused to each chain of obligate heterodimeric (asymmetric) Fc molecules. The Fc-fusion was purified by protein-A and size exclusion chromatography, and characterized by LC-MS. Receptor-mediated signaling, proliferation, and cell-surface receptor expression in cell lines, PBMCs, mixed and isolated lymphocyte subpopulations were determined by flow cytometry and ELISA to evaluate effects of IL-2, IL-2v, IL-7, MDK-202 or MDK-701 (Fc-fusions of MDK1169 and MDK1319, respectively), and combinations (mixtures) of these molecules, in comparison with the dual agonist MDK-271.

Results LC-MS analysis indicates MDK-271 is a heterodimeric molecule containing one copy each of MDK1169 (IL-2/15Rβγc-biased agonist) and MDK1319 (IL-7R agonist) fused to individual Fc-chains. Cell-based assay of MDK-271 demonstrates potent, fully efficacious phosphorylation of STAT5 in TF-1 cells expressing Rγc, and engineered to express either IL-2/15Rβ or IL-7Rα. PBMCs exposed ex vivo to MDK-271 exhibit additive, complimentary, and synergistic effects among various lymphocyte subpopulations: CD4+Tn, Teff, Treg, Tmem; C8+Tn, Teff, Tmem; and NK cells, in this analysis. The mono-specific agonists MDK-202 and MDK-701 produce proliferative effects and signaling patterns in responsive cell lines and lymphocyte subsets similar to those induced by IL-2v (an IL-2/15Rβγc-biased mutant of IL-2) and IL-7, respectively. Combining both activities in MDK-271 induces response profiles that differ in some T-cell subsets from those of mono-specific agonists of the two receptors. Animal studies designed to understand the effects of these differences are underway.

Conclusions IL-2/15Rβγc and IL-7R are both currently undergoing extensive scrutiny as potential immuno-oncology therapeutic targets. The biology of these cytokines is both overlapping and complementary in stimulating and supporting T-cell populations; and some recent evidence suggests possible superiority of the combination. Based on in vitro properties, the Fc-peptide fusion reported here, exhibiting both IL-2/15Rβγc-biased agonist and IL-7Rγc agonist activities, could be valuable in anti-tumor therapeutic applications.

Ethics Approval The use of human PBMC in this study was authorized under Minimal Risk Research Related Activities at Stanford Blood Center (SQL 79075)

AMV564, A CLINICALLY ACTIVE T CELL ENGAGER, INDUCES A TARGET-DEPENDENT ADAPTIVE IMMUNE RESPONSE

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Background AMV564 is a potent bispecific T cell engager that binds CD3 and CD33. Due to its bivalent structure, AMV564 is selective for MDSCs via clustered CD33 expressed on the cell surface both in vitro and in patients. MDSCs are responsible for local and systemic suppression of the immune response to both circulating and solid cancers. Targeting MDSC suppression allows T cell priming to be restored in both the lymph nodes and tumor microenvironment, and expands previously activated tumor-specific T cells. Here we report clinical observations and results of our ex vivo assay development.

Methods Cell lines, primary human cells, and patient samples were analyzed using flow cytometry with appropriate marker panels including AMV564 directly labeled (phycoerythrin) or detected with labeled anti-AMV564 antibodies. T cell cytotoxicity assays were conducted using primary human T cells and leukemic blast or other target cells (3:1 ratio) for 48 or 72 hours. Patient peripheral blood was sequenced for TcRbeta CDR3 variable chain on the hTsTCRBv4b.

Results AMV564 is currently under investigation in a Phase 1 clinical trial (NCT04128423). There have been no dose-limiting toxicities and clinical activity has been observed (RECIST complete response in an ovarian cancer patient) when dosed once daily as a subcutaneous injection. In patients, T cell redistribution is consistent with activation and depletion of both monocytic and granulocytic MDSCs. Immune profile changes consistent with CD8 and Th1 cell activation are observed (figure 1). Furthermore, TCR sequencing data
APX601, A NOVEL TNFR2 ANTAGONIST ANTIBODY FOR CANCER IMMUNOTHERAPY

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Background A key barrier to effective immunotherapy for cancer is the immunosuppressive tumor microenvironment (TME) characterized by infiltrating regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC). While depletion of immune-suppressive cells is a promising cancer immunotherapy strategy, current approaches are ineffective due to lack of specificity and safety concerns. Tumor Necrosis Factor Receptor 2 (TNFR2) is emerging as a novel, selective target to overcome immune suppression in TME. TNFR2 expression is generally restricted to highly immunosuppressive cell populations in the TME and the TNFR2-TNF-α pathway plays an important role in the generation and survival of these cells. TNFR2 is also an oncogene upregulated on certain tumors and can enhance tumor cell survival. Thus, targeting TNFR2 is a promising therapeutic approach with multiple potential mechanisms of action.

Methods A diverse panel of antibodies to TNFR2 was created using APXiMAB™, Apexigen’s proprietary rabbit monoclonal antibody technology. A robust assessment of over 100 antibody candidates for TNFR2 binding, TNF-α blockade and functional assays yielded APX601, a humanized IgG1 antibody, as the lead therapeutic candidate. The ability of APX601 to reverse immune suppression was assessed in Treg and MDSC suppression assays. In addition, the ability of APX601 to deplete TNFR2-expressing Treg and tumor cells was assessed both in vitro and in vivo using the mouse Colo205 xenograft model.

Results APX601 binds specifically to human TNFR2 with high affinity (Kd = 47 pM) and recognizes a unique epitope in the CRD1 domain of TNFR2. APX601 is a potent antagonist that blocks the TNFR2-TNF-α interaction in cell-based ligand binding assays (IC50 = 0.149 nM). APX601 is capable of reversing immune suppression via two mechanisms: 1) significant blockade of the immunosuppressive functions of both Tregs and MDSCs by inhibiting the binding of TNFR2 to its ligand TNF-α and 2) depletion of TNFR2-expressing Tregs, MDSC and tumor cells via antibody-dependent cell cytotoxicity (ADCC) (EC50 = 1.14 nM) and ADCP (EC50 = 0.71 nM) effector functions.

Conclusions APX601 is a potent TNFR2 antagonist antibody that reverses immune suppression by targeting TNFR2-expressing Treg and MDSC, and induces killing of tumor cells. Our data support the further development of APX601, a promising immunotherapeutic antibody with multiple potential mechanisms of action, for the treatment of a variety of solid tumors.

Ethics Approval Healthy human blood samples were obtained from Stanford Blood Center (Palo Alto, CA) from consenting donors under an approved protocol.

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NC410 IS A NOVEL IMMUNOMEDICINE FOR THE TREATMENT OF SOLID TUMORS

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Background Abnormalities in the extracellular matrix of tumor microenvironments support tumor progression, lead to
immuno dysfunction, and provide a target for cancer therapeutics. Collagens are a primary component of the extracellular matrix. Abnormal levels of collagen and of the collagen-domain containing complement component 1q (C1q) in tumor microenvironments has been proposed to disrupt anti-tumor immunity. LAIR-1 is an adhesion molecule and inhibitory receptor expressed on the cell surface of several immune cell subsets. LAIR-1 binding to collagen-like domains present in collagens and C1q inhibit immune cell function. LAIR-2 is a soluble homolog of LAIR-1 that binds to and outcompetes LAIR-1 binding to collagens and C1q and serves as a natural decoy to promote immune function.

Results NC410 has increased avidity due to Fc mediated dimerization, and blocks LAIR-1 interactions with ligands, and LAIR-1 signaling. In vivo administration of NC410 in humanized tumor models reduced tumor growth in a dose dependent fashion. NC410 increased the numbers of infiltrating human CD8+ and CD4+ T cells in the tumor, which is associated with increased levels of chemokines in the local tumor environment. Effector function was also enhanced, as denoted by increased levels of IFN-gamma and Granzyme B in the local tumor environment. In addition, NC410 increased specific collagen degradative products in the serum of humanized tumor-bearing mice, suggesting NC410 may promote tumor microenvironment remodeling and immune accessibility to further promote anti-tumor immunity.

Conclusions These data support NC410 as a novel therapeutic for targeting collagen-rich tumors and promote immune activation, infiltration and effector function.

Methods Taking advantage of a natural decoy system, we designed a protein biologic, NC410, composed of LAIR-2 fused with a functional IgG1 Fc domain to target collagen-rich tumors and promote immune activation, infiltration and effector function.

Abstracts

695 ORAL DELIVERY OF A MICROBIAL EXTRACELLULAR VESICLE INDUCES POTENT ANTI-TUMOR IMMUNITY IN MICE

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Background The small intestinal axis (SINTAX) is a network of anatomic and functional connections between the small intestine and the rest of the body. It acts as an immunosurveillance system, integrating signals from the environment that affect physiological processes throughout the body. The impact of events in the gut in the control of tumor immunity is beginning to be appreciated. We have previously shown that an orally delivered single strain of commensal bacteria induces anti-tumor immunity preclinically via pattern recognition receptor-mediated activation of innate and adaptive immunity. Some bacteria produce extracellular vesicles (EVs) that share molecular content with the parent bacterium in a particle that is roughly 1/1000th the volume in a non-replicating form. We report here an orally-delivered and gut-restricted bacterial EV which potently attenuates tumor growth to a greater extent than whole bacteria or checkpoint inhibition.

Methods EDP1908 is a preparation of extracellular vesicles produced by a gram-stain negative strain of bacteria of the Oscillospiraceae family isolated from a human donor. EDP1908 was selected for its immunostimulatory profile in a screen of EVs from a range of distinct microbial strains. Its mechanism of action was determined by ex vivo analysis of the tumor microenvironment (TME) and by in vitro functional studies with murine and human cells.

Results Oral treatment of tumor-bearing mice with EDP1908 shows superior control of tumor growth compared to checkpoint inhibition (anti-PD-1) or an intact microbe. EDP1908 significantly increased the percentage of IFNγ and TNF producing CD8+ CTLs, NK cells, NKT cells and CD4+ cells in the tumor microenvironment (TME). EDP1908 also increased tumor-infiltrating dendritic cells (DC1 and DC2). Analysis of cytokines in the TME showed significant increases in IP-10 and IFNγ production in mice treated with EDP1908, creating an environment conducive to the recruitment and activation of anti-tumor lymphocytes.

Conclusions This is the first report of striking anti-tumor effects of an orally delivered microbial extracellular vesicle. These data point to oral EVs as a new class of immunotherapeutic drugs. They are particularly effective at harnessing the biology of the small intestinal axis, acting locally on host cells in the gut to control distal immune responses within the TME. EDP1908 is in preclinical development for the treatment of cancer.

Ethics Approval Preclinical murine studies were conducted under the approval of the Avastus Preclinical Services’ Ethics Board. Human in vitro samples were attained by approval of the IntegReview Ethics Board; informed consent was obtained from all subjects.

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696 BRENTUXIMAB VEDOTIN, A CD30-DIRECTED ANTIBODY-DRUG CONJUGATE, SELECTIVELY DEPLETES ACTIVATED TREGS IN VITRO AND IN VIVO

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Background Regulatory T cells (Tregs) play an important role in maintaining immune homeostasis, preventing excessive inflammation in normal tissues. In cancer, Tregs hamper anti-tumor immunosurveillance and facilitate immune evasion. Selective targeting of intratumoral Tregs is a potentially promising treatment approach. Orthogonal evaluation of tumor-infiltrating lymphocytes (TILs) in solid tumors in mice and humans have identified CCR8, and several tumor necrosis family receptors (TNFRs), including TNFSFR8 (CD30), as receptors differentially upregulated on intratumoral Tregs compared to normal tissue Tregs and other intratumoral T cells, making these intriguing therapeutic targets.Brentuximab vedotin (BV) is approved for classical Hodgkin lymphoma (cHL) across multiple lines of therapy including frontline use in stage III/IV cHL in combination with doxorubicin, vinblastine, and dacarbazine. BV is also approved for certain CD30-expressing T-cell lymphomas. BV is comprised of a CD30-directed monomolecular antibody conjugated to the highly potent microtubule-disrupting agent monomethyl auristatin E (MMAE). The activity of BV in lymphomas is thought to primarily result from tumor directed intracellular MMAE release, leading to mitotic arrest and apoptotic cell death. The role CD30 plays in normal
immune function is unclear, with both costimulatory and proapoptotic roles described. CD30 is transiently upregulated following activation of memory T cells and expression has been linked to highly activated/suppressive IRF4+ effector Tregs.

**Methods** Here we evaluated the activity of BV on CD30-expressing T cell subsets in vitro and in vivo.

**Results** Treatment of enriched T cell subsets with clinically relevant concentrations of BV drove selective depletion of CD30-expressing Tregs > CD30-expressingCD4+ T memory cells, with minimal effects on CD30-expressing CD8+ T memory cells. In a humanized xenograft model, treatment with BV selectively depleted Tregs resulting in accelerated wasting and robust T cell expansion. The observed differential activity on Tregs is likely attributable to significant increases in CD30 expression and reduced efflux pump activity relative to other T cell subsets. Interestingly, blockade of CD25 signaling prevents CD30 expression on T cell subsets without impacting proliferation, suggesting a link between CD25, the high affinity IL-2 receptor, and CD30 expression.

**Conclusions** Together, these data suggest that BV may have an immunomodulatory effect through selective depletion of highly suppressive CD30-expressing Tregs.

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**Ethics Approval** Animals studies were approved by and conducted in accordance with Seattle Genetics Institutional Care and Use Committee protocol #SGE-024.

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**TUMOR-TARGETED CD28 COSTIMULATORY BISPECIFIC ANTIBODIES ENHANCE T CELL ACTIVATION IN SOLID TUMORS**

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**Background** T cells in the tumor micro-environment require TCR/MHC engagement and co-stimulatory receptor engagement to achieve complete activation. Solid tumors often lack expression of CD28 ligands, so we hypothesized that activation of CD28 signaling could be beneficial in solid tumors. We designed tumor-associated-antigen (TAA) x CD28 bispecific antibodies that conditionally costimulate CD28 only in the presence of TAA and TCR engagement. Clinical application of this class of antibodies has potential to enhance activity of either anti-PD(L)1 antibodies or TAA x CD3 T cell engagers.

**Methods** We designed a stability and affinity optimized anti-CD28 antibody that can be paired with TAA of choice to engage CD28 monovalently using Xencor’s XmAb 2+1 and 1+1 platforms. In vitro T cell activation with these bispecifics was measured by T cell proliferation, cytokine production, and cytotoxicity, in co-cultures of human cancer cell lines mixed with primary human CD3-stimulated T cells. In vitro activity was validated in a CMV recall assay measuring CMV+ T cell proliferation of CMV+ PBMC co-cultured with cancer cell lines ectopically treated with pp65-derived NLV peptide. In vivo anti-tumor and T cell proliferative activity of B7H3 x CD28 bispecific antibodies were determined in tumor-bearing huPBMC-NSG mice treated simultaneously with TAA x CD3 bispecific antibody. In vivo activity of PDL1 x CD28 antibodies was determined with hCD28 KI mice inoculated with MC38 tumors expressing hPDL1-antigen. Finally, safety and tolerability of B7H3 x CD28 and PDL1 x CD28 was determined in cynomolgus monkeys.

**Results** B7H3 x CD28 and PDL1 x CD28 antibodies enhanced T cell degranulation, cytokine secretion, and cancer cell cytotoxicity in concert with CD3 stimulation only in the presence of target antigen. B7H3 x CD28, alone or in combination with anti-PD1 antibody, enhanced proliferation of CMV+ T cells recognizing cancer cells loaded with pp65-derived NLV peptide. PDL1 x CD28 also enhanced CMV+ cell expansion but did not synergize with anti-PD1 antibody treatment. B7H3 x CD28 significantly enhanced in vivo anti-tumor activity of TAA x CD3 antibodies while also promoting greater T cell expansion. In hCD28 mice inoculated with MC38 tumors expressing hPDL1, PDL1 x CD28 antibody inhibited tumor growth greater than an anti-PDL1 antibody alone. B7H3 x CD28 and PDL1 x CD28 were well tolerated in cynomolgus monkeys.

**Conclusions** B7H3 x CD28 and PDL1 x CD28 bispecific antibodies show promising anti-tumor activity and warrant further development.

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**TARGETING HLA-G-MEDIATED IMMUNOSUPPRESSION WITH A FIRST-IN-CLASS ANTAGONIST ANTIBODY**

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**Background** Human leukocyte antigen-G (HLA-G) is an immune checkpoint molecule that belongs to the non-classical HLA-class I family of receptors. HLA-G restrains immune cell activation and effector function by engaging with inhibitory receptors ILT2 and ILT4. While expression of HLA-G is highly restricted under normal healthy conditions, we have demonstrated that its expression in cancer is aberrantly upregulated and broadly detected across a variety of tumor types. Tizona Therapeutics has generated a novel, fully human antibody that specifically targets HLA-G and reverses HLA-G-mediated immunosuppression. Here we present in vitro and in vivo data demonstrating the functional impact of HLA-G blockade on immune cells and evidence to support the use of TTX-080 in the clinic to treat patients with advanced solid tumors.

**Methods** Evaluation of HLA-G expression in cancer was performed using immunohistochemistry, flow cytometry, and gene profiling. Expression of ILT2 and ILT4 was assessed on tumor infiltrating leukocytes by flow cytometry. To demonstrate the suppressive function of HLA-G, primary human NK cells, T cells, and monocyte-derived macrophages were cultured with target cells expressing HLA-G. TTX-080 was then evaluated for its ability to reverse this suppression. In addition, TTX-080 was investigated in vivo using a disseminated xenograft tumor model.

**Results** Expression of HLA-G was detected on tumor cells and tumor infiltrating leukocytes across a variety of solid tumor types. TTX-080 blocked interaction of HLA-G with both ILT2 and ILT4 and restored cytotoxicity in multiple assays using either primary NK cells or NKL cell lines. Monocyte-derived macrophages expressing ILT2 and ILT4 exhibited decreased phagocytosis of HLA-G+ target cells; this inhibition was reversed with an antigen-binding fragment of TTX-080. TTX-
080 was also able to reverse HLA-G-mediated suppression of ILT2+ CD8+ T cells as assessed by degranulation and proinflammatory cytokine secretion. Notably, mice with disseminated tumors had extended median survival when treated with a single dose of TTX-080.

Conclusions TTX-080 reverses HLA-G-mediated suppression of ILT2+ and ILT4+ immune cells that are found within the tumor microenvironment. Blockade of HLA-G using TTX-080 therefore has the potential to reverse broad immune suppression in patients with advanced solid tumors by reinvigorating CD8+ T cells, enhancing NK cytolytic activity, and increasing macrophage phagocytosis.

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699 A DIFFERENTIATED ANTI-OX40 AGONIST BGB-A445 DOES NOT BLOCK BOX4-OX40L INTERACTION AND REVEALS REMARKABLE ANTI-TUMOR EFFICACY IN PRECLINICAL MODELS

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Background OX40 is a member of the tumor necrosis factor receptor super family (TNFRSF) primarily expressed on activated CD4+ and CD8+ T cells, as well as natural killer (NK) T and NK cells. It is an immune costimulatory receptor which binds to its ligand OX40L and activates downstream NF-kB pathway to induce immune cell activation, proliferation, and survival.1-3 Current agonistic anti-OX40 antibodies in clinic, which are mostly ligand-competitive antibodies, showed limited clinical responses, mainly at lower doses. Blockade of OX40-OX40L interaction might limit the efficacy of these ligand-competitive antibodies at higher doses, as OX40-OX40L interaction is essential for enhancing effective anti-tumor immunity. Here we report pre-clinical data of BGB-A445, which is a ligand non-blocking agonistic anti-OX40 humanized antibody.

Methods Cell-based flow cytometry assay was established to determine whether BGB-A445 interferes with OX40-OX40L interaction. Co-crystal structure of OX40/BGB-A445 Fab was solved to study the molecular binding mechanism. A mixed lymphocyte reaction (MLR) assay was set up to investigate the ability of BGB-A445 to activate CD4+ T-cells. The anti-tumor efficacy of BGB-A445 was evaluated in MC38 colon cancer and CT26WT colon cancer models either as a single agent or in combination with anti-PD-1 antibody.

Results The flow cytometry study showed that BGB-A445 did not interfere with the binding of OX40 to OX40L even at high concentrations. In contrast, MOXR0916, an anti-OX40 agonistic antibody developed by Genentech, completely blocked OX40 binding to OX40L. Additionally, the co-crystal structure of OX40/BGB-A445 Fab complex indicated that BGB-A445 interacts with the CRD4 region of OX40 which is distant from OX40L binding region. In the MLR assay, combined with an anti-PD-1 antibody, BGB-A445 co-stimulated CD4+ T-cells to secrete IL-2 dose-dependently, while MOXR0916 did not. In the MC38 colon cancer model in human OX40 knock-in mice, BGB-A445 demonstrated remarkable anti-tumor efficacy in a dose-dependent manner, while MOXR0916 showed a ‘hook effect’ in the same setting. In addition, BGB-A445 exhibited significant anti-tumor activity in the PAN02 pancreatic model which is resistant to anti-PD-1 treatment. Besides, BGB-A445 revealed significant combination effects with anti-PD-1 therapy in both MC38 and CT26WT models.

Conclusions In conclusion, differentiated from current clinical stage anti-OX40 antibodies, BGB-A445 is an agonistic antibody that does not block the OX40-OX40L interaction. Both in vitro and in vivo results demonstrated that BGB-A445 has remarkable immune stimulating effect and anti-tumor efficacy either as a single agent or in combination with anti-PD-1 therapy, thus warranting further clinical investigation.

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700 EPHA2/CD137 BICYCLE TUMOR-TARGETED IMMUNE CELL AGONISTS (TICAS™) INDUCE TUMOR REGRESSIONS, IMMUNOGENIC MEMORY, AND REPROGRAMMING OF THE TUMOR IMMUNE MICROENVIRONMENT

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Background Despite compelling preclinical data, agonistic anti-CD137 antibodies have been hampered by failure to delineate hepatotoxicity from efficacy in clinical studies.1 2 A new generation of both systemic and targeted CD137 agonists that are now entering clinical development rely on biologic agents with suboptimal properties for CD137 agonism due to their relatively large sizes and long circulating half-lives.3-5 These properties may limit their tissue penetration and cause sustained agonism resulting in overstimulation and activation-induced cell death of lymphocytes due to continuous exposure. BCY12491 is a tumor-targeted immune cell agonist (TICAS™) that exemplifies a new class of fully synthetic immunomodulators with constrained bicyclic peptides (Bicycles®) targeting a tumor antigen and a co-stimulatory molecule. We developed this new class of synthetic molecules with antibody-like affinities and target selectivity to circumvent the aforementioned barriers to optimal targeted CD137 agonistic therapeutics. BCY12491 (EphA2/CD137 TICA) is designed to deliver a highly potent CD137 agonist to EphA2 overexpressing tumor tissue with an intermittent dosing schedule maximizing anti-tumor activity while circumventing the need for continuous systemic exposure.

Methods BCY12491 bioactivity was assessed in vitro using a CD137 reporter assay and by measuring cytokine production from primary human PBMC/tumor cell co-cultures. BCY12491 in vivo activity was determined in huCD137-syngeneic tumor models by measuring tumor growth kinetics and using tumor immune cell and transcriptional profiling by FACS, IHC, and Nanostring.

Results BCY12491 engages EphA2 and CD137 with high affinity resulting in picomolar potency in co-culture assays consisting of EphA2-expressing tumor cell lines and CD137-expressing Jurkat NF-kappaB-luciferase reporter cells.
Moreover, BCY12491 caused EphA2-dependent CD137 agonism in primary human PBMCs co-cultured with tumor cells with varied levels of EphA2 expression. Treatment of MC38 tumors in immunocompetent mice with BCY12491 leads to a profound reprogramming of the tumor immune microenvironment including increased T cell infiltration and stimulation of NF-kappaB signaling, costimulatory signaling, cytokotoxicity and cytokine/chemokine signaling functional pathways. BCY12491 treatment leads to MC38 tumor regressions, complete responses, and immunogenic memory without continuous drug exposure in the periphery. This anti-tumor activity is dependent on CD8+ T cells, but not on NK 1.1+ cells.

Conclusions BCY12491 is a potent EphA2-dependent CD137 agonist with optimal target binding, pharmacologic, and pharmacokinetic properties that enable anti-tumor TME remodeling and complete responses in vivo with intermittent dosing. This work unleashes a new and tractable avenue to testing a novel class of therapeutic CD137 agonists in humans for the treatment of cancer.

Ethics Approval The care and use of animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec and conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

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NANOPARTICULATE MONOBENZONE (MBEH) AS A POTENTIAL DRUG CANDIDATE FOR MELANOMA

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Background Monobenzone (MBEH) is a skin depigmenting agent FDA-approved for topical applications.1 It specifically interacts with tyrosinase,2 a key enzyme in melanogenesis, to form reactive quinones that are toxic to pigmented cells, including melanoma cells. As melanoma cells express abundant tyrosinase activity,3 repurposing MBEH to target melanoma cells might serve as a treatment strategy. Furthermore, quinones can haptenize tyrosinase,4 supporting neo-antigen formation.5 Modified tumor antigens then initiate an immune cascade, engaging T cells to target tumor cells. This biphasic effect of MBEH makes it a suitable candidate to target melanoma. Although topical treatment of MBEH can suppress subcutaneous melanoma growth in vivo,6 systemic administration of the drug was toxic,7 limiting the application of MBEH for metastatic disease. To overcome this limitation, we encapsulated MBEH and its derivatives into nanoscale liposomes (~100 nm) and evaluated its anti-tumor efficacy.

Methods Liposomes were prepared8 and MBEH was loaded into the liposomes. Loading was evaluated using mass spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. In vitro cytotoxicity of liposomal MBEH to mouse and human melanoma cell lines was evaluated by MTT assays. Meanwhile, in vivo trafficking of fluorescent liposomes to B16-F10 tumors and vital organs was evaluated in tissue homogenates by flow cytometry. The anti-tumor effects of liposomal MBEH towards subcutaneously injected B16-F10 melanoma cells were evaluated in C57BL/6 mice (n=4 per group) over time.

Results Mass spectroscopy and NMR data revealed that MBEH was encapsulated into the liposomes at 2.3%mol MBEH per liposome. Liposomal MBEH was toxic to both mouse and human melanoma cells with lower half maximal inhibitory concentration (IC50) values in B16-F10 and A375 and higher IC50 values in 888-A2 and 624.38 cells. In vivo trafficking in mice revealed ~55% uptake of liposomal fluorescence by the tumor. In contrast to empty liposomes (mean tumor volume on day 21: 467.5 mm³), MBEH loaded liposomes significantly (P<0.0001) reduced B16-F10 tumor growth mice (mean tumor volume on day 21: 107 mm³). Skin depigmentation was not observed over the 21 day period of follow up after liposomal MBEH treatment, supporting that liposomal MBEH can be safely administered.

Conclusions We provide a proof-of-concept to use nanoparticulate MBEH to target the melanogenic pathway in melanoma. A detailed study of MBEH loading, nanoparticle stability, and tumor infiltrating lymphocyte can further establish nanoparticulate MBEH as a potential drug candidate for melanoma.

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Background CD73 (ecto-5′-nucleotidase) is an ecto-nucleotidase that dephosphorylate AMP to form adenosine. Activation of adenosine signaling pathway in immune cells leads to the suppression of effector functions, down-regulate macrophage phagocytosis, inhibit pro-inflammatory cytokine release, as well as yield aberrantly differentiated dendritic cells producing pro-tumorigenic molecules.1 In the tumor microenvironment, adenosine signaling pathway with altered cellular suppression is considered an important mechanism for immune evasion of cancer cells.2 3 Combination of CD73 and anti-PD-1 antibody has shown promising activity in suppressing tumor growth. Hence, we developed AK119, an anti-human CD73 monoclonal antibody, and AK123, a bi-specific antibody targeting both PD-1 and CD73 for immune therapy of cancer.

Methods AK119 is a humanized antibody against CD73 and AK123 is a tetrameric bi-specific antibody targeting PD-1 and CD73. Binding assays of AK119 and AK123 to antigens, and antigen expressing cells were performed by using ELISA, Flow, and FACS assays. In-vitro assays to investigate the activity of AK119 and AK123 to inhibit CD73 enzymatic activity in modified CellTiter-Glo assay, to induce endocytosis of CD73, and to activate B cells were performed. Assay to evaluate AK123 activity on T cell activation were additionally performed. Moreover, the activities of AK119 and AK123 to mediate ADC, CDC in CD73 expressing cells were also evaluated.

Results AK119 and AK123 could bind to its respective soluble or membrane antigens expressing on PBMCs, MDA-MB-231, and U87-MG cells with high affinity. Results from cell-based assays indicated that AK119 and AK123 effectively inhibited nucleotidase enzyme activity of CD73, mediated endocytosis of CD73, and induced B cell activation by upregulating CD69 and CD83 expression on B cells, and showed more robust CD73 blocking and B cell activation activities compared to leading clinical candidate targeting CD73. AK123 could also block PD-1/PD-L1 interaction and enhance T cell activation.

Conclusions In summary, AK119 and AK123 represent good preclinical biological properties, which support its further development as an anti-cancer immunotherapy or treating other diseases.

REFERENCES

Backgroud Exosomes are natural, abundant extracellular vesicles capable of transferring complex molecules between neighboring and distant cell types. Translational research efforts have focused on co-opting this communication mechanism to deliver exogenous payloads to treat a variety of diseases. Important strategies to maximize the therapeutic potential of exosomes therefore include payload loading, functionalization of the exosome surface with pharmacologically active proteins, and delivery to target cells of interest.

Methods Through comparative proteomic analysis of purified exosomes, we identified several highly enriched and exosome-specific proteins, including a transmembrane glycoprotein (PTGFRN) belonging to the immunoglobulin superfamily. Leveraging PTGFRN as a scaffold for exosome surface display, we developed our engExTM platform to generate engineered exosomes functionalized with a variety of structurally and biologically diverse proteins. Systemically administered exosomes are primarily taken up by macrophages in the liver and spleen. To redirect exosome uptake to other cell types, we employed our engineering platform to display functional targeting ligands, including single domain antibodies, single chain variable fragments, single chain Fab (scFabs), and receptor ligands, on the exosome surface at high density. To demonstrate that exosome surface modifications can alter cellular tropism, we generated exosomes displaying anti-Clec9A scFabs to target conventional type 1 dendritic cells (cDC1s), anti-CD3 scFabs to target T cells, and CD40 ligand to target B cells. The engineered exosomes exhibited functional antigen binding that led to greater association with the cell types expressing the cognate receptor both in vitro and in vivo.

Results In mice, systemic administration of exosomes engineered to display scFabs targeting Clec9A resulted in a 4-fold increase in the percentage of cDC1 cells in the blood that had taken up exosomes over controls, and a 6-fold increase in the number of exosomes taken up per cell. We further showed that compared to untargeted exosomes, those with altered tropism achieved increased functional payload delivery to the target cell of interest. In primary mouse dendritic cells, anti-Clec9A exosomes loaded with a cyclic dinucleotide STING agonist achieved greater pathway induction, 2.3-fold greater as measured by IFNβ production, 2-fold by IFNα, and 15-fold by IL-12, when compared to an untargeted control. Preliminary in vivo data show that intra-tumorally administered anti-Clec9A exosomes reduce the required STING agonist dose 10-fold to achieve single-agent tumor control and induce immune responses against tumor-associated antigen, compared to controls.

Conclusions These results demonstrate the potential of our engExTM platform to generate targeted exosome therapeutics capable of immune cell stimulation and tumor growth inhibition in vivo.
Preclinical Mechanism of Action and Anti-tumor Activity of CB-668, a Potent, Clinical Trial for the Treatment of Solid Tumors

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Background The tumor-associated antigen ST4 is expressed across a wide range of solid cancers. DuoBody-CD3xST4 is a bispecific antibody (bsAb) that crosslinks CD3 on T cells with ST4 on tumor cells, thereby inducing T-cell activation and T-cell mediated cytotoxicity in ST4-expressing tumor cells. Here, we tested the capacity of DuoBody-CD3xST4 to engage different T-cell subsets in vitro and investigated the mechanism of action (MoA) in vivo by combining preclinical efficacy studies with exploratory pharmacodynamic (PD) biomarker analyses.

Methods Immunohistostaining was performed on patient-derived tumor tissue-microarrays using a commercial ST4 monoclonal antibody (EPR5529). The capacity of DuoBody-CD3xST4 to engage naïve and memory T-cell subsets was assessed in co-cultures of T cells and ST4-positive tumor cells, using T-cell activation and T-cell mediated cytotoxicity as readouts. Anti-tumor activity in vivo as well as peripheral and intratumoral PD biomarkers were investigated in humanized mice bearing ST4-expressing cell line-derived xenograft (CDX) or patient-derived xenograft (PDX) tumor models.

Results High prevalence of ST4 expression (in >86% of biopsies) was observed in NSCLC, SCCHN, TNBC, bladder, esophageal, prostate and uterine cancer. In co-cultures of ST4+ tumor cells and T cells in vitro, DuoBody-CD3xST4 induced dose-dependent cytotoxicity, associated with T-cell activation, proliferation, and cytokine, perforin and granzyme production. Crosslinking of T cells with ST4-expressing tumor cells was essential as no cytotoxicity was observed in CRISPR-Cas9-generated ST4-knockout tumor cells or with control bsAbs targeting only CD3 or ST4. Importantly, naïve and memory CD4+ or CD8+ T-cell subsets had equal capacity to mediate DuoBody-CD3xST4-induced cytotoxicity, although naïve T-cell subsets showed slower kinetics. DuoBody-CD3xST4 (0.5-20 mg/kg) demonstrated anti-tumor activity in ST4+ breast and prostate cancer CDX and lung cancer PDX models in humanized mice. Treatment with DuoBody-CD3xST4 was associated with intratumoral and peripheral T-cell activation as well as elevated cytokine levels, including IFNγ, IL-6 and IL-8, in peripheral blood.

Conclusions DuoBody-CD3xST4 induced T-cell mediated cytotoxicity in ST4-expressing tumor cells, associated with T-cell activation and cytokine production in vitro. DuoBody-CD3xST4 efficiently engaged naïve and memory T cells within both CD4+ and CD8+ T-cell populations to induce T-cell mediated cytotoxicity in ST4+ tumor cells. In humanized CDX and PDX mouse models, DuoBody-CD3xST4 showed anti-tumor activity, in addition to PD biomarkers associated with T-cell activation in the tumor and periphery. Currently, DuoBody-CD3xST4 is being investigated in a first-in-human clinical trial for the treatment of solid tumors (NCT04424641), in which exploratory biomarker analyses to study the clinical MoA and PD are included.

Ethics Approval The preclinical experiments performed are in compliance with the Dutch animal protection law (WoD) translated from the directives (2010/63/EU) and are approved by the Ethical committee of Utrecht. For the PDX models, all patients had given written informed consent, and the animal experiments were carried out in accordance with the German Animal Protection Law (LaGeSoBerlin, A0452/08). The studies were approved by the local Institutional Review Board of Charite University Medicine, Germany.

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Anti-tumor Activity of CB-668, a Potent, Selective and Orally Bioavailable Small-Molecule Inhibitor of the Immuno-suppressive Enzyme Interleukin 4 (IL-4)-induced Gene 1 (IL4I1)

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Background Tumors evade destruction by the immune system through multiple mechanisms including altering metabolism in the tumor microenvironment. Metabolic control of immune responses occurs through depletion of essential nutrients or accumulation of toxic metabolites that impair immune cell function and promote tumor growth. The secreted enzyme interleukin 4 (IL-4)-induced gene 1 (IL4I1) is an L-phenylalanine oxidase that catabolizes phenylalanine and produces phenyl-pyruvate and hydrogen peroxide. IL4I1 regulates several aspects of adaptive immunity in mice, including inhibition of cytotoxic T cells through its production of hydrogen peroxide (reviewed in1). In human tumors, IL4I1 expression is significantly elevated relative to normal tissues and is notably high in ovarian tumors and B cell lymphomas. Motivated by the hypothesis that IL4I1 is an immuno-metabolic enzyme that suppresses anti-tumor immunity, we discovered CB-668, the first known small-molecule inhibitor of IL4I1.

Methods IL4I1 enzymatic activity was measured using an HRP-coupled enzyme assay. RNA in-situ hybridization was carried out on the RNAscope platform. Syngeneic mouse tumor models were used to evaluate the anti-tumor activity of CB-668. The level of phenyl-pyruvate in tumor homogenates was measured by LC/MS.

Results Our clinical candidate, CB-668 is a potent and selective non-competitive inhibitor of IL4I1 (IC50 = 15 nM). CB-668 has favorable in vitro ADME properties and showed low clearance and high oral bioavailability in rodents. Twice-daily oral administration of CB-668 was well-tolerated in mice and resulted in single-agent anti-tumor activity in the syngeneic mouse tumor models B16-F10, A20, and EG7. Oral CB-668 administration reduced the levels of phenyl-pyruvate in the tumor, consistent with inhibition of IL4I1 enzymatic activity. Anti-tumor activity of CB-668 was immune cell-mediated since efficacy was abrogated in CD8-depleted mice, and CB-668 treatment caused increased expression of pro-inflammatory immune genes in the tumor. Moreover, CB-668 had no direct anti-proliferative activity on tumor cells grown in vitro (IC50 > 50 μM). CB-668 also favorably combined with anti-PD-L1 therapy to reduce tumor growth in the B16-F10 tumor model.
Conclusions These data support an immune-mediated antitumor effect of IL4I1 inhibition by CB-668, and suggest inhibition of IL4I1 represents a novel strategy for cancer immunotherapy.

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706 BT7480, A FULLY SYNTHETIC TUMOR-TARGETED IMMUNE CELL AGONIST (TICA™) INDUCES TUMOR LOCALIZED CD137 AGONISM AND MODULATION OF TUMOR IMMUNE MICROENVIRONMENT

Background After disappointing first clinical experiences with agonistic anti-CD137 (4-1BB) antibodies, a new generation of both systemic and targeted CD137 agonists is entering clinical development.1-4 These strategies rely on biologic agents with suboptimal properties for CD137 agonism due to their relatively large sizes and long circulating half-lives. These properties may limit their tissue penetration and cause sustained agonism resulting in overstimulation and activation-induced cell death of lymphocytes due to continuous exposure. Fully synthetic constrained bicyclic peptides (Bicycles™) with antibody-like affinities and target selectivity are uniquely suited to circumvent the above barriers to optimal targeted CD137 agonistic therapeutics. BT7480 is a tumor-targeted immune cell agonist (TICA) designed to deliver a highly potent CD137 agonist to Nectin-4 overexpressing tumor tissue with a flexible dosing schedule maximizing anti-tumor activity while circumventing the need for continuous systemic exposure.

Methods BT7480 functional activity in vitro was analyzed by measuring IL-2 and IFN gamma production from primary human PBMC/tumor cell co-cultures. BT7480 in vivo activity was determined in huCD137-syngeneic tumor models using tumor immune cell and transcriptional profiling by FACS, IHC, and Nanostring as well as tumor growth kinetics as read-outs.

Results BT7480 binds potently and simultaneously to Nectin-4 and CD137 as assessed biochemically and caused Nectin-4-dependent CD137 agonism in primary human PBMC/tumor cell co-cultures. BT7480 in vivo activity was determined in huCD137-syngeneic tumor models using tumor immune cell and transcriptional profiling by FACS, IHC, and Nanostring as well as tumor growth kinetics as read-outs.

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707 DISCOVERY OF A NOVEL EP2 AND EP4 DUAL ANTAGONIST
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Background Prostaglandin E2 (PG2) is one of the most abundant prostaglandins, with crucial roles in normal and pathologic physiology. Especially, PG2 levels are abnormally elevated in many cancers, and high levels of PG2 are known to be pro-tumorigenic, likely due to the immune suppressive effect in the tumor microenvironment.1-4 There are four types of PG2 receptors; EP1, EP2, EP3 and EP4. Among them, EP2 and EP4 activate adenylyl cyclase and increase cAMP levels, which induce the cAMP-dependent protein kinase (PKA) signaling pathway. EP2 and EP4 are expressed in various immune cells (e.g. macrophages, dendritic cells, NK cells and CTLs), and genetic and pharmacological inhibition of EP2 and EP4 increases immune activity and suppresses tumor growth.

Methods To evaluate the binding affinity against EP2 and EP4, a radioligand binding assay was conducted using EP2 or EP4 transfected HEK293 cells. Cell membrane homogenates were incubated with [3H]PG2 in the absence or presence of the test compounds. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters and rinsed several times with cold Tris-HCl. The filters were dried then counted for radioactivity in a scintillation counter using a scintillation cocktail. The results were expressed as a percent inhibition of the control radioligand specific binding. The antagonistic activity against EP2 and EP4 was assessed via LANCE Ultra cAMP assay (PerkinElmer). HEK293 cells over-expressing EP2 or EP4 were seeded into the plate and treated with PG2 and compounds. After 30 minutes of incubation, cAMP levels were measured by FRET signal using Varioskan plate reader, following the manufacturer’s protocol. Anti-tumor activity of KT-00113 was evaluated using LLC1 syngeneic model. When tumor volume reached approximately 100 mm3, mice were treated PO, QD. Tumor size was measured twice every week.
Results Systematic structure-activity relationship (SAR) investigation identified novel EP2 and EP4 dual antagonists. The most promising compound KT-00113 possesses high potency against both EP2 and EP4, while maintaining high selectivity over other prostanoid receptors. In vitro and in vivo ADMET studies show that KT-00113 has a favorable profile, apt for further examination in in vivo cancer models and immune cell function in tumors.

Conclusions KT-00113, a highly potent and selective EP2/4 dual antagonist has strong potential to become the best-in-class immune suppression lifting cancer immunotherapy and may be suitable for further development in a clinical setting.

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APPLICATION OF A NOVEL MSSENS DRUG DELIVERY TECHNOLOGY FOR MRNA THERAPEUTICS
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Background Successful clinical translation of mRNA therapeutics requires an appropriate delivery strategy to overcome instability of mRNA and facilitate cellular uptake into the cells. Several lipid based nanoparticle approaches that encapsulate mRNA, notably lipid nanoparticle (LNP), have been developed, but their efficiency for delivery to certain target tissues and toxicity profiles still have room for improvement. The application of a novel polymer based nanoparticle technology platform, so called Stability Enhanced Nano Shells (SENS) for mRNA (mSENS) as a mRNA delivery platform for a cancer vaccine was demonstrated.

Methods The physicochemical properties of mSENS formulation, particle size and encapsulation efficiency, were characterized using dynamic light scattering (DLS) and gel retardation assay. Using luciferase-encoding mRNA, the protein expression levels in vitro and in vivo were evaluated by luciferase assay or bioluminescence imaging (BLI), respectively. For cancer vaccine studies, antigen (tyrosinase-related protein 2 (Trp-2)-specific T cell responses were assessed by immunophenotyping mouse splenocytes using flow cytometry and by the enzyme-linked immunosorbent spot (ELISPOT) assay. The anti-tumor efficacy was studied in B16F10 lung tumor model in C57BL/6 mice. Liver and systemic toxicity of mSENS treated mice was evaluated through blood chemistry and complete blood count (CBC) tests.

Results A library of mSENS formulations complexed with luciferase-encoding mRNA, were characterized for their particle size, surface charge, encapsulation efficiency, colloidal stability, and in vitro and in vivo luciferase protein expression level. Upon systemic administration in mice, varying biodistribution profiles were observed, implicating the potential for tailored delivery to target tissues. Particularly, cancer vaccine application was further developed leveraging the formulation with preferential spleen delivery. Following vaccination with Trp-2 mRNA encapsulated with mSENS (Trp-2 mRNA-mSENS) in B16F10 tumor bearing mice, strong Trp-2 antigen-specific IFN-γ T-cell responses were observed. Generated anti-tumor immunity also marked suppression of B16F10 lung tumors were observed in Trp-2-mSENS immunized mice compared to non-immunized controls, demonstrating the potential of mSENS as a mRNA delivery platform for the application for vaccine.

Conclusions Proprietary biodegradable polymer based-mSENS platform offers an attractive delivery strategy for mRNA by tailoring to specific therapeutic applications. Depending on the application, whether it’s a vaccine or protein replacement, a rationally designed mSENS formulation can efficiently distribute mRNA to specific tissues. In particular, application of a splenic mSENS formulation for a cancer vaccine has been demonstrated in murine tumor model. In summary, mRNA delivery through mSENS platform is expected to provide significant opportunities in clinical development for mRNA therapeutics.

Ethics Approval The study was approved by Samyang Biopharmaceuticals’ IACUC (Institutional Animal Care and Use Committee), approval number SYAU-2027.

REFERENCE

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EXOSOME SURFACE DISPLAY OF IL-12 RESULTS IN TUMOR-RETAINED PHARMACOLOGY WITH SUPERIOR POTENCY AND LIMITED SYSTEMIC EXPOSURE
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Background The promise of Interleukin-12 as a cancer treatment has yet to be fulfilled with multiple tested approaches being limited by unwanted systemic exposure and unpredictable pharmacology. To address these limitations, we generated exoIL-12™, a novel, engineered-exosome therapeutic that displays functional IL-12 on the surface of an exosome.

Methods IL-12 exosomal surface expression was achieved via fusion to the abundant exosomal surface protein PTGFRN. Potency was assessed in vitro using human PBMCs or murine splenocytes and in vivo using mouse subcutaneous tumor models. Local versus systemic pharmacology was determined with intratumoral injection in mice and subcutaneous injection in monkeys. All studies were benchmarked against recombinant IL-12 (rIL-12).

Results Exosomes engineered to express either murine or human IL-12 had equivalent potency in vitro to rIL-12 as
DEVELOPMENT OF IL-33 AS A NOVEL IMMUNOTHERAPY OF CANCER

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Background Immune-checkpoint-blockade (ICB) therapy has produced unprecedented survival benefits for cancer patients but such therapy has been limited by low response rates in most cancer. One major obstacle for ICB therapy is the reduced immunogenicity of tumor tissues due to genetically driven down-regulation of epithelial tissue cytokines. IL-33 is a member of the IL-1 gene family and its level is downregulated in many advanced carcinomas such as lung cancer, breast cancer and pancreatic cancer. It has recently been shown that IL-33 plays an important role in mediating cancer immune therapy. In addition, transgenic expression of the active form of IL-33 in tumor cells or administration of the recombinant IL-33 exerts strong antitumor effects. Mechanistically, IL-33 enhances the function of Th1 and CD8+ T cells in vitro and types 1 antitumor immune responses in vivo.

Methods In the current study, we have optimized the pharmacodynamics of IL-33 by engineering a fusion protein, called anti-HSA-IL-33, using IL-33 and an anti-human albumin antibody. We have used preclinical mouse tumor models to determine the efficacy and toxicity of this new molecule.

Results We have shown that anti-HSA-IL-33 has excellent antitumor activities alone and enhances the antitumor function of PD-1 mAbs. Despite causing increased inflammation, anti-HSA-IL-33 is well tolerated with limited toxicity in mice.

Conclusions These studies support further development of IL-33 as a novel cancer immunotherapy.

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HBM1022, A NOVEL ANTI-CCR8 ANTIBODY DEPLETES TUMOR-INFILTRATING REGULATORY T CELLS VIA ENHANCED ADCC ACTIVITY, MEDIATES POTENT ANTI-TUMOR ACTIVITY WITH KEYTRUDA

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Background CCR8-expressing CD4 and Foxp3 positive Treg (CCR8+ Treg) has been demonstrated to be a major driver for immunosuppression in solid tumors.1 Superscript. Clinical studies have shown that CCR8 is selectively up-regulated by tumor resident Tregs in several tumor types including clear cell renal cell carcinoma (ccRCC)2 Superscript and breast cancer3 Superscript. In these tumor types, CCR8 exhibit strong expression on tumor resident Tregs while it is rarely observed on Tregs in peripheral blood mononuclear cells (PBMCs). High expression of the CCR8 in tumor-infiltrating lymphocytes-Treg cells (TIL-Tregs) was associated with poor prognosis in breast cancer patients. These results suggest CCR8 as a promising therapeutic target; and anti-CCR8 mAbs could selectively inhibit a subpopulation of tumor resident Tregs in the tumor microenvironment (TME), to augment antitumor immunity.

Methods In vitro assay:HBM1022 binding on human, cynomolgus CCR8 and TIL-Tregs are evaluated via flow cytometry. Blocking and ADCC functional assay are all based on CCR8 overexpressing cell lines. In vivo efficacy study:HBM1022 anti-CCR8 antibody was administered after implantation (100 mm3) Superscript. in combination with anti–PD-1.

Results Anti-CCR8 antibody HBM1022 specifically binds to cell lines that over-express human or cynomolgus CCR8, as well as TIL-Tregs in multiple cancer types with the high affinity. HBM1022 potently blocks CCL1 binding to both human and cynomolgus CCR8. HBM1022 inhibits CCL1-induced migration and related GPCR signaling pathways. Furthermore, with enhanced antibody-dependent cell-mediated cytotoxicity (eADCC) activity, HBM1022 exhibits potent in vitro killing activity on CCR8-expressing cells. HBM1022 shows tumor growth inhibition as monotherapy in preclinical mouse syngeneic and humanized models. Moreover, HBM1022 shows enhanced antitumor activity with the combination of Keytruda in preclinical efficacy models.

Conclusions Our finding reveals HBM1022 as an innovative immunotherapy targeting intra-tumoral suppressive Treg cells to change suppressive tumor to hot tumor. HBM1022 presents its great potential as exciting mono or combo anti-tumor therapies.

The animal experiments have been approved by IACUC of University of Pittsburgh.

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Ethics Approval The animal experiments have been approved by IACUC of University of Pittsburgh.

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Ethics Approval All animals were maintained and treated at the animal care facility of Codiak Biosciences in accordance with the regulations and guidelines of the Institutional Animal Care and Use Committee (CB2017-001).
**Background**

SBT6290 is a novel therapeutic comprised of a selective TLR8 agonist conjugated to a Nectin-4-specific monoclonal antibody, designed for systemic delivery and tumor-localized activation of myeloid cells. Nectin-4 is a cell surface adhesion molecule that is overexpressed in multiple solid tumor types including triple negative breast, head and neck, lung, and urothelial cancers, with limited expression in normal tissues. Many solid tumors, including those expressing Nectin-4, are resistant to immunotherapy due to immune-suppressive mechanisms, loss of HLA, low neoantigen availability, and/or minimal T cell infiltrates. These tumors, however, are often replete with myeloid cells. Activation of these cells has emerged as a promising approach in overcoming resistance mechanisms to current cancer immunotherapies. TLR8 is highly expressed in myeloid cell types prevalent in human tumors, including conventional DCs and macrophages. Agonism of TLR8 in human myeloid cells activates a broad spectrum of anti-tumor immune mechanisms, including proinflammatory cytokine production, repolarization of suppressive myeloid cells, and the priming of CTL responses. Here, we show that SBT6290 potently activates human myeloid cells in a Nectin-4-dependent manner and that a mouse surrogate confers single agent anti-tumor activity in preclinical studies. These data support the development of SBT6290 for the treatment of patients with Nectin-4-expressing tumors.

**Methods**

SBT6290 activity was characterized in vitro using coculture systems consisting of human immune cells and Nectin-4-expressing tumor cells. The in vivo efficacy of the SBT6290 surrogate was evaluated as a single agent in mouse tumor models expressing Nectin-4.

**Results**

Studies with human immune cells show that SBT6290 potently induces multiple anti-tumor immune activities including proinflammatory cytokine and chemokine production, inflammasome activation, direct activation of DCs and indirect T and NK cell cytolytic activity. This activity requires the presence of Nectin-4 expressing tumor cells and the engagement of Fc gamma receptors on the surface of the myeloid cells by the conjugate to facilitate delivery of SBT6290 into myeloid cells. Notably, SBT6290 is >100 fold more potent than the free, unconjugated TLR8 agonist. Systemic administration of a SBT6290 surrogate in mice results in robust single agent efficacy in tumor models intrinsically resistant to checkpoint blockade, including the EMT6 model engineered to express human Nectin-4.

**Conclusions**

The preclinical data described here show the potential for SBT6290 to drive robust, single agent anti-tumor responses and support the clinical development of SBT6290 for patients with Nectin-4 expressing tumors.
Background TGFβ production by solid tumors and their microenvironment is a major mechanism used by tumors to avoid immunosurveillance. Blockade of TGFβ has been shown to promote an anti-tumor response; however, systemic blockade of TGFβ has also been associated with toxicity. We hypothesized that a PD1 x TGFβR2 bispecific antibody could selectively block the suppressive activity of TGFβ on T tumor cells and enhance their anti-tumor activity while avoiding the toxicity associated with systemic blockade.

Methods We engineered bispecific antibodies that simultaneously engage PD1 and TGFβR2 using Xencor’s XmAb platform. The anti-TGFβR2 arm was tuned for optimal activity by introducing affinity-modulating amino acid substitutions. The activity of PD1 x TGFβR2 bispecifics was evaluated in vitro using a signaling assay to measure phosphorylated SMAD (pSMAD) by flow cytometry with exogenous TGFβ in unactivated and activated PBMC. In vivo activity was evaluated by monitoring the engraftment of human PBMC in NSG mice (huPBMC-NSG). Anti-tumor activity was assessed in huPBMC-NSG mice engrafted with established human cancer cell lines. Antibodies against other T cell targets were also incorporated into TGFβR2 bispecifics, and similarly evaluated in vitro and in vivo.

Results PD1 x TGFβR2 bispecifics were confirmed to bind PD1 and block binding of TGFβ to TGFβR2. In vitro, we found that T cells from activated, serum-deprived PBMC exhibited robust induction of pSMAD in response to TGFβ and PD1 x TGFβR2 bispecifics selectively inhibited pSMAD induction in PD1-positive T cells as demonstrated by over a 100-fold potency increase compared to an untargeted anti-TGFβR2 control. Additionally, we saw an enhancement of potency when evaluating blocking activity in activated (PD1-high) vs. unactivated (PD1-low) T cells. Similar selectivity was measured when comparing inhibition of pSMAD induction for activated T cells versus other PD1-negative, TGFβ-responsive immune cells. Intriguingly, TGFβR2 bispecifics incorporated antibodies against other T cell targets allowed for the targeting of a broader population of T cells while still conferring potent selectivity against target-negative cells. In vivo, treatment of huPBMC-NSG mice with TGFβR2 bispecifics promoted superior T cell engraftment and combined additively with PD1 blockade. Furthermore, TGFβR2 bispecific treatment of huPBMC-NSG mice containing established MDA-MB-231 triple-negative breast cancer tumors promoted an anti-tumor response that was also augmented with PD1 blockade.

Conclusions Multiple PD1 x TGFβR2 bispecifics were engineered to selectively block TGFβR2 on PD1-positive T cells and evaluated in vitro and in vivo. Compelling activity, including additivity with PD1 blockade, suggests that clinical development is warranted for the treatment of human malignancies.
blockade. Soluble LAG-3 may be an important biomarker for monitoring the pharmacodynamic activity of FS118 in patients.

Ethics Approval All animal experiments were conducted under a UK Home Office Project Licence and approved by an Animal Welfare and Ethical Review Board (AWERB) in accordance with the UK Animal (Scientific Procedures) Act 1986 and with EU Directive EU 86/609

REFERENCE

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716 CELL-BASED VIROTHERAPY FOR TARGETING CANCERS

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Background Oncolytic virotherapy has been recognized as a promising new therapy for cancer for decades but only few viruses have been approved worldwide. The therapeutic potential of oncolytic viruses can be severely restricted by innate and adaptive immune barriers making oncolytic virus clinical inefficient. To overcome this obstacle, we utilized adipose-derived stem cells (AD-MSC) loaded with tumor selective CAL1 oncolytic vaccinia virus to generate a new therapeutic agent called SNV1 (SuperNova-1).

Methods CAL1 vaccinia virus was tested for its ability to replicate and selectively kill various human cancer cell lines in vitro and in vivo. Additionally, CAL1 was loaded into adipose-derived mesenchymal stem cells to generate SuperNova1 (SNV1). Both CAL1 and SNV1 were tested for their ability to kill cancer cells in the presence of active complement and neutralizing antibodies in cell culture as well as in mice. Immune cell infiltration of the treated and untreated tumors was analyzed by flow cytometry.

Results CAL1 showed preferential amplification and killed various tested human (PC3, FaDu, MDA-MB-231, RPMI) and mouse cancer cells (CT26, EMT6, TRAMP-C2, RM1). In animals, CAL1 caused tumor regression in PC3 and CT26 mouse models without signs of toxicity. SNV1 significantly enhanced protection of CAL1 virus from clearance by the immune system as compared to naked CAL1 virus, leading to higher therapeutic efficacy in animals. Five days after SNV1 administration, tumor infiltrating lymphocytes (TILs) from both treated and untreated tumors showed increased CD4 and CD8 T-cell infiltrations. Importantly, we documented a decreased frequency of Tregs, and improved effector to Treg ratios, which was associated with inhibition of tumor growth at the treated tumor site and also at distant untreated sites.

Conclusions CAL1 is potentially used as an oncolytic agent. In addition, SNV1 cell-based platform protects and potentiates oncolytic vaccinia virus by circumventing humoral innate and adaptive immune barriers, resulting in enhanced oncolytic virotherapy. Particularly, SNV1 provided instantly active viral particles for immediate infection and simultaneous release of therapeutic proteins in the injected tumors.

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717 AMG 160, A PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA)-TARGETED BIOTE® IMMUNO-ONCOLOGY THERAPY, IS ACTIVE IN MODELS OF ADVANCED PROSTATE CANCER THAT ARE RESISTANT TO RADIONUCLIDE THERAPY

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Background AMG 160 is an HLE BiTE® (half-life extended bispecific T-cell engager) that binds PSMA on prostate cancer cells and CD3 on T-cells and induces redirected T-cell lysis of PSMA-expressing cells. This mechanism may allow the BiTE molecule to be active in settings where other targeted or immune therapies have failed. Here, we evaluated the activity of AMG 160 in mouse models of advanced prostate cancer that are resistant to 177Lu-PSMA-617, a PSMA-targeted radioligand therapy that has emerged as a promising treatment modality for metastatic castration-resistant prostate cancer (mCRPC).

Methods Two prostate cancer models were tested in 6–8-week-old male NCG mice: one cohort had established subcutaneous C4-2 TP53- tumors (C4-2 cells with TP53 knockout), and the other cohort had established systemic C4-2 TP53wt tumors that mimic metastatic lesions (intracardiac injection). PSMA levels in both models (~255,000 PSMA/cell) are sufficient for tumor growth inhibition with 177Lu-PSMA-617. Mice were administered a single intravenous (IV) infusion of human T-cells. Three days later, mice were treated with 1 cycle of 177Lu-PSMA-617 (30 MBq, IV), or 3 weekly doses of AMG 160 (1 mg/kg, IV) or of a control HLE BiTE molecule (1 mg/kg, IV; target not expressed on C4-2 cells). Therapeutic efficacy was assessed by tumor burden measurements, time to progression (TTP), and survival.

Results In both prostate cancer models, AMG 160 treatment significantly improved disease control (figure 1). Median TTP was not reached in the AMG 160 group (p<0.0001), whereas it was 31d (177Lu-PSMA-617) and 23.5d (control) in the subcutaneous model, and 68d (177Lu-PSMA-617) and 50.5d (control) in the systemic model. Median survival was not reached in the AMG 160 group (p<0.0001); it was 39d (177Lu-PSMA-617) and 26.5d (control) in the subcutaneous model, and 77d (177Lu-PSMA-617) and 61d (control) in the systemic model. Following treatment with AMG 160, 2/10 mice with subcutaneous and 7/9 mice with systemic tumors had not progressed at the end of the observation period (>100 days). In contrast, all mice in the 177Lu-PSMA-617 and control groups succumbed to progressive disease.

Abstract 717 Figure 1 AMG 160 treatment extended survival in mouse models of advanced prostate cancer

Conclusions Our study demonstrates potent antitumor activity of AMG 160 monotherapy in models of metastatic CRPC that
are resistant to PSMA-targeted radioligand therapy with \(^{177}\text{Lu-PSMA-617}\). These data provide a rationale for evaluating AMG 160 in patients with mCRPC who have progressed on \(^{177}\text{Lu-PSMA-617}\). AMG 160 is currently being evaluated in a phase 1 study in patients with mCRPC (NCT03792841).

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### Trial Registration

ClinicalTrials.gov Identifier: NCT03792841

### Ethics Approval

All animal experimental protocols were approved by the UCLA Animal Research Committee (# 2005-090).

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### Abstracts

**AMG 509, A STEAP1 X CD3 BISPECIFIC XMAB\(^2\) 2+1 IMMUNE THERAPY, EXHIBITS AVIDITY-DRIVEN BINDING AND PREFERENTIAL KILLING OF HIGH STEAP1-EXPRESSING PROSTATE AND EWING SARCOMA CANCER CELLS**

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**Background** Metastatic castration-resistant prostate cancer (mCRPC) and Ewing sarcoma (EWS) are diseases for which immune therapies could potentially provide benefit. STEAP1 (Six Transmembrane Epithelial Antigen of the Prostate 1) is a cell surface protein with elevated expression in mCRPC and EWS.\(^2\)

**Methods** We designed AMG 509, a novel, half-life extended, STEAP1 x CD3 XmAb\(^2\) 2+1 bispecific antibody to induce T cell-mediated cytotoxicity against STEAP1-expressing cancer cells. AMG 509 contains two identical anti-STEAP1 Fab domains, an anti-CD3 scFv domain, and an effectorless Fc domain that extends serum half-life. We characterized STEAP1 expression in normal and tumor tissues by immunohistochemistry, and we assessed the pharmacological properties of AMG 509 including binding, T cell-mediated redirected lysis, and in vivo antitumor activity.

**Results** We detected high STEAP1 surface expression on 80% of primary prostate tumors (n=88), 89% of mCRPC lesions (n=114), including 84% of mCRPC bone metastases (n=31), and 63% of EWS samples (n=35). In contrast, in normal tissues (n=72), low STEAP1 expression was detected in only six other tissues, including the normal prostate. AMG 509 bound to recombinant human CD3\(\varepsilon\) with a K\(_D\) of 27.6 nM, and it bound specifically to 293T cells transfected with human STEAP1 with an EC\(_{50}\) of 3.8 nM. AMG 509 triggered potent T cell-redirected lysis of STEAP1-positive cancer cells, with a median EC\(_{50}\) of 37 pM across 19 cancer cell lines that endogenously express various levels of STEAP1. AMG 509-mediated cytotoxicity was specific, as it showed no activity against prostate cancer cells in which STEAP1 was knocked out. AMG 509 was 65-fold more potent in inducing the redirected lysis of prostate cancer cells in vitro than an XmAb\(^2\) molecule with a single anti-STEAP1 Fab domain. AMG 509 had greater cytotoxic activity against high STEAP1-expressing cancer cells than against low STEAP1-expressing cancer cells, and it had minimal activity against normal cells. This preferential killing of high STEAP1-expressing cells is likely driven by the avidity conferred by the dual STEAP1-binding domains, a feature that may help reduce off-target effects in the clinic. In vivo, AMG 509 induced robust anti-tumor activity in prostate cancer and EWS mouse xenograft models, with concomitant CD8+ T-cell activation and expansion in tumors.

**Conclusions** AMG 509 is a specific, first-in-class T cell-recruiting antibody with avidity-driven activity against STEAP1-positive malignancies. AMG 509 is currently being evaluated for safety, pharmacokinetics, and efficacy in a phase 1, first-in-human study in patients with mCRPC (NCT04221542).

### Acknowledgements

The authors acknowledge Micah Robinson, PhD of Amgen Inc. for medical writing support.

### Trial Registration

ClinicalTrials.gov Identifier: NCT04221542

### Ethics Approval

All animal experimental protocols were approved by an Institutional Animal Care and Use Committee (IACUC protocol number 2015-01243) and were conducted in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) (Amgen) or the standards of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (IACUC protocol number 15015x) in a facility certified with an Office of Laboratory Animal Welfare (OLAW) (UTHSA).

### References


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**CD122-DIRECTED INTERLEUKIN-2 COMPLEXES AND \(\alpha\)PD-L1 DIFFERENTIALLY REQUIRE INNATE AND ADAPTIVE IMMUNITY TO TREAT LOCAL AND METASTATIC BLADDER CANCER**

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**Background** \(\alpha\)PD-L1 bladder cancer (BC) immunotherapy is ineffective in <30% of cases.\(^3\) To address the large \(\alpha\)PD-L1-unresponsive subset of patients, we tested \(\alpha\)IL-2/IL-2 complexes (IL-2c) that block IL-2 from binding high-affinity IL-2R\(\alpha\) (CD25) for preferential \(\alpha\)IL-2R\(\beta\) (CD122) binding.\(^2\) Immunosuppressive regulatory T cells capture IL-2 by CD25 whereas antitumor CD8+ T, y6 T, and NK cells use CD122. We hypothesized that the tumor microenvironment, including local immune cells in primary versus metastatic BC, differentially affects immunotherapy responses and that IL-2c effects could differ from, and thus complement \(\alpha\)PD-L1.

**Methods** We used PD-L1+ mouse BC cell lines MB49 and MBT-2, for orthotopic, intravesical (i.e., in bladder) and intravenous challenge studies of local versus lung metastatic BC.

**Results** \(\alpha\)PD-L1 or IL-2c alone reduced tumor burden and extended survival in local MB49 and MBT-2. Using in vivo cell depletions, we found that y6 T cells and NK cells, but strikingly not CD8+ T cells, were necessary for IL-2c efficacy in bladder. We confirmed y6 T cell requirements for IL-2c, but not \(\alpha\)PD-L1 efficacy in y6 T cell-null TCR\(\beta\)KO mice. TCR\(\beta\)KO conventional T cell-null mice exhibited IL-2c, but...
TARGETING MARCO AND IL-37R ON ANTI-INFLAMMATORY MACROPHAGES IN LUNG CANCER BLOCKS REGULATORY T CELLS AND SHIFT BALANCE TO SUPPORT CYTOTOXIC LYMPHOCYTE FUNCTION

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Background The progression and metastatic capacity of solid tumors are strongly influenced by immune cells in the tumor microenvironment (TME). In non-small cell lung cancer (NSCLC) accumulation of anti-inflammatory tumor-associated macrophages (TAMs) is associated with worse clinical outcome and resistance to therapy. Numerous clinical trials aiming to recover T cell anti-tumor activity have been failing due to the persistence immune suppression in TME. Thus, there is a clinical need for alternative treatments targeting the suppressive function of the TME. We have previously shown that antibodies targeting the scavenger receptor MARCO reprograms the pro-tumoral TAMs in murine cancer models. Here, we investigated the immune landscape of NSCLC in the presence of MARCO expressing TAMs. We tested targeting MARCO or the tumor mechanisms inducing MARCO on human TAMs and hypothesized that targeting these mechanisms will remodel the suppressive environment and relive the anti-tumor responses to increase the efficacy of immunotherapy.

Methods To test our hypothesis, we first investigated the immune landscape of NSCLC in the presence of pro-tumoral MARCO+TAMs compared with tumors infiltrated by MARCO-TAMs. We next used RNAseq to analyze differential gene expression in NSCLC tumors infiltrated by MARCO positive or negative macrophages. In vitro, cytokine differentiated macrophages alternatively cultured with lung cancer cell lines were co-cultured with Natural Killer (NK) cells and T cells to mimic their interaction in the TME. Later, macrophages were treated with targeting antibodies and their phenotype and function were examined prior and following interaction with other immune cells.

Results We found that MARCO expressing TAM numbers correlated with increased occurrence of regulatory T cell and effector T cells and decreased NK cells in NSCLC infiltrated by MARCO+TAMs. Furthermore, transcriptomic data from the tumors uncovered a correlation between MARCO expression and the anti-inflammatory cytokine IL-37. Studies in vitro subsequently showed that lung cancer cells polarized macrophages to express MARCO and gain an anti-inflammatory phenotype through the release of IL-37. These human MARCO expressing TAMs blocked cytotoxic T cell and NK cell activation, inhibiting their proliferation, cytokine production and tumor killing capacity. Mechanistically, MARCO+macrophages enhanced regulatory T (Treg) cell proliferation and IL-10 production and diminished CD8 T cell activities. Targeting MARCO or IL-37 receptor (IL-37R) repolarized TAMs resulted in recovered cytolytic activity and anti-tumoral capacity of NK cells and T cells.

Conclusions In summary, our data demonstrate a novel immune therapeutic approach targeting human TAM immune suppression of NK and T cell anti-tumor activities and remodel immune suppression.

Ethics Approval The study was approved by Institutional Ethics Board, approval number Dnr 2013.977-31.1.
by enhancing adhesion and transmigration of T cells to endothelial (HUVEC) cells. AT1412 was most potently enhancing transendothelial T-cell migration, in contrast to a high affinity version of AT1412 or other high affinity anti-CD9 reference antibodies (e.g. ALB6). Enhanced immune cell infiltration is also observed in immunodeficient mice harbouring a human immune system (HIS). AT1412 strongly enhanced CD8 T-cell and macrophage infiltration resulting in tumor rejection (A375 melanoma). PD-1 checkpoint blockade is further sustaining this effect. In a second melanoma model carrying a PD-1 resistant and highly aggressive tumor (SK-MEL5) AT1412 together with nivolumab was inducing full tumor rejection, while either one of the antibodies alone did not.

Conclusions The safety of AT1412 has been assessed in preclinical development and is well tolerated up to 10 mg/kg (highest dose tested) by non human primates. AT1412 demonstrated a half-life of 8.5 days, supporting 2–3 weekly administration in humans. Besides transient thrombocytopenia no other pathological deviations were observed. No effect on coagulation parameters, bruising or bleeding were observed macro- or microscopically. The thrombocytopenia is reversible, and its recovery accelerated in those animals developing anti-drug antibodies. First in Human clinical study is planned to start early 2021.

Ethics Approval Study protocols were approved by the Medical Ethical Committee of the Leiden University Medical Center (Leiden, Netherlands).

Consent Blood was obtained after written informed consent by the patient.

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**Abstracts**

**A432**

**INHIBITION OF INTEGRIN αvβ8-MEDIATED TGF-β ACTIVATION WITH C6D4 PROVIDES IMPROVED POTENCY AND SELECTIVITY VS GENERAL TGF-β INHIBITORS FOR CANCER IMMUNOTHERAPY**

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**Background** TGF-β plays a key role in immune evasion as a critical regulator of both innate and adaptive tumor immunity and promotes broad immunosuppressive effects on numerous inflammatory cell subpopulations ultimately resulting in tumor immune tolerance and evasion.１ It has also been implicated in resistance to immune checkpoint therapies, and additive or synergistic effects of dual TGF-β and PD-1 inhibition has been reported.２３ A number of TGF-β inhibitors are in clinical development with different modes of action. Most protein-based inhibitors are designed to block diffusible TGF-β from interacting with its proximal signaling receptor TGF-βR2 and includes monoclonal antibodies (Mabs) and receptor traps. This investigation compares inhibition of TGF-β by a number of inhibitors and the integrin αvβ8 (C6D4) to assess their relative potential as cancer therapeutics.

**Methods** No reporter system currently exists to investigate the mechanistic basis of cell-intrinsic TGF-β activation, whereby the L-TGF-β presenting cell is also the cell that responds to TGF-β signaling (figure 1). To build a cell-intrinsic TGF-β activation system, TMLC cells were stably transfected with wild-type (WT) TGF-β. Without co-transfecting GARP, TMLC do not present L-TGF-β on their cell surface. When co-transfected with TGF-β and GARP, high levels of cell surface expression of L-TGF-β are detected. Additionally, to build a cell-intrinsic TGF-β activation system which express a non-releasable form of TGF-β, we mutated the L-TGF-β furin cleavage site (R249A) and similarly expressed the L-TGF-β(R249A)/GARP complex on the surface of TGF-β reporter cells (TMLC). These cell-intrinsic TGF-β activation systems were used to assess the relative abilities of Mabs αvβ8, TGF-β, TGF-βR2, GARP or TGF-βR2 receptor trap to inhibit αvβ8-mediated TGF-β activation.

Results αvβ8 exhibited superior inhibitory activity compared with other TGF-β inhibitors, which was similar in both diffusible and non-diffusible models (figure 2). The biologic relevance of these finding was confirmed using CD4+ T-cells in place of the reporter cells where TGF-β-dependent Treg generation was almost completely blocked by αvβ8 but was poorly inhibited by the other TGF-β inhibitors.

**Abstract 722 Figure 1** Novel cell-intrinsic TGF-β reporter system

**Abstract 722 Figure 2** Inhibition curves showing inhibition of αvβ8-mediated TGF-β activation by various inhibitors including anti-β8 (C6D4) in a model of diffusible (A) or non-diffusible (R249A mutant) L-TGF-β (B)
Conclusions In this study avß8 exhibited dramatic TGF-ß inhibitory activity compared with a wide range of inhibitors in development. Because integrin avß8 may direct TGF-ß signaling from within its latent complex, this may offer an advantage for target specificity and avoid the challenges faced by non-specific TGF-ß inhibitors. These findings characterize avß8 as a novel and potent immunotherapy drug for further clinical investigation.

Trial Registration NA

Ethics Approval NA

REFERENCES


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LYMPH NODE-TARGETED AMP-VACCINE ENABLES TUMOR-DIRECTED MKRAS-SPECIFIC IMMUNE RESPONSES WITH POTENT POLYFUNCTIONAL AND CYTOLYTIC ACTIVITY

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Background Subunit vaccines targeting tumor antigens have shown limited capacity for expanding cytotoxic T-cells against tumors in the clinic. Especially in the case of KRAS-driven tumors, responses elicited by conventional vaccines have been exceedingly weak. For molecular immunogens including peptides and oligonucleotides, inefficient delivery to immune cells residing in the lymphatics is a significant challenge limiting their ability to induce cancer-directed immune responses of sufficient strength and functionality to impact tumors. Improving the targeting of immunogens to lymph nodes (LN), where resident immune cells potently orchestrate immunity, can substantially amplify their ability to induce effective tumor-directed immunity. Here, we demonstrate such an approach for significantly enhancing mKRAS-directed T-cell responses by precisely targeting antigens and adjuvants directly to the draining LN through a simple one-step conjugation to albumin-binding lipids. These amphiphilic conjugates (‘Amphiphiles’, or AMP) then ‘hitch-hike’ on albumin into the LNs where they elicit strong immune responses. LN accumulation of structurally optimized amphiphiles in mice is greatly improved over soluble equivalents.

Methods C57BL/6J mice received two or more doses of benchmark or amphiphile-modified vaccines, comprised of mKRAS peptide and CpG adjuvant, subcutaneously injected into the tail base in two-week intervals. Immunological readouts were performed 7 days post dosing. For ELISpot analysis of IFNγ and Granzyme B production and flowcytometric bead array analysis of Th1/2 cytokines, splenocytes were harvested and re-stimulated with antigen overnight. In vivo, cytolytic capabilities of antigen-specific T-cells were evaluated by pulsing CFSE-stained splenocytes from naïve mice with mKRAS antigen and injecting these cells intravenously into immunized mice. Recovery of CFSE-labeled target cells from immunized mice was performed 24h later and analyzed flowcytometrically.

Results We show robust immune responses that yield strong activation against all common mutations in the mKRAS protein compared to low or undetectable responses generated by soluble or benchmark treatments. Further, this response is composed of CD4+ as well as CD8+ T-cells resulting in the production of high levels of TH1-associated cytokines upon re-stimulation with mKRAS-specific peptides in vitro. In vivo, robust cytolytic function towards mKRAS-presenting targets can be measured in T-cells.

Conclusions By targeting immunogens directly and precisely to the LNs, the Amphiphile platform can significantly amplify the potency of subunit vaccines. In the case of mKRAS, substantially improved cytolytic immune responses represent a promising therapeutic strategy for targeting mKRAS-driven tumor growth and survival in a large fraction of human tumors. Furthermore, this platform technology is simple, rapid and scalable for broad clinical application.

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724 STAT3 INHIBITION IN ACUTE MYELOID LEUKEMIA CELLS ALLOWS FOR TLR9-DRIVEN DIFFERENTIATION TO IMMUNOGENIC MONOCYTIC CELLS AND INDUCTION OF T-CELL MEDIATED IMMUNE RESPONSES

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Background Signal transducer and activator of transcription factor 3 (STAT3) is commonly activated in acute myeloid leukemia (AML) and known for supporting cancer cell proliferation and survival. Recently, we demonstrated that STAT3 also plays a critical role ensuring AML immune evasion. Intravenous injections of bi-functional decoy oligodeoxynucleotides (CpG-STAT3dODN) blocked STAT3 activity and induced TLR9 signaling in Cbfb/MYH11/Mpl (CMM) AML cells, thereby resulting in immunogenic effects and T cell-mediated immune responses and leukemia regression.

Methods To understand the molecular mechanisms of the CpG-STAT3 decoy-induced AML differentiation and immunogenicity, we performed global gene expression analysis on the in vivo treated AML cells using oligonucleotide strategy as well as an inducible STAT3 gene silencing.

Results Transcriptional profiling revealed the upregulation of myeloid cell differentiation related genes, such as Irf8, Cebpa, and Gadd45A with reduction of oncogenic Runx1 and Run1t1 in CMM leukemic cells after CpG-STAT3dODN treatment but not after control treatments. CpG-STAT3dODN treatment also upregulated set of antigen-presentation related genes, such as CIta, II12a, and Ifng in CMM AML cells. Importantly, the induction of Irf8 and Cebpa, with the concomitant suppression of Runx1 were found specifically in the subset of differentiated CD11b+ CMM cells but not in the bulk CD11b- leukemic cells. These effects were likely related to epigenetic reprogramming of AML cells as indicated by treatment-induced changes in the expression and protein levels of STAT3 regulated DNA methyltransferases, DNMT1 and DNMT3a/b. Furthermore, our initial studies suggest that STAT3 inhibition/TLR9 activation leads to immunogenic effects also in a xenotransplanted model of human FLT3-ITD MV4-11 leukemia in humanized mice. CpG-STAT3dODN alone or together with clinically-relevant demethylating agent (Decitabine) triggered differentiation of MV4-11 cells into CD11b+HLA-DR+CD86+ antigen-presenting cells (APCs) and increased ratio of CD8+ to
regulatory T cells in the bone marrow, thereby reducing leukemia burden.

Conclusions Our results suggest that eliminating STAT3 permits the TLR9-driven reprogramming of AML cells into APCs to unleash T cell-mediated responses against leukemia.

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**725** PRE-CLINICAL DEVELOPMENT OF TNFR2 LIGAND-BLOCKING BI-1808 FOR CANCER IMMUNOTHERAPY

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**Background** The pleiotropic TNF-alpha:TNFR axis plays a central role in the immune system. While the cellular expression of TNFR1 is broad, TNFR2 expression is mainly restricted to immune cells. The therapeutic potential of targeting TNFR2 for cancer treatment has been previously indicated and to gain further insight, we characterized a wide panel antibodies, generated from the n-CoDeR F.I.R.S.T™ target and antibody discovery platform. We identified parallel human and mouse TNFR2 specific, complete ligand (TNF-alpha) blocking antibodies and could show potent anti-tumor activity in severe immune-competent models, both as single agent and in combination with anti-PD1 using a BI-1808 murine surrogate. The mechanism-of-action was shown to be FcgR dependent and likely mediated through a combination of intra-tumor T reg depletion, CD8 + T cell expansion and modulation of tumor-associated myeloid cells. These findings were confirmed using BI-1808 in a humanized mouse model.

**Methods** To address safety of the human lead-candidate BI-1808 two toxicological studies were performed in cynomolgus monkeys. The first study was a dose-range-finding study and the second a GLP study where three doses (2, 20 and 200 mg/kg) were given weekly for four consecutive weeks followed by a recovery period of eight weeks. In addition, cytokine release was further studied in T cell stimulation assays and in a humanized mouse model. Moreover, the BI-1808 murine surrogate was used to study the relationship between dose, receptor occupancy (RO) and efficacy in immune competent mouse cancer experimental models.

**Results** Four weekly administrations of BI-1808 to cynomolgus monkeys were well tolerated at all doses, with no associated clinical signs, and no histopathological changes. Non-adverse and reversible increases in neutrophil counts and decreases in T cells were observed at all dose levels. No drug-related adverse events were observed and consequently the NOAEL for BI-1808 was determined to be 200 mg/kg. Pharmacokinetic studies demonstrated an expected half-life of two weeks at receptor saturation. There were no indications of cytokine release in any of the systems tested. Finally, we could show that to achieve max therapeutic effect, sustained RO was needed for approximately two weeks, covering the time it takes to generate a full adaptive Immune response.

**Conclusions** There is a clear association between RO and therapeutic effect and BI-1808 is well tolerated at doses associated with high and sustained RO. Collectively, these studies were used to determine the starting dose in upcoming phase I/II study in solid cancer aiming for first-patient in during December 2020.

**Ethics Approval** The study on cynomolgous monkeys was conducted by Citox/Charles River Laboratories in compliance with animal health regulations, in particular: Council Directive No. 2010/63/EU of 22 September 2010 and French decree No. 2013-118 of 01 February 2013 on the protection of animals used for scientific purposes. Studies in mice were approved by the Swedish Animal Experiment Ethics Board, ethical permit/ethical license numbers 5.2.18-17196/2018 and 5.8.18-03333/2020

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**726** SRF114 IS A FULLY HUMAN, CCR8 SELECTIVE IgG1 ANTIBODY THAT INDUCES DESTRUCTION OF TUMOR TREGS THROUGH ADCC

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**Background** T regulatory cells (Tregs) are potent suppressors of immune activation in the periphery and tumor microenvironment (TME). Tumor-infiltrating Tregs have also been associated with resistance to cancer therapies. Loss of peripheral Tregs can lead to widespread autoimmunity and tissue destruction; therefore, specifically depleting tumor Tregs is an attractive therapeutic approach to locally activate the immune system. CCR8 expression is highly restricted to tumor Tregs across multiple cancer types, supporting the notion that CCR8 targeting may induce tumor-specific Treg depletion while sparing peripheral Tregs. Moreover, depletion of CCR8+ Tregs leads to significant tumor growth inhibition with correlative tumor Treg depletion in established CT-26 tumors. These data provide rationale for targeting CCR8 to deplete tumor Tregs. Here, we describe the development of SRF114, a fully human IgG1 anti-CCR8 antibody that induces tumor Treg destruction through antibody-dependent cellular cytotoxicity (ADCC).

**Methods** Virus panning against the N-terminal region of CCR8 and subsequent affinity maturation process led to discovery of SRF114, a fully human monoclonal antibody that is specific to CCR8. To evaluate SRF114 specificity, binding was profiled on recombinant CCR8 N-terminus, CCR8+ and CCR8+ cell lines, and primary cell cultures. An extracellular protein target cell microarray was used to further validate specificity. SRF114 functional assays included the Promega CD16 (V/F variants) ADCC signaling assay, PBMC/293T-hCCR8+ cell co-culture experiments, and natural killer (NK)-activation assays targeting Raji-CCR8+ cell lines. To confirm tumor Treg binding and depletion, NK allogenic co-culture experiments were performed with SRF114 using isolated tumor infiltrating lymphocyte cultures from freshly resected tumors.

**Results** A tumor Treg-restricted pattern of CCR8 expression was confirmed using publicly available datasets and profiling of CCR8 expression on Tregs from fresh tumor tissues. SRF114 binds to CCR8-expressing 293T cells with pM affinity and not to parental cells. SRF114 does not bind any cell populations in PBMCs from healthy donors and has no other protein targets assessed by cell microarray. In dose-dependent ADCC assays, SRF114 induces cell killing with pM EC50 values, which is further enhanced by removing the fucose groups from the Fc-domain. Finally, SRF114 specifically binds to human tumor Tregs and induces killing of Tregs in NK co-culture experiments.

**Conclusions** The fully human anti-CCR8 antibody SRF114 specifically binds to and targets CCR8+ tumor Tregs for depletion, likely through ADCC. Through this mechanism, SRF114
INCREASED SERUM LEVELS OF EBI3 ARE ASSOCIATED WITH POOR OUTCOME IN HEPATOCELLULAR CARCINOMA PATIENTS AND SRF388, A FIRST-IN-CLASS IL-27 BLOCKING ANTIBODY, INHIBITS THE GROWTH OF MURINE LIVER TUMORS

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Background IL-27 is a heterodimeric cytokine consisting of IL-27p28 and Epstein-Barr virus-induced gene 3 (EBI3) that binds the IL-27 receptor subunit alpha and glycoprotein 130. IL-27 is produced by activated macrophages and dendritic cells and limits the intensity and duration of immune responses in the tumor microenvironment by inducing the expression of immunoregulatory receptors (PD-L1, TIM3, LAG-3, TIGIT) and inhibiting production of proinflammatory cytokines (IFNγ, IL-17, TNFα). The IL-27 subunit EBI3 is elevated in plasma from patients with certain cancers including renal cell carcinoma, where it correlates with poor outcome. Based on high expression of IL-27 transcript in tumors from patients with hepatocellular carcinoma (HCC), the role of IL-27 was further explored in patient samples and a mouse model of HCC.

Methods Gene expression profiles from the Cancer Genome Atlas (TCGA) were analyzed to identify tumors with elevated IL-27 transcripts. Serum from patients with HCC was analyzed for levels of the IL-27 subunit EBI3. The ability of SRF388, a first-in-class IL-27-blocking antibody that binds to IL-27p28, to reverse IL-27-induced inhibition of cytokine production in human immune cell cultures from patients with HCC was assessed in vitro. Finally, the anti-tumor activity of SRF388 was assessed in an orthotopic murine model of HCC.

Results TCGA expression data revealed that IL-27p28 transcripts were elevated in tumors from patients with HCC relative to other indications. Serum levels of EBI3 were: 1) elevated in a subset of HCC patients; 2) inversely correlated with survival; 3) independent of serum alpha-fetoprotein levels; and 4) elevated in both hepatitis B/C virus positive and negative patients. Treatment with SRF388 stimulated increased cytokine production in activated peripheral blood mononuclear cells from patients with HCC that was further enhanced when combined with PD-1 blockade. Furthermore, SRF388 inhibited the growth of orthotopic Hepa1-6 liver tumors. mRNA transcriptional profiling of treated tumors revealed that SRF388 profoundly altered the transcriptional landscape in this model. In particular, treatment with SRF388 inhibited expression of immunoregulatory receptors PD-L1 and TIGIT, repressed transcripts associated with TGF-β signaling, and altered myeloid and natural killer cell transcripts.

Conclusions These data indicate that elevated IL-27 subunit EBI3 is a hallmark of HCC and is associated with poor outcomes in these patients. Blockade of IL-27 with SRF388, currently being evaluated in a Phase I clinical trial in patients with advanced solid tumors (NCT04374877), may represent a promising therapy for patients with HCC where it can potentiate anti-tumor immune responses.

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ABSTRACTS

ANTIBIOTICS AND RESPONSE TO IMMUNOTHERAPY: REAL-WORLD EXPERIENCE

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Background Immune checkpoint inhibitors (ICI) have altered the therapeutic paradigm of advanced non-small cell lung cancer (NSCLC) and have become an attractive treatment strategy in several malignancies. The identification of reliable predictors associated with resistance is essential to dictate new approaches to broaden responder groups. Growing evidence has shown that the gut microbiome is an important regulator of the systemic immune system and is involved in the response to ICI. The aim of the study was to evaluate the association between antibiotics use & ICI efficacy in advanced NSCLC.

Methods A retrospective, single-centre study of unselected patients with advanced NSCLC treated with ICI between June 2016 and May 2019. We included consecutive patients who received at least one dose of PD-1 inhibitors (Nivolumab or pembrolizumab) Clinicopathological characteristics and the status of any oral or intravenous antibiotic use were evaluated. Antibiotic use was defined as antibiotic treatment at any time between 4-weeks pre- and 4-weeks post the start of ICI (table 1). Progression-Free Survival (PFS) & Overall Survival (OS) were estimated with Kaplan-Meier method & compared between Abx groups. Cox proportional model was used for multivariate analyses.

Results After a median follow-up of 8.5 months [0.3–56.4], a significant improvement in PFS was observed in untreated group compared to Antibiotics treated group. 12.4 months (95%CI, 22.9) vs 4.1 months (95%CI, 2.6–5.6) (p < 0.001; figure 1). Similarly, OS among patients with no Antibiotics usage was defined as antibiotic treatment at any time of any oral or intravenous antibiotic use were evaluated. Antibiotic use was defined as antibiotic treatment at any time between 4-weeks pre- and 4-weeks post the start of ICI (table 1). Progression-Free Survival (PFS) & Overall Survival (OS) were estimated with Kaplan-Meier method & compared between Abx groups. Cox proportional model was used for multivariate analyses.

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Abstract 728 Figure 1  Kaplan-Meier curves for PFS in patients with and without antibiotics use

Abstract 728 Figure 2  Kaplan-Meier curves for OS in patients with and without antibiotics use

Conclusions  Our results point to a detrimental effect of antibiotics on treatment outcome to ICI therapy. The antibiotics use was significantly associated with attenuated efficacy of anti-PD-1 therapies in patients with NSCLC Modulation of antibiotic-related changes of gut microbiota may be important to improve clinical outcomes in ICI for cancer treatment. In patients needing antibiotics, careful selection to avoid antimicrobial agents that modulate immune responses should also be taken into consideration. Further studies are needed to determine the regimen, length of antibiotics treatment and its relation to survival benefits.

REFERENCES


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Background  Immune checkpoint inhibitors (ICI) and targeted therapies (TT) have improved the survival outcomes in patients with advanced melanoma. However, less is known about their impact on Asian patients with melanoma. In this study, we hypothesize that patients of Asian ancestry would have improved survival for advanced melanoma since the introduction of ICI and TT in 2011.

Methods  Asian patients with melanoma were identified in the National Cancer Database (NCDB) from 2004–2016. Patient, tumor, and treatment characteristics were compared for populations treated before and after 2011 using Chi-square analyses. Overall survival (OS) was analyzed using Kaplan-Meier estimates.

Results  1,411 Asian patients with melanoma were identified. Overall, 21% were melanoma in situ, and 79% were invasive melanomas. 62% of patients did not have a documented histologic subtype. The most common reported histologies were superficial spreading (14%) and acral lentiginous (10%) melanomas. Primary locations included 41% lower extremity, 17% upper extremity, and 11% head and neck. The age at diagnosis has increased during the study period - 38% over 60 years old in 2004, to 54% in 2016 (P<0.002). Kaplan-Meier survival estimates were performed for the whole Asian melanoma population and showed worse OS for all patients diagnosed after 2011 compared with patients diagnosed before 2011 - 83% vs 84% at 24 months (P=0.0033), 71% vs 76% at 48 months (P<0.001), respectively. However, the OS for those stage IV melanomas diagnosed after 2011 is better compared to patients diagnosed before 2011 - 52% vs 26% at 24 months (P<0.001), and 20% vs. 13% at 48 months (P<0.001), respectively. (Table 1) The worse OS trend seen for all patients was driven by those with early stage disease and likely does not reflect melanoma specific survival. Utilization rates of ICI and TT in Stage IV melanoma in Asian populations was significantly higher after 2011 (9% versus 30% before and after 2011 respectively, p=0.026). This was comparable to the utilization rates of 12% vs 34% for all patients

Abstract 729 Table 1  Survival for stage IV Asian melanoma

<table>
<thead>
<tr>
<th>Time point</th>
<th>Survival Probabilities (95% Confidence Interval)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004–2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>0.51 (0.33, 0.71)</td>
<td></td>
</tr>
<tr>
<td>24 months</td>
<td>0.29 (0.20, 0.39)</td>
<td></td>
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<tr>
<td>48 months</td>
<td>0.13 (0.08, 0.27)</td>
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*Longest follow up time in group 2 (Asian Melanoma diagnosed 2012 and after)
HISTOTRIPSY FOCUSED ULTRASOUND ABLATION INDUCES IMMUNOLOGICAL CELL DEATH IN TREATED AND DISTANT UNTREATED TUMORS

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Background Histotripsy, a novel image-guided, robotically assisted sonic therapy platform, is a non-invasive and non-thermal tumor ablation modality. We have previously shown that histotripsy potentiates profound innate and adaptive anti-tumor responses in addition to direct tumor destruction. In this study, we sought to characterize the biomarkers of tumor cell death pathways immediately after histotripsy and after the induction of adaptive anti-tumor immune responses in preclinical settings.

Methods Immunocompetent C57BL/6 mice were inoculated with bilateral subcutaneous flank injections of Hepa1-6 hepatocellular carcinoma to generate 8–10 mm tumors within 8–11 days. Unilateral subtotal histotripsy was then performed. Mice were euthanized at 6h, and 1, 3, and 10–12 days post-treatment (dpt). Tumors were measured, harvested, fixed, sectioned and studied using multicolor immunohistochemistry.

Results Histotripsy decreased treated tumor growth by 50% and abscopal tumor growth by 30–40% compared to untreated tumors at 12dpt, evidencing a systemic anti-tumor immune response that inhibited growth of distant untreated tumor. Treated tumors showed immediate tissue liquefaction in the ablation zone with marked extranuclear translocation of the damage associated molecular pattern HMGB1. At 1dpt, 100% of tumor cells within the ablation zone showed HMGB1 translocation, and 70% of tumor cells at the periphery of the ablation zone showed HMGB1 translocation. Caspase 3 cleavage was not observed in the direct ablation zone, but at the junction of the ablated and non-ablated tissue ~40% cells that released HMGB1 showed cleaved Caspase 3. Caspase 9 cleavage was observed in ~50% cells that had cleaved Caspase 3, suggesting early programed cell death with mitochondrial damage and cytochrome C release 1 dpt; the presence of inflammasome integration/activation suggested pyroptosis induction. Areas of tumor well outside the zone of ablation and within untreated tumors contralateral to ablated tumors did not show early DAMP release or apoptotic cell death compared to the control tumors. However, a robust immune cell infiltration was observed in these locations at 10–12dpt, involving CD8 T-cell infiltration and areas of tumoral HMGB1 release in the vicinity of the infiltrating CD8 T cells - indicating the induction of immune rejection of treated and untreated tumors by histotripsy.

Conclusions Our results indicate that histotripsy ablation promotes tumor cell destruction through both immediate mechanical disruption, as well as possible adjacent apoptotic and pyroptotic death. Systemic CDB T-cell mobilization and immunological cell death in the treated and the contralateral tumors is a novel long term therapeutic benefit.

REFERENCE

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Regulatory, financial, and access considerations

COSTS OF CARE FOR FIRST-LINE (1L) TREATMENT OF ADVANCED NON-SMALL CELL LUNG CANCER (aNSCLC): A REAL-WORLD CLAIMS ANALYSIS

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Background Recent advances in therapy have created numerous options for the 1L treatment of aNSCLC. This study describes the total direct healthcare costs for patients treated with immunotherapy monotherapy (IO), chemotherapy (CT), or immunotherapy plus chemotherapy (IO+CT) in the 1L setting.

Methods The Ability Patient Complete claims database was used to identify US patients aged ≥ 18 years diagnosed with aNSCLC (ICD-9: 162.8; ICD-10: C34.9) initiating 1L treatment with IO, CT, or IO+CT between January 2015 and May 2019. Patients were required to have at least 6 months of continuous enrollment prior to initiation of 1L treatment, ≥ 1 inpatient or 2 outpatient claims for lung cancer, and a claim within 45 days for a secondary metastatic site. Patients with another malignant primary cancer, who participated in a clinical trial, or who received treatments consistent with small cell lung cancer or a systemic therapy not used for lung cancer were excluded. Costs were calculated on a per-patient per month (PPPM) basis from initiation of 1L treatment until discontinuation or end of study period and expressed in 2019 US dollars. A standardized cost approach was applied, with average wholesale prices for antineoplastic and other drug costs and CMS fee schedules for outpatient visits, inpatient stays, ED visits, and other medical costs (e.g. all other outpatient medical services including infusions of growth factors, radiographic studies, blood draws, etc.). All antineoplastic costs were considered individually.

Results 8,154 patients were included in the cohort: 1,319 received IO, 5,315 CT, and 1,520 IO+CT. By cohort, mean age was 65 (IO), 63 (CT), and 62 (IO+CT) years while mean Charlson Comorbidity Index was 2.12, 2.11, and 1.83, respectively. Key results by healthcare resource utilization category are provided in the table below (table 1).

Conclusions The total PPPM healthcare costs of patients receiving chemotherapy (CT or IO+CT) are higher than those only receiving IO monotherapy. These differences are driven by higher outpatient visit, other medical, and pharmacy costs. IO-containing regimens have higher antineoplastic costs than...
CT, but options with no or limited CT may be able to offset these costs through a reduction in other medical expenses.

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Tumor and stromal cell biology

CHEMERIN REACTIVATES PTEN AND SUPPRESSES PD-L1 IN TUMOR CELLS VIA A NOVEL CMKLR1-MEDIATED PATHWAY

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Background Chemokines and chemoattractants play critical roles in trafficking that help regulate leukocyte infiltrates in the tumor microenvironment. Chemokines/chemoattractants can also modulate tumor cell phenotype and function, as tumor cells express functional receptors for these agents. Chemerin (retinoic acid receptor responder 2, RARRES2) is an endogenous leukocyte chemoattractant that recruits innate immune cells through its receptor, CMKLR1. RARRES2 is widely expressed in nonhematopoietic tissues and often down-regulated across multiple tumor types compared with normal tissue. We and others have shown that augmenting chemerin in the tumor microenvironment significantly suppresses tumor growth, in part, by immune effector cell recruitment. As chemerin has various roles outside of leukocyte trafficking (eg adipocyte differentiation and metabolic processes), we hypothesized that it may have additional, tumor-intrinsic effects.

Methods We investigated the effect of exogenous chemerin on human prostate and sarcoma tumor lines. Key signaling pathway components were elucidated using qPCR, Western blotting, siRNA knockdown, and specific inhibitors. Functional consequences of chemerin treatment were evaluated using in vitro and in vivo studies.

Results We show for the first time that human tumors exposed to exogenous chemerin significantly upregulate PTEN expression/activity, and concomitantly suppress programmed death ligand-1 (PD-L1) expression. CMKLR1 knockdown abrogated chemerin-induced PTEN and PD-L1 modulation, revealing a novel CMKLR1/PTEN/PD-L1 signaling cascade. Targeted inhibitors suggest that signaling occurs through the PI3K/AKT/mTOR pathway. We found that chemerin treatment significantly reduced tumor migration, while significantly increasing T-cell-mediated cytotoxicity. Chemerin treatment was as effective as both PD-L1 knockdown and the anti–PD-L1 antibody atezolizumab in augmenting T cell mediated tumor lysis. Forced expression of chemerin in human DU145 prostate tumors significantly suppressed in vivo tumor growth, significantly increasing PTEN and decreasing PD-L1 expression. Primary prostate tumor cultures that were treated with recombinant chemerin showed significant increases in PTEN and decreases in PD-L1 expression compared to controls. Lastly, analyses of clinical trial data from human metastatic prostate cancer patients receiving treatment with ipilimumab (NCT02113657) showed higher tumoral levels of RARRES2 expression correlated with higher levels of PTEN, higher effector immune cell (eg cytotoxic T cells, NK cells) signatures, and improved clinical outcomes, suggesting a strategy to augment chemerin/RARRES2 levels in tumors may improve responses to immunotherapy.

Conclusions Collectively, our data show for the first time a novel link between chemerin, PTEN, and PD-L1 in human tumor lines. These results show that chemerin – in addition to its ability to suppress tumor growth by recruitment of immune effector cells, may also have a role in improving T-cell-mediated immunotherapies through favorable modulation of PTEN and PD-L1.

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INTEGRATIVE MOLECULAR PROFILING OF HIGH-GRADE PRIMARY PROSTATE CANCER IDENTIFIES PATIENTS WITH A BIOMARKER PROFILE THAT FAVORS THE COMBINATION OF STANDARD OF CARE (SOC) THERAPY WITH IMMUNOTHERAPY


Background Prostate cancer (PCa) is primarily driven by androgen receptor (AR) signaling and has a highly immunosuppressive microenvironment. Although genomic and histopathological differences between low- and high-grade primary PCa (lgPCa and hgPCa) have been reported, an integrative assessment of multiple molecular features in the context of disease grade and metastatic outcome is lacking. We propose that a subset of hgPCa patients who relapse under SOC may benefit from adjuvant immune-checkpoint blockade (ICB) added to SOC to overcome immune suppression.

Methods We analyzed treatment naïve prostatectomy tissue from a cohort of 124 primary PCa patients (n= 58, Gleason score ≤6; n= 66, Gleason score ≥8). We performed RNAseq expression profiling, whole-exome sequencing (WES) and immunohistochemistry. We employed digital spatial analysis in tumor vs. stromal regions to characterize differences in CD8+ T-cell topology between lgPCa and hgPCa.

Results 1.Comparisons in lg vs. hgPCA: Digital spatial analysis assessing the proximity of CD8+ T-cells to tumor cells revealed a T-cell exclusion phenotype that is more prominent in hgPCa, whereas evaluation of overall CD8+ T-cell density in tumor and stromal regions did not differentiate disease grades. HgPCa had a higher frequency of at least one functional mutation in either TP53, RHPN2, or KMT2D genes.
A NOVEL NQO1 SPECIFIC ANTI-TUMOR AGENT, SBSC-S3001, SELECTIVELY REGRESSES THE GROWTH OF TUMORS WITH HIGH NQO1 EXPRESSION

Mirhyuk Yun, Haoo Yeung Kim, Sang Woo Jo, Changhoon In, Gyu Young Moon, Seok Ko, Eun Ju Lee, Yong Rae Hong, Seewoong Lee, Helen Cho. Samyang Biopharmaceuticals Corporation, Pangyo, Korea, Republic of; Limto Therapeutics Inc, Pangyo, Korea, Republic of.

Background NAD(P)H-quinone oxidoreductase 1 (NQO1) is a cytosolic two-electron oxidoreductase overexpressed in many types of cancers, including breast cancer, pancreatic cancer, colorectal cancer, cholangiocarcinoma, uterine cervical cancer, melanoma, and lung cancer. Up-regulation of NQO1 protects cells from oxidative stress and various cytotoxic quinones and is associated with late clinical stage, poor prognosis and lymph node metastasis. NQO1 increases stability of HIF-1α protein, which has been implicated in survival, proliferation, and malignance of cancer. Therefore, accumulating evidences suggest NQO1 as a promising therapeutic target for cancer. Accordingly, we have characterized the effect of a novel synthetic NQO1 substrate SBSC-S3001, and demonstrated its selective cytotoxic effects in cancer cells with high expression of NQO1.

Methods In vitro cytotoxicity was determined by sulforhodamine B (SRB) assay in cancer cells with high NQO1 expression and CRISPR-mediated NQO1 knockout cells. The effect of SBSC-S3001 on the energy metabolism pathway was evaluated by western blot analysis of metabolism associated proteins from NQO1-overexpressed cancer cells treated with the compound for 24 hours. In vivo anti-tumor activity was evaluated in MC38 syngeneic and DLD-1 orthotopic mice models.

Results SBSC-S3001 exhibited selective cytotoxicity in cancer cells with high expression of NQO1 in a dose-dependent manner. The cytotoxicity was observed in both normoxia and hypoxia conditions, correlating with the energy metabolism, mitochondrial biogenesis, and cancer proliferative pathways. Also, stronger cytotoxicity was observed in NQO1-overexpressed cancer cells treated with SBSC-S3001 compared to beta-lapachone and analogue treatment. When evaluated in vivo, SBSC-S3001 effectively inhibited the growth of syngeneic and orthotopic tumors when administered as a monotherapy. SBSC-S3001 treatment associated with reduction in key enzymes of the glycolytic pathway (LDHa and GAPDH) and HIF-1α and increase in levels of mitochondrial oxidative phosphorylation (OXPHOS) complex.

Conclusions Treatment of SBSC-S3001, a novel, NQO1-specific substrate reduces HIF-1α and key enzymes associated with glycolysis and suppresses the growth of tumors overexpressing NQO1. Further characterization of SBSC-S3001 as a novel metabolic anti-cancer agent for cancers with NQO1 overexpression is warranted.

Ethics Approval The study was approved by Samyang Biopharmaceuticals Institution’s Ethics Board, approval number SYAU2031.

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Abstract 733 Figure 1 Unsupervised analysis of primary hgPCa that develop metastasis. Features used for unsupervised classification into Cluster 1 and Cluster 2 are: IHC CD8+ cell proximity to tumor cells (IHC_Infiltrated/Desert/Excluded), CD8+ overall cell density in tumor area, MHCI IHC H-score in tumor (% of tumor cells that are positive X (IHC_Infiltrated/Desert/Excluded), CD8+ overall cell density in tumor, Cluster 1 and Cluster 2 are: IHC CD8+ cell proximity to tumor cells

S3001, SELECTIVELY REGRESSES THE GROWTH OF TUMORS WITH HIGH NQO1 EXPRESSION

Mirhyuk Yun, Haoo Yeung Kim, Sang Woo Jo, Changhoon In, Gyu Young Moon, Seok Ko, Eun Ju Lee, Yong Rae Hong, Seewoong Lee, Helen Cho. Samyang Biopharmaceuticals Corporation, Pangyo, Korea, Republic of; Limto Therapeutics Inc, Pangyo, Korea, Republic of.

Background NAD(P)H-quinone oxidoreductase 1 (NQO1) is a cytosolic two-electron oxidoreductase overexpressed in many types of cancers, including breast cancer, pancreatic cancer, colorectal cancer, cholangiocarcinoma, uterine cervical cancer, melanoma, and lung cancer. Up-regulation of NQO1 protects cells from oxidative stress and various cytotoxic quinones and is associated with late clinical stage, poor prognosis and lymph node metastasis. Therefore, accumulating evidences suggest NQO1 as a promising therapeutic target for cancer. Accordingly, we have characterized the effect of a novel synthetic NQO1 substrate SBSC-S3001, and demonstrated its selective cytotoxic effects in cancer cells with high expression of NQO1.

Methods In vitro cytotoxicity was determined by sulforhodamine B (SRB) assay in cancer cells with high NQO1 expression and CRISPR-mediated NQO1 knockout cells. The effect of SBSC-S3001 on the energy metabolism pathway was evaluated by western blot analysis of metabolism associated proteins from NQO1-overexpressed cancer cells treated with the compound for 24 hours. In vivo anti-tumor activity was evaluated in MC38 syngeneic and DLD-1 orthotopic mice models.

Results SBSC-S3001 exhibited selective cytotoxicity in cancer cells with high expression of NQO1 in a dose-dependent manner. The cytotoxicity was observed in both normoxia and hypoxia conditions, correlating with the energy metabolism, mitochondrial biogenesis, and cancer proliferative pathways. Also, stronger cytotoxicity was observed in NQO1-overexpressed cancer cells treated with SBSC-S3001 compared to beta-lapachone and analogue treatment. When evaluated in vivo, SBSC-S3001 effectively inhibited the growth of syngeneic and orthotopic tumors when administered as a monotherapy. SBSC-S3001 treatment associated with reduction in key enzymes of the glycolytic pathway (LDHa and GAPDH) and HIF-1α and increase in levels of mitochondrial oxidative phosphorylation (OXPHOS) complex.

Conclusions Treatment of SBSC-S3001, a novel, NQO1-specific substrate reduces HIF-1α and key enzymes associated with glycolysis and suppresses the growth of tumors overexpressing NQO1. Further characterization of SBSC-S3001 as a novel metabolic anti-cancer agent for cancers with NQO1 overexpression is warranted.

Ethics Approval The study was approved by Samyang Biopharmaceuticals Institution’s Ethics Board, approval number SYAU2031.

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Abstract 733 Figure 1 Unsupervised analysis of primary hgPCa that develop metastasis. Features used for unsupervised classification into Cluster 1 and Cluster 2 are: IHC CD8+ cell proximity to tumor cells (IHC_Infiltrated/Desert/Excluded), CD8+ overall cell density in tumor area, MHCI IHC H-score in tumor (% of tumor cells that are positive X (IHC_Infiltrated/Desert/Excluded), RNAseq signatures for AR pathway in tumor, T-cell exhaustion, Interferon-γ, Macrophage M1, Neuroendocrine phenotypes (stain intensity), RNAseq signatures for AR pathway in tumor, T-cell area, MHCI IHC H-score in tumor (% of tumor cells that are positive X (IHC_Infiltrated/Desert/Excluded) and CD8 infiltration type in relation to tumor and cancer subtypes as defined by Mortensen et al, Science Reports 2015).

Compared to lgPCa. Assessment of MHC-I deficiency by IHC and mRNA revealed that hgPCa has significantly lower MHC-I protein expression compared to lgPCa. Interestingly, MHC-I loss in hgPCa associated with a T-cell exclusion phenotype. Moreover, RNAseq gene expression signatures revealed higher expression of tumor-associated macrophage (TAMs), Tregs, Cancer-Associated Fibroblasts (CAFs), DNA damage repair (DDR) genes and lower Interferon-γ (IFN-γ) expression in hgPCa compared to lgPCa. Overall, hgPCa is characterized by a combined phenotype of ‘MHCIloss/IFN-γ low/CAFhigh/TAMhigh/T-reghigh/DDRhig’. Comparisons within hgPCa that develop metastasis: Unsupervised analysis of molecular features in hgPCa patients that developed metastases identified a subset of patients that exhibit a less immunosuppressive phenotype with lower tumor AR expression, retained tumor MHCI-I expression, moderate CD8+ T-cell infiltration and a high IFN-γ RNA signature (figure 1), suggesting potential benefit from ICB therapy.

Conclusions Our analysis suggests that hgPCa is characterized by low antigenicity as assessed by loss of MHC-I protein expression and an immunosuppressive microenvironment rich in CAFs, macrophages, Tregs and T-cell exclusion phenotypes. Unlike lgPCa, hgPCa can have a poor prognosis (within 5 years relapse). However, a subset of hgPCa patients that metastasized while on SOC exhibited a biomarker profile that might benefit from combination of SOC with ICB.

Ethics Approval This study was approved by BMS Cambridge Massachusetts Institutional Biosafety Committee, approval number CAM_2020_12050_6.

Consent ‘Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.’

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0734
IMPACT OF REVERSING AN EPITHELIAL-TO-MESENCHYAL TRANSITION PROGRAM ON TUMOR METABOLISM AND IMMUNE SUPPRESSION

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Background To identify novel molecular mechanisms used by triple negative breast cancer (TNBC) to facilitate metastasis, we manipulated oncogenic epithelial-to-mesenchymal transition (EMT) by restoring the microRNA-200c (miR-200c), termed 'the guardian of the epithelial phenotype.' We identified several tumor cell catalyzing enzymes, including tryptophan 2,3-dioxygenase (TDO2) and heme oxygenase-1 (HO-1). The Richer lab has published that TDO2 promotes anchorage independent cell survival during TNBC metastasis via its catalytic kynurenine, which also induces CD8 \(^+\) T cell death. Similarly, published studies have demonstrated that HO-1 supports BC anchorage-independent survival. However, effects of the HO-1 catalytobilirubin on the tumor microenvironment had not been studied. We postulated that TNBC utilize targetable catalyzing enzymes, like HO-1, to simultaneously support tumor cell survival and dampen the anti-tumor immune response.

Methods To test our hypothesis in an immune competent mouse model, Met-1 mammary carcinoma cells from a late stage MMTV-PyMT tumor were engineered to inducibly express miR-200c. Tumor cell infiltrates were analyzed by immunohistochemistry (IHC), flow cytometry and multispectral fluorescence. RAW264.7 mouse macrophages were cultured with conditioned medium from carcinoma cells ± miR-200c or the HO-1 competitive inhibitor tin mesoporphyrin (SnMP). RAW264.7 macrophages were also treated with 0–20 \(\mu\)M bilirubin and macrophage polarization and effecrotic capacity, the ability to engulf dead tumor cells, were assessed using qRT-PCR and IncuCyte assays.

Results MiR-200c restoration to Met-1 orthotopic tumors decreased growth by 45% and increased infiltration of CD11c\(^+\) dendritic cells and activation, determined by CD44 expression, of CD68\(^+\) CD8\(^+\) cells. While the number of F4/80\(^+\) macrophages was unchanged by miR-200c, the percent of M1 anti-tumor macrophages (F4/80\(^+\)iNOS\(^+\)/total cells) increased by >6-fold in miR-200c\(^+\)tumors. RAW264.7 macrophages cultured with conditioned medium from miR-200c-restored mammary carcinoma cells had a 25–95% decrease in M2 pro-tumor genes (Arg1, Il4 and Il13) and a 15–55% increase in M1 genes (Nos2, Tnfa and Cxcl10). A similar decrease in M2 (30–50%) and increase M1 (35–160%) genes was seen in macrophages cultured with conditioned medium from SnMP treated mammary carcinoma cells. Conversely, bilirubin treatment alone enhanced M2 macrophage polarization and inhibited effecroticy in a dose-dependent manner.

Conclusions Use of miR-200c to reverse EMT revealed that HO-1 promotes simultaneous TNBC cell survival and immune suppression. These studies are the first to show that tumor cell-HO1 activity and subsequent bilirubin production may alter macrophage function in the tumor microenvironment. This finding could be clinically relevant since HO-1 inhibitors like SnMP are already FDA approved for treatment of other diseases.

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MICRONORNA EXPRESSION PATTERNS IN MELANOMAS ORIGINATING FROM GYNECOLOGIC SITES

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Background Melanomas originating from gynecologic sites (MOGS) are rare mucosal melanomas originating from the vulva, vagina, and cervix. MOGS are associated with a poor survival rate and limited therapeutic options, as patients often present an advanced disease stage. MiRNAs (miRs) are a class of small, non-coding RNA molecules composed of 21–23 nucleotides that control expression of target genes via post-transcriptional regulation and can exhibit dysregulated expression in cancer. Patterns of miR expression and their effects on disease progression have not yet been explored in the setting of MOGS. We hypothesize a unique miR expression profile exists in MOGS that can mediate disease progression via interaction with target genes.

Methods RNA was isolated from formalin fixed, paraffin embedded tissue samples of human vaginal and vulvar melanoma for comparison to normal adjacent vaginal mucosal tissue (NAT) and primary cutaneous melanoma (PCM), respectively. miR expression was then quantified using the NanoString human miRNA assay. Common experimentally validated gene targets of differentially expressed (DE) miRs were identified using miRNet, and pathway analysis was completed to examine potential downstream effects of dysregulated miR expression.

Results Comparison of miR expression in vaginal melanoma to NAT revealed 25 DE miRs (fold change > 1.5, \(p < 0.05\)), with 10 demonstrating a significant decrease in expression in vaginal melanoma tissue relative to NAT, including hsa-miR-145-5p, hsa-miR-99a-5p, and hsa-miR-1972, and 15 exhibiting a significant increase in expression including hsa-miR-17-5p, hsa-miR-19b-3p, hsa-miR-20a-5p, and hsa-miR-20b-5p. 45 DE miRs were identified between vulvar melanoma and PCM, among which 3 demonstrated a significant decrease in expression in vulvar melanoma including miR-200b-3p, miR-494-3p, and miR-200a-3p, and 44 demonstrated a significant increase in expression including miR-17-5p, miR-146a-5p, and miR-19b-3p (fold change > 2, \(p < 0.01\)). Among these DE miRs, both miR-17-5p and miR-146a-5p have recently been experimentally validated as direct or indirect regulators of PD-L1 expression in melanoma. Pathway analysis for DE miRs in vaginal and vulvar melanoma revealed significant enrichment of 35 and 30 pathways, respectively, each including TGF-\(\beta\) signaling, for which 57 genes in the pathway are validated target genes of 13 DE miRs in vaginal melanoma (\(p = 1.5e-12\)), and 59 genes in the pathway are validated targets of 17 DE miRs in vulvar melanoma (\(p = 2.4e-13\)).

Conclusions The results of this study support miRNAs as important potential regulators of gene expression in vaginal and vulvar melanomas that can contribute to tumor progression, tumor immunogenicity, and response to current immunotherapies.

Ethics Approval This study was approved by the Ohio State University Institutional Review Board, approval #2007C0015.

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A NOVEL HUMANIZED MURINE MODEL TO IDENTIFY NEOANTIGEN-SPECIFIC T CELLS IN PEDIATRIC ACUTE MEGAKARYOBLASTIC LEUKEMIA

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Background CBFA2T3-GLIS2 is a chimeric transcription factor in 18.4% of pediatric patients with non-DOWN syndrome acute megakaryoblastic leukemia (non-DS-AMKL). It remains one of the poorest prognostic subtypes amongst pediatric acute myeloid leukemia patients with event free and overall survival probabilities of 8% and 14% respectively, despite intensive multi-agent chemotherapy and stem cell transplant treatment modalities.\(^1\) Adaptive cell therapies targeting the CBFA2T3-GLIS2 fusion may provide additional treatment options for AMKL patients. We hypothesize CBFA2T3-GLIS2 fusion junction specific neoantigen(s) are presented by surface HLA class I expression and elicit functional immune responses by CD8+ T cells.

Methods To interrogate this hypothesis in silico, we used NetMHCons to predict neoantigens spanning the fusion junction.\(^2\) To test our hypothesis in vivo, we established a small cohort (total N=12) of immunodeficient NSG-SGM3 mice engrafted with patient derived xenograft (PDX) cells from a CBFA2T3-GLIS2 positive AMKL patient for 20 days followed by the introduction of PBMCs from a healthy, unrelated donor with a 6/6 HLA class I match to the PDX cells for an additional 10, 15, or 20 days (figure 1).\(^3\) Leukemia and T cell phenotype was characterized by flow cytometry. Levels of soluble analytes were measured in the serum by cytometric bead array. The TCR repertoire and transcriptional profile of the CD8+ T cells was determined through paired gene expression and 5’ single cell V(D)J sequencing using 10X Genomics.

Results NetMHCons identified two neoantigens spanning the fusion junction with intermediate binding affinities (IC50) of 304.37 and 384.09 nM. Flow cytometry analysis at Day 40 (figure 1) showed dramatic reduction of leukemic blasts (huCD45+, huCD34+) when exposed to PBMCs compared to Leukemia Only control (1.48% and 93.7%, respectively). Circulating human Granzyme A was elevated at Day 40 in the PDX mice with PBMCs (9665.4 pg/ml) compared to the PBMC Only control (789.7 pg/ml), indicating a potential leukemia-directed cytokine lymphocyte response.

Abstract 737 Figure 2 Leukemia cells targeted by clonal CD8+ T cells.

At Day 40, our humanized murine model showed reduced leukemia burden when exposed to PBMCs (a) compared to a Leukemia Only control (b). This response is mediated by the release of Granzyme A from a subset of uniform, clonally expanded leukemia-specific CD8+ T cells (a) that are not present in the highly diverse expanded TCR repertoire of the PBMC Only control (c). Colored circles represent CD8+ T cells with different TCRs. Orange symbol represents leukemia cells.

Conclusion Our novel murine model demonstrates a potential neoantigen-directed CD8+ T cell response against patient AMKL cells, highlighting the importance of adoptive cell therapy to improve patient outcomes.

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Ethics Approval The vertebrate animal studies described here were approved by the Institutional Animal Care and Use Committee (IACUC); animal welfare assurance number A3325-01. The patient derived xenograft leukemia cells and healthy donor PBMCs used in the studies described here are exempt from requiring Institutional Review Board (IRB) approval because we receive no personal identifying information about the patient/donor from which the xenograft cells/PBMCs are derived.

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A441
ESTABLISHMENT AND APPLICATION OF A SERIES OF GENETICALLY ENGINEERED MOUSE DERIVED BREAST CANCER HOMOGRAFT MODELS

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Background Breast cancer is a complex disease which is defined by an intrinsic heterogeneity at the histopathological and molecular levels, as well as in response to therapy. It remains the second leading cause of cancer death among women worldwide despite advances in screening, detection and new therapeutic options. Therefore, it is important to establish relevant preclinical mouse models to study new therapeutics and tumor biology. Genetically engineered mouse models (GEMMs) have been developed in order to understand the molecular, biochemical and cellular functions of oncogenes or tumor suppressor genes. However, the application of GEMMs is constrained due to the spontaneous nature of tumor onset and progression and high cost of breeding. Homograft tumor models, which are derived from and retain the histopathological and molecular features of GEMMs, can be used as faithful surrogates of human tumors.

Methods We generated a series of homograft tumor models from GEMMs overexpressing human epidermal growth factor receptor 2 (HER2, also known as ERBB2) or polyomavirus middle T antigen (PyMT) driven by the mouse mammary tumor virus (MMTV) promoter, or Simian Virus 40 T-antigen (SV40 Tag) under the promotion of the rat prostate steroid binding protein (C3(1)), which are commonly used GEMMs in preclinical breast cancer research.1, 2 Models were generated by transplanting the mammary tumors into donor animals. Furthermore, we characterized the homograft tumors through histopathological analysis, immunohistochemical analysis, and immune profiling, as well as immunotherapeutic, cytotoxic and targeted therapy.

Results Nine breast cancer homograft models were developed from MMTV-ERBB2, MMTV-PyMT and C3(1)-Tag GEMMs, including six hormone receptor negative and HER2 positive models (mBR9013, mBR9026, mBR9027, mBR9028, mBR9029, mBR9030), one hormone receptor positive and HER2 negative model (mBR6174) and two triple negative models (mBR6004, mBR9014). Immune profiling of six models showed enriched macrophage infiltration in the tumor microenvironment. Immunotherapy treatment with anti-mPD-1 and anti-mCTLA-4 produced tumor growth inhibition (TGI) of 98% and 110%, respectively, in the triple negative model mBR9014, accompanied by tumor regression. HER2 targeted treatment with laptinib produced robust response with TGI ranging from 48% to 97% in one HER2 negative and two HER2 positive models. Varying response to the cytotoxic treatments was observed among different models, with cisplatin producing robust response of TGI over 80% in all five of the tested models.

Conclusions We have generated and characterized a series of mouse breast cancer homograft models from GEMMs to facilitate both mechanistic investigation and preclinical testing of novel therapeutics.

Ethics Approval Animal experiments were conducted in accordance with animal welfare law, approved by local authorities, and in accordance with the ethical guidelines of Crown-Bio (Taicang).

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DEVELOPMENT OF PATIENT-DERIVED MODELS OF ESOPHAGEAL CANCER FOR GUCY2C-DIRECTED IMMUNOTHERAPEUTIC TESTING

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Background Esophageal cancer is the fifth most common cause of cancer-related death in the world1 with a 5-year survival rate of <20%.2 Current therapies result in high toxicity and low efficacy, with as many as 60% of esophageal cancer patients not responding to therapeutics.3 CAR-T cell therapy is a therapeutic that can selectively and robustly target cancer cells and eliminate bulky metastatic disease. Previous studies have shown preclinical success with CAR-T cell therapy targeting the human colorectal cancer antigen guanylyl cyclase C (GUCY2C).3–5 Interestingly, esophageal cancers arising from premalignant metaplasia resembling intestine (intestinal metaplasia, also known as Barrett’s esophagus) are highly prevalent and ectopically express GUCY2C. Thus, we hypothesize that GUCY2C will serve as an effective CAR-T cell therapy target in many esophageal cancer patients. However, the paucity of intestine-like human esophageal cancer models limits exploration of this hypothesis, necessitating development of suitable esophageal cancer models (figure 1).

Methods To develop esophageal cancer models for GUCY2C immunotherapy testing, esophageal cancer samples were collected at Thomas Jefferson University Hospital by endoscopic biopsy of treatment-naïve patients or by esophagectomy, primarily in patients previously treated with standard neoadjuvant therapy. Patient-derived xenograft (PDX) models were initiated from samples to establish in vivo models for immunotherapy testing. qRT-PCR, immunoblot, and immunofluorescence were performed to test for GUCY2C expression in primary and PDX specimens. Histopathology was performed to confirm retention of primary tumor features.

Results GUCY2C was present in only 2 of 6 esophagectomy samples. Interestingly, those patients with detectable GUCY2C were treatment-naïve, while neoadjuvant-treated patients lacked viable tumor, revealing neoadjuvant therapy as a significant barrier to esophageal cancer model generation. In contrast, of the 3 adenocarcinoma specimens collected by endoscopic biopsy in treatment-naïve patients, 2 express GUCY2C. To date, PDX models have been initiated from 6 total samples and successfully established for 3 samples. This 50% success rate may improve over time as PDX formation is often delayed in many models (>150 days). Importantly, established esophageal adenocarcinoma PDX models were histologically similar to their matched primary tumors and retained GUCY2C expression, integral to their validation as models of GUCY2C immunotherapy testing.

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Conclusions Several human esophageal adenocarcinoma models were successfully established, primarily from endoscopic biopsy of treatment-naïve patients as neoadjuvant therapy proved to be a significant barrier. These models will be useful to explore GUCY2C-directed CAR-T cell therapies and other novel therapies targeting intestine-like esophageal cancer, prior to testing in early-phase clinical trials.

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Ethics Approval The study was approved by the Thomas Jefferson University Institutional Review Board (#18D.495) and Institutional Animal Care and use Committee (#01529).

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Abstract 739 Figure 1 GUCY2C expression during tumorigenesis in adenocarcinoma of the esophagus

including POLE, were identified that are significantly mutated in TMB-high samples compared to MSI-H samples (table 1). Loss of function of these genes may result in an ultra-mutated phenotype. Contradicting the notion that POLE mutation is predominantly associated with MSS tumors and are mutually exclusive with the complete loss of MMR, 4–6 we found about half of POLE-mutant samples (8/16) were MSI high, five of which had MMR mutations (figure 2).

Conclusions The study investigated the association of DNA repair genes with TMB and MSI. We compared genes significantly altered in TMB high and MSI-H samples and identified genes pointing to a potential mechanism that induces ultramutation in a subset of cancer patients with intact MMR, which can serve as potential biomarkers for immunotherapy efficacy linked with high TMB.

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741 HIGH-GRAD SINONASAL CARCINOMAS AND SURVEILLANCE OF DIFFERENTIAL EXPRESSION IN IMMUNE RELATED TRANSCRIPTOME

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Background The skull base is the location of a wide variety of malignant tumors. Among them is sinonasal undifferentiated carcinoma (SNUC), a highly aggressive sinonasal neoplasm that was recently reclassified into subgroups of high-grade carcinomas with unique genomic events (e.g., SMARC-deficient carcinoma, nuclear protein in testis NUT carcinoma). Other high-grade carcinomas in this location are neuroendocrine carcinomas, sinonasal adenocarcinomas, and teratocarcinosarcomas. Given the rarity of these tumors, little transcriptomic data is available. The aim of this study was to characterize the immune-oncology gene expression profile in SNUC and other high-grade sinonasal carcinomas.

Methods Next-generation sequencing was performed in 30 high-grade sinonasal carcinoma samples using the HTG EdgeSeq Precision Immuno-Oncology Panel. Ingenuity pathway analysis was performed to understand the immunobiology, signaling, and functional perturbations during tumor development.

Results The samples were divided into 3 groups: 21 SNUCs and SMARC-deficient sinonasal carcinomas; 5 high-grade neuroendocrine carcinomas (HGNECs), with small cell and large cell variants; and 4 high-grade sinonasal carcinomas (HGSNCs) of mixed histology (1 NUT carcinoma, 1 teratocarcinosarcoma, and 2 sinonasal adenocarcinomas). PRAME and ASCL1 emerged as upregulated transcripts with strong protein validation for SNUC and HGNEC; other upregulated candidates EZH2 and BRCA1 offer consideration for alternative targeted therapy, and downregulation of major histocompatibility complex molecules and chemokines represent another hurdle in the development of effective immunotherapy.

Conclusions This immune-oncology gene expression analysis of 3 groups of high-grade sinonasal carcinoma with emphasis on SNUC identified a number of differentially expressed transcripts reflecting effects on tumorigenesis. Identification of immune pathways should be further investigated for possible integration of immunotherapy into a multidisciplinary approach to these cancers and personalized treatment.

Ethics Approval MD Anderson Cancer Center Institutional Review Board approval was obtained prior to the start of the study.

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HIGH LEVELS OF STROMAL TUMOR INFILTRATING LYMPHOCYTES, CD3, CD8 CELLS & IMMUNOSCORE® ARE ASSOCIATED WITH PATHOLOGICAL CR AND TIME TO PROGRESSION IN TNBC PATIENTS UNDERGOING NEO-ADJUVANT CHEMOTHERAPY

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Background The presence of high levels of stromal tumor infiltrating lymphocytes (TILs) has been associated with better prognosis in early triple-negative breast cancer (TNBC). The Immunoscore® (IS) is a prognostic tool, which categorizes the densities of spatially positioned CD3 and CD8 cells in both invasive margins (IM) and the center of the tumor (CT), yielding a five-tiered classification (0–4). High IS values have been reported to predict improved outcomes in colorectal cancer.

Methods The cohort consisted of 52 TNBC patients (pts) who previously received neo-adjuvant anthracycline and taxane based chemotherapy. Quantitative analysis of the immune cells was carried out using a computer-assisted image analysis in different tumor locations for CD3 and CD8 T-cell markers. Additionally, we measured stromal TILs according to the internationals TILs working group. Pre-treatment tumor samples were immune-stained for CD3 and CD8 T-cell markers and stromal TILs. The relationship between various clinical pathological factors including tumor size, glands, stage and immune factors were analyzed by Chi2 and Fischer exact test. The log-rank test and the Kaplan Meyer methods were used to estimate relapse free survival.

Results The median age of the patients was 50 years (27–84 years). Tumor sizes were categorised as T1 = 9 patients (17%), T2 = 41 patients (77%) and T3 = 3 patients (6%). Patients with positive glands = 19 (36%) patients and patients without gland involvement = 34 (64%). Stage grouping included stage 1 = 5 (9%), stage IA = 33 (63%) patients, stage IB = 9 (17%) patients, stage III = 6 (11%) patients. The median Ki-67 was 45% (5–90%). The median density of CD3 CT cells = 1190 mm² (range 34–4614), CD3 IM = 1855 mm² (range 57–6190), CD8 CT 508 mm² (range 17–2486) and CD8 IM 805 mm² (range 90–3156). The median percentage of stromal TILs was 5% (0–60%). Patient with an IS of 0 = 4 patients (8%), IS 1 = 3 (5%), IS 2 = 20 patients (38%), IS 3 = 24 patients (45%) and IS 4 = 2 patients (4%). The pathological complete response (pCR) rate of the entire cohort was 62%. A positive correlation was found between TILs and CD3 CT (R = 0.641, p < 0.0000), CD8 CT (R = 0.5623, p < 0.0000), CD3 IM (R = 0.6099, p < 0.0000), and CD8 IM. (R = 0.5010, p < 0.0010). TILs correlated with immunoscore (R = 0.3603, p < 0.0087). There was no correlation between TILs and Ki-67 (R = 0.1497, p < 0.2943). On univariate analysis, factors associated with higher pCR included nodal status (positive = 42,11% vs negative = 73,53% (p<0,02362) and Ki67 <40% = 33,33% vs =40% = 76,47% (p<0,00235). A high density of CD3 (> than 1100 mm²) and CD8 (> than 400 mm²) positive T-cells in the CT was associated with higher pCR (CD3 CT: 30% vs 70%, p=0.00489 and CD8 CT: 30% vs 70%, p=0.03344). Analysis of CD3 (> than 1200 mm²) (CD3 IM: 12% vs 88%, p=0.0.02367) and CD8 in the IM (> than 550 mm²) was also significant for an association with pCR (CD8 IM:23% vs 77%, p=0.03). High IS (1+4= 73%) vs intermediate (2=55%) vs low (0+1=43%) showed a numerical difference, however, did not reach a statistical significance with pCR (p=0.111). Analysis of TILs = 20% showed a pCR of 76% compared to patients with TILs < 20% with a pCR of 54% (p < 0.12295). A Ki67 =40% was associated with pCR of 76% compared to patients with Ki67 < 40% with a pCR 33% (p < 0.00235). The median time to progression of the patients not attaining a pCR was 1600 days, compared to patients who did attain a pCR with a median DFS not yet reached, but exceeds 1800 days. The median time to progression of patients with Ki67 < 40% was 1700 days while the patients with Ki67 >40% has not been reached yet (p < 0.03). At 1800 days, 95% of patients with CD3 > 1100 mm² did not relapse, compared to 75% patients with CD3 = 1100 (p < 0.03).

Conclusions This exploratory study shows that analysing CD3 and CD8 in the centre of the tumor and invasive margin might be more sensitive than examination of TILs in TNBC patients.

Ethics Approval The study was approved by Pharma-Ethics - (Institution’s Ethics Board), approval number 170516563.
intermediated by the less exhausted, GZMK-enriched CD8 population (figure 3C-D). GZMK also discriminated between 2 MAIT populations. GZMK-enriched cells had increased expression of the stem-like T cell transcription factor TCF7, and the T cell memory transcription factor EOMES. GZMK expression was associated with improved survival in de novo TCGA AML cohort (p=0.0017). scTCR clonotype assessment revealed shared clonotypes with the terminally effector CD8 CTL cells following PD-1 blockade. Following treatment, novel clones represented 38.7% (39/101) of total clones, followed by contracted clones (32.6%) and expanded (28.7%) clones (figure 3E-F). However, 76.9% and 72.4% of novel and expanded clones were contributed by the responders. Non-responders contributed only 5% and 3.4% of the novel and expanded clones, respectively.

Conclusions Chr7/7q loss is associated with resistance to PD-1 blockade. CD8 cells exist in a continuum in BMs of patients with AML and GZMK expression identifies a stem-like, memory T cell subset. The subverted T cells can be reinvigorated via PD-1 blockade and induce responses in AML driven via novel and expanded clones demonstrating AML T cell plasticity and adaptability. Further functional characterization of GZMK expressing lymphocytes in mediating antileukemic responses is underway.

Ethics Approval The study was approved by IRB at MD Anderson Cancer Center

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HIGH THROUGHPUT TISSUE IMAGING AND PHENOTYPING BY STREAMLINED 8-PLEX STAINING AND ANALYSIS OF COMPLEX TMA SAMPLES

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Conclusions In this study, we highlighted the benefits of using a combination of well-characterized TMAs, a fast, optimized 8-plex mIHC protocol, and a detailed analysis pipeline to characterize the immune-response in a broad range of cancer types and samples, leading to a better understanding of the TME as well as a streamlined workflow for further translational studies.

Results Immune cell counts and phenotypes were identified using automated analysis for cores within the tumor and within the tumor margin using a panel characterizing a range of immune cell populations, and compared across each tissue type. Deep phenotyping was performed for each core to identify unique profiles for each tissue type, with a workflow optimized for high-throughput analysis of rich-content TMAs.

Methods Each slide comprised 144 cores (1 mm) and included duplicate cores for each case (1 from invasive margin; 1 from tumor center) from 11 different tumor types including breast cancer (ER+, Her2+, TNBC), NSCLC (squamous, adenocarcinoma), SCLC, CRC, pancreatic, gastric, hepatic and esophageal cancers. TMA sections were stained using the UltiMapper I/O Immuno8 panel, which includes markers for CD3, CD4, CD8, FOXP3, CD68, PD-1, PD-L1, and a pan-CK/SOX10 cocktail as a tumor indicator. The stained TMAs were scanned at 20X magnification on a fluorescence whole slide scanner. To provide accurate marker colocalization data, marker images were aligned using the UltiStacker software, using the nuclear
counterstain images as references from multiple rounds of imaging. Image analysis was performed using Visiopharm software and generated total and negative cell phenotype counts, cell density in tumor and stroma, as well as spatial interactions maps in each of the 288 cores in the TMA set.

**Background**
Multiplex immunohistochemistry (mIHC) and associated data analysis methods are rapidly becoming invaluable tools to improve our understanding of the complex tumor micro-environment (TME) and accelerate the discovery of novel immunotherapy targets. These techniques can enable the accurate phenotyping of the immune response and checkpoint expression in the spatial context of the tumor. The goal of this study was to identify the populations of immune cells (T-cytotoxic, T-helper, T-reg, and macrophages), their functional status, as well as their interactions with the tumor, in a range of samples and indications using a carefully designed multi-tumor Tissue Micro-Array (TMA) set of 2 slides from TriStar.

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### Abstract 746

**NON SMALL-CELL LUNG CANCER CELLS AND CANCER-ASSOCIATED FIBROBLASTS DRIVE MACROPHAGE POLARIZATION IN A NOVEL CO-CULTURE MODEL**

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**Background**
The plasticity of macrophage phenotype within the tumor microenvironment (TME) correlates with prognosis in non-small cell lung cancer (NSCLC).1 M2-like macrophages promote immunosuppression and facilitate tumor progression, while M1-like macrophages may drive an inflammatory antitumor immune response.2 Through a novel co-culture model comprised of cancer cells, cancer-associated fibroblasts (CAFs), and macrophages, we investigated whether NSCLC oncogenotype impacts macrophage phenotype and postulated that the immunosuppressive activity of macrophages is mediated through tumor-secreted soluble molecules. If identified and inhibited, these may re-sensitize cancer cells to immune surveillance and enhance antitumor immunity.

**Methods**
We developed an in vitro co-culture system (patient-derived NSCLC cells, human CAFs, and mouse macrophages) to interrogate impact of NSCLC cells and CAFs on macrophage phenotype. Expression of salient macrophage genes (i.e. ARG1, NOS2, IL-1β, IL-6, CHIL-3, SOCS3) was investigated through species-specific qPCR. Whole-genome RNA sequencing (RNAseq) in select cases was conducted and cytokine arrays measuring expression of 40 inflammatory cytokines were performed. Positive controls included stimulation of macrophages with LPS and IL-4.

**Results**
More than 70 NSCLC cell lines were characterized in the co-culture assay. Three highly reproducible clusters of macrophage phenotypes were identified: high Arginase (immunosuppressive), high IL-1β (inflammatory) and high SOCS3 (inflammatory, involved in JAK-STAT3 pathway) (figure 1).3 4 Major oncogenotypes (i.e. KRAS, TP53, STK11, EGFR, BRAF mutation) did not correlate with macrophage phenotype (figure 2). Analyses of differences between the 3 clusters is ongoing. 10 exemplar NSCLC lines representing each of these 3 clusters were selected for RNA sequencing (mouse genes) and cytokine array protein (human) profiling. Across all clusters, we found suppression of macrophage endocytosis pathways and activation of scavenger receptor A (SRA) signaling, reflecting an M2-like phenotype.5 We also observed increased expression of human IL-6, IL-8, and MCP1, which are implicated in suppression of innate immune sensing of tumor cells (figure 3). RNAseq of CAF lines demonstrated mixed inflammatory and myofibroblastic phenotypes (figure 4), with increased expression of genes associated with macrophage recruitment and activation including: IL-6, CSF-1, CXCL6, CCL2, and CCL7.6

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[Image: Abstract 746 Figure 1](image1)

Three macrophage phenotypes induced in co-culture
Heatmap of mRNA expression from mouse macrophages co-cultured with human NSCLC cells and CAFs. mRNA expression of salient mouse macrophage genes depicted (x-axis) for each NSCLC cell line co-culture (y-axis).

[Image: Abstract 746 Figure 2](image2)

Macrophage phenotype independent of oncogenotype
Percentage of mutations of known human NSCLC oncogenes per mouse macrophage phenotype cluster.
Conclusions Through this novel co-culture model (figure 5), we demonstrate that patient-derived NSCLC cells reproducibly induce three major macrophage phenotypes in an oncotype-independent manner. Furthermore, cytokine release from NSCLC cells and CAFs is implicated in this process. This co-culture model provides a physiologically consistent experimental platform to identify tumor cell and CAF features that drive macrophage phenotype which may be suitable for targeted therapy.

Acknowledgements We thank the McDermott Center Next-Generation Sequencing Core at UT Southwestern. Figure 5 was created with Biorender.com

REFERENCES
cells and regulatory T cells (Treg), as PL consisted of higher numbers of T cells (791.8 Helper T cells/mm², 195.7 Treg/mm²) than LM (626.3 Helper T cells/mm², 121.3 Treg/mm²). However, cytotoxic T cells exhibiting GZB+ and CTLa-4+ were fewer in PL (140.2 cells/mm²) than in LM (203.3 cells/mm²), and the ratio is 0.69. The mean number of GZB+ TIL in PL (32.5 cells/mm²) was lower than in LM (35.3 cells/mm²), and their proportions among total TIL counts were 0.12 and 0.31, respectively. In PL, GZB+: GZB- ratio is 0.16 while the ratio is 1.91 for LM. A fewer number of TILs exhibiting GZB suggests that PL has lower efficiency in immune response than LM. Another crucial checkpoint receptor that inhibits immune response, CTLa-4, was more prevalent in PL, with CTLa-4+: CTLa-4- ratio in Treg being 0.36 in PL, compared to 0.11 in LM. The tumor proportion score (TPS) of PD-L1 was higher in PL than LM (40.0 vs. 6.6).

Conclusions In our study, we showed the differences in the numbers of TIL or regulatory T cells and expressions of immune checkpoint receptors (PD-L1, CTLa-4), which significantly influence outcomes for CIT. The study is ongoing to confirm different IME between the PL and LM groups in a larger tumor cohort.

REFERENCES

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Abstract 748 Figure 1 Mesothelin expression by primary tumor location
A+C Representative low Mesothelin expression at low (X10) (A) and higher power (X20) (C). B+D Representative high Mesothelin expression at low (X10)(B) and higher power (X20)(D). E) Log(x+1) transformed Mesothelin Expression as determined by automated cell counting, median and IQR, all data points shown. Median: 5.5, 79.5, 146.0 for ICC, H-ECC, D-ECC, respective, p-value = 0.025. F-H) Mesothelin Expression determined by visual inspection and scoring for ICC (F), H-ICC (G), and D-ECC (D).

748 MYELOID CELL INFILTRATION CORRELATES WITH PROGNOSIS AND VARIES BASED ON TUMOR LOCATION IN CHOLANGIOCARCINOMA
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Background Cholangiocarcinoma (CC) is a rare malignancy with an increasing incidence and poor prognosis. Immunotherapy represents one potential treatment for CC, however identification of immunotherapeutic targets requires a thorough characterization of the tumor immune microenvironment (TIME). Mesothelin, a tumor associated antigen, is abundantly expressed in other malignancies, though its expression in CC has not been well characterized. We hypothesized that (1) the TIME of CC would vary by primary tumor location and between primary and metastatic lesions, (2) high tumor infiltration by CD8+ T cells and low infiltration by M2 macrophages would be associated with improved survival, and (3) most CC would express mesothelin.

Methods 99 CC tumors from unique stage I-IV patients were included, of which 89 were primary tumors (24 intrahepatic (ICC), 65 extrahepatic (ECC - 30 hilar (H-ECC) and 35 distal (D-ECC))) and 10 were metastatic lesions. Tissue microarrays were constructed and immunohistochemistry (IHC) was performed for lymphoid and myeloid markers, as well as for PD-L1 and mesothelin. IHC+ cells were quantified by automated image analysis. Expression of mesothelin and PD-L1 by tumors cells were evaluated on a semiquantitative scale (0, +1, +2, or +3). Hypothesis testing was performed using Kruskal-Wallis test and survival analyses were performed with Univariate and Multivariate Cox Hazard Models.

Results Most tumors were infiltrated by myeloid cells in addition to CD4+, CD8+, and FoxP3+ T-cells. Mesothelin was expressed (≥1+) in 68% of tumors (figure 1), while PD-L1 was expressed (≥1%) in only 16% of tumors. Higher densities of M1 macrophages (CD68+) were present in D-ECC relative to ICC and H-ECC (figure 2). M1 macrophages were also found in higher densities in metastatic tumors. Mesothelin and granzyme-B expression was significantly higher in D-ECC. Increasing density of myeloid cells (CD14+) and M2 macrophages (CD163+) was associated with worse survival (p= 0.02, 0.03, respectively) (figure 3). Intraepithelial and intratumoral T cell infiltration did not correlate with OS.
Abstract 747 Figure 2  Immune infiltration based on primary tumor location.
Increase in immune infiltrate in primary tumors as distance from liver increases. P-values determined by Jonckheere-Terpstra Test with FDR corrections.

Abstract 748 Figure 3  CD14 and CD163 Correlate with OS.
A+C) Kaplan Meier Curve of OS for (A) CD14 (Median OS: 20 vs. 90 months, log-rank p-value <0.01) and (C) CD163 (Median OS: 15 vs. 32 months, log-rank p-value<0.01). B+D) Multivariate Cox Hazard Models. Assumptions of Cox Hazard Model were checked with Schoenfeld residual values, significance level <0.01.

Conclusions The TIME of CC varies significantly by primary tumor location and between primary and metastatic lesions. D-ECC has a favorable immune profile compared to ICC and H-ECC, with a better milieu for antigen presentation including increased mesothelin and less suppressive macrophages, which may support better response to checkpoint blockade. The data supported the hypothesis that higher densities of intra-tumoral M2 macrophages and myeloid cells correlated with worse OS, even after controlling for clinical variables, suggesting that these cell populations may represent promising immunotherapeutic targets in CC.

Abstracts

SPATIAL PROFILING OF THE TUMOUR MICROENVIRONMENT IN HEAD AND NECK CANCER TO IDENTIFY BIOMARKERS OF RESPONSE TO THERAPY
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Background Immune checkpoint inhibitors (ICI) have shown durable and long-term benefits in a subset of head and neck squamous cell carcinoma (HNSCC) patients. To identify patient-responders from non-responders, biomarkers are needed which are predictive of outcome to ICI therapy. Cues in the tumour microenvironment (TME) have been informative in understanding the tumour-immune contexture.

Methods In this study, the NanoString GemoMx™ Digital Spatial Profiling (DSP) technology was used to determine the immune marker and compartment specific measurements in a cohort of HNSCC tumours from patients receiving ICI therapy.

Results Our data revealed that markers involved with immune cell infiltration (CD8 T-cells) were not predictive of outcome to ICI therapy. Rather, a number of immune cell types (CD4, CD68, CD45, CD44, CD66b) were found to correlate with progressive disease.

Conclusions This study, to our knowledge, represents the first spatial analysis of HNSCC tumours.

Ethics Approval The study was approved by the Queensland University of Technology Ethics Board.

MALAT1 LncRNA CONTROLS METASTATIC REACTIVATION OF DORMANT BREAST CANCER BY IMMUNE EVASION
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Background Long non-coding RNAs (lncRNAs) are involved in various biological processes and diseases. Malat1 (metastasis-associated lung adenocarcinoma transcript 1), also known as Neat2, is one of the most abundant and highly conserved nuclear lncRNAs. Several studies have shown that the expression of lncRNA Malat1 is associated with metastasis and serving as a predictive marker for various tumor progression. Metastatic relapse often develops years after primary tumor removal as a result of disseminated tumor cells undergoing a period of latency in the target organ.1–4 However, the correlation of tumor intrinsic lncRNA in regulation of tumor dormancy and immune evasion is largely unknown.

Methods Using an in vivo screening platform for the isolation of genetic entities involved in either dormancy or reactivation of breast cancer tumor cells, we have identified Malat1 as a positive mediator of metastatic reactivation. To functionally uncover the role of Malat1 in metastatic reactivation, we have developed a knock out (KO) model by using paired gRNA CRISPR-Cas9 deletion approach in metastatic breast and other cancer types, including lung, colon and melanoma. As proof of concept we also used inducible knockdown system under in vivo models. To delineate the immune microenvironment, we have used 10X genomics single cell RNA-seq, ChIRP-seq, multi-color flowcytometry, RNA-FISH and immunofluorescence.

Results Our results reveal that the deletion of Malat1 abrogates the tumorigenic and metastatic potential of these tumors in models of metastatic reactivation and in vivo.

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and supports long-term survival without affecting their ploidy, proliferation, and nuclear speckles formation. In contrast, overexpression of Malat1 leads to metastatic reactivation of dormant breast cancer cells. Moreover, the loss of Malat1 in metastatic cells induces dormancy features and inhibits cancer stemness. Our RNA-seq and ChIRP-seq data indicate that Malat1 KO downregulates several immune evasion and stemness associated genes. Strikingly, Malat1 KO cells exhibit metastatic outgrowth when injected in T cells defective mice. Our single-cell RNA-seq cluster analysis and multi-color flow cytometry data show a greater proportion of T cells and reduce Neutrophils infiltration in KO mice which indicate that the immune microenvironment playing an important role in Malat1-dependent immune evasion. Mechanically, loss of Malat1 is associated with reduced expression of Serpinb6b, which protects the tumor cells from cytotoxic killing by the T cells. Indeed, overexpression of Serpinb6b rescued the metastatic potential of Malat1 KO cells by protecting against cytotoxic T cells.

Conclusions Collectively, our data indicate that targeting this novel cancer-cell-initiated domino effect within the immune system represents a new strategy to inhibit tumor metastatic reactivation.

Trial Registration N/A

Ethics Approval For all the animal studies in the present study, the study protocols were approved by the Institutional Animal Care and Use Committee(IACUC) of UT MD Anderson Cancer Center.

Consent N/A

REFERENCES

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Abstract 752 Figure 1 Immune traits according to BRAF mutation status. (a) Cytolytic activity score(CYT). (b) Infiltration of regulatory T cells(Tregs). (c) PD-L1 expression.

Abstract 752 Figure 2 Immune traits according to grade of differentiation. (a) Cytolytic activity score(CYT). (b) Infiltration of regulatory T cells(Tregs). (c) PD-L1 expression.

Background The use of immune checkpoint inhibitors (ICIs) in cancer treatment has been approved by the FDA, but its application is experimental in the treatment of papillary thyroid cancer (PTC). Induction of immune response via recognition of neoantigens is considered to be the basis for the treatment mechanism of ICIs. However, the neoantigen landscape has not been explored in PTC. Our aim is to investigate the immune landscape of PTC in relation to neoantigens, taking into account the BRAF mutation status and grade of differentiation as contributing factors.

Methods BRAF V600E mutation status and thyroid differentiation scores (TDSs) were gathered from the PTC cohort of The Cancer Genome Atlas (TCGA). TDS was derived from the mRNA expression levels of 16 thyroid function genes to quantify the grade of differentiation. Tumors with TDSs in the 1st quartile and 4th quartile were defined as poorly differentiated and well differentiated, respectively. The neoantigen burden for each sample was predicted using CIBERSORT. The infiltration of immune cells was calculated through CIBERSORT.

Results Among 400 patients with predicted neoantigen data, 187 (47%) had BRAF mutations. The BRAF mutated tumors showed increased cytolytic activity score (CYT, p=0.001), increased infiltration of regulatory T cells (Treg, p<0.001), and higher PD-L1 expression (p<0.001) compared to BRAF wild-type tumors (figure 1). In regard to grade of differentiation, poorly differentiated tumors showed increased CYT (p=0.002), increased infiltration of Treg (p<0.001), and higher PD-L1 expression (p<0.001) compared to well differentiated tumors (figure 2). However, BRAF mutation status or grade of differentiation did not correlate with the neoantigen burden. Also, the neoantigen burden did not show any correlations with immune landscape features such as infiltration of CD8+ T cells or Treg, CYT, and PD-L1 expression.

Conclusions Increased CYT and higher expression of PD-L1 in the BRAF mutated or the poorly differentiated tumors imply the possible role of ICI use in these subgroups of patients. However, the immune response to these subgroups does not seem to be mediated through the increase in neoantigen formation. Further studies are warranted to explore markers for immunotherapy implication.

REFERENCES

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Background Tumors with high tumor mutational burden (TMB) or defects in mismatch repair (dMMR) respond well to immune checkpoint inhibitors (ICIs). TMB and DNA repair gene mutations including dMMR are closely related to the increase of neoantigens, which are recognized by immune cells to trigger an immune response. Although not a standard of care in thyroid cancer treatment, there are ongoing clinical trials for ICI use in differentiated thyroid carcinoma. However, not much has been explored concerning the neoantigen landscape and its association with immune traits in papillary thyroid cancer (PTC). We aim to analyze the immune landscape of PTC in association with neoantigen burden, TMB, and DNA repair gene mutations.

Methods
We used the PTC cohort data from The Cancer Genome Atlas (TCGA). The mutation counts and data for neoantigen prediction were acquired from TCGA mutation calling. CloudNeo pipeline was used for neoantigen prediction. TMB was calculated as the sum of missense and indel mutation counts per megabase pairs covered by whole-exome sequencing. Tumor-infiltrating immune cells were estimated using CIBERSORT.

Results
Out of the 496 PTC patients from cBioPortal, a subset of 400 patients with available mutation counts and predicted neoantigen burden was included in the study. Immune cell infiltration estimated by CIBERSORT showed macrophage M2 as the most abundant, followed by macrophage M0 and other T cells (figure 1). The TMB ranged from 0.03 to 2.05 with a median value of 0.2. Neoantigen burden ranged from 0 to 18 with a median value of 1, which is relatively low compared to the median value of 18 in non-small cell lung cancer (NSCLC) (figure 2). One or more DNA repair gene mutations were discovered in 32 patients (8%). The mutation status of repair genes was not related to TMB or neoantigen burden. TMB or neoantigen burden was not related to immune traits such as infiltration of CD8+ T cells or regulatory T cells, cytolytic activity score, and PD-L1 expression.

Conclusions
This is the first study to report the immune landscape of PTC in the context of neoantigen. The lack of association between TMB or neoantigen burden with immune traits may be due to the relatively low number of neoantigens in PTC compared to other immunogenic cancers such as NSCLC. Our results suggest that mutations in DNA repair genes or TMB are likely to have limited value in predicting response to ICI treatment in PTC.

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treatment ICM in newly diagnosed BC patients (pts) and compare them to healthy controls.

**Methods** Soluble forms of ICM, as well as cytokines and chemokines, were measured using Multiplex® bead array and ELISA technologies. Plasma samples from 98 BC pts and 45 healthy controls were analyzed for each protein. Data was prospectively obtained. Measured levels were compared between BC pts and healthy controls using a non-parametric test (Mann-Whitney).

**Results** Soluble stimulatory molecules GITR (p < 0.000002), GITRL (p < 0.007), CD27 (p < 0.002), CD28 (p < 0.003), CD40 (p < 0.003), CD80 (p < 0.009), ICOS (p < 0.0006) as well as inhibitory molecules PD-L1 (p < 0.0000001), CTLA-4 (p < 0.005), TIM-3 (p < 0.00006), HVEP (p < 0.000002) and TLR-2 (p < 0.05) levels were significantly lower in early BC pts compared to healthy controls. When analyzed according to BC characteristics (TNBC vs. non-TNBC, tumor size, stage, nodal status and age) no significant difference was detected between the soluble levels of these ICM and between the different subsets. Additionally, serum levels of CXCL5 (p < 0.000001), CCL23 (p < 0.04), IL-16 (p < 0.00005), interferon-α (p < 0.03) and IL1-RA (p < 0.03) were significantly lower compared to healthy controls. Serum CX3CL1 or fractalkine (p < 0.024465) was significantly higher compared to healthy controls.

**Conclusions** In the current study, we identified low levels of both stimulatory and inhibitory soluble immune checkpoint molecules in newly diagnosed, non-metastatic BC pts compared to healthy controls. These results indicate that early BC is associated with a down-regulation of both soluble stimulatory and inhibitory immune-checkpoint pathways. Newly diagnosed early BC pts have a generalized immune-suppression independent of subtype and stage, which, to our knowledge, is the first study to describe soluble immune checkpoints in early BC pts.

**Acknowledgements** None

**Trial Registration** N/A

**Ethics Approval** The study was approved by The Research Ethics Committee, Faculty Health Sciences, University of Preetoria, approval number 517/2017.

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**Abstracts**

**756**

**ASSESSMENT OF THE IMMUNE CHECKPOINT LANDSCAPE IN HEAD AND NECK SQUAMOUS CELL CARCINOMA BY SINGLE-CELL RNA SEQUENCING AND MULTISPECTRAL IMAGING**

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**Background** Neutrophils are expanded and abundant in an important fraction (up to 35% of patients) in cancer-bearing hosts. When neutrophils are expanded, they usually promote exert immunomodulatory functions promoting tumor progression and the generation of metastases. Neutrophils can undergo a specialized form of cell death called NETosis that is characterized by the extrusion of their DNA to contain infections. In cancer NETs have been described to promote metastases in mouse models. IL-8, a CXCR1/2 ligand clinically targeted by blocking antibodies, has been described to induce NETosis and is upregulated in many cancer patients. Our hypothesis is that chemokines secreted by cancer cells can mediate NETosis in tumor associated neutrophils and that NETs can be one of the immunomodulatory mechanisms provided by tumor associated neutrophils.

**Methods** NETosis induction of peripheral neutrophils and granulocytic myeloid derived suppressor cells by different chemotactic stimuli, tumor cell supernatants and cocultures upon CXCR1/2 blockade. NET immunodetection in mouse models and xenograft tumors upon CXCR1/2 blockade. In vitro tumor cytotoxicity assays in the presence/absence of NETs, and videomicroscopy studies in vitro and by intravital imaging to test NETs inhibition of immune cytotoxicity by immune-cell/target-cell inhibition. Tumor growth studies and metastases models in the presence of NETosis inhibitors and in combination with checkpoint blockade in mouse cancer models.

**Results** Under the influence of CXCR1 and CXCR2 chemokine receptor agonists and other chemotactic factors produced by tumors, neutrophils, and granulocytic myeloid-derived suppressor cells (MDSCs) from cancer patients extrude their neutrophil extracellular traps (NETs). In our hands, CXCR1 and CXCR2 agonists proved to be the main mediators of cancer-promoted NETosis. NETs wrap and coat tumor cells and shield them from cytotoxicity, as mediated by CD8+ T cells and natural killer (NK) cells, by obstructing contact between immune cells and the surrounding target cells. Tumor cells protected from cytotoxicity by NETs underlie successful cancer metastases in mice and the immunotherapeutic synergy of protein arginine deiminase 4 (PAD4) inhibitors, which curtail NETosis with immune checkpoint inhibitors. Intravital microscopy provides evidence of neutrophil NETs interfering cytolytic cytotoxic T lymphocytes (CTLs) and NK cell contacts with tumor cells.

**Conclusions** CXCR1 and 2 are the main receptors mediating NETosis of tumor associated neutrophils in our in-vitro and in vivo systems expressing high levels of CXCR1 and 2 ligands. NETs limit cancer cell cytotoxicity by impeding contacts with cancer cells.

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cell-to-cell interactions were predicted between heterogeneous cell populations. Histologic inflammation was corroborated with scRNAseq and multiplex flow cytometry. Cell type-specific PD-L1 contributions within the TME were quantified using multispectral imaging.

**Results** Major cell type clusters (immune, epithelial, fibroblast and endothelial cells) were identified. Expression patterns for PD-1, TIGIT, LAG-3 and TIM-3 ligands were evaluated on these suppressive TME cell types. By modeling receptor-ligand interactions between CD8+ T cells and the rest of the major TME cell types, CD8+ T cells were predicted to form more ICR-ICL interactions with tumor-associated macrophages (TAMs) than with any other cell type. With focus on LGALS9/galectin-9 and CD274/PD-L1, flow cytometric analyses validated the scRNAseq observation that both ligands were expressed on TAMs from both inflamed and non-inflamed tumors. Furthermore, flow cytometry and multispectral imaging analyses implicated macrophages as one of the major contributors of CD274/PD-L1 within the TME.

**Conclusions** Our data suggest that in the setting of HNSCC, TAMs are one of the major contributors of ICL in the HNSCC TME. Strategies that selective target this immunosuppressive population may be necessary to break tolerance to PD-1-targeting therapies.

**Ethics Approval** The study was approved by the UPMC Hillman Cancer’s Ethics Board, approval number 99-069.

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### Late-breaking abstracts

#### Biomarkers, immune monitoring, and novel technologies

**757** INTRATUMORAL DELIVERY CD40 AGONIST ANTIBODY VIA NOVEL NANOFLUIDIC DRUG-ELUTING SEED REDUCED TUMOR BURDEN OF MURINE Pancreatic DUCTAL ADENOCARCINOMA

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**Background** Pancreatic adenocarcinoma (PDAC) is associated with extremely poor prognosis and a 5-year survival rate of 10% and remains a lethal malignancy. Surgical resection and combination with chemoradiotherapy are the current standard-of-care options, may improve long-term survival in localized disease; however, the majority of patients are diagnosed at advanced stage. The incorporation of immunotherapy in the treatment algorithm convenes a new era for PDAC treatment. Several immunotherapy approaches have been investigating for treating PDAC such as checkpoint inhibitors, vaccines, adoptive cell therapy, and so on. Immunotherapy has been shown as a promising therapeutic method for many cancer types; however, the complexity and immunosuppressive of the solid tumor microenvironment (TME) results in limited treatment efficacy for PDAC.

**Methods** To sensitize the TME in response to immunotherapy, we developed an implantable intratumoral drug delivery device, Nanofluidic Drug-Eluting Seed (NDES) can be injected via a minimally invasive trocar system that feasible for the clinical setting. NDES has shown efficiently delivered immunotherapy to murine breast cancer model and reduced tumor burden and showed low liver inflammation compared to the intraperitoneal delivery approach in the previous study.¹

² Here, we utilized NDES for the sustained intratumoral delivery of the CD40 antibody. We compared the efficacy of NDES against intraperitoneal and intratumoral administration, which mimics conventional systemic treatment. Tumor growth was investigated for treatment efficacy. Local and systemic immune responses were assessed via flow cytometry.

**Results** NDES delivered CD40 significantly reduced tumor burden, some even achieved tumor clearance. Local NDES CD40 delivery approach showed a systemic increase of CD8+ and CD4+ T cells in the tumor-draining lymph node and spleen by flow cytometry. Furthermore, NDES CD40 treated mice showed an increase of CD8+ and CD4+ central memory T cells locally and systemically. We also investigated the combination with radiotherapy, no significant difference in tumor burden was observed when compared to single-agent CD40 antibody. The results indicated CD40 promotes TME response and improved treatment efficacy.

**Conclusions** These immunological responses demonstrate ‘cold’ to ‘hot’ tumor transformation, which translated to tumor burden reduction. Overall, NDES delivery strategy offers promise for enhancing therapeutic index and transforming the landscape of PDAC tumor therapy.

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**758** IDENTIFICATION OF TUMOR ANTIGEN-SPECIFIC T CELLS IN THE PERIPHERAL BLOOD OF COLORECTAL CANCER PATIENTS

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**Background** Interactions between the immune system and the tumor are now recognized as key determinants of clinical outcome in colorectal cancer (CRC). Immune landscapes have been extensively studied within resected primary tumors and immune markers, such as T cells, have been found to be associated with CRC patients’ survival. Little is known about the immune profile of cells in peripheral blood. We hypothesize that the functional status of T cells, characterized by their response to CRC tumor-associated antigens (TAAs), can be monitored in the peripheral blood of patients and that they have prognostic relevance in CRC.

**Methods** In vitro T cell responses to pools of overlapping peptides representing the TAAs MUC-1, hTERT, NY-ESO-1 and CEA were assessed by analyzing IFN-gamma and TNF-alpha production by CD8+ T cells using flow cytometry, in 5 stage II-III CRC patients just prior to surgical resection and 3 healthy age- and sex-matched controls.

**Results** T cells responding to MUC-1, hTERT, NY-ESO-1 and CEA were present in 3, 3, 1 and 5 CRC patients, respectively, whereas only one response to TAAs (MUC-1) was found in

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one healthy control (figure 1). When TAA responses were pooled together, 83.3% of responders were patients (n=5) and 100% of non-responders were healthy controls (n=2).

Conclusions The presence of circulating T cells responding to CEA in all 5 patients, but also to MUC-1 and hTERT in 3 patients suggests that these TAAs may be good targets for immunotherapy in CRC. Our findings also provide a rationale to investigate the prognostic value of CEA-, MUC-1- and hTERT-specific T cells in the peripheral blood of CRC patients and to consider vaccination with these antigens to boost or induce responses to control residual tumor post-surgery.

Ethics Approval This study was approved by Health Sciences North’s Research Ethics Board; approval number 18-104. http://dx.doi.org/10.1136/jitc-2020-SITC2020.0758

759 DEVELOPMENT OF AN IMPLANTABLE ARTIFICIAL LYMPH NODE AS A THERAPEUTIC CANCER VACCINE

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Background Personalized therapeutic cancer vaccines aim to target and reprogram the host immune system to achieve cancer eradication in situ. Cancer vaccines deliver two main components: immunostimulants and tumor antigens to reduce tumor burden with a robust T cell response; however, none have reached broad clinical success due to difficulty in vaccine administration, ex vivo cellular manipulation, low clinical efficacy and broad administrative barriers. While most efforts to date have focused on repeated bolus administrations, biomaterial-based vaccine strategies have led to promising clinical translation.

Methods In light of these challenges, we have designed a clinically-viable platform-based vaccine strategy, termed the NanoLymph, to provide spatiotemporal elution of immunostimulants and tumor antigens locally to recruit and activate antitumor immunity for cancer eradication. Here, we aim to target the release of granulocyte macrophage colony stimulating factor (GM-CSF) and TLR-7/8 agonist Resiquimod (R848) to promote recruitment and activation of dendritic cells (DCs), a key player in antitumor cytotoxicity.

Results We demonstrate the NanoLymph as an structurally stable and biocompatible immunostimulatory niche for durable DC-driven tumor specific T-cell mediated cytotoxicity. Additionally, we demonstrate the NanoLymph’s ability to recruit and activate immune cells of interest, activating antitumor immunity against model antigen. Thus, we have provided the framework necessary to develop a personalized therapeutic cancer vaccine for tumor-specific T-cell mediated responses necessary to generate immunological memory.

Conclusions Future studies will evaluate immunostimulant and tumor antigen biodistribution in vivo and further apply the NanoLymph in a tumor bearing model to effect antitumor cytotoxicity. Ultimately, we aim to develop a personalized platform applicable for every patient of any cancer type aimed at direct clinical translation.

Ethics Approval This study was approved by the Houston Methodist Research Institute (HMRI), according to protocols approved by the Institutional Animal Care and Use Committee (IACUC). HMRI’s Animal Welfare Assurance number is A4555-01. HMRI assures strict compliance with all federal regulations and guidelines involving the use of laboratory animals in biomedical research.

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760 TROUGH LEVELS OF IPILimumAB IN SERUM AS A POTENTIAL PREDICTIVE BIOMARKER OF CLINICAL OUTCOMES FOR PATIENTS WITH ADVANCED MELANOMA AFTER TREATMENT WITH IPILIMUMAB

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Background Immune checkpoint blockade (ICB) using anti-CTLA-4 and anti-PD-1/PD-L1 has revolutionized the treatment of advanced cancer. However, ICB cures only a fraction of patients, and biomarkers such as PD-L1 expression or CXCL11 have suboptimal sensitivity and specificity. Exposure-response (E-R) relationships have been observed in other therapeutic mAbs. There are many factors that can influence E-R, yet several studies have shown that trough levels of anti-PD-1/PD-L1 correlated with clinical outcomes. Little is known about the potential utility of anti-CTLA-4 levels as a predictive biomarker.

Methods Serum was obtained after doses 2 and 4 from patients with advanced melanoma who received ipilimumab alone (3 mg/kg every 3 weeks for 4 treatments) via an expanded access program. We have successfully established a proteomics assay to measure ipilimumab concentration in serum using a versatile LC-MS/MS-based nano-surface and molecular-orientation limited proteolysis (nSMOL) approach. Serum samples from 38 patients were assessed for the mab concentrations after dose 2 were ranged between 4.44 and 33.63 ug/ml (median:16.30, IQR: 11.41 – 20.87). We found that patients with lower serum trough levels of ipilimumab were more likely to respond to ipilimumab-based ICB (Suppl 3):A1–A559
ipilimumab had poorer overall survival when we grouped patients based on the ipilimumab trough level (figure 1. Median survival: < median = 199.5 days, > median = 519.0 days. Log-rank test: p = 0.0057). A similar result was observed for ipilimumab trough levels after dose 4. We also found that trough levels of ipilimumab inversely associated with CXCL11 (p = 0.0095. R² = 0.1818), a predictive biomarker we previously identified, and soluble CD25 (sCD25) (p = 0.0038. R² = 0.2210), a prognostic biomarker for advanced melanoma but not with other biomarkers such as absolute lymphocyte counts, LDH, VEGF, sMICA, and sMICB.

Conclusions Our results suggest that the trough levels of ipilimumab might be a useful predictive biomarker for the long-term survival of the patients with advanced melanoma treated by ipilimumab. The weak association of ipilimumab trough levels with CXCL11 and sCD25 as well as no association with known biomarkers highlights the potential usefulness of trough levels of ipilimumab as the biomarker. Further studies are required to understand the mechanisms for lower levels of ipilimumab in refractory patients to improve the efficacy of ICB.

Acknowledgements This study was funded by Providence Portland Medical Foundation and Shimadzu Corporation.

Trial Registration NCT00495066

Ethics Approval All patients provided written informed consent and all studies were carried out in accordance with the Declaration of Helsinki under good clinical practice and Institutional Review Board approval.

REFERENCES


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Abstract 760 Figure 1 Poorer OS in patients with lower trough levels of ipilimumab.

Patients with lower serum trough levels of ipilimumab had poorer overall survival when we grouped patients based on the ipilimumab trough level (Median survival: < median = 199.5 days, > median = 519.0 days. Log-rank test: p = 0.0057).

Background Immunotherapy dramatically changed the landscape of melanoma treatment. Even if nearly 40% of patients has a long-term benefit from anti-PD-1 agents, nearly 30% relapse in the first year of treatment, showing in some cases very rapid disease progression. Actually, there are no effective biomarkers that could predict patient’s clinical benefit. Aim of this study is to identify gene profiling biomarkers that could help to select melanoma patients who most likely respond to anti-PD-1 therapy.

Methods We defined as fast responder (FR) or fast progressor (FP) patients who got clinical response or clinical progression within eight weeks from first cycle of therapy. We retrospectively collected data from 51 metastatic melanoma patients (25 FR and 26 FP) treated from October 2016 to June 2020 in first-line with anti-PD1 monotherapy (nivolumab or pembrolizumab) at National Cancer Institute of Naples, Italy. Gene expression profiling analysis was performed using Nano-String® IO 360 panels on PBMCs collected at baseline from 18 patients (10 FR and 8 FP). Patients with ECOG ≥2 were excluded. They were all IV stage (5 M1a, 1 M1b, 12 M1c) of which 15 were B-RAF wild-type (83%) and 3 were B-RAF mutated (17%). Statistical associations between treatment response and gene score variables were estimated through Bonferroni correction for multiple comparisons and Benjamini-Hochberg.

Results Patterns of gene expression were assessed for correlation to response. We compared PBMCs Nanostring analysis between FR and FP patients. We found a higher expression of KRas, CD39, IFI16, IL18, FCGR2A, IL1RN, MAP3K8, TLR5, TLR8, MyD88 and NF-kB in FP patients (all with p-value ≤0.005), most of them related to cell proliferation and immunosuppressive mechanism. Instead we found a higher expression of PRF1, PIK3R1, HLA-DPA1, HLA-DRB1, HLA-DOA, CD45RA, LDHB, KIR3DL2, CD2, CD28, CD7, CD27 in FR patients (all with p-value ≤0.01), most of them related to priming and cytolyis.

Conclusions Our study suggests that a specific gene signature may discriminate FR or FP patients. These preliminary data provide a rationale for further investigating gene profiling signature as a potential biomarker of response to immunotherapy.

Acknowledgements The study was supported by the Institutional Project ‘Ricerca Corrente’ of Istituto Nazionale Tumori IRCCS Fondazione ‘G. Pascale’ of Napoli, Italy.

Ethics Approval The study was approved by the internal ethics board of the Istituto Nazionale Tumori IRCCS Fondazione ‘G. Pascale’ of Napoli Italy, approval number of registry 17/17 OSS.

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A Combination of Functional Biomarkers Improves Identification of the Tumor-Specific Reactive T Cell Repertoire

Background Detecting the entire repertoire of tumor-specific reactive T cells is essential for investigating the broad range of T cell functions in the tumor-microenvironment. At present, assays identifying tumor-specific functional activation measure either upregulation of specific surface molecules, or de novo production of the most common antitumor cytokines or mobilization of cytotoxic granules.

Methods In this study, we combined transcriptomic analyses of tumor-specific reactive tumor infiltrating lymphocytes (TILs), TIL-autologous tumor cell co-cultures and commonly used established detection protocols to develop an intracellular flow cytometry staining method encompassing simultaneous detection of intracellular CD137, de novo production of TNF and IFNγ and extracellular mobilization of CD107a. In this study, we combined transcriptomic analyses of tumor-specific reactive tumor infiltrating lymphocytes (TILs), TIL-autologous tumor cell co-cultures and commonly used established detection protocols to develop an intracellular flow cytometry staining method encompassing simultaneous detection of intracellular CD137, de novo production of TNF and IFNγ and extracellular mobilization of CD107a.

Results This approach enabled the identification of a larger fraction of tumor-specific reactive T cells in vitro compared to standard methods, revealing the existence of multiple distinct functional clusters of tumor-specific reactive TILs. Publicly available datasets of fresh tumor single-cell RNA-sequencing from four cancer types were investigated to confirm that these functional biomarkers identified distinct functional clusters forming the entire repertoire of tumor-specific reactive T cells in situ.

Conclusions In conclusion, we describe a simple method using a combination of functional biomarkers that improves identification of the tumor-specific reactive T cell repertoire in vitro and in situ.

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Expanded and Activated TILs Kill Tumor Cells Enabling IO Compound Assays

Background The Tumor Microenvironment (TME) has a key role in solid tumor therapy screening. We have developed a 3D ex vivo immunosuppressive assay mimicking the TME. It enables both allogenic & autologous tumor lysis by expanded Tumor Infiltrate Lymphocytes (TILs). It is a valuable 3D assay to study the activity of immune therapy drugs in patient sample.

Methods TME-aligned immunosuppressor media was produced by conditioned media from activated human Mesenchymal Stem Cells (hMSC). The TILs were expanded from patient-derived tumor samples and used for tumor killing potential evaluation. Target tumor cells were obtained from different sources: a) Isolated from patient-derived material and frozen until use in experiments with autologous or allogenic TILs or b) Tumor cell lines purchased from ATCC. The cells were mixed according to desired Effector:Target (E:T) ratios and embedded in 3D matrix in presence of TME-aligned media and immune therapy compounds, as Immune Checkpoint inhibitors (ImmChPi). The cell retrieval was performed at the end of desired timepoints and tumor cell killing and TILs activation profile were analyzed by flow cytometry.

Results The in vitro expanded TILs were able to kill allogenic and autologous tumor cells in several different E:T ratios within 24 hours. The% tumor cell killing for allogenic samples of the same cancer type showed a similar range as autologous killing. In a representative autologous E:T experiment we observed 40% of killing at E:T ratio 10:1 (figure 1A). These same TILs showed even higher% tumor killing against allogenic tumor samples (up to 90%, data not shown). The Immune Checkpoint (ImmChP) expression during expansion may change and was followed to select proper expansion timelines. For example, in a particular ovarian cancer sample TIM3 was expressed in 75% of the expanded TILs (figure 1B) and the treatment with TIM3 blocking antibody increased nearly 2-fold tumor cell killing in a dose dependent manner (figure 1C).

Abstract 763 Figure 1

Conclusions The Novel TME-Aligned 3D IO Assay is a reliable and powerful tool to study the mode of action of tumor cells lysis by expanded TILs. Immune Therapy Drugs Screening can be performed in autologous or allogenic E:T conditions, allowing full mode of action description of Bi or Multispecific antibodies, ImmChPi and others, and opens a new door for therapy prediction studies in patient’s material.

Ethics Approval The study was approved by Hospital 12 de Octubre Ethics Board, with approval number 14/199.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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Cellular therapies

**Expansion with IL-15 Increases Cytotoxicity of Vγ9Vδ2 T Cells and is Associated with Higher Levels of Cytotoxic Molecules and T-Bet**

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**Background** Adaptive cell therapy (ACT) is an approved treatment option for certain hematological cancers and has also shown success for some solid cancers. Still, benefit and eligibility do not extend to all patients. ACT with Vγ9Vδ2 T cells is a promising approach to overcome this hurdle.

**Methods** In this study, we explored the effect of different cytokine conditions on the expansion of Vγ9Vδ2 T cells in vitro.

**Results** We could show that Vγ9Vδ2 T cell expansion is feasible with two different cytokine conditions: (a) 1000U/ml interleukin (IL)-2 and (b) 1000U/ml IL-2 + 100U/ml IL-15. We did not observe differences in expansion rate or Vγ9Vδ2 T cell purity between the conditions; however, IL-2/IL-15-expanded Vγ9Vδ2 T cells displayed enhanced cytotoxicity against tumor cells, also in hypoxia. While this increase in killing capacity was not reflected in phenotype, we demonstrated that IL-2/IL-15-expanded Vγ9Vδ2 T cells harbor increased amounts of perforin, granzyme B and granulysin in a resting state and release more upon activation. IL-2/IL-15-expanded Vγ9Vδ2 T cells also showed higher levels of transcription factor T-bet, which could indicate that T-bet and cytotoxic molecule levels confer the increased cytotoxicity.

**Conclusions** These results advocate the inclusion of IL-15 into ex vivo Vγ9Vδ2 T cell expansion protocols in future clinical studies.

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**Contextual Secretion of Nanoscale Interleukin (IL)-12 by CAR T Cells for the Treatment of Cancer**

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**Background** Interleukin (IL)-12 activates T cells and macrophages pivoting the switch that turns chronic into acute inflammation and results in cancer rejection. However, despite formidable antitumor effects in preclinical models, its clinical utilization is limited by severe systemic toxicity. Here, we present a conditional, antigen-dependent, non-editing CRISPR activation (CRISPRa) circuit (RB-2-12) that purposefully induces minimally effective doses of IL-12 for autocrine activation of CAR-T.

**Methods** RB-2-12 is a CAR T cell engineered to express the IL-12 heterodimer via conditional transcription of its two endogenous subunits p35 and p40. The circuit includes a lentiviral constructs encoding an anti-HER2 (4D5) single chain variable fragment, with CD28 and CD3ζ co-stimulatory domains linked to a tobacco etch virus (TEV) pro tease and two single guide RNAs (sgRNA) targeting the promoter region for IL-12A or IL-12B. A second constructs encodes linker for activation of T cells, complexed to nuclease-deactivated/dead Cas9 (dCas9)-VP64-p63-Rta transcriptional activator (VPR) via a TEV-cleavable linker (LdCV). Activation of CAR brings CAR-TEV in proximity to LdCV releasing dCas9 for nuclear localization to the regulatory regions and conditionally and reversibly induce nanoscale expression of the p70 heterodimer. RB-2-12 was compared in vitro to control (cRB-2-12, lacking the IL-12 sgRNAs).

**Results** RB-2-12 induced autocrine production of low concentrations of IL-12 upon exposure to HER2+ FaDu cancer cells resulting in significantly enhanced production of interferon (IFN)-γ, cytotoxic activity and proliferation (figure 1a). These effects were comparable to co-culturing conventional HER2-specific CAR-T cells with a modified FaDu cell line expressing high doses of IL-12 (figure 1b).

**Conclusions** We have previously shown that tandem suppression of PD-1 expression upon HER-2 CAR activation using CRISPR interference enhances anti-cancer properties of CAR-T cells in vivo against HER2-FaDu xenografts by promoting their persistence and long-term tumor colonization (companion abstract submitted to SITC annual meeting). We hypothesize that addition of a Th1 polarizing component such as IL-12 will exponentially increase the efficacy of reprogrammed CAR-T cells by combining enhancement of effector functions to cellular fitness. At the same time, the autocrine effects of nanoscale IL-12 production limit the risk of off-tumor leakage and systemic toxicity. Such cumulative synthetic biology approaches are currently investigated in vitro and in vivo model systems. Current work is testing the effectiveness of RB-2-12 in vivo against FaDu xenografts.

**Acknowledgements** None

**Trial Registration** N.A.

**Ethics Approval** Not Applicable

**Consent** Not Applicable

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1. None

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**NOVEL BIOLUMINESCENT BIOASSAYS FOR THE DISCOVERY AND DEVELOPMENT OF T CELL REDIRECTING CANCER THERAPIES**

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**Background** Two main approaches for T cell-based therapies involve molecular T cell redirection by CD3 bispecific molecules such as bispecific T-cell engagers (BiTE) and cellular T cell redirection by genetic modification of T cells with chimeric antigen receptors (CAR) or transgenic T cell receptors (TCR). BiTEs redirect the cytotoxic activity of endogenous polyclonal T cells by simultaneously engaging CD3 on T cells and tumor antigens on target cells. BiTE potency studies have relied on primary cells, which measure target cell killing through redirected T cell cytotoxicity (RTCC) or cytokine release. However, these primary cell-based assays suffer from high donor-to-donor variability, as well as lengthy and hard to implement protocols.

**Methods** We have recently developed a new RTCC assay and cytokine immunoassays that are simple, sensitive and can quantitatively measure the potency of BiTEs and similar biologics. In this assay, preactivated cytotoxic T cells and target cells (both in cryopreserved thaw-and-use format) stably expressing a HaloTag-HiBiT fusion protein are co-incubated with a BiTE, which results in lysis of the target cells and subsequent release of the Halotag-HiBiT protein. These HiBiT proteins then bind to extracellular LgBiT provided in the detection reagent and form functional NanoLuc Luciferase to generate luminescence.

**Results** The assay is homogenous, highly sensitive, and has a robust assay window. Use of CAR-T has demonstrated promising results in treating leukemia, while the development of TCR-engineered T cells that can recognize intracellular tumor antigens, is still in early stages. To facilitate the screening and characterization of new transgenic TCRs, we used CRISPR/Cas9 to develop two TCRαβ-null reporter T cell lines, which are CD4+ or CD8+. Reinroduction of peptide-specific TCR α and β chains into TCRαβ-null reporter T cell lines results in peptide-dependent TCR activation and luciferase reporter expression. The select expression of CD4 or CD8 in the TCRαβ-null reporter T cell lines can enable the development of transgenic TCRs for both MHCI- and MHCIIdrmed tumor antigen targets.

**Conclusions** Together, these bioluminescent bioassays represent a new set of tools for the discovery and development of T cell-based immunotherapies.

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**INTERFERON GAMMA REDUCES CAR-T EXHAUSTION AND TOXICITY WITHOUT COMPROMISING THERAPEUTIC EFFICACY IN HEMATOLOGIC MALIGNANCIES**

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**Background** Chimeric antigen receptor (CAR) T cell therapy has shown remarkable efficacy in hematologic malignancies, ultimately leading to its FDA approval for relapsed/refractory acute lymphoblastic leukemia and large cell lymphomas in 2017. Despite the success of CAR T cells in the clinic, toxicities such as cytokine release syndrome (CRS) can be severe. Attempts to mitigate these effects have primarily focused on the blockade of macrophage-derived cytokines, such as IL-6 and IL-1B. Herein, we show that the pharmaceutical blockade or genetic deletion of interferon gamma (IFNg, a CAR-T-derived cytokine that strongly correlates with CRS in the clinic, appears to be a viable target for the reduction of CAR-T-associated toxicities.

**Methods** Pharmacologic (blocking antibody) and genetic (CRISPR/Cas9) approaches were used to block IFNg signaling and/or production by CAR T cells. In vitro CAR-T function and cytotoxicity was tested using ELISA, flow cytometry and short-/long-term killing assays prior to their assessment in vivo. NSG mice were injected with Nalm6 or Jeko-1 cancer cells prior to treatment with IFNg-modified CAR-T and tumor size and IFNg production were measured. To determine how the loss of IFNg might affect innate immune cells, CAR-T, macrophages and tumor cells were co-cultured and assessed by flow cytometry, immunofluorescence, Luminex and RNA sequencing.

**Results** IFNg could be blocked using a novel anti-IFNg antibody or CRISPR/Cas9 editing of the CAR T cells without affecting T cell activation, proliferation or cytokine production (IL-2, TNFα, GM-CSF). Successful blockade of the IFNg signaling pathway was confirmed by reduced phosphorylation of JAK1, JAK2 and STAT1, even in the presence of exogenous IFNg. Loss of IFNg did not reduce the cytotoxic potential or persistence of CAR-T against hematologic malignancies in vitro or in vivo. When cultured with macrophages and cancer cells, IFNg knockout (IFNgKO) CAR-T yielded decreased levels of IL-1B, IL-6, IL-13, MCP1 and CXCL10, indicating a reduction in macrophage activation induced by CAR-T in the absence of IFNg. Serum from tumor-bearing mice treated with IFNgKO CAR-T elicited lower activation of macrophages in vitro compared to those treated with IFNg-producing CAR-T cells. Furthermore, IFNgKO CAR T cells co-cultured with tumor cells and macrophages demonstrated less exhaustion as shown by reduced expression of PD1, Tim3 and Lag3 and increased IFNgKO CAR-T expansion.

**Conclusions** Collectively, these data suggest that IFNg is not required for the efficacy of CAR-T in hematologic malignancies and can potentially be targeted to reduce toxicity and enhance CAR-T efficacy and persistence in the clinic.

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**RE-DIRECTED T CELL THERAPY TO CONTROL INVASIVE ASPERGILLOSIS**

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**Background** Opportunistic invasive fungal infections (IFI) are a major threat to immunocompromised populations such as patients with acute myeloid leukemia (AML) and allogenic hematopoietic stem cell transplant (HSCT) recipients(1,2). Specifically, Aspergillus fumigatus (AF) is responsible for high morbidity and mortality in cancer patients. As antifungal therapy has limited efficacy in immunocompromised patients, we
sought to develop fungus-specific chimeric antigen receptor (CAR) T cells as a novel immune augmentation strategy to treat IFIs including invasive aspergillosis. To target fungal pathogens, we fused the pattern-recognition receptor Dectin-1 to activate T cells via chimeric CD28 and CD3-ζ domains upon binding to β-1,3-glucan carbohydrates in the fungal cell wall(3). The generated Dectin-1 CAR+ T cells showed high specificity for β-1,3-gucan and inhibited the growth and branching of AF germlings in an in vitro co-culture assay. However, we found poor efficacy of Dectin-1 CAR+ T cells against mature AF hyphae, likely due to changes in the fungal cell wall that hamper T-cellular binding to β-1,3-glucan carbohydrates. To overcome this limitation, we have recently developed an AF-specific CAR (AF-CAR) based on a monoclonal antibody which recognizes a surface epitope of mature AF hyphae. Methods Lentiviral vectors were used to generate AF-CAR expressing T cells from human peripheral blood mononuclear cells. Heat killed AF-293 hyphae was used for co-culture studies with No DNA T cells, and AF-CAR expressing T cells. Cell clusters, binding with AF hyphae were noticed in AF-CAR incubated wells whereas no such cell cluster were observed in NoDNA T cells incubated wells. Results When co-incubated with AF hyphae, AF-CAR+ T cells efficiently targeted mature hyphae and formed lytic synapses with hyphal filaments. The released cytolytic granules damage hyphae and controls branch node formation. Furthermore, exposure to AF hyphae stimulated significant upregulation of activation markers CD69 and CD154 on AF-CAR+ T cells. The activated CAR T cell secretes proinflammatory cytokines which can boost innate immune system to fight against IFI. Conclusions In summary, these results indicate that we have successfully generated a novel anti-Aspergillus CAR construct with good in vitro targeting efficacy against mature AF hyphae. After thorough evaluation of fungicidal activity, cytokine release patterns, and release of cytotoxic mediators, we plan to embark on preclinical tolerability and efficacy studies in a murine model of invasive pulmonary aspergillosis. Thus, we report the production of Aspergillus specific CAR T cells to provide long term protection to immunocompromised patients, such as AML patients and HSCT recipients, from invasive Aspergillus infections. Acknowledgements This study was supported by NIAID-R33 AI127381. Ethics Approval This study was approved by IBC committee, University of Texas MD Anderson Cancer Center, Houston, Texas, 77030. REFERENCES 1. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld AG, Anais- sie EJ, Brouillette LM, Herrwaldt L, Ito J, Kontoyiannis DP, Lyon GM, Marr KA, Morison VA, Park BI, Patterson TF, Perl TM, Oster RA, Schuster MG, Walker R, Walsh TJ, Wannemuehler KA, Chiller TM. Invasive fungal infections among organ transplant recipients: results of the transplant-associated infection surveillance network (TRANSNET). Clin Infect Dis 2010;50(8):1101–11. 2. Bhant VR, Viola GM, Ferrajoli A. Invasive fungal infections in acute leukemia. Ther Adv Hematol 2011;2(4):231–47. 3. Kumaresan PR, Manuri PR, Albert ND, Maiti S, Singh H, Mi T, Roszik J, Rabino- vich B, Olivares S, KrishnaMurthy J, Zhang L, Najjar AM, Huls MH, Lee DA, Champlin RE, Kontoyiannis DP, Cooper LJ, Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection. Proc Natl Acad Sci U S A 2014;111(29):10660–5. http://dx.doi.org/10.1136/jitc-2020-STIC2020.0768
Abstract 771 Figure 1

Initial culturing of four primary MFS tumor cases with complete media (CM) over 4 weeks. Ten total cases were selected, five cases for each UPS and MFS sarcoma subtypes. To date, four MFS cases #164, 207, 214, and 225 have been processed. TIL populations were identified and categorized based on their growth rates and labelled as ‘fast’ or ‘slow’ growing TILs, respectively. ‘A’ and ‘B’ represent technical replicates. Population TIL 164 ‘A’ has no replicates. 15 populations were derived from the four MFS cases. TILs were cultured and expanded from tumor fragments in CM over 4 weeks in duration. CM consisted of Iscove’s Modified Dulbecco’s Medium, 6000 IU/mL IL-2, 10% human serum albumin, 25 mmol/L L-glutamine, 5.5x10-5 mol/L β-mercaptoethanol, 100 IU/mL penicillin, and 100 µg/mL streptomycin. At week 4, cells were collected and counted with a hemocytometer. Only 6 populations achieved ≥ 1x106 cells and are categorized as high initial cell count populations, 9 populations achieved <1x106 cells and are categorized as low initial cell count populations. The 9 low initial cell count populations were further numbered with specific cell counts in figure 1 for clarity. These cell yields, with the exception of TIL 207 1A and 1B, are insufficient for characterization experiments.

Background Sarcoma is a group of rare bone and soft tissue tumors with over 50 distinct subtypes. Survival rate ranges widely due to the lack of efficacious treatments. Immunotherapy, such as adoptive cell therapy (ACT), has drawn great interest due to its minimal toxicity. In ACT, tumor infiltrating lymphocytes (TILs) are isolated from patients, expanded, and autologously reinfused back. We recently observed TIL’s presence in Undifferentiated Pleomorphic Sarcoma (UPS) and Myxofibrosarcoma (MFS) tumors and found that tumor’s PD-L1 overexpression is correlated with better clinical outcome in UPS but not MFS.1 The Thelper1 inflammatory pathway was highly activated in the former subtype, which may explain the better outcome. These results illustrate the immunological differences where TILs may play a critical role. We hypothesize that there are phenotypic and functional differences between TILs of UPS and MFS that may be related to clinical outcomes. Sarcoma TILs are rare and challenging to culture.

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1Jacky Chen*, 2Jay Wunder, 3Nialan Gokgoz, 1Irene Andrus, 1Lunenfeld Tanenbaum Research Institute, Toronto, Canada; 2Sinai Health System, Toronto, Canada


770 ANALYSIS OF GUT MICROBIOME IN PATIENTS RECEIVING ADOPTIVE T-CELL THERAPY (ACT) ACROSS DIFFERENT SOLID TUMOUR TYPES

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Background Tumour Infiltrating Lymphocytes (TILs) is a modality of ACT under development in solid tumours. Unfortunately, prior lymphodepletion is a key step that frequently requires the administration of antibiotics and antifungics for long periods of time. Although there is evidence that gut microbiome may influence tumour response in patients treated with checkpoint-inhibitors, it has not been extensively studied in ACT.1

Methods Analysis of gut microbiome at three different times (T1: before lymphodepletion, T2: before TIL infusion and T3: day +15) has been performed in patients treated with ACT between 2018 and 2020. The composition and structure of the sampled microbial communities was assessed through the amplification and sequencing the V3-V4 variable regions of the 16S rRNA gene. The Illumina Miseq sequencing 300×2 approach was used. Taxonomic assignment of phylotypes was performed using a Bayesian Classifier trained with Silva database version 132 (99% OTUs full-length sequences). The following metrics were measured: observed OTUs (community richness), evenness (Pielou’s index) and Shannon’s diversity index. Differential abundance of taxa was tested using ANCOM test and Kruskal Wallis test.

Results A total of 21 patients have been treated with TILs between 2018 and 2020 at our institution. 67% were female. Median age was 43 (range 26–70 years). All patients had stage IV pre-treated solid tumours: 55% cervical cancer, 33% melanoma, 10% lung adenocarcinoma and 5% head and neck cancer. Median previous treatment lines was 3 (range 2–4). Analysis of gut microbiome has been performed in 3 of these patients: one achieved PR, one progressed and the third one suffered an unexpected death. 971 phylotypes were detected. Analysis revealed differences in terms of observed OTUs, evenness and Shannon’s index when comparing T1 and T2 with T3. At T3 a tendency towards less diversity and evenness was observed when compared with T1 and T2 (H 3.0, p-value 0.083, not statistically significant). Comparing the distribution of considered taxa in ACT responders vs. non-responders, we observed significant differences for both class (Bacteroidia, Clostridia and Gammaproteobacteria) and order (Bacteroidales, Lactobacillales, Clostridiales and Enterobacteriales) levels.

Conclusions A deep change in gut microbiome composition along TILs therapy was observed. Though preliminary, differences between responders and non-responders were observed but should be confirmed in larger populations.
Abstract 771 Figure 2
REP treatment of populations with high initial cell count over 3 weeks. This graph corresponds to Table 1. 6 out of 15 populations achieved \( > 1 \times 10^6 \) cells after 4 weeks of initial culturing and were subsequently REP-treated with anti-CD3/anti-CD28 coated magnetic Dynabeads (Life Technologies) on 96-well plates. REP-treated populations have trendlines with positive slopes across the 3 weeks of REP expansion, indicating positive growth rates. Negative controls (-) without REP treatments have near-flat trendlines indicating lack of growth. REP was successful in expanding all 6 TIL populations. At week 3 post-REP, cells were collected and counted via hemocytometer.

which impedes their studies. We first aim to robustly expand TILs to sufficient numbers.

Methods TILs are being expanded and cultured from UPS and MFS primary tumors with various PD-L1 levels. To initiate TIL culturing, bulk tumors were fragmented into 1mm, seeded at 1 fragment/well, and cultured in interleukin-2 supplemented complete media. Due to insufficient cell yields for characterization, rapid expansion protocol (REP) with anti-CD3/anti-CD28 co-stimulating beads was subsequently employed for further expansion.

Results Of 4 MFS cases processed to date, 15 TIL populations were derived and cultured (figure 1). Only 6 in 15 TIL cultures obtained \( > 1 \times 10^6 \) cells and are considered high initial cell count populations. 9 in 15 cultures obtained \(< 1 \times 10^6 \) cells and are considered low initial cell count populations. REP successfully expanded 14 out of 15 TIL populations, each obtaining between 7.8 to 268.0 \( \times 10^6 \) cells (tables 1 and 2, figures 2 and 3).

Conclusions Sarcoma infiltrates are difficult to culture and their roles remain largely unstudied. Our results demonstrate anti-CD3/anti-CD28 co-stimulation’s capability in expanding 93.3% of TILs and established a robust method of expansion. Future investigation of lineage markers, cytokine profiles, and cytotoxicity aims to identify immunological differences between UPS and MFS. TILs will be primed with memory-inducing cytokines (IL-7, IL-12, IL-15, IL-21) to modulate their capabilities or robustness. Negative controls (-) were only allocated for certain populations with sufficient initial cell availability.

Abstract 771 Table 1
REP treatment of populations with high initial cell count over 3 weeks. Table 1 corresponds to graphed analysis in figure 2. 6 populations that achieved \( > 1 \times 10^6 \) cells after 4 weeks of initial culturing were treated with anti-CD3/anti-CD28 coated beads on 24-well plates over 3 weeks. Total cells seeded before REP, total cells collected after REP, and fold-expansion calculated are shown. At week 0, all populations were seeded at a density of 1x10^6 cells per well, except for TIL 164 1A and TIL 164 1B which were seeded at 1.1x10^6 cells and 1.4x10^6 cells per well, respectively. Populations were expanded via addition of beads at a bead to cell ratio of 1:1. At week 3 post-REP, cells were collected, counted, and fold-expansion was determined. Cell counts performed via hemocytometer. Negative controls (-) were only allocated for certain populations with sufficient initial cell availability.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Total Cells Seeded (( \times 10^6 ))</th>
<th>Total Cells Collected (( \times 10^6 ))</th>
<th>Fold-Expansion</th>
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<td>TIL 164 1A</td>
<td>2.2</td>
<td>103.0</td>
<td>46</td>
</tr>
<tr>
<td>TIL 164 1B</td>
<td>1.4</td>
<td>146.0</td>
<td>103</td>
</tr>
<tr>
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<td>22</td>
</tr>
<tr>
<td>TIL 207 1B</td>
<td>7.0</td>
<td>208.0</td>
<td>38</td>
</tr>
<tr>
<td>TIL 225 1A</td>
<td>5.0</td>
<td>64.0</td>
<td>13</td>
</tr>
<tr>
<td>TIL 225 1B</td>
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<tr>
<td>TIL 207 1A (-)</td>
<td>2.0</td>
<td>8.0</td>
<td>4</td>
</tr>
<tr>
<td>TIL 207 1B (-)</td>
<td>2.0</td>
<td>12.7</td>
<td>6</td>
</tr>
<tr>
<td>TIL 225 1A (-)</td>
<td>2.0</td>
<td>4.5</td>
<td>2</td>
</tr>
<tr>
<td>TIL 225 1B (-)</td>
<td>2.0</td>
<td>4.3</td>
<td>2</td>
</tr>
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</table>

Abstract 771 Table 2
REP treatment of populations with low initial cell count over 4 weeks. Table 2 corresponds to graphed analysis in figure 3. 9 populations that achieved \(< 1 \times 10^6 \) cells after 4 weeks of initial culturing were treated with anti-CD3/anti-CD28 coated beads on 96-well plates over 4 weeks. Total cells seeded before REP, total cells collected after REP, and fold-expansion calculated are shown. At week 0, all populations were seeded at a density of 5x10^4 cells per well, except for populations with \(< 8 \times 10^4 \) cells in total, which were seeded at all available cells per well. For example, TIL214 1B was seeded at 6x10^4 cells per well. Populations were expanded via addition of beads at a bead to cell ratio of 1:1; once at week 0 and once more as re-stimulation at week 2. At week 4 post-REP, cells were collected, counted, and fold-expansion was determined. TIL225 2B did not yield any growth under 10X light microscope observation. Lack of cells and cell debris was observed; hence this population was not collected. Cell counts performed via hemocytometer. Negative controls were not established due to constraints with initial cell availability.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Total Cells Seeded (( \times 10^6 ))</th>
<th>Total Cells Collected (( \times 10^6 ))</th>
<th>Fold-Expansion</th>
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<tr>
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<td>120</td>
<td>24.3</td>
<td>203</td>
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<tr>
<td>TIL 207 2B</td>
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</tr>
<tr>
<td>TIL 214 3B</td>
<td>25</td>
<td>20.6</td>
<td>824</td>
</tr>
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<td>38.8</td>
<td>162</td>
</tr>
<tr>
<td>TIL 225 2B</td>
<td>0</td>
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</table>
determination state and enrich cellular stemness. This would enhance TILs in vivo anti-tumor activity and prolong their survival. Elucidating TILs and their relations with tumor’s PD-L1 expression would allow clinicians to appropriately recognize sarcoma’s tumor immune environments and select the most desirable infiltrates for superior ACT.

Ethics Approval
The study was approved by Mount Sinai Hospital’s Ethics Board, approval number 01-0138-U.

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A POTENT AND OFF-THE-SHELF ONK CELL THERAPY PRODUCT TARGETS HER2+ CANCER CELLS AND RESISTS SUPPRESSIVE TUMOR MICROENVIRONMENT

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Background
Autologous or allogeneic natural killer (NK) cells possess efficient cytotoxicity against tumor cells without severe side effects such as CRS or graft-versus-host disease (GvHD). In addition to chimeric antigen receptor (CAR) strategy, antibody-body conjugates (ACC) platform provides more efficient way to arm NK cells with binding specificity and enhanced potency against target cells. In this work, we develop a NK cell therapy product ACE1702, a novel NK cell line oNK conjugated with trastuzumab, and assess its potency against HER2+ solid tumors.

Methods
oNK cells were covalently conjugated with monoclonal antibody Trastuzumab after sublethal irradiation by our patented antibody-cell conjugates (ACC) platform to become our cryopreserved final product ACE1702 compliant with current good manufacturing practice (cGMP). Function of ACE1702 was validated by real-time xCELLigence analyzer and MTT assay in vitro. Efficacy of intraperitoneally (ip.) delivered ACE1702 was evaluated in tumor-bearing female immune compromised NSG mice. Characterization of ACE1702 was analyzed by flow cytometry.

Results
We demonstrated that the trastuzumab-armed oNK cells, ACE1702, exerted human epidermal growth factor 2 (HER2) binding specificity and enhanced cytotoxicity against various types of cancer cells with different grade of HER2 expressions compared to control oNK cells in vitro. In vivo results in human ovarian cancer cell line SK-OV-3-bearing xenograft mouse model further supported the in vivo observations. Of note, ACE1702 also displayed a better cytotoxicity against HER2+ cancer cells than trastuzumab and its derived antibody-drug conjugate. ACE1702 also remained cytotoxicity against cancer cells in the suppressive tumor microenvironment. Characterization revealed a preferential expression of cell membrane proteins responsible for NK activity capacitated ACE1702 with enhanced cytotoxicity. These results underscore the potency of ACE1702 in eradication of cancer cells.

Conclusions
Here we introduced a novel trastuzumab-modified oNK cell product with enhanced specificity against myriad types of HER2+ cancers. Selective conjugation of trastuzumab with membrane proteins contributing to NK activation conferred ACE1702 with enhanced cytotoxicity even in the suppressive tumor microenvironment.

Acknowledgements
None

Trial Registration
None

Ethics Approval
The animal study was conducted according to protocols approved by the Institutional Animal Care and Use Committee of Muragenics.

Consent
None

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ADOPTIVE CELL THERAPY RESPONSE IN MELANOMA IS MEDIATED BY STEM-LIKE CD8 T CELLS

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Background
Adoptive T cell therapy (ACT) utilizing ex vivo-expanded autologous tumor infiltrating lymphocytes (TILs) can result in complete regression of human cancers. Successful immunotherapy is influenced by several tumor-intrinsic factors. Recently, T cell-intrinsic factors have been associated with immunotherapy response in murine and human studies. Analyses of tumor-reactive TILs have concluded that anti-tumor neoantigen-specific TILs are enriched in subsets defined by the expression of PD-1 or CD39. Thus, there is a lack of consensus regarding the tumor-reactive TIL subset that is directly responsible for successful immunotherapies such as ICB and ACT. In this study, we attempted to define the fitness landscape of TIL-enriched infusion products to specifically understand its phenotypic impact on human immunotherapy responses.

Methods
We compared the phenotypic differences that could distinguish bulk ACT infusion products (I.P.) administered to patients who had complete response to therapy (complete responders, CRs, N = 24) from those whose disease progressed following ACT (non-responders, NRs, N = 30) by high dimensional single cell protein and RNA analysis of the I.P. We further analyzed the phenotypic states of anti-tumor neoantigen specific TILs from patient I.P (N = 26) by flow cytometry and single cell transcriptomics.

Results
We identified two CD8+ TIL populations associated with clinical outcomes: a memory-progenitor CD39-negative stem-like TIL (CD39-CD69+) in the I.P. associated with complete cancer regression (overall survival, P < 0.0001, HR = 0.217, 95% CI 0.101 to 0.463) and TIL persistence, and a terminally differentiated CD39-positive TIL (CD39+CD69+) population associated with poor TIL persistence post-treatment. Although the majority (>65%) of neoantigen-reactive TILs in both responders and non-responders to ACT were terminally differentiated CD39-positive TIL (CD39+CD69+) population associated with poor TIL persistence post-treatment. Additionally, the majority (>65%) of neoantigen-reactive TILs in both responders and non-responders to ACT were found in the differentiated CD39+ state, CR infusion products also contained a pool of CD39- stem-like neoantigen-specific TILs (median = 8.8%) that was lacking in NR infusion products (median = 23.6%, P = 1.86 x 10-5). Tumor-reactive CD8 T cells were capable of self-renewal, expansion, and persistence, and mediated superior anti-tumor response in vivo.

Conclusions
Our results support the hypothesis that responders to ACT received infusion products containing a pool of stem-like neoantigen-specific TILs that are able to undergo prolific
expansion, give rise to differentiated subsets, and mediate long-term tumor control and T cell persistence, in line with recent murine ICB studies mediated by TCF+ progenitor T cells. Our data also suggest that TIL subsets mediating ACT-response (stem-like CD39-) might be distinct from TIL subsets enriched for anti-tumor-reactivity (terminally differentiated CD39+) in human TIL.

Acknowledgements We thank Don White for curating the melanoma patient cohort, and J. Panopoulos (Flowjo) for helpful discussions on high-dimensional analysis, and NCI Surgery Branch members for helpful insights and suggestions. S. Krishna acknowledges funding support from NCI Director’s Innovation Award from the National Cancer Institute.

Trial Registration NA

Ethics Approval The study was approved by NCI’s IRB ethics board.

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Checkpoint blockade therapy

774 ANTIBIOTIC ADMINISTRATION PRIOR TO IMMUNOTHERAPY LEADS TO POOR OVERALL SURVIVAL ACROSS MULTIPLE MALIGNANCIES

Abstract 774 Figure 1 Antibiotics up to six months before ICI reduce OS.

Figure 1: Antibiotics Prior to Checkpoint Inhibitor Therapy Lead to Inferior Overall Survival: Analysis of patients treated with any type of antibiotic lead to worsened overall survival compared to those who did not receive antibiotics or who received a one time dose of cefazolin. Statistical analysis showed by both Log Rank and Wilcoxon testing p-values were <0.0001 with median survival 6.5y vs. 2.3y for those who received antibiotics prior to ICI treatment (HR 3.4, 95% CI 2.2 to 5.3).

Abstract 774 Figure 2 Antibiotics up to six months before ICI reduce PFS.

Figure 2: Antibiotics Prior to Checkpoint Inhibitor Therapy Lead to Inferior Progression-Free Survival: Analysis of patients treated with any type of antibiotic lead to worsened progression-free survival as well compared to those who did not receive antibiotics or who received a one time dose of cefazolin. Statistical analysis showed by both Log Rank and Wilcoxon testing p-values were 0.0027 and 0.0011 respectively. Median PFS from initiation of immunotherapy was 1.1y vs. 0.46y for those who received antibiotics prior to ICI treatment (HR 1.7, 95% CI 1.2 to 2.5).
common ICIs were pembrolizumab, nivolumab, followed by ipilimumab-nivolumab, ipilimumab, and durvalumab. 81 patients of 218 received antibiotics within 6 months of receiving checkpoint inhibitors. Of antibiotics administered, the most common classes were cephalosporins (86%), fluoroquinolones (28%), and glycopeptides (23%) with substantial overlap. Overall survival and progression-free survival was improved for those who did not receive antibiotics prior to ICI therapy (median OS 6.5 vs. 2.3 years, HR 0.36, p<0.0001; median PFS 1.1 vs 0.5 years, HR 0.6, p=0.0027) (figure 1 and 2 respectively). Linear regression showed no significant association between antibiotic use prior to ICI use and age, sex, race, ICI type, or ECOG status.

Conclusions This data adds to the growing body of knowledge that the use of antibiotics prior to ICI treatment leads to inferior overall and progression-free survival.

Acknowledgements We would like to thank the Roman Jan- darov, UC Cancer Center, the University of Cincinnati Division of Hematology and Oncology, Department of Internal Medicine of the University of Cincinnati, and the University of Cincinnati Medical Center for their continued support.

Ethics Approval IRB 2019-0610

REFERENCES

RARE CASE REPORTS ON THYMIC CARCINOMA PATIENTS TREATED WITH PEMBROLIZUMAB

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Background Thymomas and thymic carcinomas (TC) are intrathoracic malignancies that, although rare, represent the most common anterior mediastinal tumors in adults (comprising approximately 0.2-1.5% of all malignancies).1 Recent accumulating evidence on immune checkpoint pathways suggests that immunotherapy might be a promising therapeutic option for refractory TCs.2 We herein report two cases of a pembrolizumab-treated TC patient.

Methods Immunotherapy is accessible through individual permission for the treatment of TC in Hungary. We retrospectively collected data of two TC patients treated with pembrolizumab in one institute.

Results The first patient was a 66-year-old woman. Squamous cell TC was diagnosed in her left parasternal region. She was a former smoker, had no history of autoimmune disorders, and had no associated symptoms at the time of diagnosis. She underwent open thymectomy. The histology proved type C (WHO classification) TC with a pathological TNM of T1aN0 and microscopic-positive margins. Consequently, the patient received 60 Gy of postoperative radiotherapy. Nine months after the surgery local recurrence and multiple hepatic metastases appeared. Six cycles of chemotherapy (ADOC regimen) were introduced as first-line systemic therapy which resulted in stable disease (SD) for 8 months. Pembrolizumab was administered as second-line treatment (200 mg every 3 weeks) for 6 cycles. The best result was SD. Due to progression, third-line docetaxel treatment was initiated. Shortly after, ptosis and diplopia developed. Myasthenia gravis was diagnosed, and third-line chemotherapy was judged ineffective. The second patient was a 63-year-old man. He was diagnosed with unresectable TC and treated with chemotherapy (ADOC regimen) up-front. After six cycles the tumor regressed, and surgery was performed with R2 result. Postoperatively, the patient was given six cycles of chemotheraphy (cisplatin/etoposide) and radiotherapy. Six months later local progression was detected and pembrolizumab was commenced. Eight cycles of pembrolizumab produced SD as best response. No immune-related adverse effects (irAEs) were detected. After progression Sunitinib therapy was started. In both cases, additional immuno-histochemistry investigations were performed.

Conclusions In the literature, there is no phase 3 trial on immune-checkpoint inhibitor (ICI) therapy of TC. Phase 2 trials reported promising results with pembrolizumab.3–4 However, there are conflicting results with other ICIs.5 Before starting, it is important to rule out autoimmune disorders to evade serious, even life-threatening immune-complications. The high likelihood of irAEs in TC also underpin the importance of predictive biomarkers. Further studies are required to evaluate the efficacy and safety of immunotherapy in TC.

Acknowledgements The authors thank the multidisciplinary clinical teams involved in the treatment and management of the patient.

REFERENCES

A ROLE FOR IMMUNE CHECKPOINT BLOCKADE TO ENHANCE T CELL-MEDIATED RESPONSES IN COMBINATION WITH CHEMOTHERAPY IN OESOPHAGEAL ADENOCARCINOMA

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Background Combining immune checkpoint inhibitors (ICIs) with immunogenic chemotherapies is a promising approach in
oesophageal adenocarcinoma (OAC) to convert ‘cold’ tumours to ‘hot’ tumours expanding the efficacy of ICIs to a greater spectrum of patients. However, there is a vast array of immune checkpoints (ICs) expressed by T cells and the effect of ICIs in combination with chemotherapy regimens is largely unknown.

Methods The expression profile of a range of ICs on circulating and tumour-infiltrating T cells was assessed using flow cytometry prior to and post-neoadjuvant treatment and correlated with clinical parameters (n=20). PBMCs isolated from OAC blood were treated with single agent ICIs alone (single agent anti-PD-1, anti-PD-L1, anti-A2aR and anti-TIM-3 inhibition) and in combination with FLOT (5-Fluorouracil, oxaliplatin and docetaxel) and CROSS (carboplatin and paclitaxel) chemotherapy regimens. The production of anti-tumour cytokines by T cells was assessed in vitro by flow cytometry (n=6).

Results In the treatment-naïve and post-treatment setting, a range of ICs were expressed by circulating T cells and were significantly increased on tumour-infiltrating T cells, which included PD-L1, PD-L2, CD160, PD-1, CTLA-4, TIGIT, TIM-3, LAG-3, A2aR and ICOS (p<0.05) (figure 1). Pre-treatment circulating PD-1+ T cells positively correlated with pathological nodal status (p<0.05), (figure 2). Whereas tumour-infiltrating CD3+CTLA-4+ cells positively correlated with nodal metastasis and lymphovascular invasion (p<0.05). The percentage of tumour-infiltrating CD3+CTLA-4+ and CD3+ICOS+ cells was significantly lower post-neoadjuvant treatment (p<0.05) (figure 3). However, post-neoadjuvant treatment circulating CD3+PD-1+ cells and CD3+CD4+TIGIT+ cells positively correlated with a better treatment response, determined by PET/CT (p<0.05), (figure 4). ICIs enhanced T cell production of anti-tumour cytokines IL-2 and IFN-y alone and in combination with chemotherapy in vitro from treatment-naïve OAC patients (p<0.05).

Conclusions T cells expressing ICs in circulation and infiltrating OAC tissue were adverse prognostic markers in the pre-treatment setting, perhaps due to their role in enabling tumour immune evasion and subsequent tumour progression. In contrast, T cells expressing ICs post-chemotherapy treatment in peripheral circulation were favorable prognostic markers. ICs are typically expressed by ‘hot’ tumours there-
Abstract 778

PRECLINICAL STUDY USING A GLUTAMATERGIC SIGNALING AND IMMUNE-CHECKPOINT INHIBITORS IN A SPONTANEOUS MELANOMA PRONE MOUSE MODEL

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Background Much progress has been made in understanding melanoma pathogenesis within the last few years through targeted therapies and immunotherapies. However, resistance to small molecule inhibitors remains an obstacle. Immunotherapies such as checkpoint inhibitors against PD-1/PD-L1 lead to durable responses but only in a subset of melanoma patients. Mouse models reflecting human cancers provide invaluable tools towards the translation of basic science discoveries to clinical therapies, but many of these in vivo studies are short-term and do not accurately mimic patient circumstances. Our lab has a melanoma-prone transgenic mouse model which is driven by ectopic expression of a normal neuronal receptor, metabotropic glutamate receptor 1 (GRM1). This mouse model recapitulates melanoma development and progression frequently associated with melanoma patients, where aberrant GRM1 expression is detected. We have shown that in >90% of late-stage melanoma patients, there is atypical GRM1 mediated signaling and expression.

Methods In this study, we are using these mice, TGS, to determine the long-term, 18-week, therapeutic consequences of troriluzole, a prodrug for riluzole, which is an inhibitor of glutamatergic signaling plus anti-PD-1, an immune-checkpoint inhibitor. Tumor burden is monitored every 6 weeks for 18 weeks using a small imaging system, IVIS and tumor burden is quantified using ImageJ software. Blood, lymphoid, and tumor samples were collected at several time points during the study for molecular, and immune analyses.

Results Preliminary results suggest a gender-biased treatment response and that the combination of troriluzole and anti-PD-1 is more efficacious than either agent alone. In males, a 43.9% reduction in tumor burden was observed while in females there was a 29.6% increase in tumor burden in the combination group compared to vehicle. In concordance, after the removal of the treatment modality, the male mice in the combinatorial group survived 42 days longer compared to females there was a 29.6% increase in tumor burden in the combinatorial group survived 42 days longer compared to vehicle controls with sustained tumor reduction by 68.3%. In female mice no significant advantage in survival or reduction in tumor burden was noted.

Conclusions N/A

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Abstract 779

IMMUNE CHECKPOINT BLOCKADE IMPACTS THE SUPPRESSIVE PHENOTYPE AND FUNCTION OF REGULATORY T CELLS IN AN ENDOGENOUS MOUSE LYMPHOMA MODEL

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Background Antibodies against programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have become established part of anti-cancer therapy.

REFERENCES

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However, the mechanisms contributing to the therapeutic success have not been entirely uncovered by now. Here we focus on the impact of PD-1/CTLA-4-blocking antibodies on regulatory T cells (Tregs), which are known to be involved in tumor immune evasion in many cancer types.

Methods To evaluate how Tregs are affected by anti-PD-1/CTLA-4 therapy, we used a MYC-transgenic mouse model of spontaneously arising B-cell lymphoma, which can be effectively treated by immune checkpoint inhibition. Data were acquired by flow cytometry.

Results As earlier shown, Tregs were involved in immune escape of MYC tumors. The Treg to effector T cell (Teff) ratio was elevated within the CD4-positive cell compartment. Tumor-infiltrating Tregs were predominantly thymic Tregs, which recognized overexpressed tumor-derived self-peptides in an MHC class II-restricted manner and showed upregulated expression of activation markers, Foxp3, CD25 and IL-10. To examine whether these phenotypic alterations correlated with the immunosuppressive capability of Tregs, an in vitro suppression assay was established. In this setting, MYC Tregs turned out to suppress proliferation and IFN-γ release. To investigate if immune checkpoint blockade interferes with these Treg-dependent immunosuppressive pathways, MYC mice were treated with a combination of anti-PD-1 and anti-CTLA-4 antibodies. Tregs from treated MYC mice showed decreased expression of CD69, CD137, Foxp3, CD25 and IL-10 as compared to Tregs from untreated MYC mice. This correlated with a lower suppressive capacity of Tregs from treated animals in the in vitro suppression assay.

Conclusions Taken together, the data show that immune checkpoint blockade impairs the development of the suppressive phenotype of intratumoral Tregs. Thus, apart from the initially described Teff reactivation, other mechanisms are also relevant for unfolding the therapeutic effect of immune checkpoint inhibitors.

Ethics Approval All animal experiments were approved by Regierung von Oberbayern, approval number 55.2-1-54.

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VALIDATION OF THE COMBINATORIAL EFFECT OF BLINATUMOMAB AND NIVOLUMAB IN CANCER THERAPY

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Background Cancer immunotherapies, including immune checkpoint inhibitors, CAR-T, cancer vaccines and bispecific antibodies, have been brought to spotlight in recent years as several therapeutic strategies targeting the immune system have produced exciting clinical results. Bispecific antibody typically play dual roles in blocking the immune checkpoint and redirecting/re-boosting the function of the immune effector cells. Blinatumomab belongs to CD3 bispecific T cell engager (CD3 BiTE), which was engineered to harbor two arms binding with CD3 and CD19 simultaneously and direct CD8+ T cells to specifically recognize CD19 positive lymphoma cells to execute cytotoxicity. Approval of Blinatumomab for patients with relapse/refractory B cell acute lymphoblastic leukemia (ALL) has driven remarkable increase in combination studies of Blinatumomab with other immunotherapies such as checkpoint inhibitors.

Methods In this study, we developed CD8+ T cytotoxic system targeting different B lymphoma cell line and fully validated the function of Blinatumomab in promoting target tumor cell lysis by primary CD8+ T cells (figure 1). In addition, we established a mixed lymphocyte and tumor system to
mimic physiological TME to dissect the combinational role of Nivolumab and Blinatumomab (figure 2).

**Results** The result suggest that combinatory therapy is highly depend on the dosage of Blinatumomab and also T cell number in the TME, which might give an instruction for ongoing clinical trial design. Finally, we have employed humanized mouse models bearing Raji or Daudi tumor cells to further validate this combination treatment in vivo. Both In-vivo and In-vitro data support that Blinatumomab is dominant in activating T cell and Nivolumab can only exhibit synergistic effect under suboptimal dosage of Blinatumomab.

**Conclusions** CD3 BiTE Opdivo could further promote T cell activation under the treatment of ALDHi. Using polyclonal stimulation of murine splenocytes and human PBMC, we observed that ALDHi promote T cell proliferation, especially of CD8 T cells. Furthermore, exposure of naïve CD4 cells to Th1 and Treg differentiation conditions leads to increased production of INFγ and reduced number of Foxp3+ iTregs, respectively. Further, in a co-culture of iTreg and stimulated splenocyte, ALDHi treatment diminishes the iTreg’s capacity to induce immune suppression. Ex vivo treatment of ovarian cancer ascites cells with various ALDHi leads to significant decrease of CD14+ cells, an effect associated with downregulation of NR4A1 (NUR77), a nuclear receptor that interacts RAR/RXR, downstream of RA signaling. Our results support the use of ALDHi as immune modulators in ovarian cancer and adjuvants to immunotherapy.

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**Abstract 782 Figure 1** Establishment of In vitro co-culture system for CD3 BiTE establish in vitro human PBMC based system to validate CD3 BiTE function

**Abstract 782 Figure 2** Opdivo and CD3 BiTE Combination Opdivo could further promote T cell activation under the treatment of CD3 BiTE

**Conclusions** Successfully establish in vitro system to evaluate the function of CD3 BiTE and also take advantage of MLR/tumor co-culture system to demonstrate PD1 antibody could further promote T cell activation under appropriate dosage of CD3 BiTE.

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**Abstracts**

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**ESTROGEN-DEPRIVATION PROMOTES TH1 POLARIZATION OF TUMOR-ASSOCIATED T CELLS IN A MOUSE MODEL OF HIGH GRADE SEROUS OVARIAN CANCER**

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**Background** Immunotherapy has achieved long-term survival in patients with melanoma and other tumors, introducing a new paradigm in cancer treatment. Differential outcomes among men and women receiving immune checkpoint inhibitors implicate sex steroids as modulators of treatment response. Estrogen signaling has a profound impact on T cell function of cancer deaths in women, are clearly needed. Aldehyde dehydrogenase-1A (ALDH1A) enzymes represent a novel therapeutic target for OvCa. ALDH1A is upregulated in OvCa initiating cells and mediate the biosynthesis of retinoic acid (RA) to regulate numerous cellular processes, including proliferation, metastasis, and chemotherapy resistance. We recently identified novel pan-ALDH1A family inhibitors (ALDHi) that induce necroptosis in OvCa stem-like cells and synergize with chemotherapy, leading to tumor eradication in vivo. Here, we hypothesize that, in addition to controlling tumor progression, ALDHi trigger immunogenic cell death (ICD) via necroptosis and can potentiate anti-tumor immunity.

**Methods** We performed RNA-Sequencing on four human OvCa cell lines (A2780, CAOV-3, OVCAR-5, OVASHO) treated for 8 hours with two different ALDHi. To measure the impact on T cell immunity we performed flow cytometry to measure cell proliferation assays and CD4 naïve differentiation into Th1/ Th17/Treg subsets. Molecular targets in the RA pathway were confirmed by western blot.

**Results** ALDHi triggered significant changes in (i) ER stress unfolded protein response and regulators of the ER stress response, such as ATF4 and EIF2AK3 (PERK), (ii) inflammatory pathways, (iii) cell death, survival, and (iv) gene transcription-RAR signaling. Treatment of cancer cell lines with ALDHi induced expression of Phospho-eIF2α, a marker for the ICD, along with increased expression of ATF3 and ATF4, and calreticulin, suggesting cancer cells undergoing ICD.

Using polyclonal stimulation of murine splenocytes and human PBMC, we observed that ALDHi promote T cell proliferation, especially of CD8 T cells. Furthermore, exposure of naïve CD4 cells to Th1 and Treg differentiation conditions leads to increased production of INFγ and reduced number of Foxp3+ iTregs, respectively. Further, in a co-culture of iTreg and stimulated splenocyte, ALDHi treatment diminishes the iTreg’s capacity to induce immune suppression. Ex vivo treatment of ovarian cancer ascites cells with various ALDHi leads to significant decrease of CD14+ cells, an effect associated with downregulation of NR4A1 (NUR77), a nuclear receptor that interacts RAR/RXR, downstream of RA signaling.

**Conclusions** ALDHi induce immunogenic cell death in cancer cells. Immune cells respond to ALDHi in a cell specific manner. ALDHi support CD8 T cell proliferation and CD4 Th1 induction, while inhibiting iTregs. Exposure to ALDHi leads to downregulation of NR4A1 and reduction in suppressive macrophage numbers. Our results support the use of ALDHi as immune modulators in ovarian cancer and adjuvants to immunotherapy.

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and has been shown to upregulate FoxP3 expression, promoting a suppressive regulatory phenotype. Conversely, estrogen deprivation promotes Th1 skewing, including increased IFN-γ production in response to antigen-specific stimulation. We hypothesize that immuno-modulatory effects of estrogen deprivation will enhance immunotherapy outcomes.

Our lab has previously demonstrated that IFN-γ levels in the TME predict response to immune checkpoint blockade (ICB) regimens in ovarian cancer models. CTLA4 but not PD1/PDL1 ICB combined with PARP inhibition (PARPi), an oral chemotherapeutic, significantly increased IFN-γ in the TME. Furthermore, IFN-γ was required for the durable survival benefit achieved with PARPi/anti-CTLA4. Here we test whether estrogen deprivation enhances IFN-γ production in the TME and response to PARPi/anti-PD1.

Methods Five-week-old female FVB mice underwent oophorectomy, laparotomy without oophorectomy (sham), or no surgery (n = 5 per group). On day 10, mice were intraperitoneally challenged with 200,000 BR5-Akt syngeneic OC cells and randomly assigned to receive either PARPi/anti-CTLA4, PARPi/anti-PD1 or vehicle control treatment. PARPi (40mg/kg/day) was administered days 13-30 and 100 mg anti-CTLA4 or 300 mg anti-PD1 was administered on D14. A second dose of anti-PD1 was given on D24. On day 30, peritoneal cells were analyzed by flow cytometry. Tumor burden was measured by IVIS.

Results Oophorectomy was associated with a significant increase in IFN-γ production by tumor-associated CD4+ T cells [30.4% vs 8.2%, p = 0.016] and an increase in the proportion of central memory CD8+ T cells [59.3% vs 34.9%, p = 0.007] in response to PARPi/anti-PD1 compared with sham and no-surgery controls. In contrast, no differences in T cell phenotype or function was noted among groups receiving PARPi/anti-CTLA4. These changes were associated with a decrease in tumor burden in response to PARPi/anti-PD1 on D30.

Conclusions Estrogen deprivation promotes Th1 polarization among tumor-associated T cells in response to PARPi/anti-PD1 treatment. With evidence that high levels of IFN-γ in the TME strongly correlate with survival, we predict that these effects will enhance treatment outcomes in response to PARPi/anti-PD1. This work presents a rationale for testing estrogen receptor modulators in combination with immune therapy agents and provides a potential mechanism to account for observed differences in patient outcomes.

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786 DISTINCT EFFICACY AND IMMUNOLOGICAL RESPONSES TO APD-1, APD-L1 AND APD-L2 IMMUNOTHERAPY IN B16 MELANOMA IN AGED Versus YOUNG HOSTS INCLUDES T-CELL STEM CELL EFFECTS AND PD-L2 EXPRESSION DIFFERENCES

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Background Aging is the biggest risk factor for cancer, yet little is known about cancer immunotherapy effects. ?PD-1 can block PD-L1 and PD-L2 while ?PD-L1 blocks PD-1 and CD80. A recent key finding in young hosts including humans is that melanoma response to aPD-1/aPD-L1 correlates with CD8+TCF-1+ T cell stem cell (TCSC) generation.2

Methods We tested aPD-1 (100 or 200 μg/mouse), aPD-L1 (100 μg/mouse) or aPD-L2 (200 μg/mouse) in aged (18-33 months) and young (3-8 months) mice challenged orthotopically with B16 (WT or PD-L1ko) melanoma (SQ) or ID8agg ovarian cancer (IP). Tumors were analyzed by flow. Bone marrow-derived DC were generated with GM-CSF.

Results We reported that aPD-1 treats young and aged with B16 and aPD-L1 treats young not aged.3 aPD-L2 treated B16 in aged but, remarkably, not young, the first anti-cancer single agent immunotherapy exhibiting this property [figure 1]. Efficacy in young (aPD-1, aPD-L1) and aged (aPD-L2) correlated with increased tumor TCSC [figure 3], but TCSC differed by age and treatment (e.g., distinct CCR2, CXCR5, CXCR3) [figure 2]. aPD-L2 efficacy against B16 in aged mice required host IFN-γ and IL-17 [figure 4]. IP ID8agg ovarian cancer did not respond to aPD-L2 in aged or young mice. Aged expressed up to 40-fold more PD-L2 versus young on various immune cells suggesting high PD-L2 helps aPD-L2 response [figure 6]. Host IFN-g contributed to aged PD-L2 expression, which did not appear cell-autonomous [figure 6]. PD-L1KO aged but not young mice challenged with PD-L1KO B16 responded to aPD-1 [figure 5], consistent with PD-L2 block as a mechanism.

Abstract 786 Figure 1 aPD-L2 treats B16 in aged but not young mice

Abstract 786 Figure 2 aPD-1, aPD-L1 and aPD-L2 elicit distinct TCSC

Abstract 786 Figure 3 Treatment efficacy correlate with increased TCSC
Conclusions Treatment differences in aged versus young could depend on immune checkpoint or TCSC differences, which could be related to CD8+ T-cell infiltration, including TCSC. aPD-1 efficacy in aged PD-L1KO mice challenged with PD-L1KO B16 suggests that aPD-1 efficacy is through PD-L2 block in aged. PD-L2 expression differences and anatomical compartment differences in tumor microenvironment may also contribute to treatment efficacy differences. We are now identifying mechanisms for increased PD-L2 and other mechanisms for aPD-L2 efficacy in aged, and testing TCSC effects. Our work can improve cancer immunotherapy in aged hosts and provides insights in treatment failures, including in young hosts.

Acknowledgements South Texas MSTP training grant (NIH T32GM113896), TL1TR002647, Graduate Research in Immunology Program training grant (NIH T32 AI138944), R01 CA231325, Samuel Waxman Cancer Research Foundation Grant

Ethics Approval The study was approved by UTHSA IACUC, approval number 20180021.

REFERENCES


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squamous carcinoma=1, OS 3+yrs; MET-exon14+ NSCLC=1, OS 9 mos.), 1 PR (7.7%, OS 6 mos), and 2 SDs (15.4%) in the CNS. Median CNS-PS and OS were 2.9 mos (95% CI: 1.3-NR) and 4.9 mos (95% CI: 3.7-NR), respectively. There were no unacceptable safety signals. Sensitivity for LMM detection by t-DNA was 84.6% (95% CI: 54.6-98.1%), and 46.2% (95% CI: 19.2-74.9%) by cytopathology. Pre and on-therapy CSF cytokine analysis showed complete responders clustered together, while progressors clustered differently.

Conclusions Patients with LMM from solid tumors have a dismal prognosis and limited treatment options. In this phase 2 trial, we identified an impressive 38% CNS response rate for pembrolizumab in patients with LMM, deep and durable responses in selected patients with ICI-responsive tumors, and that pembrolizumab was well-tolerated. CSF t-DNA may be more sensitive for detection of LMM than cytopathology, and immunologic subsets of ICI-response based on cytokine profiles warrant further study. These data support investigation of pembrolizumab in larger populations with LMM.

Trial Registration NCT03091478
Ethics Approval The study was approved by John’s Hopkins University’s Institutional Ethics Board, approval number J1655
Consent All participants provided informed consent as per the study protocol

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**INTRATUMORAL PLASMID IL-12 EXPANDS CD8+ T CELLS AND INDUCES A CLINICALLY VALIDATED CXCR3 SIGNATURE IN TRIPLE-NEGATIVE BREAST CANCER**

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**Background** Triple-negative breast cancer (TNBC) is an aggressive disease with limited therapeutic options. Immune checkpoint inhibitors (ICI) have entered the therapeutic landscape in TNBC, but only a minority of patients benefit. Interleukin-12 (IL-12) is a pro-inflammatory cytokine involved in the generation of an inflammatory tumor microenvironment and is critical in eliciting a productive anti-tumor immune response. It has been investigated as an anti-cancer therapeutic using various delivery routes, but intratumoral injection of plasmid IL-12 (tavokinogene telseplasmid; tavo) followed by electroporation is a gene therapy approach with minimal systemic immune-related toxicity.

**Methods** Intratumoral injection of tavo was tested in several preclinical models of TNBC and single cell RNA sequencing (scRNAseq) was used to evaluate changes in the tumor microenvironment following treatment. These findings were then applied to the analysis of patient samples from a single arm, prospective clinical trial of tavo monotherapy (OMS-I140; NCT02531425).

**Results** A comprehensive analysis of cellular networks using ligand-receptor interactions identified CXCR3 (expressed by APCs) to be positively correlated with CXCL9/10/11 secreted by CD8 T cells. Further investigation of tavo treated murine tumors resulted in a 50-gen CXCR3 gene expression signature that is associated with a decrease in granulocytes, enhanced antigen presentation, increased T cell infiltration, and induction of PD-1/PD-L1. A deeper look at paired TCR alpha and beta chains on tumor infiltrating T cells (TILs) demonstrated a dramatic shift in TIL clonality and frequency following tavo treatment. There was a significant increase in not only the number of expanded (>10) clones, but also a robust activation signature that was absent in control tumors. Treatment of mice with tavo prior to anti-PD1 therapy led to complete tumor regression and long-term survival in a significant proportion of mice, while none of the mice treated with anti-PD1 alone exhibited this therapeutic efficacy. As a proof of concept, we utilized nanostring data from OMS-I140 to show a significant enhancement in this signature in patients who demonstrated a greater than 2-fold increase in CD8 TILs by IHC post-treatment. Further, we show a single patient who had previously been non-responsive to ICI that went on to receive anti-PD1 as their immediate next treatment after participating in OMS-I140, and demonstrated a significant clinical response.

**Conclusions** Together these data identify a clinically relevant CXCR3-associated gene signature that represents both a potential biomarker for response to ICIs and a potentially targetable pathway for therapeutic intervention in TNBC.

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**A PHASE II STUDY (TACTI-002) OF EFTILAGIMOD ALPHA (A SOLUBLE LAG-3 PROTEIN) WITH PEMBROLIZUMAB IN PD-L1 UNSELECTED PATIENTS WITH METASTATIC NON-SMALL CELL LUNG(NSCLC) OR HEAD AND NECK CARCINOMA(HNSCC)**

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**Background** Eftilagimod alpha (efti) is a soluble LAG-3 protein that binds to a subset of MHC class II molecules to mediate antigen presenting cell (APC) and CD8 T-cell activation. The stimulation of the dendritic cell network and subsequent T cell recruitment may lead to stronger anti-tumor responses in combination with pembrolizumab than observed with pembrolizumab alone. We report results from stage 1 of all parts of the study (NCT03625323).
Methods Patients (pts) with selected PD-L1 expression were recruited into 3 cohorts: part A; 1st line, immunothera-
py naïve NSCLC; part B; 2nd line, immunotherapy refrac-
tory NSCLC and part C; 2nd line immunotherapy naïve HNSCC. The study uses a Simon’s 2-stage design, with
objective response rate (ORR) by iRECIST as the primary
endpoint (EP). Secondary EPs include tolerability, disease
control rate (DCR), progression free survival (PFS), overall
survival (OS), PK, PD and immunogenicity. Fifty-eight (58)
pts were recruited into stage 1. Up to additional 31 pts will
be recruited if a pre-specified ORR threshold is met for the
respective part. Efti is administered as 30 mg subcutaneous
injection every 2 wks for 8 cycles and then every 3 wks for
9 cycles; pembrolizumab is administered at 200 mg intrave-
nous infusion every 3 wks for up to 2 yrs. The study was
approved by ethic committees and institutional review
boards.

Results Between Mar 2019 and Jul 2020 88 pts were
enrolled. The median age was 67 yrs (range 53-84) and
70% were male. ECOG PS 0:1 was 42% and 58% respecti-
vely. Pts from all PD-L1 tumor expression subgroups were
recruited. Pts received a median of 4 (1-25) pembrolizumab
and 5.5 (1-22) efti administrations. The most common (≥
15%) treatment emergent adverse events (TEAEs) were
asthenia (28%), cough (27%), decreased appetite (22%),
dyspnea (21%), fatigue (18%) and diarrhea (15%). Three
(3) pts discontinued due to treatment related AEs. The
ORR (acc. to iRECIST) of the 58 patients enrolled into
stage 1 is shown in (table1). PK profiles after the first or
repeated efti dosing were in line with previous studies, with
a mean Cmax at 7 ng/ml reached 24h. Circulating TH1
biomarkers 2 weeks after the last efti administration were
increased (3 months vs. baseline) by a mean 61% and
209% for CXCL10 (Student paired t-test, p=0.02, n=31).

Abstract 790 Table 1
ORR (acc. to iRECIST) of the 58 patients enrolled into
stage 1

<table>
<thead>
<tr>
<th>Response parameter</th>
<th>Part A (1st line NSCLC, PD-L1-naive)</th>
<th>Part B (2nd line NSCLC, PD-L1 refractory)</th>
<th>Part C (2nd line HNSCC, PD-L1-naive)</th>
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</thead>
<tbody>
<tr>
<td>No.</td>
<td>17</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Median follow-up, months</td>
<td>16.4</td>
<td>5.5</td>
<td>9.5</td>
</tr>
<tr>
<td>ORR (n, %)</td>
<td>9 (52.9)</td>
<td>1 (4.4%)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td></td>
<td>[27.4–77.0]</td>
<td>[0.1–21.9]</td>
<td>[17.3–64.3]</td>
</tr>
<tr>
<td>CR (n, %)</td>
<td>3 (18.3)</td>
<td>0 (0)</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>ORR (n, %)</td>
<td>14 (76.5)</td>
<td>7 (40.0)</td>
<td>9 (50.0)</td>
</tr>
<tr>
<td>Responses with low PD-L1 (n, %)</td>
<td>4 (44.4)</td>
<td>3 (15.8)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td></td>
<td>[15–50 %] PD-L1</td>
<td>[0–25 %]</td>
<td>[15–50 %]</td>
</tr>
<tr>
<td>Median PFS, months (5% C)</td>
<td>11.8</td>
<td>2.1</td>
<td>4.26</td>
</tr>
<tr>
<td></td>
<td>[3.2–16.6]</td>
<td>[1.8–8.0]</td>
<td>[1.4B–8.0]</td>
</tr>
<tr>
<td>OS rate at 9/12/15 months</td>
<td>85 % / 71 % / 64 %</td>
<td>not yet reached</td>
<td>67 % / 50 % / not yet reached</td>
</tr>
</tbody>
</table>
an opportunity to be followed for at least 16 weeks) is shown in the table 1. At data cutoff, 91.8% of responders remained ongoing and still on treatment. Treatment-related adverse events (TRAEs) occurred in 93.6% of pts (G3 in 13.8% [13/94], no G4 or G5, treatment discontinuation in 2.1% [2/94]). Treatment-related SAEs occurred in 3.2%. Most frequent TRAEs (>15%) were fever (24.5%), hypothyroidism (21.3%), upper respiratory tract infection (18.1%), and ALT elevations (17.0%). Grade ≥3 TRAEs reported in ≥2 pts were platelet count decreased (2.1%). Immune-related AEs were reported in 42.6% of pts (G3 in 2.1%: psoriasis [n=1], IgA nephropathy [n=1]).

Conclusions Penpulimab was shown to be highly active resulting in a high CR rate in pts with R/R cHL. With longer follow-up, CR rate for penpulimab in R/R cHL could be further increased. Penpulimab demonstrated notably lower rates of SAE, TRAE leading to discontinuation, and Grade 3 immune-related AEs in pts with R/R cHL.

Trial Registration NCT03722147

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0791

Background Specific immune cell responses against oncogenic antigens are major determinants to achieve long-term disease control for HPV-related malignancies. We developed TG4001, a viral based vaccine against the HPV E6 and E7 antigens. Following the demonstration of its safety in phase Ib, we aimed to evaluate the antitumor activity and immune priming effects of TG4001 in combination with the PD-L1 inhibitor avelumab in HPV-related malignancies in phase II (NCT03260023).

Methods Patients (pts) with previously treated R/M HPV-16+ cancers received TG4001 at 5x107 pfu SC weekly for 6 weeks, every 2 weeks up to M6, and every 12 weeks thereafter in combination with avelumab IV at 10mg/kg every 2 weeks. PBMC and tissue samples were collected longitudinally prior to and during the treatment period. Specific T cell response was assessed using ex-vivo IFNg-ELISPOT, and changes in the tumor microenvironment by phenotyping of immune infiltrate and transcriptomic analyses of immune related genes.

Results 34 pts with anal (15), oropharyngeal (8), cervical (6) or vulvar/vaginal (5) cancer, were enrolled. Median age was 61 years; the majority (88%) had received at least 1 prior line of chemotherapy (CT) with 32% having received ≥ 2 lines. 8 pts achieved confirmed response according to RECIST 1.1 (1 CR, 7 PR, ORR 23.5%). Responses were observed in all primary tumor types and across all lines of prior therapy. Liver metastases had a profound impact on outcome: ORR was 34.8% and PFS 5.6 months in pts without liver metastases (n=23) versus 0% and PFS of 1.4 months in pts with liver metastases (n=11). Consistent with phase Ib data, the combination had a favorable safety profile.

11 pts were evaluable for T-cell response at day (D) 43. 7/11 patients had vaccine-induced reactive T cells against E6, E7 or both. In particular, in the patient with CR, lesions disappearance was accompanied by the development of a strong T-cell response against E6 and E7. This response developed as early as D43 and sustained at 6 months after initiation of therapy, consistent with the durable disease-control. Increased infiltrates, expression of immune related genes and higher PD-L1 protein expression were observed across all patients suggesting a remodeling of the tumor microenvironment consistent with a switch toward a ‘hot tumor’ phenotype.

Conclusions Our study suggests that immunotherapeutic combination of TG4001 and avelumab shows valuable tumor activity in pts with previously treated advanced HPV-16+ cancers. These results warrant validation in a larger cohort of patients.

Trial Registration NCT03260023

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0793
Conclusions The combination of intratumoral MK-4621 plus intravenous pembrolizumab had a manageable safety profile. At the highest dose level, modest antitumor activity was observed in patients treated with combination therapy.

Trial Registration ClinicalTrials.gov identifier, NCT03739138

Ethics Approval An independent institutional review board or ethics committee approved the protocol at each study site, and the trial is being conducted in compliance with Good Clinical Practice guidelines and the Declaration of Helsinki.

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Conclusions The combination of ipilimumab and nivolumab in metastatic or unresectable angiosarcoma is a rare cancer of endothelial cells that can be aggressive and carries a high mortality. A subset of angiosarcomas are characterized by high tumor mutational burden (TMB) and UV light exposure DNA mutational signature. Isolated case reports have suggested clinical efficacy of immune checkpoint blockade in angiosarcoma; no prospective studies of immune checkpoint inhibition in angiosarcoma have been reported. We report efficacy analysis results for patients with advanced or unresectable angiosarcoma treated with ipilimumab and nivolumab as a cohort of an ongoing phase II clinical trial for rare cancers (NCT02834013).

Methods This is a prospective, open-label, multicenter phase II clinical trial of ipilimumab (1mg/kg IV q6weeks) plus nivolumab (240mg IV q2weeks) for patients with metastatic or unresectable angiosarcoma treated with ipilimumab and nivolumab as a cohort of an ongoing phase II clinical trial of ipilimumab (1mg/kg IV q6weeks) plus nivolumab (240mg IV q2weeks) for patients with metastatic or unresectable angiosarcoma. Primary endpoint is objective response rate as assessed by RECIST v1.1, including measurable cutaneous disease that can be followed by photography. Secondary endpoints include PFS, OS, stable disease at six months, and toxicity. A two-stage design is used with six patients in the first stage and an additional ten patients in the second stage.

Results At data cutoff, 16 patients with angiosarcoma were enrolled. Median age was 68 years (25-81 years). Median number of prior lines of therapy was 2 (0-5). 9 patients had cutaneous primary tumors of any cutaneous site, 7 had non-cutaneous primary tumors. ORR for all patients was 25% (4/16, table 1, figure 1). Subgroup analysis revealed that 60% (3/5) of patients with primary cutaneous tumors of the scalp or face had a confirmed objective response. 6-month PFS was 38%. 75% of patients experienced an adverse event (AE), and 25% experienced a grade 3-4 AE. 68.8% experienced an immune related AE (irAE), and 2 (12.5%) developed grade 3 or 4 irAEs. Grade 3-4 irAEs were ALT and AST increase and diarrhea. There were no grade 5 toxicities.

Conclusions The combination of ipilimumab and nivolumab was well tolerated and had an ORR of 25% in angiosarcoma regardless of primary site, with 3 of 5 patients with cutaneous tumors of the scalp or face responding. Ipilimumab and nivolumab as a combination therapy confer clinical benefit. In 3-week cycles, patients received intratumoral MK-4621 0.4, 0.6, or 0.8 mg on days 1, 8, and 15 for 6 cycles (delivered via jetPEI®, Polyplus Transfection, Illkirch, France) plus intravenous pembrolizumab 200 mg on day 1 for 35 cycles. Treatment continued until disease progression or unacceptable toxicity. The primary objective was to establish a preliminary recommended phase 2 dose based on DLTs (cycle-1), AEs, and treatment discontinuations due to AEs; AEs were graded per NCI CTCAE v4.0. Tumor imaging was performed Q9W; response was assessed by the investigator.

Results As of May 14, 2020, 30 participants received therapy with MK-4621 0.4 (n=7), 0.6 (n=5), or 0.8 mg (n=18). Median time on therapy was 57 (range, 1-365) days. The most frequent tumor types were breast (20%) and melanoma (17%); 90% of patients received 2 prior lines of therapy. One patient in the 0.8-mg group experienced a DLT (grade 3 treatment-related pleural effusion), which resulted in treatment discontinuation; no other patient discontinued owing to AEs. Grade 3 treatment-related AEs occurred in 1 patient (14%) at the 0.4-mg dose (pyrexia), 1 patient (20%) at the 0.6-mg dose (hypertension), and 5 patients (28%) at the 0.8-mg dose (anemia [n=2], dyspnea/pleural effusion [n=1], lymphopenia [n=1], pyrexia [n=1]). No treatment-related grade 4/5 AEs occurred. Across dose levels, the most frequently occurring treatment-related AEs were pyrexia (63%), chills (37%), cytokine-release syndrome (20%), and nausea (20%). Efficacy outcomes are shown on table (1). Significant changes in blood interferon-gamma inducible protein-10 and monocyte chemotactic protein-2 were observed at each dose level, consistent with the mechanism of action of MK-4621.

Abstract 794 Table 1 Efficacy outcomes

<table>
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<tr>
<th>Table: Efficacy Outcomes</th>
<th>MK-4621 0.4 mg + Pembrolizumab (n=7)</th>
<th>MK-4621 0.6 mg + Pembrolizumab (n=5)</th>
<th>MK-4621 0.8 mg + Pembrolizumab (n=18)</th>
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</thead>
<tbody>
<tr>
<td>Overall response, % (n)</td>
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<td>0/1 (0)</td>
</tr>
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<td>0/1 (0)</td>
<td>0/1 (0)</td>
<td>0/1 (0)</td>
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<td>Partial response (PR)</td>
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<td>0/1 (0)</td>
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<td>Progressive disease (PD)</td>
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<td>5/10 (21)</td>
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<tr>
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<td>0.0 (0-52)</td>
<td>0.0 (11-35)</td>
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Abstracts

975 A MULTICENTER PHASE II TRIAL (SWOG S1609, COHORT 51) OF IPILIMUNAB AND NIVOLUMAB IN METASTATIC OR UNRESECTABLE ANGIOSARCOMA: A SUBSTUDY OF DUAL ANTI-CTLA-4 AND ANTI- PD-1 BLOCKADE IN RARE TUMORS (DART)

1Michael Wagner*, 2Megan Othrus, 3Sandip Patel, 4Christopher Ryan, 5Ashish Sangal, 6Benjamin Powers, 7George Budd, 8Adrienne Victor, 9Chung-Tsen Hsueh, 10Rashmi Chugh, 11Surees Nair, 12Kirsten Leu, 13Mark Agulnik, 14Edward Mayerson, 15Melissa Plets, 16Charles Blanke, 17Howard Steticher, 18Young Kwang Chai, 19Razelle Kurzrock, 20University of Washington, Seattle, WA, USA; 21SWOG/FHCRC, Seattle, WA, USA; 22UCSD Moores Cancer Center, La Jolla, CA, USA; 23OHSU, Portland, OR, USA; 24CTCA at Western Regional Medical Center, Phoenix, AZ, USA; 25Kansas MU-NCORP, Overland Park, KS, USA; 26Cleveland Clinic, Cleveland, OH, USA; 27University of Rochester, Rochester, NY, USA; 28Loma Linda University, Loma Linda, CA, USA; 29University of Michigan, Ann Arbor, MI, USA; 30Michigan CRC N CORP, Allentown, PA, USA; 31University of Rochester, Rochester, NY, USA; 32University of Nebraska Medical Center, Omaha, NE, USA; 33Northwestern University, Chicago, IL, USA; 34University of Nebraska, Omaha, NE, USA; 35Cancer Therapy Evaluation Program (CTEP), Bethesda, MD, USA; 36SWOG Group Chair’s Office, OHSU, Portland, OR, USA

Background Angiosarcoma is a rare cancer of endothelial cells that can be aggressive and carries a high mortality. A subset of angiosarcomas are characterized by high tumor mutational burden (TMB) and UV light exposure DNA mutational signature. Isolated case reports have suggested clinical efficacy of immune checkpoint blockade in angiosarcoma; no prospective studies of immune checkpoint inhibition in angiosarcoma have been reported. We report efficacy analysis results for patients with advanced or unresectable angiosarcoma treated with ipilimumab and nivolumab as a cohort of an ongoing phase II clinical trial for rare cancers (NCT02834013).

Methods This is a prospective, open-label, multicenter phase II clinical trial of ipilimumab (1mg/kg IV q6weeks) plus nivolumab (240mg IV q2weeks) for patients with metastatic or unresectable angiosarcoma treated with ipilimumab and nivolumab as a cohort of an ongoing phase II clinical trial for rare cancers (NCT02834013).

Acknowledgements Funding: National Institutes of Health/National Cancer Institute grant awards CA180888, CA180819, CA180868; and in part by Bristol-Myers Squibb Company

Trial Registration NCT02834013

Ethics Approval This study was approved by the NCI CIRB.

http://dx.doi.org/10.1136/jitc-2020-STIC2020.0795
A PHASE I/II TRIAL COMBINING AVELUMAB AND TRABECTEDIN FOR ADVANCED LIPOSARCOMA AND LEIOMYSOSARCOMA

1Michael Wagner*, 1Qianchuan He, 1Yuhang Zhang, 1Lee Cranmer, 2Elizabeth Loggers, 2Sabrina McDonnell, 3Shannon Maxwell, 4Seth Pollack, 4University of Washington, Seattle, WA, USA; 2Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Background Leiomyosarcoma (LMS) and liposarcoma (LPS) are soft tissue sarcoma subtypes that frequently express PD-L1 and are infiltrated with T cells. They are generally resistant to PD-1/PD-L1 inhibition, possibly due to infiltration with high levels of immunosuppressive tumor-associated macrophages (TAMs). Trabectedin is FDA-approved for refractory LMS and LPS. Prior studies demonstrated trabectedin activity against TAMs. We hypothesized that PD-L1 inhibition by avelumab would be enhanced by trabectedin through its inhibition of immunosuppressive TAMs.

Methods This is a single-arm, open-label, Phase I/II study of avelumab combined with trabectedin for patients with advanced LMS and LPS. In the phase I portion, we evaluated safety and feasibility at 3 trabectedin doses (1, 1.2 and 1.5 mg/m2) with avelumab at standard dosing (10 mg/kg) in a 3+3 design. Primary endpoint of the phase II portion was the objective response rate (ORR) by RECIST 1.1. 24 patients were included for phase II endpoints. Secondary objectives were duration of response, progression-free survival (PFS), clinical benefit rate (CBR) at 12 weeks, and overall survival.

Results 37 patients enrolled; 34 were evaluable. 23 had LMS, 11 had LPS. In Phase 1, there were DLTs in 2 of 6 patients at both higher doses of trabectedin including grade 3 GGT elevation, bilirubin and alanine aminotransferase (ALT) elevation, small bowel obstruction, and reduced ejection fraction. The recommended Phase 2 dose was 1.0 mg/m2 trabectedin and 10 mg/kg avelumab. At the Phase 2 dose, the most common adverse events (AEs) attributed to study drug were fatigue, ALT increase, diarrhea, anorexia, nausea, and infusion reaction. There were 8 instances of PORT inflammation or infection. The most common Grade 3 AEs attributed to study drug were neutropenia and ALT increase. There were no grade 4/5 AEs at the Phase 2 dose. 23 patients were evaluable for primary ORR endpoint. 2 (8.7%) had partial response (1 confirmed), 11 had stable disease as best response. CBR (PR+SD) at 12 weeks was 56%; 6 month PFS was 50.1%; median PFS was 23.4 months. 9 patients remain on study treatment. In a secondary analysis of all patients, ORR was 8.6% (3/35 with PR), median PFS was 6.1 months.

Conclusions Administration of this combination was feasible with acceptable toxicity. The recommended Phase 2 dose was 1.0 mg/m2 trabectedin and 10 mg/kg avelumab. The combination failed to meet the primary endpoint of ORR, however the PFS appears favorable compared to prior studies of trabectedin in this population and warrants further study.

Trial Registration NCT03074318

Ethics Approval The study was approved by the Fred Hutch IRB, number 9717.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0796

A CLASS I HISTONE DEACETYLASE INHIBITOR (HDACI) IN COMBINATION WITH NIVOLUMAB (NIVO) IN ANTI-PD1 THHERAPY-NAÏVE ADVANCED MELANOMA (TN-MEL)

1Nikhil Khushalani*, 1Andrew Brohi, 1Joseph Markowitz, 2Udymila Bachanova, 2Gregory Daniels, 2Heather Yeckes-Rodin, 2Sajing Fu, 1Lori McCormick, 1Michael Kurman, 1Mireille Gillings, 1Gloria Lee, 1Jayynep Erguol, 3Medford Cancer Center, Tampa, FL, USA; 2University of California at San Diego, San Diego, USA; 1Hem-Onc Associates of the Treasure Coast, Port St. Lucie, FL, USA; 4M. D. Anderson Cancer Center, Houston, TX, USA; 5HUYA Bioscience International, San Diego, CA, USA

Background Anti-PD1 based therapy has been the mainstay of treatment for advanced melanoma for several years. HBI-8000 is a Class I selective oral HDACi with immunomodulatory effects including enhanced cell-mediated toxicity, enhanced tumor infiltration by cytotoxic T-cells and reduced tumor infiltration by T-regulatory cells. In a phase 1b/2 trial in melanoma, kidney cancer and non-small cell lung cancer, the recommended phase 2 dose of HBI-8000 was determined to be 30mg orally twice weekly (BIW) combined with nivolumab administered at the approved dosing schedule (JITC 2018; P346). This report describes the tolerability of this combination in all enrolled melanoma patients, and efficacy in the expansion cohort of anti-PD1 TN-MEL.

Methods Patients with unresectable or advanced melanoma and measurable disease, of ECOG performance status 0-1, and with adequate hematologic and biochemical parameters were enrolled. Treated brain metastases not requiring steroids were permitted. Tumor response was assessed by RECIST v1.1 and iRECIST with staging every 8 weeks; treatment continued for 24 months, disease progression or unacceptable toxicity. Data cut-off was Jan 31, 2020 for the reported analyses.

Results Forty-nine patients (32 anti-PD1 naïve, 17 with prior anti-PD1 therapy) were treated with HBI-8000 (47 patients at 30 mg BIW; 2 patients at 40mg BIW in Phase 1b) in combination with nivolumab. The median age was 63 years (range 28-84); 57% were male. In the anti-PD1 naïve cohort, most (30/32) had normal LDH. The most common all grade treatment related adverse events (AEs) included fatigue (n=23), diarrhea (n=24), abdominal pain (n=14), and lymphopenia (n=13). Although HBI-8000 related thrombocytopenia (n=25) and neutropenia (n=15) were common, clinically significant bleeding or febrile neutropenia were not observed. The most frequent >/= G3 AEs related to HBI-8000 were hypophosphatemia (n=7), neutropenia (n=4), thrombocytopenia (n=3) and lymphopenia (n=2). Twelve patients discontinued treatment for AEs. Among 31 anti-PD1 naïve patients evaluable for response, there were 23 objective responses (4 CR, 19 PR; ORR 74%), 5 stable disease (disease control rate 90%), and 3 progressive disease. Median time to response was 1.9 months. At a median follow-up for this cohort of 8.9 months (range, 0.9-33.5 months), the median duration of response and median progression-free survival have not been reached.

Conclusions The combination of HBI-8000 and nivolumab is well tolerated and demonstrates very encouraging efficacy in patients with anti-PD1-naïve advanced melanoma. Follow-up to assess durability of response is ongoing, and further investigation of this promising combination is planned.

Trial Registration NCT02718066
SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF mRNA-4157 IN COMBINATION WITH PEMBROLIZUMAB IN SUBJECTS WITH UNRESECTABLE SOLID TUMORS (KEYNOTE-603): AN UPDATE

1Julie Bauman*, 2Howard Burris, 3Jeffrey Clarke, 4Manish Patel, 5Daniel Cho, 6Martin Gutierrez, 7Rickie Julian, 8Aaron Scott, 9Pamela Cohen, 10Joshua Frederick, 11Celine Robert-Tissot, 12Hongchong Zhou, 13Kinjial Mody, 14Karen Keating, 15Robert Meehan, 16Justin Gairner. 1University of Arizona, Tucson, AZ, USA; 2SCRT, Nashville, TN, USA; 3Duke, Durham, NC, USA; 4Florida Cancer Specialists, Sarasota, FL, USA; 5NYU, New York, NY, USA; 6Hackensack, Hackensack, NJ, USA; 7Moderna, Tewksbury, NJ, USA; 8Mass General Hospital, Boston, MA, USA

Background T-cell targeting of mutation-derived epitopes (neoantigens) has shown to drive anti-tumor responses. Immunizing patients against such neoantigens in combination with a checkpoint inhibitor (CPI) may elicit greater anti-tumor responses than CPI alone. Mutations are rarely shared between patients, thus requiring a personalized approach to vaccine design. mRNA-4157 is a lipid encapsulated mRNA based personalized cancer vaccine encoding neoantigens selected using a proprietary algorithm to induce neoantigen specific T cells and associated anti-tumor responses. This report includes updates from the mRNA-4157 Phase1(P1) study. The initial data was presented at ASCO2019.

Methods This study evaluates the safety and efficacy of mRNA-4157 as monotherapy in patients with resected solid tumors (Part A) and in combination with pembrolizumab in patients with advanced/metastatic solid tumors (Parts B). The selected solid tumors in Part A-B includes melanoma, bladder carcinoma, HPV-negative (HPV-neg) HNSCC, NSCLC, SCLC, MSI-High (MSI-h), or TMB-High cancers. Expansion cohorts includes patients with CPI-naïve MSS-CRC and HPV-neg HNSCC (Part C) and with resected melanoma (Part D). Patients receive up to 9 cycles (Q3W) of mRNA-4157 by intramuscular injection at up to 1 mg alone (Part A) or in combination with pembrolizumab (200 mg IV Q3W, Parts B-D). Pembrolizumab is administered for two cycles before the first dose of mRNA-4157 and may continue after 9 cycles of combination. Endpoints include safety, tolerability, efficacy and biomarker assessments.

Results 79 patients received mRNA-4157; 16 as monotherapy and 63 in combination with pembrolizumab. Only low grade and reversible treatment related AEs were reported. 14/16 Part A patients (3 melanoma, 11 NSCLC, 2 MSI-h CRC) remained disease free on study. 28 patients in Part B (6 blader, 2 HNSCC, 3 melanoma, 10 NSCLC, 2 SCLC, 4 MSI-h tumor, 1 TMB-h tumor), 27 patients in Part C (10 HNSCC and 17 MSS-CRC), and 8 patients with resected melanoma (Part D) received combination. 3 CR (1 HNSCC, 1 MSI-h CRC and 1 MSI-h prostate), and 8 PR (1 bladder, 4 HNSCC, 2 SCLC and 1 MSI-h endometrial) were observed with combination. Of 10 CPI-naive HPV-neg HNSCC patients, the response rate was 50% (1CR, 4PR, 5SD) mPFS 9.8months, which compared favorably to published rates of ~14.6% mPFS 2.0months for pembrolizumab monotherapy. 1 3 Biomarker assessments including immune gene expression profiling will be presented.

Conclusions mRNA-4157 has an acceptable safety profile along with observed clinical responses in combination with pembrolizumab. Preliminary efficacy analysis from CPI-naïve relapsed/refractory HPV-neg HNSCC cohort suggests activity of this combination. Study is ongoing.

Ethics Approval The study was approved by each participating sites’ local IRB.

REFERENCES


DURABLE RESPONSES AND IMMUNE ACTIVATION WITH INTRATUMORAL ELECTROPORATION OF PIL-12 PLUS PEMBROLIZUMAB IN ACTIVELY PROGRESSING ANTI-PD-1 REFRACTORY ADVANCED MELANOMA: KEYNOTE 695 INTERIM DATA

1Pablo Fernandez-Penas, 1Matteo Carlini, 2Katy Tsai, 3Victoria Atkinson, 4Monaster Shaheen, 5Sajeev Thomas, 6Catalin Mihaiiciou, 7Tom Van Hagen, 8Rachel Roberts-Thomson, 9Andrew Haydon, 10Andrew Mant, 11Marcus Butler, 12Gregory Daniels, 13Elizabeth Buchbinder, 14John Hynghstrom, 15Mecker Moller, 16Igor Puzanov, 17C Lance Cowey, 18Eric Whitman, 19Carmen Ballesteros-Merino, 20Shawn Jensen, 21Bernard Fox, 22Emmett Schmidt, 23Clemens Kiepler, 24Scott Diebold, 25Erica Browning, 26Keneta Hermitz, 27Lauren Svenson, 28Jon Salazar, 29Jack Lee, 30Christopher Baker, 31Donna Bannavong, 32Jordy Sell, 33Kellie Malloy Foerter, 34David Canton, 35Sandra Aung, 36Christopher Twitty, 37Adil Daud. 1University of California, San Francisco, CA, USA; 2Princess Alexandra Hospital, University of Queensland, Wooloongabba, Australia; 3University of Arizona, Tucson, AZ, USA; 4UF Health Cancer Center at Orlando Health, Orlando, FL, USA; 5McGill University Health Centre, Montreal, Canada; 6St. John of God Hospital, Subiaco, Australia; 7Adelaide Oncology and Haematology, Adelaide, Australia; 8The Alfred Hospital, Victoria, Australia; 9Box Hill Hospital, Box Hill, Australia; 10Princess Margaret Cancer Centre, Toronto, Ontario, Canada; 11University of California, San Diego, La Jolla, CA, USA; 12Dana Faber Cancer Institute, Boston, MA, USA; 13University of Utah Healthcare Huntsman Cancer Institute, Salt Lake City, UT, USA; 14University of Miami Sylvester Cancer Center, Miami, FL, USA; 15Roswell Park Cancer Institute, Buffalo, NY, USA; 16Baylor University Medical Center, Dallas, TX USA; 17Atlantic Health System, Morristown, NJ, USA; 18Earle A. Chiles Research Institute, Portland, OR, USA; 19Memc and Co., Inc., Kennewick, WA, USA; 20Onconess Medical Incorporated, San Diego, CA, USA.

Background Electroporated plasmid IL-12 (TAVO or tavokinogene telseplasmid) is a novel pro-inflammatory intratumoral therapy with substantial single agent activity in melanoma, which has been shown to synergize with anti-PD-1 antibodies in patients predicted as non-responders to anti-PD-1.

Interim data from patients with stage III/IV melanoma actively progressing on anti-PD-1 antibody are presented herein.

Methods Patients with confirmed disease progression by RECIST v1.1 after at least 12 weeks of treatment on pembrolizumab or nivolumab (or combination checkpoint blockade) and within 12 weeks of last dose (with no intervening

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0797

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0798
therapies) were enrolled. There was no limit on the number of prior lines of therapy. At least one accessible lesion was electroporated with plasmid IL-12 (pIL-12-EP) on days 1, 5 and 8 every 6 weeks and pembrolizumab was administered every 3 weeks. Tumor response in treated and untreated lesions was assessed by RECIST v1.1 every 12 weeks. Endpoints include ORR, safety, PFS, OS, and DOR.

Results The first 56 patients treated of 100 planned were included in this interim analysis. Of these, 84% had Stage IV disease, 30% had M1c or M1d disease, and 27% had prior exposure to ipilimumab. In 54 efficacy evaluable patients the investigator-assessed ORR was 30% (3 CR/13 PR), 5 patients had 100% reduction of target lesions. All responses have been confirmed, only two responding patient progressed while on study, 2 patients completed the study with ongoing responses confirmed, only two responding patient progressed while on study, 2 patients completed the study with ongoing responses.

In patients with M1c/M1d disease, the ORR was 35.2% (n=6/17). Tumor reduction was observed in untreated lesions in 12 of 12 patients who had unaccessible lesions or accessible untreated lesions. The median overall survival (mOS) and duration of response (mDOR) has not been reached, with a median follow-up time of 13 months. Grade 3 treatment-related adverse events (TRAEs) were seen in 5.4% of patients, and there were no grade 4/5 TRAEs. The rate of grade 3 treatment-emergent (TEAEs) regardless of cause was 23.2%. The median time for pIL-12-EP treatment was 10 minutes (range 2,46). Consistent with prior studies of single-agent pIL-12-EP, tumor IHC, and transcriptomic assessments revealed hallmarks of antigen-specific antitumor immunity in this study. Additional analyses including microbiome, TCR clonality, and peripheral blood biomarker assays will be presented.

Conclusions In this rigorously defined PD-1 antibody refractory patient population, the addition of pIL-12-EP to PD-1 antibody therapy induced deep, durable, systemic response in local treated and distant visceral metastatic untreated lesions with nominal systemic toxicity.

Trial Registration Trial Registration: NCT#03132675

Ethics Approval The study was approved by a central IRB and/or local institutional IRBs/Ethics Committees as required for each participating institution.

Consent Written informed consent was obtained from the patients participating within the trial, the current abstract does not contain sensitive or identifiable information requiring an additional consent from patients.

REFERENCES

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Abstracts

800 A PHASE I DOSE ESCALATION AND EXPANSION STUDY OF INTRATUMORALLY ADMINISTERED CV8102 AS A SINGLE-AGENT OR IN COMBINATION WITH ANTI-PD-1 ANTIBODIES IN PATIENTS WITH ADVANCED SOLID TUMORS

1Thomas Eigentler*, 2Lucie Heinzerling, 3Jürgen Krauss, 4Carsten Weishaupt, 5Peter Mohr, 6Sebastian Ochsenreither, 7Patrick Terheyden, 8Juan Martin-Liberal, 9Marc Oliva, 10Michael Fluck, 11Peter Brossart, 12Jose Manuel Trigo Perez, 13Ulrike Gnad-Vogt.

Background CV8102 is a non-coding, non-capped RNA complexed with a carrier peptide activating the innate (via TLR7/8, RIG-I) and adaptive immune system. An ongoing phase I trial is investigating i.t. CV8102 either as a single agent or in combination with systemic anti-PD-1 antibodies in patients with advanced melanoma (MEL), squamous cell carcinoma of the skin (cSCC) or head and neck (hSCC) and adenoid cystic carcinoma (ACC).

Methods An open-label, cohort-based, dose escalation and expansion study in patients with advanced cutaneous melanoma (cMEL), cutaneous squamous cell carcinoma (cSCC), head and neck squamous cell carcinoma (hSCC) or adenoid cystic carcinoma (ACC) is ongoing investigating i.t. CV8102 as single agent and in combination with anti-PD-1 antibodies.

Abstract 799 Figure 1

Best confirmed overall response by RECIST v1.1 after confirmed progression on anti PD-1

Abstract 799 Figure 2

Percent change in sum of target lesions over time

800 A PHASE I DOSE ESCALATION AND EXPANSION STUDY OF INTRATUMORALLY ADMINISTERED CV8102 AS A SINGLE-AGENT OR IN COMBINATION WITH ANTI-PD-1 ANTIBODIES IN PATIENTS WITH ADVANCED SOLID TUMORS

1Thomas Eigentler*, 2Lucie Heinzerling, 3Jürgen Krauss, 4Carsten Weishaupt, 5Peter Mohr, 6Sebastian Ochsenreither, 7Patrick Terheyden, 8Juan Martin-Liberal, 9Marc Oliva, 10Michael Fluck, 11Peter Brossart, 12Jose Manuel Trigo Perez, 13Ulrike Gnad-Vogt.

Background CV8102 is a non-coding, non-capped RNA complexed with a carrier peptide activating the innate (via TLR7/8, RIG-I) and adaptive immune system. An ongoing phase I trial is investigating i.t. CV8102 either as a single agent or in combination with systemic anti-PD-1 antibodies in patients with advanced melanoma (MEL), squamous cell carcinoma of the skin (cSCC) or head and neck (hSCC) and adenoid cystic carcinoma (ACC).

Methods An open-label, cohort-based, dose escalation and expansion study in patients with advanced cutaneous melanoma (cMEL), cutaneous squamous cell carcinoma (cSCC), head and neck squamous cell carcinoma (hSCC) or adenoid cystic carcinoma (ACC) is ongoing investigating i.t. CV8102 as single agent and in combination with anti-PD-1 antibodies.
8 intratumoral injections of CV8102 are being administered initially over a 12 week period, while patients benefiting from the single agent therapy may receive further treatment. In an initial dose escalation part the maximum tolerated dose and recommended phase 2 dose for subsequent cohort expansion will be defined.

Results As of September 16, 2020, 29 patients have been treated with CV8102 as a single agent (25-900 μg) and 21 patients have received CV8102 (25-900 μg) in combination with anti-PD-1 antibodies. Most frequent treatment related adverse events were mild to moderate fever, fatigue, chills and headache. One patient treated at the 900 μg single agent experienced a dose limiting toxicity (G3 transaminase increase in the context of G2 cytokine release syndrome).

Regression of injected and distant noninjected lesions was observed in several patients in the single agent and the anti-PD-1 combination cohorts. Updated safety and efficacy results will be presented.

Conclusions CV8102 showed an acceptable tolerability and preliminary evidence of clinical efficacy as single agent and in combination with anti-PD-1 antibodies.

Trial Registration NCT03291002

Ethics Approval The study was approved by the Central Ethics Committees in Tuebingen, Germany under 785/2016AMG1, in Ethics Approval CV8102 showed an acceptable tolerability and Conclusions PD-1 combination cohorts. Updated safety and efficacy results will be presented.

References

Abstract 801 Table 1 Summary of Patients

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<tr>
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</tr>
<tr>
<td>Head &amp; Neck</td>
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</tr>
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Tumor types for the 17 patients with advanced metastatic disease included in this clinical trial (NCT03815682)

We describe preliminary results from the ongoing first-in-human Phase 1 trial.

Methods Autologous anti-TAA T cells are generated with a proprietary dendritic cell priming process and then loaded with an IL15-Fc nanogel. TAA used in cassette: PRAME, NY-ESO-1, SSX2, Survivin and WT1. Thawed RPTR-147 is delivered by infusion. Pre- and post-treatment biopsies were collected for biomarker analysis by immunohistochemistry (IHC) and transcriptome sequencing. Serial blood collections were obtained for measuring IL-15 pharmacokinetics and pharmacodynamic parameters including plasma cytokine levels and immunophenotyping by flow cytometry. T cell receptor sequencing (TCRSeq) was used to characterize the T cell repertoire from manufactured T cell product and the patient’s blood.

Results Interim clinical and biomarker data from 17 patients with advanced metastatic disease refractory to SOC who received monthly infusions of 20-360 million cells/m², were reviewed (table 1). There were no no dose-limiting toxicities and no evidence of cytokine-release syndrome. The 360M/m² dose contained 3X more IL15-Fc than the MTD of systemically administered IL15-Fc, but produced less than a tenth of the systemic exposure to free IL15-Fc. Currently, 360M cells/m² is considered safe and well-tolerated. Further dose escalation is planned.

Matched evaluable biopsies were obtained in 7 patients. Tumor-infiltrating T cell lymphocytes was observed in 5 cases for CD8 T cells and 4 cases for CD4 T cells. A dose dependent increase in both inflammatory cytokines and NK & CD8 + T cells was observed, consistent with expected MOA and PK. TCRSeq analysis demonstrated that product specific T cell clones could be tracked in both patient’s blood and tumor over time. Further analysis to decode the specificity of those cells and demonstrate that tumor antigen specific T cells can be found in patient’s blood and tumor biopsies is ongoing.

Of the 17 patients who received RPTR-147 infusions 10 were noted to have stable disease (SD) and in 4 patients SD lasted > 6 months.

Conclusions Interim results with RPTR-147 have shown it to be well-tolerated and have a favorable safety profile. Dose-escalation is proceeding. Ongoing biomarker analysis will inform future clinical strategies in matching patients to an optimized PRIME IL-15 T cell product.

Trial Registration NCT03815682

Ethics Approval The study was approved by local institutional IRBs after acceptance of the IND by the FDA.
Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0801

802 RAMUCIRUMAB PLUS ATEZOLIZUMAB IN PATIENTS WITH STAGE IV NON-SMALL CELL LUNG CANCER PREVIOUSLY TREATED WITH IMMUNE CHECKPOINT BLOCKADE

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Background T-cell trafficking to the tumor is inhibited via vascular endothelial growth factor (VEGF)-mediated down-regulation of important adhesion molecules on tumor-associated blood vessels and progression of disease on immune checkpoint blockers (ICBs) has been associated with decreased tumor-infiltrating immune cells. We hypothesized that inhibiting VEGF signaling through its receptor, VEGFR2, would increase intratumoral T cells. Therefore we sought to evaluate the combination of ramucirumab, an anti-VEGF receptor 2 monoclonal antibody, and atezolizumab in patients with advanced-stage, non-small cell lung cancer (NSCLC) patients who have previously progressed on at least one line of ICB. Here, we report on the first twelve patients enrolled on trial.

Methods Advanced stage NSCLC patients with an ECOG performance status of ≤1 who had previously been treated with ICBs were eligible with no limitation on prior lines of ICB therapy. Patients with untreated brain metastasis, recent hemoptysis, gastrointestinal bleeding or perforation or fistula were excluded. The study was conducted with a two-stage MiniMax design. Peripheral blood and repeated biopsy, when feasible, were collected for correlative analysis.

Results Twelve patients were enrolled in the first stage of the trial. The median age was 68 (range 47-78), 10 of the patients are female, and 10 had no-squamous histology. Patient had an average of 3.5 prior lines of therapy and 1.6 lines of prior immunotherapy. Overall, treatment was well-tolerated with no grade 3 or 4 adverse events. The most common adverse events were grade 1 or 2 hypertension (35%), nausea (25%) and vomiting (25%). There were no objective responses and 11 patients (91%) achieved stable disease. The median progression-free survival is 3 months with 3 patients (25%) on trial for more than 12 months. The median overall survival (OS) at the time of the latest data cutoff on 9/15/20 is 11.5 months.

Conclusions The preliminary data from our study showed that combination of ramucirumab and atezolizumab is well-tolerated and associated with prolonged overall survival in a subset of heavily pretreated patients who progressed on prior ICB. The trial is still accruing patients and exploratory analyses are planned.

Ethics Approval The study was approved by the Washington University Institutional Review Board.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0802

803 PHASE 1/2 STUDY USING ENB-003, A FIRST-IN-CLASS SELECTIVE ETBRI, IN COMBINATION WITH PEMBROLIZUMAB IN SUBJECTS WITH ADVANCED REFRactory SOLID TUMORS

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Background The endothelin B receptor (ETBR) is upregulated in many types of cancer and is associated with poor overall survival and a paucity of TILs (tumor infiltrating lymphocytes). The ETBR prevents T-cell extravasation and tumor infiltration by a mechanism involving adhesion molecule downregulation in the tumor vasculature. Thus ETBR expression may mediate resistance to immunomodulatory therapy. ENB-003 is a small molecule ETBRI (ETBR inhibitor) which overcomes resistance to anti-PD1 across multiple cancer types in preclinical studies. Part 1 of this study seeks to evaluate the safety and tolerability of ENB-003 in combination with pembrolizumab in refractory advanced ETBR+ solid tumors. Part 2 of the study is an expansion cohort basket trial assessing the efficacy of ENB-003 in combination with pembrolizumab in anti-PD1 refractory melanoma, platinum resistant ovarian cancer and refractory pancreatic cancer.

Methods Study ENB-003-101 (MK-3475-951) is a multicenter, Phase 1/2, open-label study of ENB-003 in combination with pembrolizumab in adult subjects with advanced solid tumors. The part 1 dose escalation is enrolling subjects with ETBR+ tumors and includes 5 doses of ENB-003 in combination with a fixed dose of pembrolizumab. The primary objective of part 1 is to assess safety and tolerability, the secondary objective is to evaluate anti-tumor effect (RECIST 1.1 and iRECIST). Exploratory objectives are to examine biomarkers/pharmacodynamics.

Results ENB-003, as a single agent and in combination with anti-PD1, was investigated in a variety of syngeneic preclinical models. ENB-003 enhanced the anti-tumor activity of anti-PD1 in anti-PD1 resistant models of melanoma, ovarian cancer, pancreatic cancer, bladder cancer and SCC. For example, the combination of ENB-003 plus anti-PD1 in an anti-PD1-resistant melanoma model resulted in complete tumor eradication in 21 days as well as the formation of TLOs (tertiary lymphoid organs). The combination of ENB-003 plus pembrolizumab was well tolerated in the first 2 cohorts of the ongoing Phase 1 trial in patients with advanced refractory solid tumors that are ETBR+. Best overall responses from the first 2 cohorts (n=6) demonstrates disease stabilization (SD) in 2 patients as well as a partial response (PR) in an ovarian cancer patient with ~60% reduction in target lesions.

Conclusions ETBRi is a novel approach to overcoming immunotherapy resistance. The combination of ENB-003 and pembrolizumab is well tolerated thus far and is demonstrating promising early signals of anti-tumor efficacy. Trial updates will be reported.
Trial Registration NCT04205227
Ethics Approval This study was approved by an institutional Review Board at each investigational site.

REFERENCE

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0803

A PHASE II STUDY OF THE ANTI-PROGRAMMED CELL DEATH-1 (PD-1) ANTIBODY PENPULIMAB IN PATIENTS WITH METASTATIC NASOPHARYNGEAL CARCINOMA (NPC) WHO HAD PROGRESSED AFTER TWO OR MORE LINES OF CHEMOTHERAPY

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Background NPC is rare but has a distinct geographic distribution, with a predominance in Southeast Asia. Favorable results with PD-1 inhibitors in NPC provide a strong rationale to investigate penpulimab in this disease. Penpulimab was engineered to eliminate FcγR binding and ADCC/ADCP completely, where ADCC/ADCP effects can induce T-cell apoptosis and clearance and then compromise anti-tumor activity. Penpulimab demonstrated a slower PD-1 antigen binding off-rate than marketed PD-1 antibodies, which results in better cellular activity and higher receptor occupancy. Penpulimab also showed numerous contacts with NS58 glycosylation on the BC loop of PD-1 which could be an advantage to facilitate interaction of PD-1 antibody and may contribute to slower binding off-rate. These structural differentiations offer more robust biological effect and enhance anti-tumor activity of penpulimab.

Methods AK105-202 (NCT038666967) is a multicenter, single-arm, open-label study of penpulimab in metastatic NPC patients (pts) with disease progression after ≥2 prior lines of therapy including platinum-containing chemotherapy. All patients received penpulimab 200 mg q2w until progression or unacceptable toxicity. The primary endpoint was ORR based on RECIST v1.1 as assessed by an independent review committee (IRC). Key secondary endpoints included DCR, PFS, duration of response (DoR). Archived tissues were retrieved for the analysis of PD-L1 (Shuwen SAB-028). PD-L1 expression of tumor proportion score (TPS)≥50% was regarded as positive. Plasma Epstein-Barr virus DNA were obtained for biomarker correlative analysis.

Results As of 18 September 2020, the median follow-up was 7.9 months (range 0.9 to 16.9). The anti-tumor activity of penpulimab in the 111 pts with disease progression after ≥2 prior lines of therapy evaluable for efficacy (defined as pts who had an opportunity to be followed for at least 16 weeks and had measurable disease at baseline per RECIST v1.1) is shown in the table 1.

Conclusion Penpulimab demonstrated encouraging anti-tumor activity and favorable safety profile in pts with disease progression after ≥2 prior lines of therapy. A higher proportion of objective responses was observed in NPC pts with PD-L1–positive tumors receiving penpulimab than those with PD-L1–negative tumors.

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SAFETY AND EMERGING EVIDENCE OF IMMUNE MODULATION OF THE LIVE BIOThERAPEUTIC MRX0518 IN THE NEOADJUVANT SETTING FOR PATIENTS AWAITING SURGICAL REMOVAL OF SOLID TUMOURS

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Background The gut microbiome has emerged as a promising innovative therapeutic target for immune-stimulation treatment of solid tumours. MRX0518 is a novel, gut microbiome- derived oral live biotherapeutic. It has potent anti-tumorigenic efficacy in the preclinical setting including murine models of lung (LLC1), kidney (Renca) and breast (EMT6) cancer.1 In these models, a significant reduction in tumour growth has been demonstrated, including induction of immunostimulatory responses with tumour infiltration of NK cells, CD8+ and CD4+ T-cells. MRX0518 is under investigation in various oncological settings, including in combination with immune checkpoint inhibitors (NCT03637803) and radiotherapy (NCT04193904).

Methods Treatment naïve patients were recruited from April 2019 to February 2020. Patients were eligible if they received a histologically confirmed diagnosis of cancer (solid tumours) scheduled for surgical resection. Patients received 1 capsule of MRX0518 (1x1010 to 1x1011 CFU) twice daily from

Abstract 804 Table 1

<table>
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<th>IRC-assessed (N=111)</th>
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<tr>
<td>confirmed ORR * (% (95% CI))</td>
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<tr>
<td>ORR for PD-L1 positive b</td>
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<tr>
<td>ORR for PD-L1 negative b</td>
</tr>
<tr>
<td>DCR c (% (95% CI))</td>
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<tr>
<td>DoR d (median (range), months)</td>
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<td>time: DoR d (% (95% CI))</td>
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Table 1: Summary of clinical outcomes

a. Including 1 complete response and 29 partial response. At data cutoff, 90% of respondents remained ongoing.

b. 43 pts were PD-L1 positive (TPS≥50%) and 66 pts were PD-L1 negative (TPS<50%)

c. Including 1 ongoing response awaiting confirmation classified under SD.
Changes in T cell clonality in AWARE-1 study, a window-of-opportunity study with atezolizumab and the oncolytic virus Pelareorep in early breast cancer

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Abstract

A previous phase 2 study in metastatic breast cancer demonstrated a statistically significant improvement in overall survival (OS) in patients treated with pelareorep (pela) in combination with paclitaxel (PTX) versus PTX alone. Given that pela is an intravenously delivered immuno-oncolytic reovirus, we hypothesized that the OS benefit from pela + PTX may be attributed to an adaptive T cell response triggered by pela. To examine if pela can mediate the priming of an anti-tumor immune response, we are conducting together with the SOLTI group the AWARE-1 study (a window-of-opportunity study of pela in early breast cancer), which is currently enrolling and for which initial translational research results are presented.

Methods AWARE-1 is a window-of-opportunity study to evaluate the safety and effect of pela ± atezolizumab on the tumor microenvironment (TME) in 38 women with early breast cancer. Patients are treated with pela on days 1, 2, 8, and 9, while atezolizumab is administered on day 3. Tumor biopsies are collected at diagnosis, day 3, and day ~21. Five cohorts will be examined: Cohort 1: HR+/HER2-neg (10 patients) receiving pelareorep + letrozole; Cohort 2: HR+/HER2-neg (10 patients) receiving pelareorep + letrozole + atezolizumab; Cohort 3: TNBC (6 patients) receiving pelareorep + atezolizumab; Cohort 4: HER2+/HR+ (6 patients) receiving pelareorep + trastuzumab + atezolizumab; Cohort 5: HER2+/HR- (6 patients) receiving pelareorep + trastuzumab + atezolizumab. The primary endpoint of the study is CellTIL score, a metric for quantifying the changes in tumor cellularity and infiltration of TILs, where an increase in CellTIL is associated with a favorable response to treatment. Tumor tissue is being examined for pela replication, and changes to the TME are being assessed by immunohistochemistry and T cell receptor sequencing (TCR-seq). Peripheral blood is also being examined by TCR-seq.

Results Detailed TCR-seq results from peripheral blood and tumor tissue are presented for the ten-patients enrolled into Cohort 1 who received pela and letrozole. In tumor tissue, T cell clonality increased in day 21 biopsies relative to baseline biopsies, with similar increases in T cell fraction (the number of T cells) in the majority of patients. In general, most of the tissue-expanded T cell clones were also seen in the peripheral blood.

Conclusions Overall, these preliminary data from cohort 1 of AWARE-1 demonstrate that pela mediates priming of a T cell-based immune response that occurs both systemically and within breast cancer tissue.

Trial Registration NCT04102618

Ethics Approval This study was approved by the Spanish Health Authority, protocol number 2018-003345-42.

REFERENCES


807 A MULTICENTER OPEN-LABEL PHASE I/LB STUDY OF SO-C101 AS MONOTHERAPY AND IN COMBINATION WITH PEMBROLIZUMAB IN PATIENTS WITH SELECTED ADVANCED/METASTATIC SOLID TUMORS

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Background IL-15 is a member of the common y-chain family of cytokines that shares functional activities with IL-2. SO-C101 is a superagonist fusion protein of IL-15 and the IL-15 receptor α sush+i domain. SO-C101 stimulates the proliferation and the cytotoxic activity of NK cells and memory CD8+ T cells.

In pre-clinical studies SO-C101 promoted expansion and activation of human, murine and cynomolgus monkey NK and CD8+ T cells. NK and CD8+ T cell activation correlated with potent monotherapy anti-cancer activity of SO-C101 in metastatic and solid tumor models. The combination of an anti-PD-1 or of anti-cancer monoclonal antibodies with SO-C101 augmented the anti-tumor responses in mouse models. First clinical study was initiated in June 2019 to investigate SO-C101 as monotherapy and in combination with pembrolizumab.

Methods The phase 1/1 b study currently on-going is a multicenter, open-label, dose escalation study of selected advanced/metastatic solid tumors. The study consists of 2 parts: Part A - dose escalation of SO-C101 as monotherapy; Part B - dose escalation of SO-C101 in combination with pembrolizumab. Study objectives are to define the maximum tolerated dose (MTD) and/or recommended phase 2 dose (RP2D) of SO-C101 in both parts.

Results As of September 22nd, 19 subjects were treated in part A in 6 escalating dose levels, and 3 subjects were treated in part B, at dose level 1.

SO-C101 was well tolerated. No DLT was observed, the main AEs related to SO-C101 were injection site reactions, fever, chills, flu-like syndrome, all G1- G2, and transient lymphopenia in 5 subjects, Grade 2 to 4, all resolved within few days.

Preliminary PK results showed the PK profile to be dose-proportional, with a Tmax of approx. 5 – 6 hours after administration and T1/2 approx. 4 hours.

Preliminary PD analysis showed dose dependent NK and CD8+ T cell activation.

A preliminary efficacy signal has been observed in a patient refractory to anti-PD1 therapy, who showed a RECIST PR with initial 20% shrinkage of target lesions at 6 weeks and 49% shrinkage at 12 weeks on CT-scan.

Conclusions To date, SO-C101 has been well tolerated, with a manageable toxicity and encouraging signs of clinical activity. The study will proceed to reach a RP2D in both monotherapy and combination with Pembrolizumab. Expansion of the study in selected indications is warranted.

Trial Registration https://clinicaltrials.gov/ct2/show/NCT04234113?term=sotio&draw=3&rank=12

The study was approved to proceed by FDA – IND 140011 -and by the sites ECs.

Ethics Approval The NCT04234113 clinical trial was approved by each investigational site health agency and ethical committee.

Consent Written informed consent of patients was obtained prior enrollment in the NCT04234113 clinical trial.

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808 EXPLORING RESISTANCE MECHANISM TO PEMBROLIZUMAB AND ANG-2 INHIBITOR TREBANANIB (NCT03239145) USING HIGH-DIMENSIONAL SINGLE-CELL MASS CYTOMETRY (CYTOF)

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Background Angiogenesis is mediated by both the vascular endothelial growth factor (VEGF) family and the angiopoietin (Ang1-2)/Tie-2 pathway.1-3 We demonstrated that increased soluble VEGF and ang-2 is associated with decreased benefit to immune checkpoint inhibitors (ICIs).4 We then initiated a clinical trial combining pembrolizumab and ang-1/2 inhibitor (trebananib) (NCT03239145) with expansion cohorts in microsatellite stable (MSS) colorectal cancer (CRC), ovarian and renal cell cancer.5 We present the correlative analysis using high-dimensional single-cell mass cytometry (CyTOF) to characterize the effects of the combination therapy and examine differences between patients according to clinical benefit.

Methods We used two separate CyTOF panels to monitor 48 markers of innate and adaptive immune populations in 26 evaluable patients who received the PR2D of trebananib (30mg/kg). Mass cytometry assay was performed on peripheral blood mononuclear cells of 26 patients at baseline (C1D1), 16 patients at cycle 3 day 1 (C3D1), and 4 patients at cycle 9 day 1 (C9D1). We compared immune cell markers between patients with clinical benefit (CB) and patients with no clinical benefit (NCB).

Results Of 26 patients (16 CRC, 8 ovarian, 2 RCC), 11 patients had confirmed PR (3) or SD (8) resulting in CB of 42.3% while 15 patients had NCB. Independent of CB, there were statistically significant decreases from C1D1 to C3D1 in naive CD8+ T cells (p=0.03), CD4+ T central memory cells

Abstract 808 Figure 1 T cell subset analysis by cycle and clinical benefit

Detection of T cell subsets at C1D1, C3D1, and C9D1. (A) CD3+ T cells decrease from C1D1 to C3D1 in patients with no clinical benefit (p=0.009, n=16). CD3+ T cells are significantly higher at C3D1 in patients with clinical benefit (p=0.02, n=16). (B) CD4+ T cells decrease from C1D1 to C3D1 in patients with no clinical benefit (p=0.01, n=16). (C) CD8+ T cells decrease in patients with no clinical benefit from C1D1 to C3D1 (p=0.03, n=16). (D) CD8+ T effector memory cells decrease significantly in patients with no clinical benefit between C1D1 and C3D1 (p=0.03, n=16). (E) CD4+CXCR3+ cells decrease significantly in patients with no clinical benefit from C1D1 to C3D1 (p=0.02, n=16). (F) CD8+CXCR3+ cells decrease significantly from C1D1 to C3D1 in patients with no clinical benefit (p=0.02, n=16).
(p=0.05), and PD-1+CD4+ and CD8+ T cells (p<0.0001). In NCB patients, there was a statistically significant decrease from C1D1 to C3D1 in CD3+ T cells (p=0.009), CD4+/CXCR3+ T cells (p=0.02), CD8+/CXCR3+ T cells (p=0.02), and CD8+ T effector memory cells (p=0.03) while no significant changes in these T cell populations were observed in CB patients (figure 1). In NCB patients, monocyct myeloid-derived suppressor cells (p=0.003) and classical monocytes increased from C1D1 to C3D1 (p=0.01) while there was no significant change in this population in CB patients (figure 2). Interestingly, CB patients had higher activated CD56+NKp30+ at baseline (p= 0.03) with increased cytolytic CD56 dim CD16+ population from C1D1 to C3D1 (p= 0.04) compared to NCB patients (figure 3).

Conclusions Our findings suggest that the activity of anti-PD-1 and ang-2 peptibody (trebananib) combination is hindered by an increase in immunosuppressive myeloid cells leading to decrease in memory and effector T cell populations. The association between baseline activated NK cell and the expansion of cytolytic NK cells with favorable outcomes should be further explored.

**Trial Registration** NCT03329145

**Ethics Approval** The study was approved by the Institutional Review Board (IRB) at Dana-Farber Cancer Institute for NCT03329145.

**Consent** Written informed consent was obtained from the patient for participation in this study and publication of data.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0808

**Abstract 808 Figure 2** Myeloid cell subset analysis by cycle and clinical benefit
Detection of myeloid cell subsets at C1D1, C3D1, and C9D1. (A) Myeloid cells increase significantly from C1D1 to C3D1 in patients with no clinical benefit (p=0.01, n=16). (B) Monocyct myeloid-derived suppressor cells increase significantly in patients with no clinical benefit from C1D1 to C3D1 (p=0.003, n=16). (C) Dendritic cells are significantly higher at C1D1 (p=0.02, n=26) and C3D1 (p=0.02, n=16) in patients with no clinical benefit. (D) Total monocytes significantly increase in patients with no clinical benefit from C1D1 to C3D1 (p=0.02, n=16). (E) Classical monocytes increase in patients with no clinical benefit from C1D1 to C3D1 (p=0.01, n=16). (F) M2 macrophages trend higher in patients with no clinical benefit at C3D1 (p=0.07, n=16).

**Abstract 808 Figure 3** NK cell subset analysis by cycle and clinical benefit
Figure 3: Detection of NK cell subsets at C1D1, C3D1, and C9D1. (A) There is a trend towards increased CD56dim/CD16- cells in NCB patients compared to CB patients at C3D1 (p = 0.08). (B) CD56dim/CD16+ cells are significantly higher in CB patients compared to NCB patients at C3D1 (p=0.04). (C) There were no significant differences in CD56bright cells according to cycle or clinical benefit. (D) CD3+/CD19-/CD56+ cells are significantly higher in CB patients compared to NCB patients at C3D1. There is a trend towards decrease of this cell subset from C1D1 to C3D1 in NCB patients (p=0.06). (E) NKp30+/CD56+ cells are significantly higher in CB patients compared to NCB patients at C1D1, but this was not significant at C3D1 (p=0.23).
inhibitor pembrolizumab would improve 1-year DFS for patients with resectable, loco-regionally advanced (clinical T3/4 and/or ≥2 nodal metastases) HNSCC (NCT02641093).

**Methods** Eligible patients received pembrolizumab (200 mg I.V. x 1) 1-3 weeks before resection. Adjuvant pembrolizumab (q3 wks x 6 doses) was administered with weekly cisplatin (40mg/m2 X 6) and radiation (60-66Gy) for those with high-risk features and radiation alone for patients with intermediate-risk features. The primary endpoint was DFS, which was compared by log-rank test to historical controls (RTOG 9501). Evidence of pathological response to neoadjuvant pembrolizumab was evaluated by comparing pre- and post-surgical tumor specimens for treatment effect (TE) defined as tumor necrosis and/or histiocytic inflammation and giant cell reaction to keratinaceous debris. Response was classified as none (NPR, <20%), partial (PPR, ≥20% and <90%) and major (MPR, ≥90%) pathological response. Gene expression analysis in paired tumor specimens was evaluated by Nanostring.

**Results** Sixty-six of 84 enrolled patients had received adjuvant pembrolizumab and therefore were evaluable for DFS at the time of interim analysis. Patient characteristics included: median age 59 (range of 27 – 76) years; 30% female; 85% oral cavity, 11% larynx, and 2% human papillomavirus negative oropharynx; 85% clinical T3/4 and 68% ≥2N; 41(51%) high-risk (positive margins, 49%; ECE, 80%). At a median follow-up of 16 months, 1-year DFS was 66% (95%CI 0.48-0.84) in the high-risk group (p=1) and 91% (95%CI 0.79-1) in the intermediate-risk group (versus 69% in RTOG 9501, p=0.05) (figure 1). Among 70 patients evaluable for pathological response, TE was scored as NPR in 40, PPR in 27, and MPR in 3 patients. Patients with pathological response that were also evaluable for DFS (PPR + MPR) had significantly improved 1-year DFS when compared with those with NPR (100% versus 57%, p=0.0033; HR = 0.18 [95%CI 0.05-0.64]) (figure 2). PPR/MPR was associated with robust macrophage infiltration via Nanostring.

**Conclusions** Neoadjuvant and adjuvant pembrolizumab led to high DFS in intermediate-risk, but not high-risk, resected HNSCC patients. Pathological response to neoadjuvant pembrolizumab was associated with high 1-year DFS.

**Acknowledgements** We’d like to acknowledge the UCCC clinical trials office for their hard work on this study as well as our patients. We’d also like to acknowledge Merck & Co, Inc as they partially funded the clinical trial.

**Trial Registration** NCT02641093

**Ethics Approval** This study was approved by the University of Cincinnati IRB with approval number 2015-6798

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0809
URELUMAB (ANTI-CD137 AGONIST) IN COMBINATION WITH VACCINE AND NIVOLUMAB TREATMENTS IS SAFE AND ASSOCIATED WITH PATHOLOGIC RESPONSE AS NEOADJUVANT AND ADJUVANT THERAPY FOR RESECTABLE PANCREATIC CANCER


Background Data analysis of specimens from prior clinical trials identified the immune co-stimulatory molecule CD137 within the tumor microenvironment (TME) of pancreatic ductal adenocarcinoma (PDAC) that remain to be activated following vaccine induced T cell and PD-1 inhibitory treatments. The requirement of CD137 was subsequently supported by preclinical studies. Therefore, we conducted a clinical trial of combining anti-CD137 agonist antibody urelumab, anti-PD-1 antagonist antibody nivolumab and a GM-CSF-secreting allogeneic tumor cell vaccine (GVAX) as neoadjuvant and adjuvant therapy for resectable PDAC.

Methods Patients of >=18 years old with radiographic evidence of resectable PDACs were eligible for Arm C of this trial (NCT02451982) with an accrual goal of 10 evaluable subjects. The primary objective was to evaluate changes in numbers of tumor infiltrating CD137+CD8+ T cells. Secondary objectives were safety, overall survival, disease free survival, and other immune parameters. Patients who underwent R0/R1 resection were considered evaluable. All subjects received 480mg nivolumab and 8 mg urelumab both intravenously one day prior to receiving GVAX intradermally and two weeks before surgical resection (figure 1). After surgery, eligible patients continued to receive 5 combination immunotherapy cycles in addition to standard of care chemotherapy. Treatment-related toxicity and perioperative complications are monitored.

Results Between February 2019 and August 2020, we completed planned enrollment and treated 10 evaluable patients, who underwent R0 surgical resection of their PDACs. Nausea is the most common adverse event attributed to urelumab (table 1). Other adverse events and perioperative complication

Abstract 812 Table 1

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<th>Adverse Event</th>
<th>CTCAE Term</th>
<th>Grade</th>
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<td>2</td>
<td>1 (10%)</td>
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<td>2</td>
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<tr>
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<td>LFTs increased (ALT, AST, Alk Phos)</td>
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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0810
were observed in a type, frequency and degree similar to other treatment arms. After repeated dosing, 1 patient demonstrated grade 1 arthritis; 1 patient demonstrated self-limited, transient grade 2 elevated LFTs; 1 patient developed grade 3 rashes, which responded quickly to oral steroid and did not recur after re-dosing. Interestingly, two out of 10 resected patients demonstrated CAP grade 2 pathologic responses in the resected PDACs after a single neoadjuvant treatment; this was not observed with other treatment cohorts (GVAX alone or GVAX+nivolumab) in this neoadjuvant platform trial. Nine out of 10 resected patients remain disease free after a median follow up of 12 months. Immunology endpoints are being analyzed by multiplex immunohistochemistry, DNA sequencing for neoantigen loads, and RNA/TCR sequencing.

Conclusions Previous observations of liver toxicity with urelumab or other T cell agonists and severe immune-related adverse events were not observed in this trial, suggesting urelumab(8 mg) is safe as neoadjuvant/adjuvant therapy in this resectable PDAC patient population. Immune and clinical efficacy of anti-CD137 agonist-based combinations warrant further investigation.

Acknowledgements This is an investigator initiated clinical trial and supported by the funding from the Rare Disease Program at Bristol-Myers Squibb.

Trial Registration NCT02451982

Ethics Approval The study was approved by the Johns Hopkins Medical Institution Institutional Review Board, approved number IRB00050517

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Combination immunotherapies

MCLA-145 (CD137xPD-L1): A POTENT CD137 AGONIST AND IMMUNE CHECKPOINT INHIBITOR THAT DOES NOT SHOW SIGNS OF PERIPHERAL TOXICITY

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Background Only a fraction of cancer patients benefit from currently available immune checkpoint inhibitors (ICI). Attempts to improve efficacy of ICI by combining with costimulatory receptor agonists such as CD137 (4-1BB) have led to greater anti-tumor activity preclinically but have shown systemic toxicity in the clinic. MCLA-145 is a human CD137xPD-L1 bispecific common light chain antibody (bAb), identified through functional screening of agonist and ICI bAb combinations. Further, MCLA-145 can overcome Treg and macrophage suppression to potently activate T cells in these immune suppressive conditions. In two ICI insensitive xenograft models, MCLA-145 demonstrated good anti-tumor activity and CD8+ T cells were enriched in tumors post treatment (indicative of intratumor expansion and recruitment). No signs of GvHD were observed in mice following treatment with MCLA-145 in contrast to that seen in animals treated with other ICI mAbs.

Methods The EC30 from an in vitro T cell transactivation assay based on IFNg was used as an estimate of the MABEL for MCLA-145. A 2 compartment PK model coupled to a target-mediated drug disposition component was generated based on the available cynomolgus monkey PK data.

Results Repeated doses of MCLA-145 up to 100 mg/kg/wk in cynomolgus monkeys were well tolerated without major adverse effects, and dose-dependent increases in serum MCLA-145 concentrations were observed. Following allometric scaling, the model was used to predict exposure in humans following MCLA-145 IV given over 2-hours every 2 weeks, including the starting dose for the FIH trial.

Conclusions Conditional activation of CD137 signaling by MCLA-145, triggered by a neighboring target cell expressing PD-L1, may provide both improved efficacy and safety. MCLA-145 is currently undergoing clinical investigation (NCT03922204).

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EVALUATING THE POTENTIAL OF HARNESSING ANTI-LEUKEMIA T CELLS FOR THE TREATMENT OF T CELL ACUTE LYMPHOBlastic LEUKEMIAS (T-ALL)

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Background T cell Acute Lymphoblastic Leukemia (T-ALL) is a devastating malignancy found primarily in pediatric populations. Standard of care for T-ALL has not progressed from intensive regimens of chemotherapy. Another therapeutic strategy for treating T-ALL is to harness anti-leukemia T cells by immunotherapy. Currently, whether T-ALL is sufficiently immunogenic to generate anti-leukemia T cells is unknown. Furthermore, it is unclear how differences in the immune milieu of distinct tissue types (lymphoid vs non-lymphoid) that become infiltrated by T-ALL impacts T cell interactions with leukemia.

Methods These studies utilized primary T-ALL cells from a murine model that were transplanted into immune-competent, congenic (CD45.1) recipient mice. Tissues were evaluated by flow cytometry at distinct stages of disease to help determine if T cells respond to T-ALL. In addition, frozen tissue sections were analyzed using NanoString’s GeoMX Digital Spatial Profiling platform to evaluate T cells in specific regions of varying proximity to T-ALL.

Results Drastic changes to the composition of the TME were found at distinct stages of tumor burden. Evaluation of changes to the hosts’ (CD45.1+) T cells revealed a higher frequency of CD8 T cells with an activated phenotype. Furthermore, this increase largely correlated with tumor burden (figure 1). As this may represent anti-leukemia T cell responses, we next determined if they could be harnessed with immunotherapies directed against T cell co-signaling receptors. Although PD1 and OX40 monotherapies had no discernable effect, the combination of anti-PD1 with anti-
DT095895, A SELECTIVE EP4 RECEPTOR ANTAGONIST WITH MONOTHERAPY EFFICACY IN SYNGENEIC MOUSE MODEL(S) AND BEST-IN-CLASS PROPERTIES

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Background Elevated levels of Prostaglandin E2 (PGE2), an eicosanoid notably synthesized by the cyclooxygenase-2 (COX-2), exert strong immunosuppressive effects in the tumor microenvironment. COX-2-positive solid tumors have the ability to use this pathway as a resistance mechanism, especially to escape from the host immune system, thus limiting the anti-tumor effects of immune checkpoint inhibitors (ICI). These immunosuppressive effects are largely mediated by the EP4 receptor, expressed on multiple immune cells.

Methods A novel series of EP4 receptor antagonists has been developed, with improved pharmacokinetic properties when compared to the EP4 receptor antagonists currently being evaluated in clinical trials. An intensive lead optimization program led to the identification of DT095895, a small molecule development candidate with a ‘best-in-class’ potential. DT095895 was assessed in multiple syngeneic mouse tumor models selected for their COX-2 expression profile.

Results DT095895 preclinical package will be presented in the poster. Efficacy was seen both in a monotherapy setting, as well as in combination with an ICI. Additionally, a specific biomarker program was implemented and validated in order to show target engagement. A phospho-flow murine whole blood assay was set-up to assess the ability of DT095895 to inhibit CREB phosphorylation induced by a selective EP4 receptor agonist in CD3+ cells. This biomarker was further developed for human whole blood to support Phase 1 and clinical trials studies.

Conclusions DT095895 is a selective EP4 receptor antagonist and demonstrates strong anti-tumor effects in multiple syngeneic mouse tumor models, both as a monotherapy and in combination with ICI, through the inhibition of the PGE2-induced immunosuppression. DT095895 progresses in regulatory development.

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SYNERGISTIC CANCER IMMUNOTHERAPY USING TUMOR TISSUE-DERIVED EXOSOMES AND ARTIFICIALLY PRODUCED BACTERIAL OUTER MEMBRANE VESICLES

Kyoung-su Park*, Jan Lütèwall, Kristina Svennerholm, Rossella Crescitieli, Cecilia Lässer, Inta Gribonika. University of Gothenburg, Gothenburg, Sweden

Background Checkpoint inhibitors work only in cancers that host inflammatory cells, and ‘cold’ tumors normally do not respond. Therefore, making ‘cold’ tumors ‘hot’ is required to increase the response rate to immunooncology therapies in general. Bacteria and bacterial products have been utilized for cancer immunotherapy for more than 100 years, but currently no such treatment is available because of the severe side effects that are observed. In this study, we produced artificial outer membrane vesicles (aOMVs) from Escherichia coli outer membrane, and injected them together with cancer tissue-derived exosomes to boost an immune response to the malignancy.

Abstract 816 Figure 1  Increase in memory CD8+ T cells in response to T-ALL

Changes to the T cell compartment were evaluated by transplanting primary T-ALL cells (CD45.2+) into immune-competent CD45.1 congenic recipient mice. T cells were then evaluated in the spleens at distinct stages of disease. As shown below, an increase in the frequency of CD8+ T cells that are memory (CD44+) and effector memory largely correlated with tumor burden in the spleens of transplanted mice that could indicate anti-leukemia T cell responses. Data is representative of a cohort from 1 of 3 independent experiments.

OX40 led to a drastic reduction in T-ALL burden. Importantly, control of tumor growth was accompanied by a concomitant increase in cytotoxic CD8 T cells actively undergoing proliferation specifically in response to combination therapy. To gain better insight into T cell interactions with T-ALL, frozen tissue sections were used for comprehensive digital spatial profiling using NanoString’s GeoMX platform. This analysis revealed strong correlations between immune markers indicative of anti-leukemia responses as well as suppressive factors. Interestingly, regions enriched for activation markers were largely constrained to certain regions indicating the formation of ‘immunological hotspots’ in the context of T-ALL.

Conclusions The results from these studies suggest that T-ALL is recognized by T cells. As immune responses were not uniform within an organ, it will be important to specifically evaluate these ‘immunological hotspots’ in order to identify targets to activate T cells found in these regions. Ongoing studies are therefore aimed at comparing T cell interactions with T-ALL and their responses to immunotherapy between tissue types.

Acknowledgements Analysis of tissue section was supported in part by a SITC-NanoString Technologies Spatial Profiling Award given to T.A.T.

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Methods Outer membranes were obtained from E. coli by chemical means, followed by ionic stress and applied mild energy to generate aOMVs. The yield and purity of aOMVs were analyzed by nanoparticle tracking analysis and transmission electron microscopy. The protein and RNA contents were examined by label-free quantitative mass spectrometry and bioanalyzer. Inflammation was evaluated in macrophage cell line (RAW 264.7) and mice in vivo, and bone marrow-derived dendritic cells were used to assess the immunomodulatory functions of the aOMVs. For the study of antitumor activity, mice were subcutaneously inoculated with B16F10 cells and then subcutaneously immunized with aOMVs and melanoma exosomes five times at 3-day intervals. Also, anti-mouse PD-1 antibody was intraperitoneally injected into mice 1 day prior to immunization to investigate the effects of combination therapy. To elucidate the immunogenic mechanism, blood and spleen were obtained for antibody titer and splenocyte function study.

Results Bacterial aOMVs presented nanosized spherical shape with closed membranes and exhibited high yield and purity with very few cytosolic components. These aOMVs do not cause pro-inflammatory cytokine responses in RAW 264.7 cells and mice in vivo, despite high exposure levels. The aOMVs could be taken up by dendritic cells to stimulate cytokine and maturation marker expression. Co-immunization with aOMVs and melanoma tissue-derived exosomes elicited tumor regression in melanoma-bearing mice through Th-1 type T cell immunity and anti-tumor exosome IgG antibody production. Also, the immunotherapeutic effect of aOMVs was synergistically enhanced by anti-PD-1 inhibitor.

Conclusions Bacterial aOMVs can be produced in a large quantities with high purity, but are ‘detoxified’ compared to naturally released OMVs. The non-toxic aOMVs are powerful adjuvants for eliciting specific anti-tumor response, suggesting that aOMVs may be novel bacterial vesicle-mimetics clinically applicable as cancer treatment.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0818

Abstract 819 Figure 1 Overexpression of VIP in PDAC tumors
(A) VIP mRNA expression in several solid malignancies, with red arrow pointing at the levels in pancreatic cancer, as obtained from The Cancer Genome Atlas. (B) Blood collected from healthy volunteers (n=26) and consented untreated pancreatic cancer patients before surgery/chemotherapy (n=41) were quantified for levels of VIP. (C) Cell free supernatant collected from B16F10, SM1 and D4M (murine melanoma cell lines) or KPC, MT5 or Panc02 (murine PDAC cell lines) or BXPC3 and Panc1 (human PDAC cell lines) were quantified for VIP (D) C57BL/6 mice were implanted with 1 million B16F10 cells (n=4), D4M cells (n=4) or MT5 cells (n=3) subcutaneously, or with KPC cells in the tail of the pancreas after laparotomy (n=3). When tumor volumes reached 500mm3 for subcutaneous tumors or the tumor flux reached 2 x 1010 photons/sec for orthotopic KPC tumors, mice were sacrificed and the concentration of VIP in the blood was determined. P value for B was calculated using student t-test and C and D were calculated by one-way ANOVA followed by Tukey’s post-test. Error bars show mean with standard deviation. *p<0.0001

Abstract 819 Figure 2 PDAC cell lines express receptors for VIP
(A) Western blot analysis showing constitutive expression of VPAC1, VPAC2 and PACAP receptors in murine and human PDAC cell lines and B16F10, a murine melanoma cell line. GAPDH was used as the loading control. (B) Percentage viability of KPC and Capan02 cells treated with varying concentrations of ANT008 (0.5-5µM) relative to cells treated with 0µM ANT008 for 24, 48 and 72 hours as measured by MTT assay.
Results

Increased human and mouse PDAC expression correlates with elevated blood levels (figure 1). While the PDAC cancer cell lines express VIP receptors, ANT008 does not have direct cytotoxic effect on cell growth in vitro (figure 2). However, in orthotopic KPC model, treatment with ANT008 & anti-PD-1 significantly decreased tumor growth rate and burden (figure 3) while increasing the intratumoral levels of CD4 and CD8 proliferating T cells (figure 4) via a T cell dependent mechanism (figure 5). Additionally, in ex vivo cultures of T cells isolated from PDAC patients, ANT008...

Abstract 819 Figure 3  Efficacy of ANT008+aPD-1 therapy in in-vivo model
5x10^5 KPC.luc cells on a matrigel were implanted in the tail of pancreas of C57BL/6 mice. Seven days after tumor implantation, mice were randomized and treated with ANT008 and/or aPD-1 until day 25, when they were sacrificed. (A) From each treatment group, one mouse with biggest non-ulcerated tumor was imaged via IVIS imaging (top), sacrificed and imaged with 9.4T MRI scanning (MRI) and their paraffin embedded pancreatic tissues were stained via H&E (bottom). (B) The tumor flux measured via IVIS imaging is plotted with respect to days after tumor implantation. 'o' represent mice that were imaged via MRI on day 28 and '+' represent mice that were sacrificed before day 25 due to ulceration. (C) On day 25 mice were sacrificed and the weight of the pancreas was plotted. The brown dashed line represent average weight of pancreas from naïve age matched mice. P value was calculated using one-way ANOVA followed by Tukey’s post-test. Error bars show mean with standard deviation with **p<0.01.

Abstract 819 Figure 4  ANT008+aPD-1 therapy increases T cell infiltration
5x10^5 KPC.luc cells were implanted in the tail of the pancreas following laparotomy. Once the tumors were detectable via bioluminescent imaging, mice were randomized into four treatment groups and treated with isotype antibody and scrambled peptide or ANT008 and/or anti-PD-1 until day 25, when the mice were sacrificed, tumor tissues harvested, and formalin fixed. The tissues were stained for nucleus, CD4, CD8 and Ki67 via multiplex immunohistochemistry (A) A representative multiplex IHC image showing T cell infiltration in the different treatment groups. (B) Numbers of CD4, CD8, Ki67+ CD4 and Ki67+ CD8 T cells/mm2 with respect to weight of the pancreas is shown.

Abstract 819 Figure 5  ANT008+aPD-1 therapy is T cell dependent
5x10^5 KPC.luc cells were subcutaneously implanted in C57BL/6 or CD4KO (A) and C57BL/6 or CD8KO (B) mice and treated with scrambled +IgG (control) or ANT008+aPD-1 (treated) for 10 days from day 5 or 7 when the tumors were palpable. Mice were monitored for tumor growth and sacrificed when the tumor volume reached 500mm^3 or when the tumors ulcerated. P values were calculated using Log rank test. *p<0.0001.

Abstract 819 Figure 6  ANT008 improves effector properties of T cells
Cryopreserved PBMCs from peripheral blood of consented PDAC patients were thawed and rested overnight at 37°C in a CO2 incubator followed by T cell isolated via negative magnetic sorting. Isolated T cells were seeded on human anti-CD3 coated plates and cultured in media supplemented with 30U/ml human recombinant IL-2 and 3uM of scrambled peptide (SCRAM) or ANT008 for 9 days. On day 9 the cells were counted and stained for CD3, CD4, CD8, Tim-3, Lag-3, PD-1, CD25, FoxP3 and with aqua live/dead viability stain and analyzed via flow cytometry. (A) The gating strategy, (B) yield on da 9 with respect to number of cells seeded on day 0 and C) the percentage of T cells expressing co-inhibitory molecules are shown. P values were calculated by student t test. *p<0.001.

Abstract 819 Figure 7  Survival correlates with VPAC1 expression (TCGA)
Survival probability analysis and the respective Kaplan Meier curves from TCGA data sets for pancreatic ductal adenocarcinoma (PAAD), stomach adenocarcinoma (STAD), and colorectal adenocarcinoma (CAAD). (A) Combined survival analysis of multiple GI malignancies (PAAD, STAD and COAD) demonstrates increased VPAC1 expression is associated with a 1-year 50% survival benefit (75 vs 50%) (n=560 (high) & 557 (low)), (B) while VIP expression is not (n=540 (high) and 540 (low)). (C) In pancreatic adenocarcinoma, increased VIP receptor (VIPR1) expression trends toward an overall survival benefit (n=91 (high) & 92 (low)), while (D) VIP expression does not.
improved the effector properties of T cells via decreasing expression levels of co-inhibitory molecules and decreasing frequency of regulatory T cells (figure 6). Clinically, VIPR1 receptor expression, but not VIP, provides a survival benefit (figure 7).

Conclusions VIP is a targetable mechanism of immune escape in PDAC. Inhibiting VIP receptor signaling improves effector properties of T cells and synergistically improves the anti-tumor response to checkpoint inhibitors in mouse PDAC models.

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0819

820 MCLA-145 IS A BISPECIFIC IG/I ANTIBODY THAT INHIBITS PD-1/PD-L1 SIGNALING WHILE SIMULTANEOUSLY ACTIVATING CD137 SIGNALING ON T CELLS


Background MCLA-145 is a CD137 x PD-L1 bispecific antibody that releases PD-L1 mediated T-cell inhibition and activates and expands T cells through agonism of CD137. Immune checkpoint inhibitors (ICI) against PD-(L)1 have demonstrated anti-tumor efficacy in a fraction of patients across a broad range of cancers. CD137 (4-1BB, tumor necrosis factor receptor superfamily 9) is an inducible costimulatory receptor transiently expressed on T cells after TCR engagement. CD137 signaling is triggered by receptor clustering and leads to enhanced cytokine production; T cell proliferation, survival, and effector function; and immunological memory formation. Targeting of PD-L1 and CD137 with MCLA-145 may achieve synergistic activity by simultaneously blocking the inhibitory checkpoint-stimulation and activating tumor specific T cells through co-stimulation.

Methods We performed combinatorial functional screening of bispecific antibodies generated from high affinity inhibitory Fab binding PD-L1 combined with a large and diverse panel of agonistic CD137 Fabs.

Results MCLA-145 was selected based on its in vitro potency in multiple primary human immune cell assays. Further, it displays an ability to reverse T cell suppression mediated by M2 macrophages or Tregs. MCLA-145 binds to a unique epitope in the cysteine rich domain 2 of CD137 that overlaps with the CD137L binding region, and all potent hAbs in the screen were able to bind to this region. MCLA-145 drives activation of T cells and the degree of CD137 agonistic activity in T cells correlated with the expression level of PD-L1 on neighboring cells. Using proximity ligation assays and confocal microscopy we demonstrated that MCLA-145 clusters CD137 on the surface of T cells resulting in internalization. The binding location of MCLA-145 on CD137 may be optimal for the formation of ‘immunological synapses’ with PD-L1 expressing antigen presenting cells or tumors resulting in the potent activation of tumor specific cytotoxic T cells.

Conclusions These experiments demonstrate the dual anti-cancer activity of MCLA-145 in preclinical models: release of T-cell checkpoint inhibition through PD-L1; and activation and expansion of T cells through CD137, therefore overcoming T-cell exhaustion and increasing T-cell presence/activity (infiltration) in tumors. MCLA-145 is currently undergoing clinical development in an ongoing trial (NCT03922204).

Ethics Approval Animal experiments were performed according to guidelines for animal care of the local Animal Experiments Committee; Use of human blood cells from healthy volunteers was approved by the blood bank’s Ethical Advisory Council.

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821 CLASS SPECIFIC HDAC INHIBITION DIFFERENTIALLY AFFECTS THE FUNCTION OF SPECIFIC T CELL SUBSETS

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Background Histone deacetylase inhibitors (HDACi) are currently being used in the clinic to treat a variety of cancer types. Despite their wide use, the mechanism by which they exert anti-tumor effects is largely unknown. Although originally posited to abrogate tumor proliferation via regulating tumor suppressor genes, responses to monotherapies of HDACi have been shown to be dependent on an adaptive immune system and to enhance responses to immunotherapy. However, whether this mechanism is driven by enhancing tumor immunogenicity or enhancing anti-tumor immune responses is unclear. Understanding this could help identify optimal combination regimens for augmenting immunotherapies. Given the role of epigenetics in regulating T cell differentiation upon antigen encounter into discrete subsets, these studies sought to determine whether HDACi differentially impact naïve from memory T cell subsets.

Methods PBMCs from healthy donors were stimulated with either anti-CD3/anti-CD28 or PMA/Ionomycin in the presence of a broad range of HDACi agents. Cytokine production was measured by ELISA and the frequency of regulatory T cells by flow cytometry. The impact of HDACi on T cell subsets was assessed by comparing responses of T cell subsets for changes in cytokine production, protein acetylation and other functional responses. Supernatant was collected for comprehensive cytokine bead arrays.

Results Cytokine analysis of supernatants showed clear differences in response to HDACi as while most cytokines decreased, others were either unaffected or increased. We next performed ICS with surface markers to determine if these changes in cytokine production levels were subset specific. Comparisons of naïve and memory subsets found decreased IL-2 levels was primarily attributed to loss of production by naïve T cells. Furthermore, gain of TNFα was almost completely restricted to naïve cells. The preferential responses by naïve T cells was further verified during global changes in acetylated protein levels. Lastly, we found differences between
LOCAL RADIOTHERAPY SYNERGIZES WITH TUMOR-SPECIFIC TCR REDIRECTED T CELLS IN THE REJECTION OF PROSTATE CANCER

Background Adaptive T cell therapy (ACT) has become a promising option for cancer patients. While tumor-infiltrating lymphocytes were initially exploited as a source of tumor reactive lymphocytes, T cells genetically redirected to the tumor by TCR/CAR gene transfer are now in clinical validation. In the case of solid tumors, unfavorable immunosuppressive microenvironments remain recognized barriers to therapeutic efficacy. We have recently reported that the therapeutic activity of ACT against poorly immunogenic and indolent prostate cancer is improved by the concurrent targeting of the tumor stroma by means of T cells redirected to an ubiquitously expressed minor histocompatibility antigen or a tumor vessel targeted TNF derivative. We have now taken the concept further and hypothesized that local radiotherapy (RT), might also synergize with ACT by promoting lymphocyte endothelial transmigration and tumor recognition, and ultimately favor ablative effects.

Methods We investigated the combination of local RT and ACT in TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) mice and in mice bearing subcutaneous B16/B16-OVA (MO4) or TRAMP-C2/ TRAMP-C2-OVA tumors. Local RT was delivered by X-RAD SmART (the Small Animal Radiomicro) in single dose or hypo-fractionated regimens. ACT consisted of T cells engineered with tumor-specific TCRs. Immunogenic consequences were analyzed by Real-Time PCR, and flow cytometric (FACS) analyses. Prostate tumor debulking was evaluated by histological analyses.

Results We found that local hypofractionated RT and ACT, while individually ineffectual in controlling tumor growth, concurred to the debulking of advanced prostate adenocarcinoma when used in combination in treating TRAMP mice. Mechanistically, exposing isolated tumor cells, or the TRAMP mouse prostate to hypo-fractionated RT regimens induced stronger type-I interferon (IFN-I) responses, when compared to single high dose. Acutely, hypofractionated RT promoted better immune tumor infiltration, among which TCR redirected effector cells.

Conclusions Data support feasibility and efficacy of combining hypo-fractionated local RT with ACT in the form of TCR engineered T cells to promote prostate cancer recognition and eradication. Tumor debulking was observed in the absence of treatment-related toxicity. Systemic recirculation of TCR redirected T cells was observed. We are now investigating therapeutic effects at distal (metastatic) sites.

Acknowledgements The authors acknowledge the support of the Italian Association for Cancer Research (AIRC) and the Italian Association for Cancer Research (AIRC) with the Italian Association for Cancer Research (AIRC)

Ethics Approval The studies involving animals were approved by The Institutional Ethical Committee (IACUC#999).

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CD4 T CELLS ARE ESSENTIAL FOR AN ANTI-TUMOR EFFECT IN A B78 MURINE MELANOMA TUMOR MODEL


Background Mice bearing B78 melanoma tumors can be cured using an in situ vaccine (ISV) regimen that includes radiation (RT) together with immunocytokine (tumor-targeting mAb conjugated to IL-2). B78 melanoma cells, derived from B16 cells, express minimal to no MHC-I but express MHC-II upon IFN-γ/TNF-a stimulation. Although B78 cells are primarily MHC-I-deficient, an increased CD8 T cell infiltration into the tumor microenvironment (TME) has been shown following ISV. We further investigate the potential role of specific immune cell lineages in the B78 anti-tumor response to ISV, immune subset depletion studies and flow cytometric analyses were performed.

Methods C57BL/6 mice bearing B78 tumors were depleted of immune cell subsets with mAbs (anti-CD4, anti-CD8, anti-NK1.1, or Rat IgG control) for 3 weeks during the course of treatment. Treatment groups included no treatment, RT (12 Gy), or ISV (RT D0 and immunocytokine D5-D9). 6 mice/group (repeated three times) were followed for survival/tumor growth, and flow cytometry studies included 4 mice/group, sacrificed on D8 and D13 following the start of ISV.

Results Mice depleted of CD4 T cells during the course of ISV showed a significant reduction of anti-tumor effect as compared to mice treated with ISV/Rat IgG (p

Conclusions These studies suggest that CD4 T cells are essential for an anti-tumor response in the B78 melanoma model. In vivo depletion data show that CD4 T cells, but not CD8 or NK cells, are required for a decrease in tumor growth via ISV. Flow cytometric analyses suggest an interplay between CD4 and CD8 T cells as indicated by a decrease in CD8/IFN-γ expression following ISV in the absence of CD4 T cells. The role that MHC-I and MHC-II expression plays in this anti-tumor response is under investigation. In future studies, B78 melanoma may serve as a critical syngeneic model for development of more effective immunotherapy treatment regimens.

Ethics Approval All animal experiments were performed in accordance with protocols approved by Animal Care and Use Committees of the University of Wisconsin-Madison.

REFERENCE


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0823
MODULATION OF TLR3 PROTEIN IN RESPONSE TO RADIATION IN SQUAMOUS CELL LUNG CARCINOMA

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Background Squamous cell lung cancer (SCLC) is the second most common type of lung cancer. Treatment is complicated due to the lack of mutated molecular targets. Radiotherapy (RT) is commonly used to treat SCLC, but relapse and tumor progression are common. The combination of immunotherapy (IT) with RT can enhance the effect observed with RT alone. Effective combination of IT and RT requires an understanding of the pathways that synergize to enhance tumor cell kill in SCLC. Our lab has identified Toll-like receptor 3 (TLR3) as a molecule that is regulated by RT and can be targeted with IT. Toll-like receptors serve a crucial role against tumor cells by activating innate and adaptive immune responses that boost antitumor immunity. TLR3 is the only receptor whose molecular mechanism functions independent of MyD88, leading to NF-kB mediated apoptosis. We hypothesized that increased TLR3 expression would be associated with improved response to RT. We further hypothesized that RT can downregulate TLR3 and that this effect can be reversed with TLR3 agonists leading to enhanced tumor antigen recognition. We aim to use this data to formulate further studies using combined RT and IT.

Methods Mouse (KLN205) and human (SW900) squamous cell carcinoma (SCC) cell lines were used to study the effect of radiation on TLR3 expression. Irradiation was performed using the gammacell 3000 elan irradiator. Cells were irradiated with 0, 5, 10 and 20 Gy. Protein extraction was performed 48 and 72 hours after RT. Protein extracts were analyzed by Western Blot. Further, TLR3 mRNA expression and 5-year overall survival of SCLC patients was obtained from public databases. Kaplan-Meier method was used to correlate between TLR3 mRNA expression and survival.

Results In vitro studies and western blot analysis demonstrated a decrease of TLR3 expression in response to increasing doses of radiation. This observation was consistent in mouse and human SCC cell lines. In silico analysis of SCLC patients who received RT showed that increased TLR3 mRNA expression was associated with improved overall survival and disease-free survival.

Conclusions Our findings point to an important role for TLR3 in SCLC. Combining RT with TLR3 agonists may enhance the tumor response to RT. Several complementary experiments are underway in our lab to use the TLR3 agonist, Poly I:C, which will allow a better understanding of the effect of RT on TLR3.

REFERENCES

COVID and immunotherapy

DEEP IMMUNE PROFILING OF SARS-COV-2 ASSOCIATED IMMUNE MICROENVIRONMENT IN CANCER TISSUES FROM RECOVERED COVID-19 PATIENTS

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Background Persistence of SARS-CoV-2 virus particles in recovered COVID-19 patients remains a challenge as we continue to fight the ongoing pandemic. For instance, despite three negative consecutive nasopharyngeal swab PCR tests, residual SARS-CoV-2 was reported in the lungs of a deceased patient. Moreover, viral RNA could also be detected in rectal tissues that were obtained during incubation period. To date, there is no data regarding residual viral particles present in tissues from recovered COVID-19 patients. Hereby, we reported our findings of SARS-CoV-2 viral antigen in liver tissues from a recovered COVID-19 patient. These findings raise concern for potential transmissibility in recovered individuals.

Methods A 49-year-old South Asian male diagnosed with COVID-19 in June 2020, with incidental discovery of hepatitis B virus (HBV)-associated R0 Grade 2 hepatocellular carcinoma (HCC), was consented for our study. He did not develop significant acute respiratory symptoms throughout the course of the disease. He underwent curative resection of HCC 85 days after being tested COVID-19 negative where his blood, normal tissue and tumour samples were obtained for further analysis (figure 1). We performed deep immunopanomapping profiling on the specimens using multiplex immunohistochemistry and 25-colour flow cytometry to study SARS-CoV-2-elicited immune response.

Abstract 825 Figure 1 Study design, methodology and brief summary of the findings

Blood, normal tissue and tumour samples were obtained from a 49-year-old South Asian male who was diagnosed with COVID-19 and hepatocellular carcinoma. Normal tissue and tumour samples were analysed with multiplex immunohistochemistry, while dissociated cells from blood and tissue samples were subjected to SARS-CoV-2 peptide stimulation and analysed with 25-colour flow cytometry. Multiplex immunohistochemistry detected SARS-CoV-2 proteins in both tumour and adjacent normal tissues, while flow cytometry identified distinct immune microenvironment involving memory-like T cells.
Abstract 825 Figure 2 Immunohistochemical staining of the SARS-CoV-2 nucleocapsid protein and immune profiling with 25-colour flow cytometry in normal colon and liver tissue. a, Liver tissues were immunostained with SARS-CoV-2 nucleocapsid protein (NP), nuclei were counterstained with haematoxylin. Positive SARS-CoV-2 nucleocapsid staining in benign hepatocytes and sinusoidal Kupffer cells. Scale bar represents 50 μm. b, Multiplex immunohistochemistry of normal liver tissue. From left to right, top to bottom: DAPI (blue), CD3 (magenta), CD38 (green), granzyme B (yellow), interferon-gamma (red) and composite. Co-localisation were observed as shown by the white arrows. Scale bar represents 100 μm. c, Colon tissues were immunostained with SARS-CoV-2 nucleocapsid protein, nuclei were counterstained with haematoxylin. Positive SARS-CoV-2 nucleocapsid staining in colonic crypts, with granular supranuclear cytoplasmic pattern. Scale bar represents 50 μm. d, Multiplex immunohistochemistry of colon tissue. From left to right, top to bottom: DAPI (blue), CD3 (magenta), CD38 (green), granzyme B (yellow), interferon-gamma (red) and composite. Co-localisation was observed as shown by the white arrows. Scale bar represents 100 μm. e, Flow cytometry immune profiling of blood from colorectal cancer patient with COVID-19 following stimulation with SARS-CoV-2 peptides. Highlighted populations showed CD3 cells expressing CD38, supporting the CD3+ CD38+ co-localization findings observed in (c).
generated an exact match of control patients from the MGB database on age, sex, and Charlson comorbidity index. For both groups, we cross referenced COVID-19 infection data through June 19, 2020 from the Massachusetts Department of Public Health using date of birth, last name, and first four letters of the first name. We calculated odds ratios (OR) for COVID-19 diagnosis using a multivariate logistic regression adjusting for age, sex, race, CCI, zip code income, and local infection rate.

Results Twenty-one patients (1.3%) prescribed ICIs and 527 controls (2.0%) were identified as COVID positive in the Massachusetts department of health database. When controlling for local infection rate, age, sex, race, CCI, and zip code income, there were no significant differences in COVID infection between ICI recipients and matched controls (OR: 0.7, 95% CI: 0.45 – 1.09, p=0.1; table 1).

Conclusions In our experience, patients who were prescribed ICI were not more likely to contract COVID-19 than matched controls, which may assist in decision-making around continuation of therapy during the pandemic. More research needs to be conducted to determine potential behavioral and testing factors that may affect COVID-19 diagnosis.

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Immune cell biology

ELUCIDATING THE ROLES OF PIK3IP1/TRIP IMMUNE REGULATION ON DISTINCT T CELL SUBSETS IN THE CONTEXT OF CANCER

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Background The signaling pathways involving phosphoinosito-tide-3-kinases (PI3Ks) are highly conserved and tightly regulated to influence the activation, proliferation, and survival of all cell types. PI3K signaling plays a major role in T cell responses to antigen due to its position directly downstream of T cell receptor (TCR)/CD28 ligation. Our lab has recently shown that the cell surface protein TrIP (Transmembrane Inhibitor of PI3K, gene name: Pik3ip1) is capable of downregulating PI3K signaling in CD4+ T cells and can act as a negative regulator of T cell immune responses.1 This negative immune regulation was just recently reported to promote antitumor T-cell immunity, implicating TrIP as a potential immunotherapeutic target.2 Interestingly, although all effector subsets express TrIP to varying degrees, public expression data shows that Treg cells maintain higher TrIP message than other T-effector subsets.3

Methods Using a conditional TrIP knockout mouse model developed in our lab, we have begun to interrogate how TrIP expression regulates the opposing activities of CD8+ T cells vs. Treg and how these affect the overall tumor immune landscape. With TrIP-specific TrIP KO, we assessed the effects on syngeneic tumor growth in vivo, as well as analyzed primary and tumor-derived Treg phenotypes ex-vivo.

Results Thus far, we have found that TrIP knockout in the Treg compartment leads to no detectable differences in tumor burden. However, the lack of TrIP expression on Treg does have some effect on the effector phenotype of Treg cells isolated from the tumor.

Conclusions We describe preliminary data on the role of TrIP in Treg function and phenotype and have begun to explore its effects on the tumor microenvironment. To build on this work we are currently developing TrIP over-expressing lentiviral constructs to complement the knockout approaches described here. We have also now obtained mice with tamoxifen-inducible TrIP KO in Treg, so we will determine whether the timing of TrIP deletion affects the impact of TrIP deficiency.

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IMPROVED GROWTH PROPERTIES AND IMMUNE SURVEILLANCE IN K-RAS G12V-TRANSFORMED CELLS THROUGH OVEREXPRESSION OF BIGLYCAN

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Background The extracellular matrix protein biglycan (BGN) plays an essential role in matrix assembly, cellular migration, adhesion, proliferation and apoptosis. Recently, BGN expression has been shown to be impaired upon HER-2/neu overexpression, which was associated with an up-regulation of MHC class I expression. However, there exists no information about the link between K-RAS-mediated immune escape and BGN expression.

Methods In vitro models of human K-RAS G12V transformed mouse fibroblasts and two human colorectal carcinoma (CRC) cell lines carrying a K-RAS G12V mutation (RKO and SW480) were used for the analysis of BGN expression by qPCR and Western blot. At the same time, the major histocompatibility complex (MHC) class I surface expression, as well as CD4+ and CD8+ cells, were determined by flow cytometry. The different K-RAS G12V cells and respective controls were stably transfected with BGN. Growth properties were analyzed by proliferation, migration and invasion assays. Luciferase reporter assays were used to determine the transcriptional regulation of MHC class I and components. Tumorigenicity of BGN transfectants in comparison to control cells was evaluated by injection of respective transfectants s.c. into mice and tumor growth was monitored over time.

Results Both murine and human K-RAS G12V cells express low levels of BGN compared to control cells. Overexpression of BGN caused an inhibition of cell proliferation, a diminished anchorage-independent growth and a reduced migration rate. The altered in vitro growth properties of BGN-high K-RAS G12V+ correlated with a delayed tumor growth and a reduced frequency of tumor formation in vivo. Restoration of BGN expression increased the expression of decorin as well as enhanced MHC class I expression in K-RAS G12V-transformed cells. This is due to a BGN-induced transcriptional upregulation of major components of the MHC class I antigen processing machinery (APM), such as the transporter associated with antigen processing TAP1, TAP2 and LMP2, in BGN transfectants of K-RAS G12V+ cells. The results were further...
supported by the fact that mice bearing tumors induced by BGNhigh K-RAS G12V+ cells showed a reduced MHC class I expression, which was associated with an enhanced frequency of CD8+ and CD4+ cells in the peripheral blood.

Conclusions Our data provide evidence that (i) proteoglycan signatures are modulated by K-RAS G12V transformation, (ii) loss of proteoglycan expression is directly or indirectly involved in immune escape of K-RAS G12V overexpressing tumor cells and (iii) BGN overexpression and enhanced basal decorin expression results in altered growth properties of K-RAS G12V cells. Thus, the reduced migration rate and restoration of MHC class I surface expression by BGN or other proteoglycans is important features for their anti-tumorigenic properties in K-RAS G12-transformed tumor cells including colorectal cancers.

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TUMOR ALPHA-FETOPROTEIN INHIBITS CHOLESTEROL AND STEROID METABOLISM IN MONOCYTE-DERIVED DENDRITIC CELLS

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Background Hepatocellular carcinoma (HCC) is a particularly lethal malignancy in part due to the potently immune-suppressive tumor microenvironment. The weak immune response is due in part to the presence of tumor alpha-fetoprotein (tAFP), a fetal glycoprotein that is produced by a majority of HCC tumors. Previously, we showed that tAFP potently inhibited the differentiation of monocytes to dendritic cells when compared to cord blood-derived normal AFP (nAFP) and ovalbumin (OVA). Additionally, we demonstrated that tAFP inhibits lipid metabolism by limiting the expression of fatty acid metabolic enzymes. To identify the mechanism whereby tAFP alters dendritic cell metabolism, we analyzed microarray data by a functional enrichment pathway analysis with g: Profiler.

Methods Monocytes from healthy donors (n=4) were isolated with CD14 magnetic beads and differentiated for five days in the presence of IL-4 and GM-CSF with OVA, nAFP, or tAFP. After five days, we isolated RNA for microarray analysis using an Affymetrix HG-U133A array. R studio generated principal component analysis. Differentially expressed (DE) genes were identified as a 1 log fold change and had adjusted p values of 0.05.

Results Principal component analysis of the gene expression data revealed that tAFP clustered separately from OVA and nAFP based on PC1 (p = 0.016) and PC2 (p = 0.009) (figure 1). In total, 688 DE genes were identified with 495 upregulated and 193 downregulated (figure 2). Downregulated DE genes between tAFP versus nAFP yielded significantly down regulated pathways including cholesterol (p = 10e-7.5), steroid (p = 10e-7.5), and lipid biosynthesis (p = 10e-6) (figure 3). Interestingly, upregulated DE genes between tAFP versus nAFP included many pathways specific to stress response to metal ions including zinc (p = 10e-10.5) and copper (p = 10e-10) (figure 4).

Conclusions In addition to validating previous data demonstrating tAFP inhibited lipid biosynthesis generally, this is the first report to our knowledge of tAFP inhibiting gene signatures associated with cholesterol and sterol synthesis specifically. Furthermore, we identified significant upregulation of
gene pathways corresponding to the response gene to metal ions. Notably, functional assays are underway to confirm these gene pathway data. These findings shed new insight into how tAFP perturbs monocyte and DC metabolism and thereby limits differentiation of monocytes to immature dendritic cells. Future insights into how tAFP limits innate immunity could lead to improved immunotherapies for HCC.

**Ethics Approval**
Samples were collected with informed consent at the University of Pittsburgh (Pitt IRB #UPCI 04-001 and UPCI 04-111).

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### 830

**TARGETING CELLULAR SENESCENCE TO INCREASE CAR-T CELL FITNESS**

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**Background**
Immunosenescence refers to the age-associated decline of the adaptive immune system, which results in increased incidence and severity of infections, cancers, and autoimmunity. The elderly show reduced numbers of naïve T cells, skewed CD4:CD8 ratio, reduced proliferative and functional capabilities, and increased expression of senescence markers. These phenomena have strong repercussion in adoptive immunotherapy.

Notably, the ex vivo manufacturing process of CAR-T cells per se induces senescence extremely quickly; 15 days of T cell expansion age cells 30 years, as measured by telomere length, T cells differentiation and CDKN2a mRNA levels.

To circumvent this problem, we here propose the modulation of USP16, an epigenetic regulator of stem cells and senescence in multiple tissues. Downregulation of USP16 rejuvenates T cells, offering a powerful tool to dramatically improve the efficacy of CAR-T treatments.

**Methods**
During ex vivo CAR-T cell manufacture, cells age very rapidly, strongly decreasing T cell fitness. Importantly, we observed that cellular senescence is an early event that precedes T cell exhaustion upon CD3/CD28 T cell stimulation, making it a very interesting pathway to target. In line with this hypothesis, we demonstrated that reducing cellular senescence increases CAR-T cell functions both in vitro and in vivo.

**Results**
We identified an epigenetic regulator, USP16, whose mRNA levels increase during T cell expansion and correlate with the expression of the aging marker par excellence, CDKN2a. Genetic modulation of USP16 in CD19 and GD2 CAR-expressing T cells not only reduces senescence markers but also expands the naïve (CD45RA+CD62L+) population and enhances cell self-renewal, without negative effects on T cell expansion. USP16 modulation also results in increased killing, polyfunctionality, and expansion upon in vitro stimulation with tumor cells. Notably, the delay of cellular senescence induces long-lasting cellular fitness (figure 1) as T cells are less exhausted upon multiple tumor challenges. In vivo, T cells rejuvenated by USP16 modulation, are 60% more efficient in controlling tumor growth in a mouse model of leukemia (NALM-6) and neuroblastoma (CHLA-255).

**Conclusions**
We demonstrated that modulation of USP16 prevents cellular senescence and increases self-renewal in T cells. This approach can significantly improve CAR-T therapy in multiple diseases, including leukemias and solid tumors. Development of small molecules against USP16 could offer a viable solution to improve T cell fitness during manufacturing.

**Ethics Approval**
The study was approved by Institutional Animal Care and Use Committees (IACUC), approval number CR-0104.

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### 831

**E3 UBIQUITIN LIGASE CBL-B DEFICIENT CD8+ T CELLS OVERCOME TREG CELL-MEDIATED SUPPRESSION THROUGH IFN-γ AND INDUCE ROBUST ANTI-TUMOR IMMUNITY**

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**Background**
Adoptive T cell therapy (ACT) is reaching its potential in multiple malignancies. However, anti-tumor T cell responses can be attenuated by suppressive cells in the tumor microenvironment, such as CD4+FoxP3+ regulatory T (Treg) cells. Depletion of Treg cells can be technically challenging in ACT and may be associated with unwanted adverse effects. Alternatively, studies suggest that specific modifications in T cell signaling network may render T cells resistant to regulation by Treg cells. Here, we investigated the role of Casitas B-Lineage Lymphoma-b (Cb1-b), an E3 ubiquitin ligase and a negative regulator of TCR signaling pathways, in rendering CD8+ T cells resistant to the effects of Treg cells to bolster ACT.
Methods In vitro stimulated Cbl-b+/- or Cbl-b-/- Thy1.1+ P14 TCR-transgenic CD8+ T cells were adaptively transferred into B16-gp33 melanoma-bearing Thy1.2+ FoxP3-GFP/DTR transgenic mice treated with or without diphtheria toxin (n = 15). Tumor size and overall survival were measured. Congenially labelled T cells from tumor, draining lymph node, and spleen were comprehensively profiled using flow cytometry. To further examine the biological mechanism of Treg resistance, we performed in vitro Treg suppression assays and RNA-sequencing.

Results Adoptively transferred tumor-specific Cbl-b-/- effector CD8+ T cells mediated superior control over tumor growth and increased overall survival in comparison to the wild-type counterpart. Depletion of FoxP3+ cells increased the quantity and percentage of CD25+ 4-1BB+ expressing P14 Thy1.1+ CD8+ T cells in the tumor, whereas the effect of FoxP3+ cell depletion was negligible with Cbl-b deficient CD8+ T cells. Cbl-b deficiency also attenuated sensitivity to Treg cell-mediated suppression in vitro. Transcriptomic analyses suggested that Cbl-b regulates pathways associated with cytokine production and cellular proliferation. Specifically, hyper-secretion of IFN-γ by Cbl-b deficient CD8+ T cells attenuated suppression by Treg cells. In murine models of adoptive T cell therapy, Cbl-b deficient CD8+ T cells were less susceptible to suppression by Treg cells in the tumor through the effects of IFN-γ.

Conclusions We demonstrate that adoptively transferred effector CD8+ T cells are susceptible to regulation by Treg cells in the tumor, and that ablation of Cbl-b abolates Treg cell-mediated suppression. We highlight the therapeutic implications of targeting Cbl-b in the context of ACT.

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related to mesenchymal transition in GBM such as NF-kB and CEPPB were accessible from normal to tumor-associated microglia. On the other hand, tissue-associated macrophages exhibited enhanced calcium-regulated NFAT TF accessibility. Tumor-associated IWP and IWR myeloid cells also showed a gain of DGE of apoptosis and a reduction of proliferation-related genes.

Conclusions Our studies demonstrate that in addition to the previous dogma of myeloid mediated immune suppression that contributes to tumor immune escape, epigenomic reprogramming in the brain TIME leads to unexpected activation of transcriptional pathways that can trigger transdifferentiation and cell death of myeloid cells further promoting tumor progression. In summary, we provide an unparalleled epigenomic landscape of glioma-associated myeloid cells that may have translational implications.

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Trial Registration NA

Ethics Approval The brain tumor/tissue samples were collected as per MD Anderson internal review board (IRB)-approved protocol numbers LAB03-0687 and, LAB04-0001. One non-tumor brain tissue sample was collected from a patient undergoing neurosurgery for epilepsy as per Baylor College of Medicine IRB-approved protocol number H-13798. All experiments were compliant with the review board of MD Anderson Cancer Center, USA.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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834 CRISPR-MEDIATED IN SITU EDITING OF LIVER RESIDENT MACROPHAGES FOR TREATING LIVER CANCERS

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Background Liver cancer is one of the leading cause of cancer death worldwide with limited treatment options. The liver accommodates the largest population of tissue resident macrophages in the body, namely Kupffer cells. Immune deviation of hepatic immune responses from anti-tumor towards pro-tumor is crucial for cancer progression. This process is closely correlated with the functional polarization of these macrophages. In situ genome editing of liver resident macrophage with intention to shift macrophage function to stimulate anti-tumor immune responses is promising in treating liver cancers.

Methods We have previously shown that Kupffer cells quickly capture and phagocytose circulating bacteria, making bacteria as a potential liver macrophage-specific deliver vector. Taking advantages of the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology, we have established a bacteria mediated genome editing methods for liver resident macrophages in vivo.

Results We used a non-pathogenic Escherichia coli (E. coli) strain as a deliver vector for the CRISPR-Cas9 plasmids, essentially all liver resident macrophages but neither liver sinusoids endothelial cells nor hepatocytes were shown to take up the bacteria, indicating the robustness and specificity of E. coli-mediated plasmid delivery. To test the genome editing efficiency, we chose VSG4, Tim-4 and F4/80 that were highly expressed by Kupffer cells and validated the gene knockout/knockdown effects using intravital imaging. Expression of these receptors by Kupffer cells diminished by more than 90%. Simultaneously editing of multiple genes was also achieved with a slightly decreased efficiency when compared to single gene editing. The acute inflammatory responses and the hepatotoxicity caused by bacteria were ameliorated by pre-immunization with the same E. coli strain, and can be further minimized by using a mutant E. coli strain that processed a modified LPS structure, which dramatically decreased the TLR-4 mediated inflammatory signaling and improved the safety of this method. Moreover, we have shown that not only embryonically-derived Kupffer cell but also monocyte-derived liver macrophages could be edited. The applications of this approach in treating primary liver cancers and liver metastasis are under investigation.

Conclusions Taken together, we have established a rapid, efficient and convenient method to achieve in situ genome editing of liver resident macrophages in vivo. By targeting essential genes that instruct macrophage polarization, this method could be used as immunotherapy for liver diseases, including cancers.

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835 STRUCTURAL DIFFERENCE CAUSED BY MUTATED RESIDUES IS CORRELATED WITH IMMUNOGENICITY OF NEOANTIGENS AND SPECIFICITY OF REACTIVE T CELLS

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Background Host T-cell response is limited to only a small fraction of nonsynonymous mutations; however, the molecular properties of those immunogenic neoantigens remain elusive.

Methods Here, we interrogated the HLA class I ligandome of a microsatellite instability (MSI)-type cancer cell line using a proteogenomic approach, and found an immunogenic 9-mer neoantigen, AKF9. The AKF9 was a non-anchor type neoantigen that harbored a single amino-acid substitution (Asn > Lys) at position 8, which did not affect the HLA-binding affinity.

Results In order to assess a determinant of the immunogenicity, we prepared a panel of AKF9 variants with substitutions at position 8, and found that CD8+ T-cell responses were biased toward residues with structural difference from the wild-type. Interestingly, a substitution with moderate structural change (Asp) also induced reactive T cells; however, in contrast to the others, induced T cells frequently cross-reacted to the wild type HLA ligand. To validate these findings, we used in silico prediction of accessible surface areas and scored the difference between neoantigens and wild types (ASA). Evaluation of reported clinical datasets demonstrated that patient T-
cell induction was positively correlated with ΔASA values, while cross-reactivity of induced T cells was inversely correlated.

Conclusions Our results indicate that dissimilarity is key for both T-cell induction and discrimination from self. ΔASA may help predict immunogenic non-anchor type neoantigens inducing specific T-cell response from a variety of cancer mutation pools.

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836  RELEASING THE RESTRAINTS OF VγVδ2 T-CELLS IN CANCER IMMUNOTHERAPY
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Background VγVδ2 T-cells are a subset of cells with a crucial role in immunosurveillance which can be activated and expanded by multiple means to stimulate effector responses, often exploited in cancer immunotherapy. Little is known about the expression of checkpoint molecules on this cell population and whether the ligation of these molecules can regulate their activity. The aim of this study was to assess the expression of activatory and inhibitory markers on VγVδ2 T-cells to assess potential avenues of regulation to target with immunotherapy.

Methods PBMCs were isolated from healthy donors and the expression of activatory and inhibitory receptors was assessed on VγVδ2 T-cells by flow cytometry at baseline, following 24 hours activation and 14 days expansion using zoledronic acid (ZA) and Bacillus Calmette-Guerin (BCG), both with IL-2. Activation and expansion of Vδ2 cells was assessed by expression of CD69 and by frequency of Vδ2 cells, respectively. Production of effector molecules was also assessed following coculture with various tumour cell targets. The effect of immune checkpoint blockade on VγVδ2 T-cells was also assessed.

Results VγVδ2 T-cells constitutively expressed high levels of NK-associated activatory markers NKG2D and DNAM1 which remained high following stimulation with ZA and BCG. VγVδ2 T-cells expressed variable levels of checkpoint inhibitor molecules at baseline with high levels of BTLA, KLRG1 and NKG2A and intermediate levels of PD1, TIGIT and VISTA. Expression of checkpoint receptors were modulated following activation and expansion with ZA and BCG with decreased expression of BTLA and upregulation of numerous markers including PD1, TIGIT, TIM3, LAG3 and VISTA. Expression of these markers is further modulated upon coculture with tumour cell lines with changes reflecting coexpression of IFN-γ and IL-10 that increased during disease progression. These cells turned out to be T regulatory type 1 (Tr1) cells, which are known to be immunosuppressive. When exposing homogeneous IFN-γ-producing T helper type 1 (Th1) cells to a MYC tumor milieu in vitro, part of these cells started to express both, IFN-γ and IL-10, and showed an increased level of programmed cell death protein 1 (PD-1). Notably, these changes diminished when an IL-10 neutralizing monoclonal antibody (mAb) was added to the coculture, indicating that IL-10 is necessary for the Tr1 development and is involved in the upregulation of PD-1. In line with these results, we treated λ-MYC mice with anti-IL-10 mAb. This therapy not only led to significantly prolonged survival but also decreased expression of PD-1 on effector T cells and increased proliferation of cytotoxic T cells.

Conclusions In summary, these results showed the importance of IL-10 for the tumor immune escape in lymphoma. IL-10 induced a conversion from Th1 to Tr1 cells and elevated levels of PD-1. Both effects were diminished after IL-10 ablation. Thus, targeting IL-10 might be a promising new approach of immunotherapy.

Ethics Approval All animal studies were approved by Regierung von Oberbayern, approval number 55.2-1-54.

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838  PHENOTYPIC AND FUNCTIONAL SIGNATURES OF PERIPHERAL AND TUMOR-RESIDENT γδ T CELLS ARE INFORMATIVE FOR OUTCOME OF CHECKPOINT BLOCKADE IN MELANOMA
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Background Immune checkpoint blockade (ICB) set a milestone in cancer immunotherapy, but still only a fraction of patients responds. Thus, there is an urgent need for biomarkers predicting outcome, and also for understanding the responsible mechanisms. γδ T cells constitute a numerically minor subset of 1-10% of the peripheral T cell compartment in healthy people and have a major role in defense against multiple microbial and non-microbial challenges. Unlike the majority of T cells, γδ T cells bind their ligands in an MHC-
independent manner. We previously studied γδ T cells, that also express checkpoint molecules, in patients in the pre-checkpoint blockade era and thereafter, and identified correlations between subset frequencies of these unconventional T cells and patients’ overall survival (OS). Here, we present a detailed phenotyping and functional investigation of tumor-resident as well as peripheral γδ T cells.

Methods Phenotyping was performed in stage IV melanoma patients before and under PD-1+/−/CTLA-4 blockade using as basis our published OMIP-20 protocol.1 Cytokine expression patterns and proliferative capacities were determined as described according to our established protocols.2 Primary flow cytometry data analysis was performed using FlowJo (BD) and correlations with clinical meta data were determined using Prism (GraphPad) and SPSS (IBM).

Results We found previously that low frequencies of peripheral Vδ1 γδ T cells were associated with prolonged OS. Here, we investigated functional aspects and abundance of γδ T cells within the tumor as well as in the blood. The peripheral Vδ1 but not the Vδ2 differentiation signature revealed significantly lower proportions of naive and effector cells, as well as an accumulation of late differentiated cells in patients with high Vδ1 frequencies. The cytokine expression pattern (IFNγ, TNF and IL-17) and the degranulation marker CD107a were different in patients with versus low peripheral Vδ1 frequencies. The proliferative capabilities of Vδ1 cells in melanoma were limited in comparison to healthy subjects. Both Vδ1 and Vδ2 cells were found in tumor tissues, and these analyses are ongoing, including analyses of replicative senescence through CD57 expression.

Conclusions Our data provide novel insights into the role of γδ T cells in cancer rejection. The previously found negative correlation of Vδ1 T cells with OS is likely due to an accumulation of mal-functioning, probably exhausted Vδ1 T cells in patients with poor outcome of IC7. Thus, we suggest that Vδ1 T cells are promising candidates for future exploitation in novel ICB-approaches.

Ethics Approval This study was approved by K. Wistuba-Hamprecht’s Ethics Committee (approval nos. 490/2014BO1 and 792/2016BO2).

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839 TRANSCRIPTOMIC PROFILING OF T-CELL POPULATIONS IN NON-MUSCLE INVASIVE AND MUSCLE INVASIVE BLADDER CANCER

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1Lund University, LUND, Sweden; 2Alligator Bioscience AB, Lund, Sweden; 3Malin Lindstedt, 4Leif Håkansson. 5Skåne University Hospital, Lund, Sweden; 6Canimguide Therapeutics AB, Lund, Sweden; 7Canimguide Therapeutics AB, Lund, Sweden; 8Canimguide Therapeutic AB, Lund, Sweden

Background Bladder cancer is categorized as non-muscle invasive (NMIBC) or muscle invasive (MIBC). NMIBC makes up around 70% of the cases and although it is less aggressive, the recurrence rate is 50-70%, thus requiring extensive monitoring. Additionally, there is a risk of progression into MIBC with a 5-year survival of only 50% even when treated with radical cystectomy. Immune checkpoint inhibitors have shown promising results for treatment of bladder cancer; however, only around 30% of patients have a therapeutic effect and novel therapies are thus required. With the aim of pinpointing novel targets for T-cell based therapy, we have performed transcriptomic profiling of specific T cell populations in MIBC and NMIBC, as well as in control bladder tissue.

Methods Muscle-invasive (n=7) as well as non-muscle invasive (n=13) bladder tumor biopsies were obtained from untreated patients and control bladder tissue (n=7). Upon digestion, cells were stained with an antibody panel to enable sorting of CD8+ cytotoxic T-cells (CD8T), CD4+ T-helper cells (Th) and regulatory T-cells (Treg) using fluorescence activated cell sorting. RNA was extracted and subject to sequencing. Differential gene expression analysis was performed, using DESeq2 (genes with padj)

Results Principal component analysis demonstrated that CD8T, unlike Th and Tregs, cluster according to the invasiveness of the disease. Accordingly, many genes were significantly differentially expressed between CD8T in MIBC and NMIBC compared to control, and also between CD8T in MIBC compared to NMIBC. Several genes associated with CD8 T-cell exhaustion were significantly upregulated in MIBC compared to both NMIBC and control. Further, GSEA results indicated biological differences of the CD8T compartment between different tumor stages.

Conclusions The gene expression profiles of CD8 T-cells were significantly different in NMIBC, MIBC and control. The transcriptional profiles give clues on biological differences and disease progression and can be relevant for development of novel treatment strategies.

Ethics Approval The study was approved by the Regional Ethics Committee (EPN - Regionala Etikprövningsnämnden i Lund), approval number 2017/34.

Consent Written informed consent was obtained from all patients included in the study.

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840 PRESENCE AND DISTRIBUTION OF IMMUNOSUPPRESSIVE PEPTIDE P3028 IN RELATION TO IMMUNE PHENOTYPE OF TONSILLAR CANCER

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Background A cancer lesion may avoid detection by the immune system through a variety of immunosuppressive mechanisms involving myeloid-derived suppressor cells and regulatory T-cells. One such mechanism recently discovered is the immuno–suppressice effect of a specific peptide, i.e., P3028, produced through degradation of albumin. In this study, involving biopsies obtained from patients with tonsillar cancer (TC), P3028 is assessed in relation to overall immune phenotype, as indicated by presence and distribution of CD8+ T-cells as well as to other specific immune cells.

Methods Immunohistochemistry was performed on fresh frozen biopsies. CD8+ T-cells were used to classify the cancer lesions into immune phenotypes: ‘inflamed’ (lympho–cytes infiltrating cancer cell areas), ‘immune excluded’ (lymphocytes in surrounding stroma, but few within cancer cell areas) and ‘desert’ (few
lymphocytes in cancer cell areas and in surrounding stroma). P3028 was graded (semi-quantitatively) as high or low. Quantitative flow cytometry was performed focusing on CD8+, CD3+, and CD4+ T-cells, macrophages and dendritic cells.

The study was approved by the Swedish Ethical Review Authority (no. 2017/580). Written informed consent was obtained from the patients. A copy of the written consent is available for review by the Editor of this journal.

Results Based on immunohistochemistry focusing on presence and distribution of CD8+ T-cells, most TC lesions were found to be of an ‘inflamed’ immune phenotype. This particular phenotype also featured low expression of immunosuppressive peptide P3028 (cf. other immune phenotypes). Flow cytometry verified that ‘immune excluded’ and ‘inflamed’ cancer lesions were associated with high levels of CD8+ T-cells (cf. desert lesions). The presence of CD3+ and CD4+ T-cells as well as macrophages and dendritic cells in relation to immune phenotypes were indicated.

Conclusions TC lesions may be classified into ‘inflamed’, ‘immune excluded’, and ‘desert’ phenotypes based on presence and distribution of CD8+ T-cells. Other immune cells may be associated with these immune phenotypes, including CD3+ and CD4+ T-cells, macro-phages, and dendritic cells. P3028 is present in TC lesions: low levels of this immunosuppressive peptide are observed in the ‘inflamed’ phenotype. Arguably, P3028 prevents successful recruitment of immune cells in TC. Inferentially, presence and distribution of P3028 may be considered as a prognostic marker as well as a treatment target of this condition.

Ethics Approval The study was approved by the Swedish Ethical Review Authority (no. 2017/580).

Consent Written informed consent was obtained from the patients. A copy of the written consent is available for review by the Editor of this journal.

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Immune-stimulants and immune modulators

841 CRISPR CAS9 LIBRARY SCREEN IN PRIMARY T CELLS AND DIFFUSE LARGE B CELL LYMPHOMA CELLS TO IDENTIFY MODULATORS IN TUMOR-IMMUNE INTERACTION
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Background Immunotherapy, especially checkpoint blockers targeting programmed cell death protein 1 (PD-1) pathways, has transformed cancer treatment. Current checkpoint blockers are limited by low response rate, side effect and treatment relapse. The emergence of CRISPR Cas9-based screen provides a superior and powerful tool in gene function profiling. The application of CRISPR Cas9 screen in primary immune cells and tumor cells such as diffuse large B cell lymphoma (DLBCL) cells will accelerate the identification of key regulators in tumor-immune interaction.

Methods CRISPR screen using membrane protein-focused sgRNA library and genome-scale sgRNA library; primary T cell and tumor cell co-culture

Results First of all, we developed a CRISPR-Cas9 gene targeting method that can achieve efficient gene disruption in primary CD8+ T cells isolated from mouse (~60% efficiency) or human (~70% efficiency). We have applied this method to a pooled CRISPR library screen for key modulators of T cell-induced cytotoxicity against cancer cells in vitro. This customized library contains sgRNAs targeting nearly all membrane proteins expressed in both murine and human T cells. For our in vitro screen, mouse colorectal cancer cell line MC38 expressing chicken ovalbumin (Ova) were co-cultured with Ova-specific CD8+ T cells isolated from OT-I transgenic mice. The proliferation and function of CD8+ T cell were dampened by tumor cells in an antigen-dependent way. On the other hand, we successfully developed a genome-scale CRISPR screen platform on the difficult-to-transduce DLBCL cells. The platform is currently deployed to validate modulators involved in bispecific antibody-mediated tumor cell killing by T cells.

Conclusions We have established CRISPR Cas9 pooled screen platforms for identification of modulators of tumor-immune interaction by either target primary T cells or difficult-to-transduce DLBCL cells.

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842 A NOVEL AGONISTIC ANTI-CD40 TARGETING STRATEGY WITH AN AFFINITY PEPTIDE BINDING FEATURE FOR ANTIGEN CARGO FUNCTIONALITY: IMPROVING PEPTIDE STABILITY AND T CELL PROLIFERATION
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Background To induce a prominent anti-tumor T-cell response, a viral or tumor derived agonist epitope imbedded in a longer synthetic peptide (SLP) can be used, which also requires internalization and processing by antigen presenting cells (APCs) to enable T cell priming. Herein we present the design and evaluation of a CD40 targeting tetravalent bispecific antibody, binding peptides through affinity as an antibody-drug conjugate. APC activation as well as in vitro and in vivo T-cell proliferation studies demonstrate retained agonistic activity as well as improved T cell proliferation/expansion in vitro and in vivo, compared to non-linked peptide/antibody mixes.

Methods T-cell priming was evaluated with B3Z assay or a cytomegalovirus (CMV) model and displayed superior uptake to non-bound peptide in the co-stimulatory independent B3Z assay. In addition, intracellular peptide release in APCs was analysed using a unique quenching strategy displaying peptide release after around 4-6 hour post antigen.

Results Peptide stability in vitro, when bound to the antibody, was analysed by mass spectrometry and displayed prolonged peptide stability in serum, increasing the peptide half-life by 15 times in vitro (Conclusions) Data support that the novel delivery system can improve antigen targeting to dendritic cells, but can also provide a prolonged peptide half-life as well as a peptide delivery to APCs. Combined this improves the efficiency of both antigen delivery and CD40 agonistic functionality.

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843 REPRODUCIBLE, MOA-REFLECTING REPORTER-BASED BIOASSAYS TO ENABLE DRUG DEVELOPMENT OF BIOSIMILARS AND BIOBETTERS
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Background Cytokines and growth factors are small immunomodulatory proteins secreted by a wide variety of cells (e.g.
fibroblasts, endothelial and stromal cells) that regulate surrounding cells via autocrine, paracrine or endocrine mechanisms. Immunocytokines are a promising class of activators of the immune system, with the potential to be used alone or in combination with other therapeutic agents to treat a variety of disease including autoimmunity and cancer. This class of biologics includes FDA-approved cytokine therapies (e.g. IFN, IL-2 and Epo) as well as an increasing number of biologics designed to block cytokine activity. The latter class of biologics includes basiliximab (IL-2R), tocilizumab and sarilumab (IL-6R), siluximab (IL-6), ustekinumab and its biosimilars (IL-12/IL-23 p40), secukinumab (IL-17A), bevasizumab (VEGF), and denosumab (RANKL). Pharmaceutical pipelines include an increasing number of biosimilar and biobetter molecules with sustained and targeted activities with a goal to improve drug potency, patient tolerance and clinical response.

Methods Quantitative and reproducible functional bioassays are critical for the development and manufacture of biologics drugs targeting cytokine and growth factor pathways. In many cases, existing bioassays rely on the use of primary cells and measurement of complex endpoints. These assays are highly variable, difficult to implement, and often fail to yield data quality required for drug development in a quality-controlled environment. To address this problem, we have developed a suite of bioluminescent luciferase-based reporter bioassays that can be used to quantitatively measure the activity of specific cytokines and growth factors, including: IL-2, IL-6, IL-12, IL-15, IL-17, IL-23, VEGF and RANKL.

Results These mechanism of action (MOA) reflecting bioassays exhibit the required performance metrics for use in potency and stability studies. Importantly, these bioassays have been optimized in a thaw-and-use cell format, which eliminates the need for cell culture and ensures high reproducibility, convenience and transferability.

Conclusions In summary, bioluminescent reporter-based bioassays offer significant advantages over primary cell-based bioassays and are valuable tools for the development and manufacturing of novel biologics targeting cytokine and growth factor pathways.

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845 DEVELOPING MORE POTENT INHIBITORS OF VASOACTIVE INTESTINAL PEPTIDE SIGNALING WITH ENHANCED EFFICACY IN MOUSE MODELS OF LEUKEMIA

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Background Vasoactive intestinal peptide (VIP) is an immunosuppressive neuropeptide that significantly affect proliferation and anti-tumor properties of T cells.1,2 VIP overexpression is a potential mechanism of immune escape in solid tumors with paracrine VIP production. Our published work shows that inhibiting VIP receptor (VIP-R) signaling via VIPhyb, an antagonist fusion peptide between neurotensin and VIP, improves T cell dependent anti-tumor response in mouse models of acute myeloid leukemia (AML) and T lymphoblastic leukemia comparing the activity of DNA hypomethylating agents (DHA) with histone deacetylases inhibitors (HDACi) and EZH2 inhibitors (EZH2i), alone or combined with DHA, in mesothelioma cells.

Methods Four mesothelioma cell lines were treated with the DHA guadecitabine 1µM, or with the HDACi, Valproic Acid (VPA) 1mM, or the EZH2i, EPZ-6438 1µM, alone or combined with guadecitabine. We investigated the expression of HLA class I molecules by flow-cytometry and of PD-L1, cancer testis antigens (CTA: NY-ESO, MAGE-A1), Natural Killer Group 2 member D Ligands (NKG2DLs: MIC-A, MIC-B, ULBP2) and EMT-regulating cadherins (CDH1, CDH2) by quantitative Real-Time PCR. Fold change (FC) expression for each treatment vs untreated cells was reported as mean values (FCm) among investigated cell lines. A positive modulation of the expression was considered if FCm>1.5.

Results Guadecitabine upregulated the expression of HLA class I antigens (FCm=1.73), PD-L1 (FCm=2.38), NKG2DLs (MIC-A FCm=1.96, MIC-B FCm=2.57, and ULBP2 FCm=3.56), and upregulated/induced CTA expression. Similarly, VPA upregulated HLA class I antigens (FCm=1.67), PD-L1 (FCm=3.17), NKG2DLs (MIC-A FCm=1.78, MIC-B FCm=3.04, and ULBP2 FCm=3.75) expression; however, CTA expression was modulated only in 1 mesothelioma cell line. Conversely, EPZ-6438 up-regulated only NY-ESO-1 and MIC-B expression in 1 mesothelioma cell line.

The addition of both VPA and EPZ-6438 to guadecitabine strengthened its immunomodulatory activity. Specifically, guadecitabine plus VPA or EPZ-6438 upregulated the expression of HLA class I antigens FCm=2.53 or 2.69, PD-L1 FCm=8.04 or 2.65, MIC-A FCm=3.81 or 2.26, MIC-B FCm=8.00 or 3.03, ULBP2 FCm=6.24 or 4.53, respectively. Higher levels of CTA upregulation/induction were observed with combination treatments vs guadecitabine alone.

Cadherins modulation was mesothelioma histotype-related: CDH1 expression was induced in the 2 constitutively-negative sarcomatoid mesothelioma cells by guadecitabine alone or combined with VPA or EPZ-6438; CDH2 expression was upregulated by VPA alone (FCm=1.53) or plus guadecitabine (FCm=2.54).

Conclusions Combination of DHA-based immunotherapies with other classes of epigenetic drugs could be an effective strategy to be pursued in the mesothelioma clinic.

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844 IMMUNOMODULATORY ACTIVITY OF EPIGENETIC DRUGS COMBINATIONS IN MESOTHELIOMA: LAYING THE GROUND FOR NEW IMMUNOTHERAPEUTIC STRATEGIES

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Background Growing evidence are demonstrating the therapeutic efficacy of immune checkpoint inhibitors (ICI) in mesothelioma; however, a limited percentage of patients benefits from this therapeutic approach. Epigenetic modifications play a relevant role in negatively regulating the cross-talk between neo- and immune cells, and in contributing to the highly immunosuppressive mesothelioma microenvironment. A better understanding of mesothelioma epigenetic landscape could open the path to novel and potentially more effective approaches combining ICI and epigenetic drugs. We investigated the immunomodulatory potential of epigenetic agents by

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Abstracts

NEOSPORA CANINUM – AN IMMUNOTHERAPEUTIC PROTOZOAN AGAINST SOLID CANCERS

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Background Immunotherapy induces, provides, and/or reactsivate anti-tumor immune responses. Some microorganisms also can initiate response that lymphocytes infected tumor and/or stimulates systemic immunity. Attenuated viruses or bacteria are well studied as oncotherapeutics, but not protozoa except Toxoplasma gondii. We assessed the effect on tumors of other protozoa that were naturally non-pathogenic to humans. Thus, we discovered the ability to use Neospora caninum (Nc) in a manner and form that demonstrated a synergistic array of pertinent immunotherapeutic characteristics against solid cancers. Our first Article on Neospora as Onco-immunotherapeutic is currently under revision after review by the JITC. We report on the most recent data notably from Nc engineered to secrete human IL-15 within the tumor.

Methods In vitro, the immunostimulatory properties of Nc strains wildtype and engineered to secrete human IL-15 were

(A504). In this study, we developed novel VIP-R antagonists with enhanced efficacy when compared to VIPhyb, to generate a significantly more robust anti-tumor response in mouse models of AML.

Methods We created a combinatorial library of 300 peptide sequences that contain the six charged N-terminal residues of the neurotensin present in VIPhyb (first-generation VIP antagonist) with two or more amino acid substitutions within the C-terminal amino acid sequence of VIP (table 1). We performed in-silico screening to identify 10 novel VIP-R antagonists that were predicted to have increased binding affinity to VIP receptors VPAC1 and VPAC2 when compared to VIP or VIPhyb. The efficacy of these peptides where tested in vitro using T cells from luciferase transgenic mice seeded and expanded on anti-CD3 monoclonal antibody coated plates for three days. Enhanced potency of the novel antagonists in vivo, was tested in a mouse AML model, by treating C1498-bearing mice with subcutaneous administration of VIP, VIPhyb, scrambled peptide or the second-generation VIP-R antagonists (labeled as ‘ANT’) from day 6-12 after tumor implantation.

Results T cell proliferation using 0.3 μM of a novel VIP-R antagonist was increased up to 216% + 20% of control cultures without added peptides versus 197% + 38% in cultures with VIPhyb at 1 μM (table 1). Furthermore, the novel VIP-R antagonists increased median survival times (MST) by up to 57 days and rendered 40% of mice leukemia-free at 60 days compared to MST of 34 days and 5% long-term survival with VIPhyb (figure 1).

Conclusions In this study, we have identified novel and more potent VIP-R antagonists when compared to VIPhyb, with enhanced potency to activate and proliferate T cells and generate an effective anti-tumor response in mouse models of leukemia. These novel antagonists can lead to peptide-based immunotherapy for the treatment of various solid and liquid cancers, such as the cancer of the colon and pancreas, that overexpress VIP intratumorally.

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Abstract 846 Table 1 Novel second generation VIP-R antagonists

Predicted binding affinity of VIP, VIPhyb and VIP antagonists to VPAC1 and VPAC2 based upon in silico screening is shown along with the proliferation of luciferase+ mouse T cells and their anti-leukemia activity in mice. The level of T cell bioluminescence vs. control T cells stimulated with anti-CD3 alone is shown along with the lowest peptide concentration that achieved the maximal effect on T cell proliferation. Median survival times and the fraction of mice alive at day 60 along with the numbers of animals tested with each VIP antagonist are shown.

<table>
<thead>
<tr>
<th>Peptide Name</th>
<th>Docking score (VIP vs. VIPhyb)</th>
<th>Docking score (VIP vs. VIP antagonist)</th>
<th>Relative T cell proliferation vs no peptide control and most effective concentration</th>
<th>Percentage of mice alive at day 60 and median survival times (MST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>60.8</td>
<td>92.6</td>
<td>77% (P&lt;0.05, 96d)</td>
<td>27% MST 25 days, n=38, n=6</td>
</tr>
<tr>
<td>VIPhyb</td>
<td>60.2</td>
<td>51.01</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>27% MST 25 days, n=38, n=20</td>
</tr>
<tr>
<td>SCRAM1</td>
<td>-22.84</td>
<td>-37.10</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>Pending</td>
</tr>
<tr>
<td>ANT006</td>
<td>64.27</td>
<td>64.45</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>Pending</td>
</tr>
<tr>
<td>ANT007</td>
<td>65.17</td>
<td>65.06</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>Pending</td>
</tr>
<tr>
<td>ANT008</td>
<td>-76.11</td>
<td>59.60</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>Pending</td>
</tr>
<tr>
<td>ANT009</td>
<td>-68.10</td>
<td>55.35</td>
<td>121% (P&lt;0.05, 96d)</td>
<td>Pending</td>
</tr>
<tr>
<td>ANT010</td>
<td>70.44</td>
<td>70.00</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>25% MST 35 days, n=36, n=20</td>
</tr>
<tr>
<td>ANT011</td>
<td>-62.00</td>
<td>-68.07</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>25% MST 35 days, n=36, n=20</td>
</tr>
<tr>
<td>ANT012</td>
<td>-65.35</td>
<td>-67.07</td>
<td>121% (P&lt;0.05, 96d)</td>
<td>Pending</td>
</tr>
<tr>
<td>ANT013</td>
<td>-75.36</td>
<td>-75.60</td>
<td>121% (P&lt;0.05, 96d)</td>
<td>25% MST 35 days, n=36, n=20</td>
</tr>
<tr>
<td>ANT014</td>
<td>-69.37</td>
<td>-69.12</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>25% MST 35 days, n=36, n=20</td>
</tr>
<tr>
<td>ANT015</td>
<td>-72.6</td>
<td>-71.66</td>
<td>107% (P&lt;0.05, 96d)</td>
<td>25% MST 35 days, n=36, n=20</td>
</tr>
</tbody>
</table>

Abstract 845 Figure 1 Prolonged survival with second generation peptides

C57BL/6 mice were injected intravenously with 1 x 106 C1498 myeloid leukemia cells on day 0. The presence of engrafted was confirmed on day 6 by flow cytometry and leukemia-bearing mice were treated with daily subcutaneous injections of PBS, 10 μg VIP scrambled peptide (SCRAM1), VIP, VIPhyb, or a second-generation VIP antagonist (ANT008, etc...) for 10 days. Survival of all groups treated with a VIP antagonist was significantly better than groups treated with PBS, VIP, or SCRAM at p < 0.001. Survival between mice treated with ANTS00 was significantly better than those treated with VIPhyb (p<0.02).
studied. In vivo experiments of treatment with Neospora caninum tachyzoites administered locally (intra and peri tumoral) or remotely (subcutaneous) in a murine thymoma EG7 tumor and in human Merkel cell carcinoma (MCC).

**Results** We demonstrated that the treatment of thymoma EG7 by Neospora caninum strongly inhibited tumor development. Analysis of immune responses and interactions between Neospora caninum and tumor cells showed that Neospora caninum had the ability to lyze infected cancer cells, reactivated immune competence within the tumor microenvironment (TME), and activated the systemic immune system by promoting the recruitment of immune cells to the site of tumor. We also established in a NOD/SCID mouse model that Neospora caninum was able to induce a strong regression of human MCC. Recently, to further enhance oncotherapeutic effect, we engineered an Neospora caninum strain to secrete human IL-15 (cross reactive with mouse cells), associated with alpha subunit of IL-15 receptor, increasing its stability. This strain induced proliferation of human PBMCs and their secretion of IFN-γ.

In the EG7 model, human IL-15 secreting Neospora caninum showed greater protection against tumor development, confirming enhancement of immunotherapy by engineering Neospora caninum to deliver/secrete IL-15.

**Conclusions** These results highlight Neospora caninum as a potentially extremely efficient, and non-toxic anti-cancer agent, capable of being engineered to express at its surface or to secrete bio-drugs, like human IL-15 cytokine. Our work has identified the broad clinical possibilities of using N. caninum as an oncolytic protozoan in human medicine capable of vectoring molecular therapy, overcoming TME defenses.

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**Abstract 847**

**INFLAMMASOME ACTIVATION IN M2 MACROPHAGE RESTRAIN THE IMMUNE SUPPRESSIVE FUNCTION**

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**Background** Macrophage is an important component in tumor microenvironment (TME) and plays multiple roles in tumor initiation, progression and metastases. In response to various stimuli within TME, macrophage exhibits high level of functional heterogeneity. There are two distinct groups of macrophages: M1 macrophage exhibits pro-inflammatory phenotype with high levels of TNF-α, IL-6, and IL-1β, while M2 macrophage displays immune suppressive phenotype with high levels of anti-inflammatory cytokines such as IL-10 and TGF-β. In response to the M2 cytokines, myeloid cells within the TME further acquire higher expression of PD-L1 and thus inactivate T cells. M2 cytokines can also directly inhibit T cell activation. As a result, re-polarizing M2 macrophages becomes a key concept for cancer immunotherapy. The NLRP3 inflammasome is acquired by macrophages to fight against endogenous danger signals. Macrophage NLRP3 activation has been observed in several tumor models, but the function of NLRP3 on macrophage polarity remains controversial. Inflammasome activation with IL-1β/IL-18 secretion was reported to promote M1 polarization. However, NLRP3 activation was also reported to promote M2 polarity through up-regulation of IL4 in asthma model.

**Methods** Here, we have established an in vitro human macrophage NLRP3 activation system (figure 1), coupled with M2 macrophage polarization assay, to dissect the role of NLRP3 in macrophage phenotype.

**Results** Our results indicate that NLRP3 activation restrained M2 phenotype and further enhanced T cell activation in an M2/T cell co-culture system (figure 2).

**Conclusions** Inflammasome could be the potential target for cancer by modulating T cell activation through macrophage polarization regulation.

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Abstracts

PRIME-BOOST VACCINATION FOR THE TREATMENT OF TRIPLE NEGATIVE BREAST CANCER
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Background Triple negative breast cancer (TNBC) is currently only treated with surgery and chemotherapy.1 TNBC has higher rates of genetic mutations and contains more tumor infiltrating lymphocytes.2 These characteristics provide a strong rationale to use novel immunotherapies such as immunogenic autologous tumor cell vaccines to therapeutically target TNBC. We have demonstrated that an infected cell vaccine (ICV) that is made from irradiated and oncolytic virus tumor infected cells induces beneficial innate and adaptive immune responses in a syngeneic mouse model of TNBC. Moreover, the efficacy of ICV is improved when combined with checkpoint blockade (anti-PD-1).3 Our goal is to further improve ICV by applying a prime-boost cancer vaccination strategy to further enhance anti-tumor immune responses in preclinical and translational studies.4 5

Methods We will choose the best ‘prime vaccine’ based on the immunogenicity of TNBC cell lines after treatment with immunomodulators such as chemotherapeutic agents, irradiation, toll-like receptor agonists and anti-viral vaccines. We will measure the release of damage-associated molecular patterns (DAMPs), which act as danger signals to initiate tumor-targeted immune responses,6 after the treatment of TNBC cell lines. We will test the polarization of human monocytes when co-cultured with conditioned media (CM) from treated TNBC cells. We will also analyze the migration of human immune cells (CD56+NK cells) and CD11C+ dendritic (DC) cells differentiated from mouse bone marrow cells when co-cultured with the cell lysate of the mouse TNBC cell line treated with ‘prime vaccine’ candidates. For in vivo studies, we will test our best prime vaccine followed by the ICV as a boost vaccine in our BALB/c-4T1 mouse model. We will analyze the cytotoxicity of T lymphocytes and the secretion of cytokines, and overall survival.

Results From measuring DAMP levels and analyzing immune functions, our preliminary results suggest that oxaliplatin and the seasonal influenza vaccine are the best candidates compared to other treatments. DCs differentiated from isolated bone marrow cells exhibited a higher percentage of markers of maturation when co-cultured with the cell lysate of the mouse TNBC cell line treated with ‘prime vaccine’ candidates. For in vivo studies, we will test our best prime vaccine followed by the ICV as a boost vaccine in our BALB/c-4T1 mouse model. We will analyze the cytotoxicity of T lymphocytes and the secretion of cytokines, and overall survival.

Conclusions These results demonstrate the therapeutic potential of oncolytic virus-based immunogenic tumor vaccines could be improved by applying the prime-boost’ cancer vaccination approach to treat TNBC.

Ethics Approval ‘The study was approved by the CRCHUS Human Ethics Board, approval number 2018-2414.’

Consent ‘Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.’

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OPTIMIZATION OF A GM3-CONTAINING LIPOSOMAL VACCINE THAT DELIVERS ANTIGEN TO CD169+ SPLENIC MACROPHAGES

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Background Although promising developments in cancer vaccination have been made, therapeutic effectiveness is often insufficient. Liposomal vaccine effectiveness could be enhanced by antigen encapsulation and incorporation of molecules that actively target to antigen presenting cells to enhance T cell activation. CD169+ expressing splenic macrophages are located in the marginal zone and efficiently capture particulate antigens such as viruses and exosomes from the blood circulation. Upon antigen capture CD169+ macrophages transfer antigen to cross-presenting dendritic cells that are responsible for the activation of CD8+ T-cells.

Methods Here we prepared liposomes that contain a physiological ligand for CD169, the ganglioside GM3, to facilitate uptake by CD169+ macrophages. We assessed how various amounts of targeting molecule GM3, decoration with PEG and liposomal size affected binding and uptake by CD169+ macrophages in vitro and in vivo. In addition, we evaluated the stability of liposomal preparations in plasma. As a proof of concept, we prepared GM3-liposomes with a long ovalbumin peptide and tested the capacity of these liposomes to induce CD8+ and CD4+ T cell activation and compared it to control liposomes and soluble peptide.

Results These data indicate that targeting of splenic CD169 macrophages can be optimized by careful selection of constituents of the liposomal delivery vehicle. Moreover, optimized GM3-mediated liposomal targeting to CD169 macrophages results in potent immune responses.

Conclusions GM3-mediated liposomal targeting to CD169 macrophages presents as a promising strategy for cancer vaccines.

Ethics Approval All animal experiments were approved by the local animal welfare body.

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IMMUNOLOGICAL CONSIDERATIONS FOR DEVELOPING OPTIMAL WHOLE TUMOR CELL-DERIVED CANCER VACCINES

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Background Immunotherapies based on checkpoint blockers (ICB), targeting inhibitory immune pathways such as cytotoxic T
lymphocyte-associated protein 4 (CTLA-4) or programmed cell death protein 1 (PD-1), have shown significant success in promoting tumor regression and prolonging survival in cancer patients, particularly in melanoma and other solid tumors. However, many patients do not respond or develop resistance to these interventions, bringing the scientific community to focus their efforts in combinatorial therapies. A major factor involved in initial resistance to ICB is lack or weak T cell tumor infiltration, characterizing the so-called ‘cold tumors’. In fact, high lymphocyte infiltration and interferon (IFN)-γ status related to a T cell infiltrated phenotype (‘hot tumors’) constitute key factors for effective anti-PD-1/PD-L1 therapies. For this reason, immunological treatments that induce adaptive cellular responses in cold tumor-patients may be a desirable goal. In this context, tumor vaccines become once again an attractive alternative and/or complement for cancer treatment.

**Methods**

Here, a prototype for a generic melanoma vaccine, named TRIMELVax, was tested using B16F10 mouse melanoma model. This vaccine is made of heat shock-treated tumor cell lysates named TRIMEL combined with the Concholepas concholepas hemocyanin as adjuvant. TRIMEL is derived from a mix of equal amounts of Mel1, Mel2 and Mel3 cells, which were taken to a final concentration of 8×10⁶ cells/mL, HS-treated at 42°C for 1 hour plus 2 hours at 37°C and then lysed through three cycles of freeze/thaw in liquid nitrogen.

**Results**

While B16F10 lysate provides appropriate melanoma-associated antigens, both a generic human melanoma cell lysate and hemocyanin adjuvant contributes with danger signals promoting conventional dendritic cells type 1 (cDC1), activation, phagocytosis and effective antigen cross-presentation. TRIMELVax inhibited tumor growth and increased mice survival, inducing cellular and humoral immune responses. Furthermore, this vaccine generated an increased frequency of intratumor cDC1s but not cDC2s. Augmented infiltration of CD3+, CD4+ and CD8+ T cells was also observed, compared with anti-PD-1 monotherapy, while TRIMELVax/anti-PD-1 combination generated higher tumor infiltration of CD4+ T cells. Moreover, TRIMELVax promoted an augmented proportion of PD-1lo CD8+ T cells in tumors, a phenotype associated to prototypic effector cells required for tumor growth control, preventing dysfunctional T cell accumulation.

**Conclusions**

The therapeutic vaccine TRIMELVax efficiently controls the weak immunogenic and aggressive B16F10 melanoma tumor growth, prolonging tumor-bearing mice survival even in the absence of ICB. The strong immunogenicity shown by TRIMELVax encourages clinical studies in melanoma patients.

**Ethics Approval**

All animal experiments were performed in accordance with institutional guidelines for animal care and were approved by the Ethical Review Committee at the Universidad de Chile, Ethical Number CBA0885 (approval date: May 2016).

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**Immmuno-conjugates and chimeric molecules**

**851 POTENT TUMOR-DIRECTED T CELL ACTIVATION AND IN VIVO TUMOR INHIBITION INDUCED BY A 4-1BB X ST4 ADAPTR™ BISPECIFIC ANTIBODY**

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**Background** 4-1BB (CD137) is an activation-induced co-stimulatory receptor that regulates immune responses of activated CD8+ T cells and NK cells, by enhancing proliferation, survival, cytolytic activity and IFN-γ production. Its ability to induce potent anti-tumor CD8+ and NK cell activity makes 4-1BB an attractive target for designing novel therapeutics for immuno-oncology. However, clinical development of a mono-specific 4-1BB agonistic antibody has been hampered by dose-limiting hepatic toxicities. To minimize systemic immune toxicities and enhance activity at the tumor site, we have developed a novel 4-1BB x ST4 bispecific antibody that stimulates 4-1BB function only when co-engaged with ST4, a tumor-associated antigen. The combined preclinical dataset presented here provides an overview of the mechanism of action and the efficacy and safety profile of ALG.APV-527, supporting its advancement into the clinic.

**Methods** ALG-APV-527 was built based the ADAPTR™ platform with binding domains to 4-1BB and ST4 generated using the ALLIGATOR-GOLD™ human scFv library. ALG-APV-527 was tested using primary cells in the presence or absence of cells expressing ST4. Cell Trace-labelled PBMC sub-optimally stimulated with anti-CD3, to induce 4-1BB expression, cells were gated using flow cytometry. T cell cytotoxicity was assessed by quantifying cell death in CD8+ T cell/tumor co-cultures, and images were obtained using a cell live imaging system (Cytation 5). For tumor inhibition studies, human 4-1BB knock-in mice were injected subcutaneously with MB49 cells transplanted with human ST4. Cured mice were subsequently used in a toxicity study and liver pathology was evaluated.

**Results** In vitro, ALG.APV-527 enhances primary CD8+ T cell and NK cell function and proliferation in the presence of ST4-expressing cells. Using imaging, ALG-APV-527 in combination with a bispecific T cell engager caused increased cell death in T cell/tumor cell co-cultures. ALG-APV-527 inhibited growth of established tumors at doses as low as 2 µg/mouse in a syngeneic bladder cancer model. Following recovery, mice exhibited a memory response when rechallenged with tumor. In a high dose safety study in human 4-1BB knock-in mice, ALG-APV-527 did not cause significant systemic immune activation, whereas urelumab analogue treated mice induced dermatitis, elevated serum cytokines, CD8+ T-cell liver infiltration and systemic T-cell proliferation.

**Conclusions** ALG-APV-527 induces potent CD8+ T cell and NK cell co-stimulation and T-cell cytotoxicity and has potent in vivo anti-tumor activity, without inducing systemic toxicity. Based on preclinical data, ALG-APV-527 is a promising anti-cancer therapeutic for the treatment of a variety of ST4-expressing solid tumors.

**Ethics Approval** All studies were review and approved by the Internal Animal Care and Use Committee (IACUC) of Aptevo Therapeutics.

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**852 DIFFERENTIAL EXPRESSION OF SURFACE PROTEIN-ENCODING GENES HIGHLIGHTS THERAPEUTIC VULNERABILITIES OF FOUR SCLC SUBTYPES**

Elizabeth Park*, Carl Gay, C Allison Stewart, Kasey Cargill, Lixia Diao, Qi Wang, Robert Cardnell, Jing Wang, John Heymach, Lauren Byers. The University of Texas MD Anderson Cancer Center, Houston, TX, USA

**Abstracts**

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851 POTENT TUMOR-DIRECTED T CELL ACTIVATION AND IN VIVO TUMOR INHIBITION INDUCED BY A 4-1BB X ST4 ADAPTR™ BISPECIFIC ANTIBODY

852 DIFFERENTIAL EXPRESSION OF SURFACE PROTEIN-ENCODING GENES HIGHLIGHTS THERAPEUTIC VULNERABILITIES OF FOUR SCLC SUBTYPES
Background Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine malignancy that accounts for 15% of lung cancer diagnoses. The severity of this disease is exacerbated by the fact that there are few therapeutic options, which mostly offer limited clinical benefit, culminating in a 5-year survival rate of 10%.

Methods To identify transcriptional subtypes, we used non-negative matrix factorization of gene expression data from 81 SCLC tumors and identified four subtypes largely based on differential expression of the transcription factors ASCL1, NEUROD1, and POU2F3. We hypothesized that these subtypes may underlie unique therapeutic vulnerabilities. We examined differential expression of genes that encode surface-expressed proteins that may be targetable by reagents such as therapeutic antibodies or antibody-drug conjugates (ADCs).

Results Our four subtypes are defined either by high expression of ASCL1 (SCLC-A), NEUROD1 (SCLC-N), POU2F3 (SCLC-P), or an absence of those transcription factors and instead a prevalence of immunological factors (SCLC-Inflamed, or SCLC-I). We curated a list of approximately 60 candidate genes encoding surface proteins that are differentially expressed across the four subtypes. Within these 60 candidates, we have identified a few specific to each subtype for which there exist clinically available, targeted ADCs. The most prevalent subtype, SCLC-A, showed high expression of targets such as DLL3 (SCLC-A) and CEACAM5 (SCLC-A). SCLC-N highly expressed SSTR2, a somatostatin receptor that is being actively targeted in SCLC clinical trials. The two non-neuroendocrine subtypes, SCLC-P and SCLC-I shared some common hits such as the NK cell ligand MICA and B7H6. All of the identified and highlighted hits have been or are actively being pursued in clinical trials, highlighting the importance of understanding their expression levels pre- and post-treatment so that novel therapies can be developed that will be effective over the course of disease progression.

Conclusions The underlying biology defining our four identified subtypes of SCLC has revealed a striking number of targetable, standing their expression levels pre- and post-treatment so that novel therapies can be developed that will be effective over the course of disease progression. The two non-neuroendocrine subtypes, SCLC-P and SCLC-I shared some common hits such as the NK cell ligand MICA and B7H6. All of the identified and highlighted hits have been or are actively being pursued in clinical trials, highlighting the importance of understanding their expression levels pre- and post-treatment so that novel therapies can be developed that will be effective over the course of disease progression.

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Immune-related adverse events (irAEs) are serious side effects of immune checkpoint inhibitors (ICIs) for patients with advanced cancer. Understanding the epidemiology and risk factors for severe irAEs would be beneficial for patients and clinicians.

Methods We performed a retrospective review of cancer patients treated with ICIs using un-identifiable claims data from a nationwide US health insurance plan from January 3rd, 2011 to December 31st, 2019. Patients with an identified primary cancer and at least one administration of an ICI were included in the study. We defined severe irAE as any inpatient hospitalization with new immunosuppression within 2 years after initiation of ICI. The main outcomes were incidence of severe irAE in ICI therapy and factors associated with severe irAE occurrence. Multivariable logistic regression, including Charlson comorbidity index, age, gender, primary cancer, region, and zip code average income and unemployment, was used to model risk factors for severe irAE (table 1).

Results There were 14,378 patients followed over 19,177 patient-years identified with a primary cancer and at least 1 administration of ICI. 504 (3.5%) patients developed a severe irAE. The incidence of severe irAEs per patient ICI treatment year was 2.6%, rising from 0% (0/71) in 2011 to 3.7% (93/2486) in 2016 (figure 1). Combination immunotherapy (OR: 2.44, p < 0.001) and younger age (OR: 0.77, p < 0.001) were associated with increased odds of developing severe irAEs, whereas patients with non-lung cancer were associated with decreased odds of irAEs (melanoma OR: 0.70, p = 0.01, renal cell carcinoma OR: 0.71, p = 0.03, other cancers OR: 0.50, p < 0.001; figure 1). Sex, region, zip code income, and zip-code imputed unemployment were not associated with severe irAE incidence. Prednisone (72%) and methylprednisone (25%) were the most common immunosuppressive treatments identified in irAE hospitalizations.

Conclusions We found that 3.5% of patients initiating ICI therapy experienced severe irAEs requiring hospitalization and immunosuppression. The odds of severe irAEs were higher with younger age, treatment with combination ICI therapy (CTLA-4 and PD-1 or PD-L1), and lower for other cancers compared with patients on PD-1 or PD-L1 inhibitors with lung cancer. This evidence from the first nationwide study of severe irAEs in the US identified the real-world epidemiology, risk factors, and treatment patterns of severe irAEs in the US which may guide treatment selection and decisions for patients and clinicians.
Background Immune checkpoint inhibitor (ICI) therapy has revolutionized cancer treatment and has become a standard of care. There are now numerous FDA approved indications for ICIs and an increasing number of patients receiving these treatments, which has led to an increase in the risk of immune-related adverse events (irAEs) including endocrinopathies. Diabetes mellitus is a rare irAE of ICI therapy with an approximate incidence of 1-2%. There is paucity of data in literature about incidence, characteristics and possible predictive factors of ICI-induced diabetes mellitus. Due to limited data on ICI-induced diabetes, we conducted a retrospective review of patients who received ICI therapy at RPCCC and developed diabetes mellitus. The goal of this study is to report incidence and characteristics of new onset and worsening of diabetes in patients treated with ICI therapy.

Methods We conducted a retrospective chart review of patients who received ICIs treatment from January 1st, 2010 to May 15th, 2020. We identified patients with newly diagnosed diabetes and worsening of preexisting diabetes. Newly diagnosed diabetes was defined as fasting blood glucose ≥ 126 or hemoglobin A1c (HbA1c) ≥ 6.5, random blood glucose ≥ 200 mg/dL with symptoms or 2-hour blood glucose ≥ 200 mg/dL on oral glucose tolerance test. Worsening of preexisting diabetes, defined as more than 0.5% increase in absolute HbA1c value in preceding 3-6 months or need for insulin in stable patients with diabetes on oral hypoglycemic agents. Subjects with pre-existing type 1 diabetes mellitus or on systemic corticosteroids for more than 1-week duration prior to diagnosis of diabetes mellitus were excluded.

Results Of the 1,857 patients treated with anti-PD-1 ICIs, there were 1,079 patients treated with nivolumab, 821 patients treated with pembrolizumab, and 43 patients treated with both pembrolizumab and nivolumab. There were 254/1857 (13.7%) patients that developed one of the 28 different dermatoses identified from literature review following anti-PD-1 ICIs. Compared with the general population, patients treated with anti-PD-1 had a greater risk for development of mucositis (OR 65.7, 95% CI 35.0-123.3), xerostomia (OR 11.3, 95% CI 8.9-14.3), and lichen planus/lichenoid dermatitis (OR 10.7, 95% CI 8.4-16.8), pruritus (11.3, 95% CI 8.9-14.3), mucositis (OR 65.7, 95% CI 35.0-123.3), xerostomia (OR 11.3, 95% CI 8.9-14.3), and lichen planus/lichenoid dermatitis (OR 10.7, 95% CI 8.4-20.7) compared to the control group.

Conclusions We report the frequency of dermatoses encountered in the setting of ICI therapy, both commonly (pruritus, rash, vitiligo) and more rarely reported (scleroderma, urticaria). As nivolumab and pembrolizumab currently make up over 90% of the market, the analysis relevant to current market trends. Furthermore, this analysis sets the stage for future in-depth investigation of these cutaneous toxicities, including dose-response relationships, prognostic information from cutaneous events, and optimal treatment strategies.

Ethics Approval This study did not require IRB approval, due to the use of anonymized and de-identified aggregate-level data.

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PRELIMINARY REVIEW OF DIABETES MELLITUS INCIDENCE IN PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITORS (ICI) THERAPY – ROSWELL PARK COMPREHENSIVE CANCER CENTER (RPCCC) EXPERIENCE

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Novel single-agent immunotherapies

LAMP1 TARGETING OF THE LARGE T ANTIGEN OF MERKEL CELL POLYOMAVIRUS ELICITS POTENT CD+ T CELL RESPONSES AND PREVENTS TUMOR GROWTH

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Background The majority of Merkel cell carcinomas (MCC), a rare and highly-aggressive type of neuroendocrine skin cancer, are associated with Merkel cell polyomavirus (MCPyV) infection. MCPyV integrates into the host genome, resulting in expression of a truncated form of the viral large T antigen (LT) in infected cells, and making LT an attractive target for therapeutic cancer vaccines. While induction of tumor-reactive CD8+ T cells is a major goal of cancer therapy, CD4+ T cells provide essential support to CD8+ T cells by promoting their expression of cytotoxic effector molecules and increasing their migratory capacity. Cytokines secreted by CD4+ T cells, such as IFNy, can also exert desirable effects on the tumor microenvironment. Therefore, we set out to design a cancer vaccine that promotes potent, antigen-specific CD4+ T cell responses to MCPyV-LT.
Methods To activate antigen-specific CD4+ T cells in vivo, we utilized our nucleic acid platform, UNITE (UNiversal Intracellular Targeted Expression), which fuses a tumor-associated antigen with lysosomal-associated membrane protein 1 (LAMP1). This lysosomal targeting technology results in enhanced antigen presentation and a balanced T cell response. LTS220A, encoding a mutated form of MCPyV-LT that abrogates its pro-oncogenic properties, was introduced into the UNITE platform. LTS220A-UNITE, known as ITI-3000, was administered to female C57BL/6 mice intradermally in the ear with electroporation.

Results ITI-3000 promoted a potent, antigen-specific CD4+ T cell response to MCPyV-LT. Vaccination with ITI-3000 significantly delayed and slowed growth of B16F10 tumors expressing LTS220A in prophylactic and therapeutic settings, respectively. ITI-3000 induced a favorable tumor microenvironment (TME), including significantly enhanced numbers of CD4+ T cells, CD8+ T cells, NK cells, and NKT cells. Tumor-infiltrating myeloid cells were reduced in frequency in vaccinated mice and polarized towards an anti-tumor phenotype. Cytokine analysis of the TME showed significantly enhanced levels of cytokines associated with anti-tumor immune responses in ITI-3000-vaccinated mice, including IFNγ, TNFα, IL-2, and IL-1β. Additionally, ITI-3000 synergized with PD-1 blockade, further reducing tumor burden and enhancing survival in mice receiving combination therapy.

Conclusions We find that DNA vaccination with ITI-3000 using the UNITE platform enhances CD4+ T cell responses to MCPyV-LT and results in anti-tumor immune responses in a mouse model of Merkel cell carcinoma.

Ethics Approval This study was approved by Immunomic Therapeutics’ Institutional Animal Care and Use Committee, protocol number 16-11-002.

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A BISPECIFIC ANTIBODY TARGETING CD40 AND EPCAM INDUCES SUPERIOR ANTI-TUMOR EFFECTS COMPARED TO THE COMBINATION OF THE MONOSPECIFIC ANTIBODIES

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Background Alligator has developed a new concept, Neo-X’, to enable antigen presenting cells to efficiently enhance priming of neoantigen-specific T cells, which may be the missing aspect in tumors that lack T cell infiltration. We hypothesize that binding of the CD40 x EpCAM bsAb (4224) to CD40 on DCs and EpCAM on tumor exosomes or tumor debris leads to i) activation of the DC, ii) uptake of the tumor material, iii) cross-presentation of tumor-derived neoantigens (present in exosomes or debris) and iii) priming of tumor neoantigen-specific T cells, resulting in an increased quantity and/or quality of the tumor-targeting T cell pool. CD40 cross-linking by engagement with a tumor antigen on a tumor cell is required to achieve a functional agonistic effect, and subsequent DC activation will therefore only be achieved in the presence of tumor antigens.

Methods 4224 evaluated in vitro using human monocyte-derived DC, co-cultured with cells expressing EpCAM. In addition the functional effects were evaluated using tumor cell lines and B-cell lines expressing CD40. In vivo, the anti-tumor efficacy of the CD40 x EpCAM bsAb was determined in human CD40 transgenic mice bearing MB49 bladder carcinoma tumors transplanted with human EpCAM or controls.

Results In vitro, we have demonstrated that the CD40 x EpCAM bsAb induces tumor target dependent activation of dendritic cells, as analyzed by flow cytometry measuring HLA-DR and CD86 expression on the DC and by measuring IL-12p40 levels in the supernatant. Further, the ability of bsAbs within the Neo-X’ concept to mediate co-localization of tumor debris and CD40 expressing antigen presenting cells depends on the receptor density of the tumor target. In vivo, 4224 displayed a potent, EpCAM-dependent anti-tumor effect with significantly reduced tumor growth and improved survival compared to an equivalent dose of the combination of the monospecific CD40 Ab and EpCAM targeting antibody. The tumor-localizing property of 4224 also shows potential for improved safety compared to CD40 monospecific antibodies. A biodistribution analysis demonstrated that the bspecific 4224 in the RUBY-format displayed similar half-life as the monospecific CD40 mAb in mice.

Conclusions In conclusion, the Neo-X’ concept, by targeting CD40 and a tumor specific antigen, has the potential to mediate an expansion of the tumor-specific T cell repertoire, resulting in increased T cell infiltration and potent anti-tumor effects.

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INHIBITION OF THE KINASE ACTIVITY OF HEMATOPOIETIC PROGENITOR KINASE 1 ENHANCES ANTI-PD-1-INDUCED REINVIGORATION OF HUMAN TUMOR-INFILTRATING CD8+ T CELLS

1Yongjoon Lee, 1Seung Hyuck Jeon, 2A Yeong Park, 5Suyeon Jo, 1Jinhwa Lee, 1Su-Hyung Park, 1Jamie Jae Eun Kim*, 1Eui-Chel Shin. 1KAIST, Daejeon, Korea, Republic of; 21ST Biotherapeutics, Inc., Seongnam, Korea, Republic of

Background Immune checkpoint inhibitors (ICIs) including anti-CTLA-4, anti-PD-1, and anti-PD-L1 have been clinically used for the treatment of various types of cancer. However, ICIs have a limited efficacy, and it is required to develop a strategy to enhance the efficacy of ICIs. Hematopoietic progenitor kinase 1 (HPK1) was recently known to inhibit T cell receptor (TCR) signaling by targeting SLP76 thus suppress T-cell effector functions.

Methods In the present study, we examined the expression of HPK1 and SLP76 in tumor-infiltrating lymphocytes (TILs) obtained from renal cell carcinoma tissues, in relation with the expression of PD-1 and other immune checkpoint receptors by performing flow cytometry analysis. In addition, we examined if inhibition of the kinase activity of HPK1 by CMPD0914, that is a potent, selective and orally available HPK1 inhibitor, enhanced effector functions of tumor-infiltrating CD8+ T cells in the presence of anti-PD-1 blocking antibodies.

Results First, we found that HPK1 and SLP76 are expressed in both CD8+ and CD4+ T cells including Foxp3+ regulatory T cells irrespective of PD-1 expression. Intriguingly, the expression levels of HPK1 and SLP76 were significantly higher in the PD-1bright population compared to the PD-1- or PD-1dim populations. Further characterization revealed that HPK1 and SLP76 were highly expressed in CD8+ T-cell populations expressing TOX, a transcription regulator of T-cell exhaustion,
TARGETING IMMUNOSUPPRESSIVE MACROPHAGES OVERCOMES PARP-INHIBITOR RESISTANCE IN BRCA1-ASSOCIATED TRIPLE-NEGATIVE BREAST CANCER


Background Despite objective responses to PARP inhibition and improvements in progression-free survival compared to standard chemotherapy in patients with BRCA-associated triple-negative breast cancer (TNBC), benefits are transitory.

Methods Using high dimensional single-cell profiling of human TNBC, here we demonstrate that macrophages are the predominant infiltrating immune cell type in BRCA-associated TNBC. Through multi-omics profiling we show that PARP inhibitors enhance both anti- and pro-tumor features of macrophages through glucose and lipid metabolic reprogramming driven by the sterol regulatory element-binding protein 1 (SREBP-1) pathway.

Results Combined PARP inhibitor therapy with CSF-1R blocking antibodies significantly enhanced innate and adaptive anti-tumor immunity and extends survival in BRCA-deficient tumors in vivo and is mediated by CD8+ T-cells.

Conclusions Collectively, our results uncover macrophage-mediated immune suppression as a liability of PARP inhibitor treatment and demonstrate combined PARP inhibition and macrophage targeting therapy induces a durable reprogramming of the tumor microenvironment, thus constituting a promising therapeutic strategy for TNBC.

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DEVELOPMENT OF FPA157, AN ANTI-CCR8 DEPLETING ANTIBODY ENGINEERED TO PREFERENTIALLY ELIMINATE TUMOR-INFILTRATING T REGULATORY CELLS

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Background The clinical success of PD-1- and CTLA-4-immune checkpoint inhibitors highlights the key contribution of immunosuppression to limiting effective anti-tumor responses. However, as many patients do not respond to anti-PD1 or CTLA4 therapy, novel therapeutics that target additional immune-suppressive mechanisms are needed. Regulatory T cells (Tregs) inhibit immune responses in the tumor micro-environment via multiple suppressive mechanisms. Existing Treg-targeting agents lack specificity for intratumoral Tregs and can also deplete effector cells, a property that has likely contributed to the lack of clinical activity observed to date. CCR8 (C-C chemokine receptor 8) is selectively expressed on highly activated intratumoral Tregs, its high expression correlates with poor prognosis in multiple human tumor types and depletion of CCR8+ Tregs in preclinical models elicited potent anti-tumor activity. These observations provided rationale for the development of a CCR8-specific human depleting antibody.

Methods Human FOXP3 and CCR8 expression was correlated across multiple tumor types using TCGA datasets and expression of CCR8 evaluated in primary tumor explants and PBMCs by flow cytometry. The efficacy of anti-CCR8 antibody treatment was evaluated in the MC38 and CT26 murine tumor models. The depletion of Tregs following anti-CCR8 treatment was assessed by flow cytometry. Flow cytometric binding assays were performed using cell lines expressing human or cynomolgus CCR8. Purified human NK cells were co-cultured with CCR8+ target cells and flow cytometry used to evaluate antibody-dependent killing activity.

Results CCR8 expression was highly correlated with FoxP3 across multiple cancer subtypes and was low to absent on effector T cells. Importantly, CCR8 was not detected on any peripheral human leukocyte subset. In murine tumor models, anti-CCR8 antibody treatment reduced tumor growth in a dose- and Fc-gamma-receptor-dependent manner and resulted in complete regressions and the development of memory. Tumor shrinkage was associated with a reduction in intratumoral Tregs and increased representation of intratumoral CD8 T cells. FPA157 is a highly specific human and cynomolgus crossreactive CCR8 antibody that does not bind closely related chemokine receptors. FPA157 was engineered to enhance antibody-dependent cell-mediated cytotoxicity (eADCC) and elicited potent NK-mediated killing of target cells expressing CCR8 at levels observed on human intratumoral Tregs.

Conclusions FPA157 is a CCR8-specific monoclonal antibody with eADCC activity that is being developed for the treatment of cancer. Depletion of CCR8+ Tregs induced substantial anti-tumor activity in pre-clinical models, thus supporting the clinical evaluation of FPA157 as a novel approach to alleviate immune suppression in the microenvironment of human solid tumors.

REFERENCES


TARGETING PSGL-1, A NOVEL MACROPHAGE IDENTIFICATION AND CHARACTERIZATION OF AN INFLAMMATORY RESPONSE IN COMPLEX MULTI-CELLULAR ASSAYS, INCLUDING SEB STIMULATED PBMC ASSAYS AND MIXED-LYMPHOCYTE REACTIONS (MLRs).

To establish a pre-clinical proof-of-concept for targeting PSGL-1, we turned to ex vivo cultures of fresh patient-derived primary tumors, where the complexity of the TME can be most preserved. RNA-seq data show that ex vivo cultures treated with anti-PD-1 antibody recapitulate TME changes in anti-PD-1 treated patients, including a strong T-cell IFN-gamma signature and a reduction in oncogenic pathway activation. Blocking PSGL-1 resulted in a robust pro-inflammatory signature driven by TNF-alpha/NF-kappa-B and chemokine-mediated signaling. The increase in TNF-alpha signaling was accompanied by reduction in oxidative phosphorylation and fatty acid metabolism. The increase in pro-inflammatory cytokine and chemokine production was confirmed by measuring the re-polarization of macrophages within a tumor setting.

Lastly, we employed a humanized mouse PDX model of melanoma and show that anti-PSGL-1 treatment resulted in suppression of tumor growth favorably compared to anti-PD-1. At the cellular and molecular levels, anti-PSGL-1 treatment lead to a more enhanced inflammatory microenvironment, including a reduced M2:M1 macrophage ratio, increased antigen presentation, pro-inflammatory mediators, and effector T cell infiltration and activation.

Conclusions Our data support anti-PSGL-1 as a macrophage repolarizing agent and an effective macrophage-targeted therapy for Immuno-Oncology.
immunodominant SARS-CoV-2-specific CD8 T cell response revealed a regulated activation program that maintains CD8 T cell survival while halting their effector function and migratory capacity.

**Conclusions** The ORF1ab, that was found to be the source of an immunodominant SARS-CoV-2-specific CD8 T cell epitope, is not included in the majority of vaccine candidates in development, which may influence their clinical activity. Furthermore, these data may be a cautious indication that SARS-CoV-2-specific CD8 T cells – unlike CD4 T cells – are less likely to contribute to the immunopathology observed in severely and critically ill COVID-19 patients.

**Ethics Approval** The samples from both COVID-19 patients were collected in accordance with the Declaration of Helsinki after approval by the institutional review boards.

**Consent** Each participant signed informed consent.

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**Tumor and stromal cell biology**

**THE MESENCHYIMAL STROMAL COMPARTMENT IN COLORECTAL CANCER GREATLY ALTERS THE INNATE TUMOUR IMMUNE MICROENVIRONMENT BOTH IN 2D AND 3D CULTURE SYSTEMS**

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**Background** Colorectal cancer is the fourth most common occurring cancer and despite new treatment options it remains the third leading cause of cancer related deaths worldwide. Of the four Consensus Molecular Subtypes (CMS), the mesenchymal stromal rich CMS4 tumours are shown to have the worst disease free progression survival. However the role mesenchymal stromal cells (MSC) play in the tumour immune microenvironment has yet to be fully elucidated. Understanding the complex communication in this stromal cell rich multicellular environment is challenging but may reveal novel targets for the treatment of colorectal cancer patients.

**Methods** Tumour cell secretome (TCS) was generated from colon cancer cells with/without the addition of TNF-α, an inflammatory stimulus using both human and mouse cell lines. MSCs were then conditioned with the TCS and inflammatory TCS and changes in surface and secreted immunomodulatory molecules were assessed using RNA-seq, flow cytometry and ELISA analysis. Macrophage antigen processing and migration following co-culture with the TCS conditioned MSCs was observed using DQ-ova and transwell experiments. A Gelatin Methacryloyl hydrogel, 3D culture systems was established to study the role of MSC in the colon tumour immune microenvironment. HCT116 colon cancer cell line with THP1 monocytic cell line and primary bone marrow derived MSCs were embedded in the hydrogel and incubated for 10days, changing the media on Day 8 with/without the addition of TNF-α. Cell proliferation viability and protein secretion were assessed from the 3D CRC system.

**Results** Bioplex analysis revealed secretion of potent chemokines and cytokines from the cancer cells. This inflammatory TSC resulted in increased expression of cell surface MSC immunomodulatory markers PD-L1 and CD47 and a variety of secreted molecules. These conditioned MSCs reduced macrophage-mediated antigen processing and increase monocyte migration. A triple culture 3D model of CRC was successfully developed, and while the addition of MSC to the system did not alter spheroid size they increased the release of potent chemokines (CCL2, CXCL12), cytokines (IL-6, IL8) and growth factors (GM-CSF) from the culture system.

**Conclusions** The inflammatory tumour cell secretome can alter MSC surface expression and secretion of a variety of immunomodulatory makers. These tumour conditioned MSCs can alter innate immune cell antigen processing and migration. When MSCs are combined in 3D with monocytes and colon cancer cells the MSC significantly alter the secretion of immune modulating and tumour promoting factors from the culture system. Targeting MSC immune suppression in the colon tumour microenvironment could be a novel therapeutic target.

**Ethics Approval** Human MSC (hMSC) were isolated from the bone marrow of three healthy volunteers at Galway University Hospital under an ethically approved protocol (NUIG Research Ethics Committee, Ref: 08/May/14) according to a standardized procedure.

**REFERENCE**


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**SUGAR HIGH: DOES THE SIALIC ACID PROFILE OF CANCER-ASSOCIATED FIBROBLASTS INDUCE A MORE TUMOUR-PERMISIVE MICROENVIRONMENT?**

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**Background** Immunosuppressive tumour microenvironments (TME) inhibit the effectiveness of cancer immunotherapies. Sialic acids, which exist as terminal sugars of glyco-conjugates, are highly expressed on cancer cells and are involved in various pathological processes including increased immune evasion, tumour invasiveness and tumour cell metastasis. Siglecs (Sialic acid-binding immunoglobulin-type lectins) are expressed on immune cell surfaces and bind sialic acid. Siglec binding to hypersialylated tumour glycan blocks immune cell activation to promote immunosuppression. Intestinal stromal cells (iSCs), precursors to cancer-associated fibroblasts (CAFs), are a key component of the TME and play a vital role in tumour progression by enhancing a tumour-promoting microenvironment. The aim of this study was therefore to investigate if iSC/CAF sialylation contributes to enhanced immunosuppression in the TME.

**Methods** iSCs were isolated from colorectal cancer patient biopsies and cultured ex vivo. Informed consent was obtained from all patients prior to sampling. Tumour-derived iSCs were termed CAFs while control iSCs, isolated from tumour-adjacent non-cancerous tissue, were termed normal-associated
fibroblasts (NAFs). NAFs/CAFs were then co-cultured with healthy allogeneic PBMCs and their immunosuppressive properties were assessed by flow cytometry.

**Results** CAFs significantly suppressed the proliferation of CD8+ and CD4+ T-cells and induced a more exhausted T-cell phenotype as evidenced by increased expression of the exhaustion markers TIM-3, LAG-3 and PD-1 when compared to co-culture with control NAFs, thereby demonstrating their potent immunosuppressive properties. Strikingly, CAFs also induced significantly higher expression of both Siglec-7 and Siglec-9 receptors on CD8+ T-cells specifically.

To elucidate the role of sialylation on CAF-mediated immunosuppression, NAFs/CAFs were treated with the sialyltransferase inhibitor (SI) P-3FAX-Neu5Ac prior to co-culture. Reduction of sialic acid expression on NAFs/CAFs was confirmed by flow cytometry and the SI-treated NAFs/CAFs were then co-cultured with allogeneic T-cells to assess the functional consequences of reduced NAF/CAF sialylation. SI-treated CAFs induced significantly less CD4+TIM-3+ and both CD4+LAG-3+ and CD8+LAG-3+ T-cells compared to their untreated counterparts. Interestingly, SI-treated CAFs also induced significantly less Siglec-7 and -9 receptor-expressing CD8+ T-cells.

**Conclusions** These results demonstrate that non-haematopoietic stromal cells in the tumour-microenvironment can suppress activated T-cells and that this immunosuppressive effect can be significantly reversed through the modulation of sialylation on the stromal cell surface. These results support the hypothesis that stromal cell sialylation plays a role in their immunosuppressive properties. Understanding how sialylation of stromal cells is regulated and functions to enhance immunosuppression in the TME could uncover novel immune checkpoints to reactivate anti-tumour immunity, allowing for tumour cell clearance.

**Ethics Approval** This study was approved by Galway University Hospitals’ Clinical Research Ethics Committee, approval number C.A 2074.

**Consent** N/A

**REFERENCES**


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**DISTINCT GENOMIC FEATURES ACROSS CYTOLYTIC SUBGROUPS IN SKIN MELANOMA**

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**Background** Skin melanoma is a highly immunogenic cancer. The intratumoral immune cytolytic activity (CYT) reflects the ability of cytotoxic T cells and NK cells to eliminate cancer cells, and is associated with improved patient survival. Despite the enthusiastic clinical results seen in advanced-stage metastatic melanoma patients treated with immune checkpoint inhibitors (ICI), a subgroup of them will later relapse and develop acquired resistance. We questioned whether CYT associates with different genomic profiles in skin melanoma.

**Methods** We explored the TCGA-SKCM dataset and stratified patients to distinct subgroups of cytolytic activity. We calculated the tumor immune contexture, somatic mutations, recurrent copy number aberrations, chromothripsis, cancer neoepitopes, immunophenoscore, mutational signatures, kataegis and strand asymmetry in each cytolytic subgroup.

**Results** CYT was higher in enriched in immune-related gene sets metastatic tumors. Distinct mutational and neoantigen loads, primarily composed of C>T transitions, along with specific types of copy number aberrations, characterized each cytolytic subgroup. More chromothripsis events were found across CYT-low tumors SBS7a/b, SBS5 and SBS1 were the most prevalent mutational signatures in both cytolytic subgroups, but SBS1 differed significantly between them. SBS7a/b were mutually exclusive with SBS5 and SBS1 in both CYT subgroups. Mutational strand asymmetries related to the processes of DNA transcription and replication differed between CYT-high and CYT-low tumors. CYT-high patients had markedly higher immunophenoscore and should consequently, display an expected clinical benefit compared to CYT-low patients who either received or not, ICI.

**Conclusions** Our data highlight the existence of distinct genomic features across cytolytic subgroups in skin melanoma patients, which could affect their relapse rate or resistance to ICI.

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**PREL-P-FACILITATED ENHANCEMENT OF MHC CLASS I SURFACE EXPRESSION IN B16F10 MELANOMA CELLS**

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**Background** PRELP (proline arginine-rich end leucine-rich repeat protein; also Prolargin), a small leucine-rich proteoglycan, functions as a molecule anchoring basement membranes to connective tissues via the interaction with collagens and heparin. PRELP facilitates the binding of cells to glycosaminoglycans as an important regulator of cell adhesion and thus displays pathophysiological features. Melanoma is an immunogenic tumor, whose relationship with immune cells resident in the microenvironment significantly influences cancer cell proliferation, progression and metastasis. Evasion from immune surveillance is a hallmark of melanoma progression. While our laboratory reported that the proteoglycan biglycan (BGN) was enhancing MHC class I in tumor cells, the role of PRELP in tumor immunology has not been studied.

**Methods** The murine metastatic melanoma cell line B16F10, characterized by a reduced expression of MHC class I surface antigens was chosen for this study. B16F10 cells were transiently transfected with PRELP as well as co-transfected with BGN. Expression of antigen processing machinery (APM) components and PRELP was determined by qPCR and MHC class I surface expression by flow cytometry. Promoter activity of APM components was analysed by luciferase reporter assays. XTT assays were used to determine cell proliferation. The association of PRELP and MHC class I was studied by bioinformatics in a mixed melanoma dataset of 83 samples.

**Results** Over-expression of PRELP in B16F10 cells enhanced the expression of MHC class I surface antigens, which was...
due to a PRELP-mediated transcriptional upregulation of components of the MHC class I APM components TAP1, TAP2 and TAPBP as determined by qPCR and promoter assay in PRELP transfectants versus mock controls. Furthermore, MHC class I surface expression was even more pronounced upon BGN co-transfection with PRELP. PRELP overexpression was able to inhibit the proliferation of the B16F10 cells. Bioinformatics analyses demonstrated a positive correlation of PRELP with HLA-A, -B and -C alleles in human melanoma.

Conclusions Our findings demonstrated that overexpression of PRELP correlates with higher MHC class I expression and inhibits cell proliferation. For the first time, co-transfections of the two proteoglycans PRELP and BGN had a synergistic effect on upregulating MHC class I expression. Therefore, PRELP can serve as a novel therapeutic strategy that deserves further investigation.

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Abstracts

869 ANTI-LUNX TARGETING THERAPY FOR LUNG CANCER
Xiaochu Zheng*, Weihua Xiao, Zhigang Tian. USTC

Background The identification of novel therapeutic targets in lung cancer for the generation of targeted drugs is an urgent challenge. Lung-specific X (LunX) is a member of the palate, lung, and nasal epithelium clone (PLUNC) protein family. Some reports have suggested that the human PLUNC gene (also named LUNX) might be a potential marker for NSCLC, and PLUNC mRNA has been identified in peripheral blood and mediastinal lymph nodes from NSCLC patients. It is unclear whether LunX expression is associated with the pathological type and pathological severity in lung cancer patients. The utility of LunX as a potential therapeutic target in NSCLC is uncertain.

Methods Clinically, 80% of lung cancers are non-small-cell lung cancers (NSCLCs). Here, we analyzed 158 NSCLC samples and detected LunX expression.

Results It showed that the expression of LunX was elevated in 90% (108/150) lung cancers by IHC staining, which accompanied with significantly lower rate of post-surgery survival. Further evaluation of LunX expression in invasive tumor cells in subclavicular lymph nodes, draining lymph nodes, hydrothorax of lung cancer patients, turned out that LunX is highly expressed in invasive lung cancer cells. These data indicated that LunX overexpresses in lung cancer and associates with tumorigenesis and tumor progression.

Mechanistically, we discovered that LunX bound to 14-3-3 protein and facilitated their activation by maintaining these proteins in a dephosphorylated state, thereby contributing to the activation of pathways downstream of 14-3-3 protein, such as the Erk1/2 and JNK pathways. Thus, LunX promoted tumor growth and metastasis.

Furthermore, we generated a therapeutic antibody specific for lung cancer, which not only inhibited lung cancer growth and reduced Ki67 staining and angiogenesis in xenograft model of subcutaneously transplanted tumor, but also blocked tumor metastasis and invasion, improved the survival of these mice. We also detected that antibody treatment induces LunX antigen-antibody complex endocytosis and the degradation of LunX protein.

Conclusions Our study suggests that LunX is a novel therapeutic target in lung cancer and that the LunX-targeted therapeutic antibody may have considerable clinical benefit.

870 INVESTIGATING SEXUAL DIMORPHISM IN THE TUMOR IMMUNE MICROENVIRONMENT OF NON-MUSCLE INVASIVE BLADDER CANCER

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Background While the incidence of non-muscle invasive bladder cancer (NMIBC) is four times higher in men than women, female patients display earlier recurrence than their male counterparts following treatment with Bacillus Calmette-Guerin (BCG) immunotherapy. While patient sex (biological differences) and gender (social/behavioral differences) have long been associated with NMIBC incidence and clinical outcome, these factors remain the most understudied phenotypes in biomarker and treatment design. We hypothesized that sexual dimorphism in the pre-existing tumor immune microenvironment (TIME) may contribute to the poor clinical outcomes observed in female NMIBC patients.

Methods To test this hypothesis, we interrogated the expression patterns of genes associated with specific immune cell populations and immune checkpoint pathways using tumor transcriptome profiles from n=460 NMIBC patients (357 males and 103 females). Based on this interrogation, we utilized multiplex immunofluorescence to selectively evaluate the density and spatial distribution of CD79a+ (B), CD163+ (M2-like tumor associated macrophages), and PD-L1+ (programmed death ligand 1) cells in an independent cohort of 510 NMIBC tumors collected from n=390 patients (305 males and 85 females).

Results We observed significantly higher expression of immune checkpoints genes CTLA4, PDCD1, TIGIT, LG3 and ICOS in tumors from female patients. Importantly, transcript levels of the B cell recruiting chemokine CXCL13 and the B cell surface molecule CD40 were significantly increased in tumors from female patients. Multiplex immunofluorescence revealed that CD163+ cells were significantly higher in epithelial and stromal compartments of high-grade tumors (p = 0.0011, p = 0.00034, respectively) from female patients compared to males. While no sex-associated differences were observed in the density of CD79a+ B cells, this population was found to be significantly increased in the epithelial and stromal compartments (p = 6.9e-9, 9.4e-10, respectively) from high-grade tumors compared to low-grade tumors. PD-L1 expression was significantly higher in the epithelial compartment of high-grade tumors from female
Background Macrophages are immunological cells that sense microenvironmental signals that may result in the polarized expression of either proinflammatory (M1) or anti-inflammatory (M2) phenotype. Macrophages M2 are present in tumor microenvironment and their presence in patients with cervical cancer (CeCa) is related with less survival. Mesenchymal Stromal Cells (MSCs) are also present in tumor microenvironment of cervical cancer (CeCa-MSC), which have shown immunoregulatory effects over CD8 T cells, decreasing their cytotoxic effect against tumoral cells. Interestingly, MSCs from bone marrow (BM-MSC) decrease M1 and increase M2 macrophage polarization in an in vitro coculture system. Macrophages and MSCs are present in microenvironment of cervical cancer, however it is unknown if MSCs play a role in macrophage polarization. In the present study, we have evaluated the immunoregulatory capacity of CeCa-MSCs to induce macrophage polarization.

Methods CD14 monocytes were isolated from peripheral blood and cultivated in the absence or presence of MSCs from BM, normal cervix (NCx) and CeCa. Two culture conditions were included, in the presence of induction medium to favors M1 (GM-CSF, LPS and IFNγ) or M2 (M-CSF, IL-4 and IL-13) macrophage polarization. M1 (HLA-DR, CD80, CD86 and IFNγ) or M2 (CD14, CD163, CD206, IDO and IL-10) macrophage molecular markers were evaluated by flow cytometry. Finally, we evaluated concentration of IL-10 and TNFα in conditioned medium form all coculture conditions.

Results We observed that CeCa-MSCs and BM-MSCs in presence of M1 induction medium, decreased M1 macrophage markers (HLA-II, CD80, CD86 and IFNγ), and increase the expression of CD14 (M2 macrophage marker). Interestingly, in presence of M2 induction medium, BM-MSCs and CeCa-MSCs but not NCx-MSC increased CD163, CD206, IDO and IL-10 (M2 macrophage markers). We observed a decreased concentration of TNFα in the supernatant medium from all cocultures with MSCs, but only in presence of CeCa-MSCs, increased IL-10 concentration was detected in such cocultures.

Conclusions In contrast to NCx-MSCs, CeCa-MSCs similarly to BM-MSCs have in vitro capacity to decrease M1 and increase M2 macrophage phenotype.

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chemoradiation (CRT) on the tumor immune microenvironment (TIME) of PDAC.

Results When comparing non-treated (NT) to neoadjuvant chemoradiation (CRT) tumors, the proportion of tumor within the overall tissue sample was markedly lower in treated tumors (figure 1; Mann Whitney U, U=25, p<0.0001). Additionally, the overall density of Ki67+ cells throughout all tissue was significantly lower in samples that received CRT (figure 1; Mann Whitney U, U=52, p=0.0067). An overall influx of CD3+ cells was noted in CRT samples. T cell influx was accompanied by upregulation of inflammatory genes. When considering T cell subsets, an increase in the CD8+ (Cytotoxic) and CD4+FOXP3+ (Treg) cell densities in the tumor of CRT samples was found. CD4 +FOXP3- (T helper) cell density was found to be increased in the tumor, stroma, and overall tissue in CRT samples (figure 2). When comparing samples from patients who lived longer than 2 years to sample s from patients who did not within the CRT group, a notably higher ratio of Tregs to CD3+ cells was observed in patients who lived less than 2 years (Mann Whitney U, U=0, p=0.0006). When used as a predictor, the ratio of Tregs to CD3+ cells also correlated closely to patient survival (figure 3; Mantel-Cox, p=0.0121).

Conclusions We find that CRT greatly alters the TIME of PDAC, altering distributions of tumor cells within the microenvironment and inducing an overall influx of T cells, including cytotoxic, helper, and Treg T cell subsets. In patients receiving CRT, it appears as though the proportion of T cells infiltrating the tumor that are Tregs is closely associated with patient outcome, with a higher proportion of Treg infiltration correlating with a poor outcome. This data suggests that therapies targeting regulatory T cells should be explored in combination with chemo-radiotherapy in PDAC.

Ethics Approval The study was approved by Columbia University’s Ethics Board, approval number AAAQ7337.

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PHARMACOLOGIC MACROPHAGE DEPLETION AFFECTS METASTASIS FORMATION BY MODULATING SYSTEMIC IMMUNE RESPONSES IN A GENETIC PANCREATIC CANCER MODEL

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Background Tumour-associated macrophages (TAM) play an important role in mediating tumour progression. In pancreatic cancer, infiltrating macrophages have been identified not only in invasive tumours, but also in early preinvasive pancreatic intraepithelial neoplasias and are known to mediate tumour progression.

Methods We aimed to study the impact of pharmacological macrophage depletion by liposomal clodronate in the genetic mouse model of pancreatic cancer (KPC mouse: LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre). KPC mice were treated with liposomal clodronate or control liposomes from week 8 to week 20. Tumour and metastasis formation as well as alterations in local and circulating immune cells and cytokines were analysed.

Results Treatment with liposomal clodronate effectively reduced CD11b-positive macrophages both in the pancreas and other organs such as liver, lung and spleen. Tumour incidence and size was only slightly reduced. However, metastasis formation in the liver and lungs was markedly diminished after macrophage depletion. Reduced macrophage count was associated with significant alterations in circulating growth factors and mediators known to be secreted by macrophages and associated with angiogenesis, most prominently VEGF. Moreover, application of liposomal clodronate led to marked alterations in circulating immune cells, among them reduced regulatory T cells.

Conclusions Pharmacological depletion of macrophages in a genetic mouse model of pancreatic cancer markedly reduced metastasis formation and is associated with modulated profile of both secreted mediators and regulatory T cells. Pharmacological modulation of infiltrating macrophages represents a promising avenue for antimetastatic therapeutic approaches.

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