Spring Scientific

sitc Society for Immunotherapy of Cancer

Host Immunity in Immunotherapy Responses: From Discoveries to Precision Oncology Abstracts

March 15–17, 2023 | Denver, CO and Virtually

Denver Hyatt Regency at Colorado Convention Center

sitc Society for Immunotherapy of Cancer

Table of Contents

Abstract List

Oral Abstract Presentations		
Abstract Number	**Presenter Name**	**Abstract Title**
5	Svetlana Atasheva, PhD – *Emory University*	Lung cancer-associated myeloid cells dictate the immunologic response to oncolytic adenovirus.
11	Denise Cecil, PhD – *University of Washington*	Bacteria specific IL-10 secreting T-cells derived from the gut are cross-reactive with tumor antigens and accelerate tumor growth in mouse models.
13	Lanette Christensen, PhD – *Children's Hospital of Philadelphia*	Estrogen signaling via estrogen receptors alpha and beta impacts the function of Foxp3+ T-regulatory cells
23	Andrew Frisch, BS – *University of Pittsburgh*	Redirecting in vitro glucose flux improves the in vivo anti-tumor function of cellular immunotherapies
30	Masao Hashimoto, MD, PhD – *Emory University*	PD-1 combination therapy with IL-2 modifies CD8+ T cell exhaustion program
37	Ryan Lacinski, BS – *West Virginia University School of Medicine*	Immunotoxicology analysis of IL-12ns reveals biomarkers of beneficial and maladaptive immune responses in healthy BALB/c mice
46	Thomas Marron, MD, PhD – *Icahn School of Medicine at Mount Sinai*	Flt3L-primed *in Situ* Vaccination and pembrolizumab induce systemic tumor regressions of non-immunotherapy responsive tumors
49	Golnaz Morad, DDS, PhD – *The University of Texas MD Anderson Cancer Center*	Distinct microbial communities play a role in metastatic brain tumors
57	Elizabeth Park, PhD – *The University of Texas MD Anderson Cancer Center*	Treatment with metronidazole improves response and reduces toxicity to treatment with immune checkpoint blockade
65	Nathan Reticker-Flynn, PhD – *Stanford University*	Lymph node colonization promotes distant tumor metastasis through the induction of tumor-specific immune tolerance
72	Rahul Shinde, DVM, PhD – *The Wistar Institute*	The microbiome-derived metabolite TMAO drives immune activation and boosts responses to immune checkpoint blockade in pancreatic cancer
73	Tanaya Shree, MD, PhD – *Oregon Health & Sciences University*	Clinical and correlative results from a trial of in situ cancer vaccination in lymphoma
74	Akansha Singh, PhD – *Oklahoma State University*	Adrenergic receptor signaling regulates the CD40-receptor mediated anti-tumor
79	Zuzana Tatarova, PhD – *Brigham and Women's Hospital*	Spatial analysis of local drug induced changes in tumor microenvironment predicts effective treatment combinations in breast cancer
80	Tarun Tyagi, PhD – *Yale University*	TLT-1, a platelet released protein, suppresses CD8 T cell based systemic immunity, and exhibits prognostic and therapeutic significance in non-small cell lung cancer
87	Kyuson Yun, PhD – *Houston Methodist/ Weill Cornell Medical College*	Sex-biased Yap1 function in immune suppression

Poster Abstract Presentations		
Abstract Number	**Presenter Name**	**Abstract Title**
1	Amanda Kirane, MD – *Stanford University*	Neoadjuvant Intralesional Therapy for High Risk Primary Melanoma
2	Riyam Al Msari, BS – *Moores Cancer Center*	CD47 Blockade as a Targeted Immune Oncology Agent in Head and Neck Squamous Cell Carcinoma Model

Abstract List

Poster Abstract Presentations		
3	Stephanie Alden, MD – *The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine*	Cytokine levels with immune checkpoint inhibitors: does obesity matter?
6	Samuel Brill, BS – *Colorado State University*	Development of Canine CAR-T Cells Targeting the Disialoganglioside GD2
8	Maria Cardenas, PhD Student – *Emory University*	Stem like CD4 T cell differentiation controls the magnitude of the CD8 T cell effector response in cancer
9	Samuel Cass, MD – *The University of Texas MD Anderson Cancer Center*	A Single Dose of Prophylactic Perioperative Cefazolin Is Associated with Changes in Gut Microbiome Composition and Diversity in Patients Undergoing Surgery for Melanoma
10	Samuel Cass, MD – *The University of Texas MD Anderson Cancer Center*	Clinical Trial in Progress: Association Between Gut Microbiome and Dietary Determinants of Vaccine Response
12	Manoj Chelvanambi, PhD – *The University of Texas MD Anderson Cancer Center*	Metagenomic sequencing reveals unique gut microbial features associated with tertiary lymphoid structures in response to immune checkpoint blockade in solid cancers
14	Kristin DePeaux, BS – *University of Pittsburgh*	Oncolytic vaccinia requires infection of tumor infiltrating lymphocytes for treatment efficacy
16	Anushka Dongre, PhD – *Cornell University*	Sensitizing quasi-mesenchymal breast tumors to immune checkpoint blockade therapy by targeting CD73
17	Mark Duchow, BS – *Dell Med*	Potentiating Immunotherapy and Attenuating Tumor Cell Growth Through Combination of MEK and HDAC Inhibition in Microsatellite Stable (MSS) Colorectal Cancer (CRC)
18	Corey Dussold, MA – *Northwestern University*	Targeting wild-type IDH1 in the tumor microenvironment as a novel therapeutic intervention in glioblastoma
20	Arielle Elkrief, MD – *University of Montreal*	Fecal microbial transplantation in combination with first-line immune checkpoint inhibition in patients with melanoma and lung cancer: interim analysis from a phase II clinical trial
21	Jessica Filderman, BS – *University of Pittsburgh*	Antagonism of regulatory ISGs enhances the anti-melanoma efficacy of STING agonist ADU-S100
25	Thomas Gallup, BS – *EMPIRI, Inc*	Early insights into clinical trials through single-cell RNA sequencing from biopsy cores
26	Abigail Gault, MBBS – *Northern Centre for Cancer Care*	Distinct gut microbe metabolites differentially impact proliferative responses in human T cells: Potential mechanism for checkpoint inhibitor response heterogeneity
27	Prajna Guha, PhD – *Trisalus Life Sciences, Inc.*	Pressure-enabled intravascular delivery of SD-101 into the liver with systemic or subcutaneous checkpoint inhibitor for control of liver metastases in a murine model
31	Ilse Hernandez Aguirre – *Nationwide Children's Hospital*	Tumor antigen expressing oncolytic HSV elicits temporally different anti-tumor effects in T cell subsets
32	Heta Jadhav, MS – *Dana Farber Cancer Institute*	STING agonism enhances anti-tumor immune responses and therapeutic efficacy of PARP inhibition in *BRCA*-associated breast cancer
34	Amanda Kirane, MD – *Stanford University*	The impact of mesenchymal transition and macrophage phenotype switching in immunotherapy-resistant melanoma
36	Ashley Kroll, PhD – *Amgen*	Phagocytosis increases an oxidative metabolic and immune suppressive signature in tumor macrophages
38	Yan Leyfman, MD – *Icahn School of Medicine at Mount Sinai South Nassau*	Impact & Efficacy of a Virtual Streaming Platform to Provide Equitable Access for Immuno-Oncology Education
39	Yan Leyfman, MD – *Icahn School of Medicine at Mount Sinai South Nassau*	Impact of a Global Virtual Platform Providing Immuno-Oncology Education to Healthcare Professionals

Abstract List

Poster Abstract Presentations		
40	Yan Leyfman, MD – *Icahn School of Medicine at Mount Sinai South Nassau*	Severe SARS-CoV-2 & Cancer: Unraveling the Immunological Paradigm & a Promising Cellular Anti-COVID-19 Therapy for the Immunocompromised
41	Yan Leyfman, MD – *Icahn School of Medicine at Mount Sinai South Nassau*	Improving Immunotherapeutic Clinical Trial Access for BALT Patients with COVID-19
42	Matthew Lin, BS, MSc – *Icahn School of Medicine at Mount Sinai*	Enhancing regionally-restricted fas-mediated T cell bystander killing to mitigate tumor antigen escape
43	Taja Lozar, MD – *University of Wisconsin, Madison*	Spatial transcriptomic profilling reveals distinct dynamics in the tumor microenvironment relative to stress keratin 17 expression in immune check-point blockade treated head and neck carcinoma
47	Yusra Medik, MD – *Rutgers Heatlh Community Medical Center*	Use of Immune Checkpoint Inhibitors in Hospitalized Patients
48	Jade Moore, PhD – *University of California, San Francisco*	Loss of TGFβ signaling promotes genomically unstable cold cancers that are susceptible to immune checkpoint inhibition
50	Elizabeth Moser, MD, PhD – *Deka Biosciences*	DEKA-1 a dose-finding Phase 1 trial: observing safety and biomarkers using DK210 (EGFR) for inoperable locally advanced and/or metastatic EGFR+ tumors with progressive disease failing systemic therapy
51	Vincenzo Nasca, MD – *Fondazione IRCCS Istituto Nazionale dei Tumori*	The carry-over effect of immune-checkpoint inhibitors (ICIs) in microsatellite instability-high (MSI-H) metastatic colorectal cancer (mCRC): tumor response dynamics as predictive factors for post-progression survival
52	Levi Nelemans, MSc – *UMCG, Groningen University*	Signal Regulatory Protein Beta 2 is a novel positive regulator of innate anticancer immunity
53	Aik Seng NG, BSc – *University of Oxford*	Tumour microenvironment-mediated metabolic shifts in colorectal cancer regulate PD-L1 expression
54	Hong-My Nguyen, B. Pharm – *Texas Tech University Health Sciences Center*	Therapeutic efficacy of a Listeria-based vaccine targeting interferon-stimulated gene 15 (ISG15) in colorectal cancer
55	Bryan Oronsky, MD, PhD – *EpicentRx Inc*	Low dose but not high dose RRx-001 repolarizes tumor associated macrophages from an M2 to M1 phenotype in colorectal cancer
58	Ronal Peralta, BS – *University of Pittsburgh*	Lactic acid uptake through MCT11 enforces dysfunction in terminally exhausted T cells
59	Fanny Polesso, MSc – *Oregon Health and Science University*	Androgen deprivation therapy allows for effective anti-TIGIT immunotherapy in murine model of castration resistant prostate cancer
60	Timothy Rajakumar, MD, PhD – *Hummingbird Diagnostics GmbH*	A blood based qPCR assay is prognostic for immunotherapy in advanced stage non-small cell lung cancer
63	William Redmond, PhD – *Earle A. Chiles Research Institute*	Multimodal single-cell analysis of human TILs across multiple tumor types reveals heterogeneity and potential opportunities for personalized immunotherapy
64	Tony Reid, MD, PhD – *EpicentRx*	Trial in Progress: A Phase 1-2, First-in-Human, Open Label, Dose Escalation and Expansion Study of AdAPT-001, An Oncolytic Adenovirus That Expresses A TGF-β Trap, Which Binds To TGF-β and Inhibits TGF-β Receptor Binding, In Patients With Advanced Solid Tumors
66	Samuel Ricci, BS – *National Institutes of Health*	Assessing toxicity and anti-tumor activity of CD40 agonist monoclonal antibody combined with mesothelin-directed immunotoxin in a murine model of pancreatic cancer
67	Annah Rolig, PhD – *Providence Cancer Institute*	Intratumoral bacteria influence immunotherapy efficacy
69	Yongwoo Seo, MD – *MD Anderson Cancer Center*	VirMAP for Cancer: Characterization of the intratumoral virome in virally-associated cancers and a resource for investigators

Abstract List

Poster Abstract Presentations		
75	Egbert Smit, MD – *Netherlands Cancer Institute and Leiden University Medical Center*	Initial assessment of utility of a serum proteomic test to predict outcomes of patients with advanced non-small cell lung cancer (NSCLC) treated in first line with immune checkpoint inhibitors (ICIs)
77	Kellie Spahr, BS – *University of Pittsburgh*	Mitochondrial citrate transport modulates functional exhaustion in CD8+ T cells
78	Nathan Standifer, PhD – *Tempest Therapeutics*	TPST-1120, a first-in-human peroxisome-proliferator activated receptor-alpha (PPAR-α) antagonist: scientific rationale, clinical activity and biomarker assessments
81	Nasreen Vohra, MD – *East Carolina University, Brody School of Medicine*	Distinct immune signatures in sentinel lymph nodes may provide insights to guide breast cancer immunotherapy
82	Patrick Wagner, MD – *Allegheny Health Network Cancer Institute*	Simultaneous assessment of the tumor microbiome and immune contexture in colorectal cancer using a metaproteomics approach in archival tissue
84	Niels Werchau, MSc – *Miltenyi Biotec B.V. & CO. KG*	Exploring latent TGF-β as a tumor microenvironment target using MACSima™ imaging cyclic staining technology and Adapter CAR™ T-cells
85	Kai Wu, MD, PhD – *UCSF Fong Lab*	Short lived responses to anti-BCMA CAR-T cells are associated with expansion of activated cytotoxic CD4+ CAR-T cells
90	Yumeng Zhang, MD – *Moffitt Cancer Center*	High pretreatment DHEA level is associated with shorter overall survival in newly diagnosed metastatic non-small cell lung cancer patients receiving immune checkpoint inhibitors

*ePosters can be viewed any time during the program on the virtual meeting platform.
(Abstract list as of March 9, 2023)

(Odd numbered posters will be presented on Wednesday, March 15, 2023)

(Even numbered posters will be presented on Thursday, March 16, 2023)

Abstracts

1

Neoadjuvant intralesional therapy for high risk primary melanoma

M. Usman Ahmad, MD[1], Beatrice Sun, MD[1], Cameron Gaskill, MD, MPH[2], Emanual Maverakis, MD[2], Sepideh Gholami, MD[2], Michael Lowe, MD, MA[3], Amanda Kirane, MD[1*]

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[3]*Emory, Atlanta, GA, United States*

Background
Although survival remains significantly poorer for thicker, ulcerated melanoma, with less than 40% survival at 10 years, these patients are not currently candidates for modern immunotherapy. Talimogene Laherparpvec (TVEC) is the only FDA approved intralesional therapy for melanoma and has demonstrated significant overall response rate (64% complete pathologic response of injected lesions) and bystander effect (34% in uninjected lesions) in late stage melanoma. We hypothesize neoadjuvant intra-lesional therapy with TVEC in stage II melanoma will treat local and subclinical regional disease and still allow for standard of care surgery.

Methods
This is an open-label, multicenter, Phase 2 study (NCT04427306) of TVEC in stage II melanoma patients prior to wide excision and sentinel lymph node biopsy (SLNB). Patients with melanoma >2.0mm in thickness with residual disease are included. Primary objectives are to assess the safety and efficacy of TVEC measured by conventional assessments of histologic response used in other neoadjuvant studies. Secondary objectives include identification of immunologic and molecular predictors of response and measurement of SLNB positivity. A sample size of 62 patients will provide 91% power to detect a difference (P_1-P_0) of 19% in major response rate using a one-sided binomial test, with target significance level of 0.05.

Results
To date 5 patients have enrolled and been treated. 3 patients presented with acral disease, 1 truncal, and 1 extremity tumor. All patients completed therapy, adverse events were limited to grade I fever and injection site pain. All patients completed wide local excision and sentinel lymph node biopsy, there were no surgical complications. 3 patients demonstrated at least partial pathologic response (<50% viable tumor) with 1/5 patients demonstrating complete clearance of primary tumor. Only 1 of 5 patients harbored positive sentinel disease. Digital Spatial Profiling analysis is ongoing to identify immunologic and tumor markers associated with response in the primary tumor and draining sentinel node.

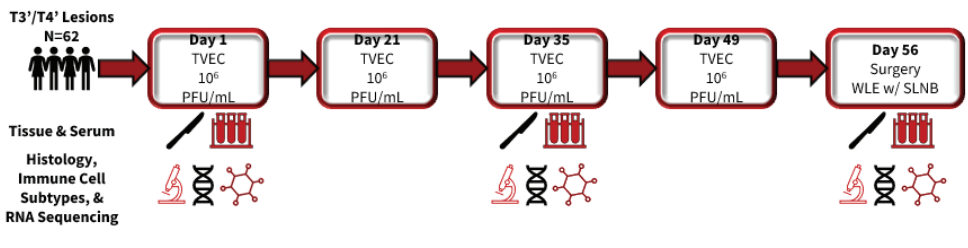
Conclusions
This study is the first to target earlier stage melanoma in the neoadjuvant setting with intratumoral immunotherapy using correlative endpoints to evaluate immune mechanisms of response and mechanistic dissection of the factors responsible for bystander effect in draining nodes. In this preliminary cohort, neoadjuvant TVEC was safe and well tolerated. We anticipate neodjuvant intralesional therapy will present a viable strategy to significantly improve outcomes in this high risk population.

Trial Registration
NCT04427306

Ethics Approval
This study was approved by Stanford University Institutional Review Board; approval number 68118.

Consent
Informed consent was obtained as part of trial admission and surgery.

Figure 1. Study Scheme



2

CD47 blockade as a targeted immune oncology agent in head and neck squamous cell carcinoma model

Riyam Al Msari, BS[1*], Robert Saddawi-Konefka, MD PhD[1], Joseph Califano, MD[1]

[1]*Moores Cancer Center, La Jolla, CA, United States*

Background
Myeloid antigen presenting cells (APCs) are critical to PD1i and radiotherapy responses in Head and Neck Squamous Cell Carcinoma (HNSCC), but are inhibited by the "don't eat me" immune checkpoint molecule CD47 overexpressed on tumor cells that interacts with SIRPα receptor found on myeloid cells, impeding their immune function. Evorpacept is a high affinity CD47 blocking fusion protein with a SIRPα binding domain coupled to an inactive Fc domain that avoids anemia and hematologic toxicities. We hypothesize that Evorpacept maximizes PD1i and tumor-targeted stereotactic body radiation therapy (SBRT) responses in HNSCC by expansion of tumor-antigen specifc cytotoxic T cells and enhancing antigen presentation by APCs.
Methods
To explore this, we employed our tobacco-signature, orthotopic murine oral squamous cell carcinoma (OSCC) models , one of which matches the immune infiltrate and PD1i response of human OSCC and the other of which is immune-cold with no response to PD1i. We also used Selective CRISPR Antigen Removal (SCAR) lentiviral vector system to knock out CD47 from the model and eliminate the remaining immunogenic cas9 editing machinery. Using these models, we investigated the synergistic effects and immune mechanism of Evorpacept on PD1i and tumor-targeted SBRT responses.
Results
First, we find that CD47 knock out from our OSCC preclinical model leads to complete tumor regression, emphasizing CD47 as an immune oncology target in HNSCC. Then, we show that Evorpacept potentiates both PD1i and tumor-targeted SBRT responses and leads to durable immunity in our immune-responsive and immune-cold OSCC preclinical model. Using IRDye-tagged Tilmanocept to map the tumor draining lymph nodes (tdLNs), we find that Evorpacept increases cytotoxic tumor-antigen specific T cells populations in the tdLNs and the tumor microenvironment (TME) with PD1i or tumor-targeted SBRT administration. Lastly, within the tdLNs, we determine increased tumor-specific antigen presentation by professional APCs, including macrophages, dendridic cells, and B-cells.
Conclusions
We demonstrate that Evorpacept synergizes with PD1i and tumor- targeted SBRT and enhances anti-tumor immunity by promoting APC tumor-specific antigen presentation from the TME to the tdLNS to prime and expand tumor-specific cytotoxic T cell populations. This work highlights CD47 as a bridge between innate and adaptive antitumor immunity in HNSCC.

Figure 1. Evorpacept and PD1i Synergy

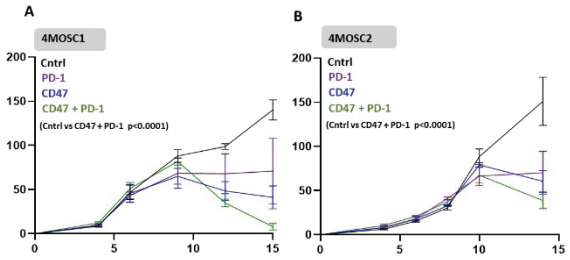


Figure 1. Evorpacept potentiates PD1i response in our immune-responsive (A) and immune-cold (B) OSCC preclinical model.

Abstracts

Figure 2. SCARless CRISPR 4MOSC1 CD47 Knockout

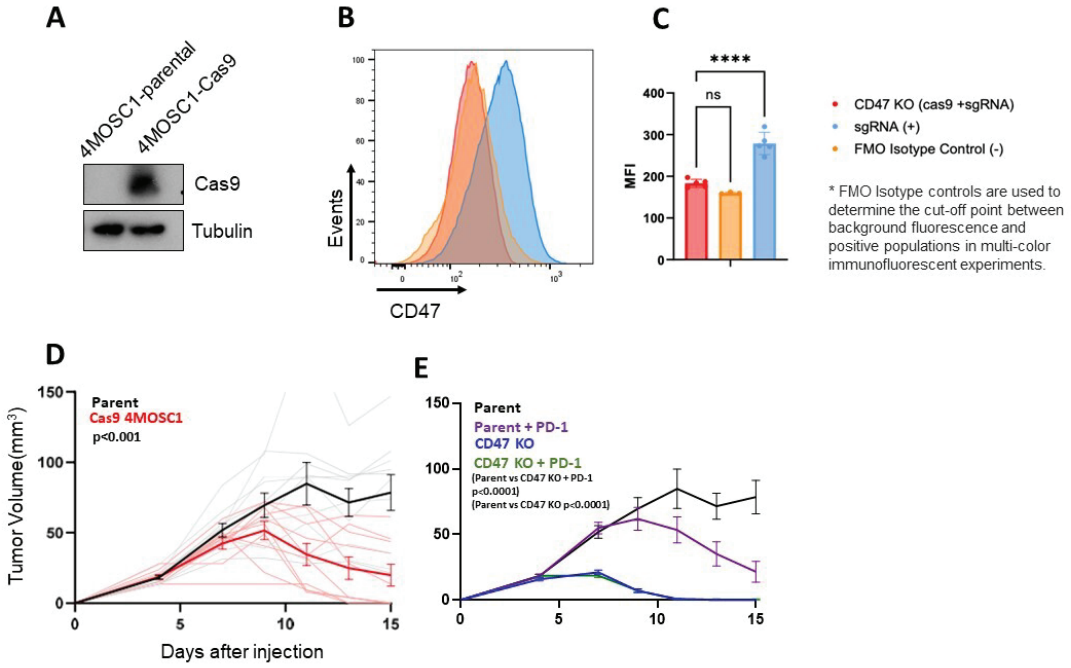


Figure 2. A) Western blot verifying Cas9 expression B) & C) Flow cytomerty analysis verifying CD47 Knockout. D) Tumor Volume Kinetics demonstrating the immunogenicity of Cas9 in 4MOSC1 model in vivo. E) CD47 knock out leads to complete tumor regression.

Figure 3. Tumor-targeted SBRT Increases Antigen Presentation

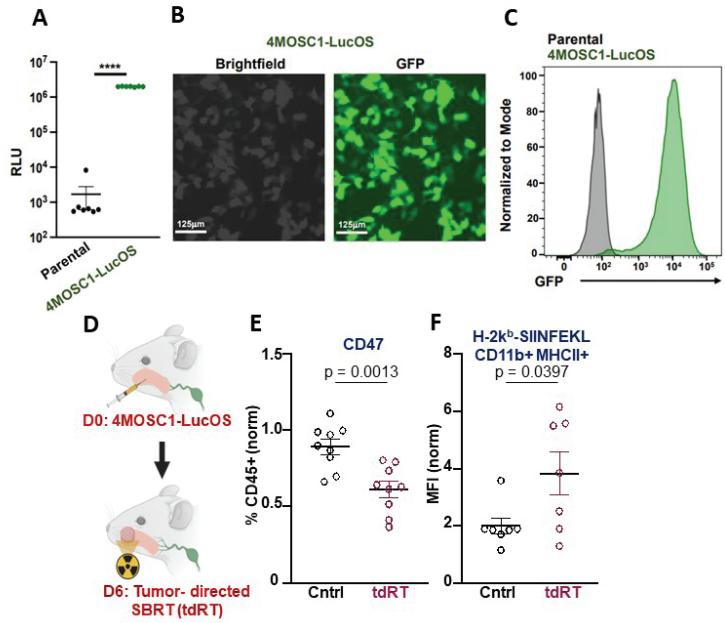


Figure 3. (A-C) Expression of GFP in the novel 4MOSC1 pLenti-GFP-LucOS model, as indicated by quantified RLU (A; n = 7 independent samples/group), representative images (B), and flow cytometry (C). Tumor-directed Stereotactic Body Radiation Therapy (tdRT) (D) leads to decreased tumor CD47 expression (E) and increased antigen presentation by activated myeloid cells (F).

Figure 4. Evorpacept Mechanism of Action

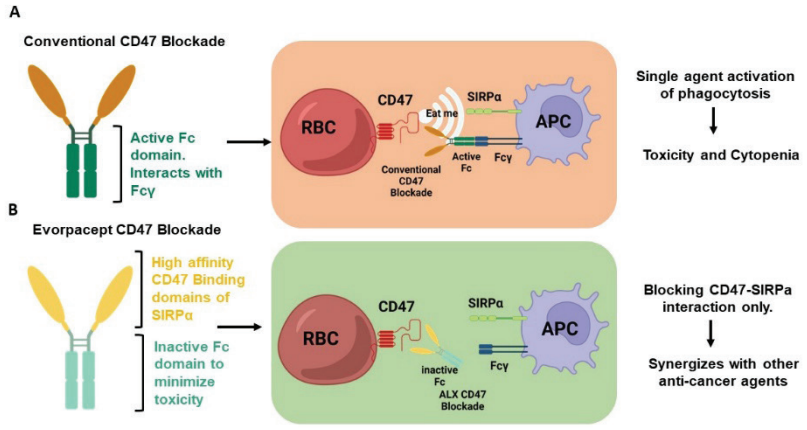


Figure 4. Conventional CD47 Blockade Mechanism of Action (A) vs Evorpacept (B).

Figure 5. Lymphatics Mapping

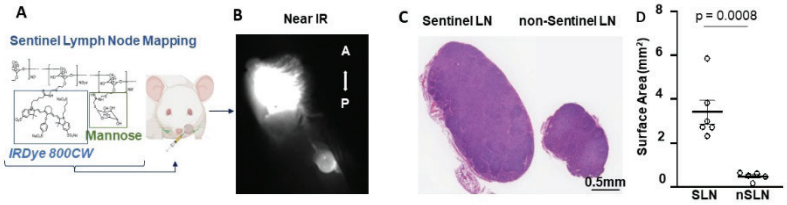


Figure 5. A) Tilmanocept chemical structure. Mannose substrate binds to CD206, a receptor expressed on the surface of reticuloendothelial cells such as macrophages and immature dendritic cells found in lymphatics. B) IRDye 800cw in Tilmanocept is detected under Near IR. Size comparison of sentinel and non sentinel lymph node C)& D).

3

Cytokine levels with immune checkpoint inhibitors: does obesity matter?

Stephanie Alden, MD[1*], Howard Li, BA[1], Aanika Balaji, MD[1], Yasser Ged, MBBS[1], Jeannie Hoffman-Censits, MD[1], Evan Lipson, MD[1], Tanguy Lim-Seiwert, MD[1], Marina Baretti, MD[1], Burles Johnson, MD, PhD[1], Jarushka Naidoo, MD[1], Mark Yarchoan, MD[1], Chester Kao, MD[1]

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Background
Multiple studies suggest that patients with obesity have improved outcomes when treated with immune checkpoint inhibitors (ICIs) as compared to those with normal body mass index (BMI), although the mechanism is poorly understood [1-3]. Differential serum cytokines have been observed in obese patients, but changes after ICI have not been fully elucidated. In this study, we assessed whether there are on treatment cytokine differences between obese and non-obese patients after ICI treatment.

Methods
We conducted a pan-tumor single-center, prospective study in patients treated with ICIs as standard of care. All patients consented to provide blood/tissue samples at baseline and up to 12 months after treatment initiation. Cytokine concentration (pg/mL) was determined using Luminex multiplex panels containing 39 different cytokines of clinical interest based on prior studies. Clinical outcomes assessed included progression free survival (PFS) and overall survival (OS). We defined obesity as BMI greater than or equal to 30 kg/m^2. Wilcoxon ranked sum test was performed to compare log2 transformed concentration of cytokines at baseline and on treatment. Cox proportional hazard ratios shown are adjusted for age and cancer type.

Results
We identified 70 patients, of whom 22 (31.4%) were obese. Among 39 cytokines evaluated, we found significantly differences in the levels of IL-6, IL-8, IL-15, MIG, and MIP-1B ($p < 0.05$) while on treatment in obese as compared to overweight or normal BMI patients. With respect to clinical outcomes, when comparing obese to non-obese patients, there was a significant difference in PFS (median (95% CI): not reached (10.7-not reached) vs. 4.1 (2.3-not reached), p = 0.004; HR: 0.33 (95% CI: 0.14-0.78, p = 0.01) (**Figure 1A**), with a trend towards significance in overall survival (OS) (not reached (not reached-not reached) vs. not reached (10.8-not reached), p = 0.08; HR: 0.29 (0.08-1.04), p = 0.06) (**Figure 1B)**.

Conclusions
In our cohort, patients with obesity had significantly improved PFS, with a trend towards improvement in OS. While on treatment, all cytokines that differed significantly promoted inflammation and/or T- and B-lymphocyte differentiation and were suppressed in obese patients, suggesting a potential mechanism for improved clinical benefit of ICI in obesity. In future analyses with larger patient cohorts, we hope to further elucidate cytokine changes and how they relate to treatment response and adverse events.

Acknowledgements
This study was funded by Genentech.

References
1. Cortellini A, Bersanelli M, Buti S, et al. A multicenter study of body mass index in cancer patients treated with anti-PD-1/PD-L1 immune checkpoint inhibitors: when overweight becomes favorable. *j immunotherapy cancer*. 2019;7(1):57. doi:10.1186/s40425-019-0527-y
2. Kichenadasse G, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Association Between Body Mass Index and Overall Survival With Immune Checkpoint Inhibitor Therapy for Advanced Non–Small Cell Lung Cancer. *JAMA Oncol*. 2020;6(4):512. doi:10.1001/jamaoncol.2019.5241
3. Yoo SK, Chowell D, Valero C, Morris LGT, Chan TA. Outcomes Among Patients With or Without Obesity and With Cancer Following Treatment With Immune Checkpoint Blockade. *JAMA Netw Open*. 2022;5(2):e220448. doi:10.1001/jamanetworkopen.2022.0448

Ethics Approval
This study was approved by the Johns Hopkins School of Medicine Institutional Review Board; approval number IRB00267960.

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.

Figure 1. Survival Characteristics based on Obesity

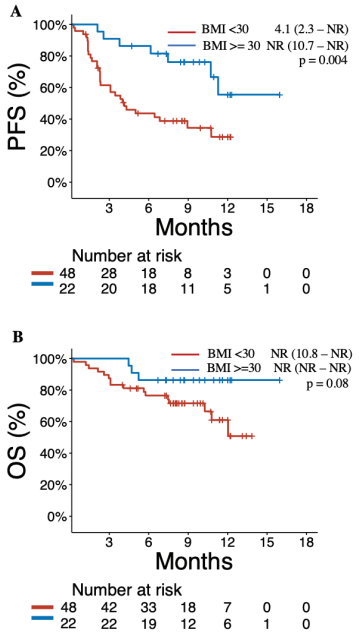

Figure 1. (A) Progression free survival (PFS) and (B) overall survival (OS) for the overall cohort, shown in months, comparing obese and non-obese patients.

5

Lung cancer-associated myeloid cells dictate the immunologic response to oncolytic adenovirus.

Svetlana Atasheva, PhD[1*], Jia Yao, PhD[1], Dmitry Shayakhmetov, PhD[1]

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Background
One of the appeals of oncolytic virotherapy is the capacity of viruses to change the tumor microenvironment, frequently interpreted as the de-polarization of tumor-associated myeloid cells from highly pro-tumorigenic to less pro-tumorigenic phenotypes. This study aimed to analyze how therapy with oncolytic adenovirus AVID-317 modulates the complexity, phenotypes, and pro-tumorigenic function of tumor-associated myeloid cells.
Methods
We utilized single-cell RNAseq approaches and extensive complementary cytokine measurements in ex vivo cultures of primary human lung adenocarcinoma and a mouse model of orthotopic metastatic lung cancer. To explore the phenotypic and functional states of distinct myeloid cell populations before and after virotherapy, we performed large-scale correlation analysis to reveal cytokines and myeloid cells functional markers that play central roles in the tumor microenvironment after systemic virotherapy.
Results
Our analyses showed that myeloid cells in lung tumors consist of complex cluster populations of macrophages, dendritic cells, and granulocytic cells in different polarization states. Oncolytic virotherapy led to increased expression of MCHII, CD86, CD80, and IDO1 on myeloid cells indicating activation of these populations. However, virus-mediated activation resulted in increased expression not only of type I IFN signature genes and pro-inflammatory cytokines, but also potentiated expression of pro-tumorigenic genes and cytokines, such as PDGFB, IL4I1, cathepsins, Lif, and IL-5. This simultaneous activation of M1 and M2 functional states of myeloid cells does not support the universal assumption of de-polarization of tumor-associated myeloid cells to lesser pro-tumorigenic phenotypes in response to oncolytic virotherapy.
Conclusions
The clinical success of oncolytic virotherapy will require careful consideration of the tumor-associated myeloid cells activation in response to virotherapy and finding effective drug combinations to suppress the pro-tumorigenic function of myeloid cells prior to administration of viruses with potent anti-tumor activity.

6

Development of canine CAR-T cells targeting the disialoganglioside GD2

Samuel Brill, BS[1*], Michael Yarnell, BS[2], Lillie Leach, BS[2], Terry Fry, MD[2], Douglas Thamm, VMD[1]

[1]*Colorado State University, Fort Collins, CO, United States*
[2]*University of Colorado Anschutz Medical, Aurora, CO, United States*

Background
Chimeric antigen receptor (CAR)-T cell therapy is a form of immunotherapy which has demonstrated remarkable efficacy in blood cancers, boasting up to 90% remission rates in some forms of lymphoma. Despite success in blood cancers, CAR-T cell efficacy in solid tumors is lacking. There are myriad forces driving the immune-tumor microenvironment that lead to CAR-T cell dysfunction in the context of solid tumors. While mouse models have been integral to CAR-T cell development to date, modeling the complex tumor-immune dynamics scales poorly in mice. Dogs provide a natural animal model to evaluate cell-based immunotherapy owing to their similar immune systems, natural history of cancer, and clinical care.
Methods
We have developed a system for generating canine CAR-T cells as a first step to develop a canine model for CAR-T cell therapy. GD2 expression on canine tumor cell lines was assessed via flow cytometry. CAR-T cell immunophenotype and transduction efficiency was evaluated with flow cytometry. CAR-T functional assays included luciferase activity, Incucyte live cell videomicroscopy, and ELISA-based cytokine evaluation.
Results
We demonstrate that the tumor associated antigen, disialoganglioside GD2, is expressed on canine osteosarcoma and melanoma cell lines. We have optimized culture conditions for T cell proliferation and activation, and demonstrated efficient CAR transduction using a gamma-retroviral vector. Finally, we demonstrate that primary canine T cells transduced with a GD2-targeting CAR can specifically lyse GD2+ tumor, while sparing GD2- cells.
Conclusions
Together, these data establish a platform for designing and evaluating canine derived CAR-T cells, demonstrate that GD2 is a targetable antigen on canine osteosarcoma and melanoma, and that canine CAR-T cells can specifically target GD2+ tumor cells.

8

Stem like CD4 T cell differentiation controls the magnitude of the CD8 T cell effector response in cancer

Maria Cardenas, PhD student[1*], Nataliya Prokhnevska, BS[1], Caroline Jansen, PhD[1], Ewelina Sobierajska, BS[1], Viraj Master, MD, PhD[1], Haydn Kissick, PhD[1]

[1]*Emory University, Atlanta, GA, United States*

Background
CD4 T cells can differentiate into multiple effector subsets that can mediate variable functions. In this work we wanted to understand how CD4 T cell differentiation shapes the CD8 T cell response in cancer.
Methods
Tumor tissue was collected from patients undergoing surgery at Emory University Hospital and processed for flow cytometry and ex vivo functional analyses. For in vivo studies, prostate cancer mouse model expressing the LCMV glycoprotein (TRAMPC1-GP) was used, as well as LCMV Armstrong infection. FOXP3-DTR (DEREG) mice were used for in vivo Treg depletion experiments. Additionally, we generated Ifngr1 KO P14 T cells using an HSC BM chimera system. Donor HSCs from CAS9 x P14 mice were expanded and infected with a lentivirus carrying a Ifngr1 sgRNA to generate P14 Ifngr1 KO mice. P14 Ifngr1 KO or WT cells were then transferred into TRAMPC1-GP bearing FOXP3-DTR mice for functional experiments.
Results
We identified a population of tumor specific CD4 T cells that maintain an activated, undifferentiated phenotype throughout the cancer response. These CD4 T cells were the dominant population within tumor draining lymph nodes (TDLNs), characterized by high expression of TCF1 and little to no expression of other lineage defining transcription factors. Tumor specific TCF1+ CD4s exhibited functional stem-like properties, as they self-renewed and differentiated almost exclusively into Tregs in tumor bearing mice. These cells, however, were not limited to Treg differentiation, as sorted TCF1+ CD4s from a 5-week tumor response expanded and gave rise to TH1 and TFH effectors in response to LCMV Armstrong infection. Interestingly, we also found a similar population of activated PD1+ TCF1+ CD4s with stem-like capabilities infiltrating various human tumors, indicating this phenotype of CD4 T cells is comparable in human cancer and mouse models. Importantly, the choice of lineage differentiation of TCF1+ CD4 T cells regulated the quality of the CD8 T cell anti-tumor response. When Tregs were selectively depleted, TCF1+ CD4 T cells differentiated into TH1s, which enhanced tumor specific CD8 T cell effector differentiation in both tumor and TDLNs, resulting in reduced tumor burden. Using a bone marrow chimeric CRISPR-Cas9 approach, we found that IFNg from TH1s was intrinsically required for CD8 stem to effector differentiation within TDLNs, promoting an improved anti-tumor response.
Conclusions
Overall, this work shows that CD4 differentiation dictates tolerance or immunity in cancer by regulating the phenotype, magnitude and location of the CD8 T cell effector response.
Ethics Approval
Patients were recruited in accordance with an approved IRB protocol, and all patients provided informed consent. All mice were used in accordance with National Institutes of Health and the Emory University Institutional Animal Care and Use Committee guidelines

9

A single dose of prophylactic perioperative cefazolin is associated with changes in gut microbiome composition and diversity in patients undergoing surgery for melanoma

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Background
Antibiotics profoundly impact the gut microbiome and prior antibiotic treatment in cancer patients has been associated with worse response to immunotherapy (ICB) and overall survival. Oral, broad-spectrum antibiotics have been shown to induce gut dysbiosis, diminishing diversity and altering taxonomic composition. Antibiotics are routinely administered prior to surgery although for many low-risk operations, including many procedures for early-stage melanoma, surgical-site infections are uncommon. The long-term impact of a single dose of intravenous antibiotic on the microbiome is unknown. In this randomized trial, we aimed to assess the impact of perioperative, prophylactic antibiotics on gut microbiome diversity and composition.

Methods
Patients with clinical stage I/II melanoma undergoing wide excision +/- sentinel lymph node biopsy were randomized to receive preoperative cefazolin or no antibiotic. Stool samples were collected before surgery and on postoperative days (POD) 3, 14, and 90 for 16S rRNA sequencing (Figure 1).

Results
22 patients were enrolled and randomized to receive preoperative cefazolin (ABX: n=11) or no cefazolin (No ABX: n=11). Age, sex, BMI, and clinical AJCC 8th edition staging at diagnosis were similar between arms. Analysis of 16S data revealed no difference in alpha- or beta-diversity between groups at baseline (Figure 2A-B). A trend towards decreased alpha-diversity was observed in the ABX group at POD90 compared to the No ABX group (Figure 2C). Over time, no significant shifts in beta-diversity compared to baseline were identified by principal coordinates analysis or weighted UniFrac distance in either cohort. Several compositional changes occurred over time in the ABX compared to the No ABX group. Notably, reduced differential abundance of *Akkermansia* at POD14 and POD90 was identified in the ABX group relative to the No ABX group (Figure 2D-E).

Conclusions
Preliminary results from this exploratory pilot trial suggest that prophylactic, perioperative cefazolin may negatively impact the diversity and composition of the gut microbiome in patients undergoing surgery for early-stage melanoma. Alpha-diversity, which has previously been described as a predictor of ICB response, was altered through POD90 following cefazolin treatment. Furthermore, abundance of *Akkermansia*, which has also been linked to ICB response in renal cell and non-small cell lung cancer, was diminished at POD14 and POD90 after cefazolin treatment. As even subtle shifts in taxonomic communities can result in significant functional repercussions in anti-tumor immunity and host health, future work will include whole metagenomic sequencing in order to further elucidate structural and functional shifts occurring in the gut microbiome of study participants.

Trial Registration
NCT04875728

Ethics Approval
This study was approved by the Institutional Review Board of the University of Texas MD Anderson Cancer Center, per protocol 2020-0265. All participants provided written, informed consent prior to taking part in this study.

Abstracts

Figure 1. Study schematic

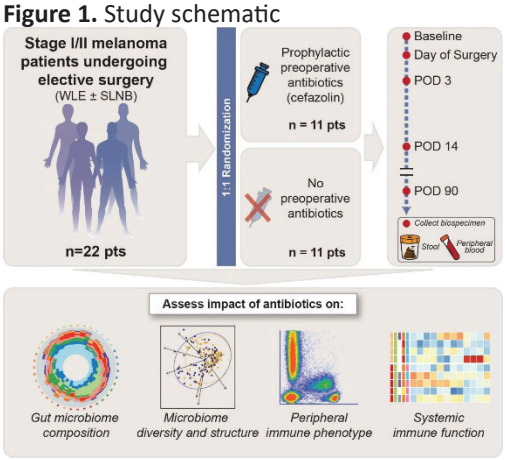
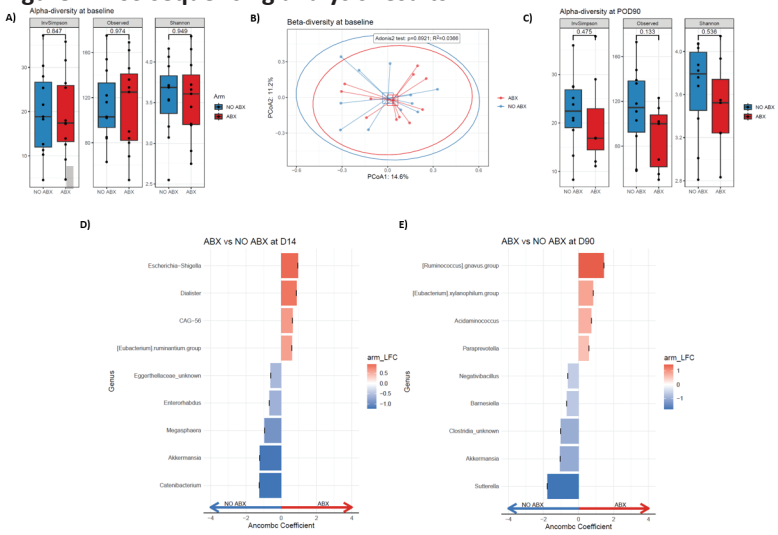


Figure 2. 16S sequencing analysis results



10

Clinical trial in progress: association between gut microbiome and dietary determinants of vaccine response

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Background
Response to immune checkpoint blockade (ICB) has been associated with gut microbiome diversity and abundance of specific fiber-fermenting bacteria (*e.g.* Ruminococcaceae family).[1] Diet is a known modulator of gut microbiome structure and function, and high-fiber intake has been associated with improved progression-free survival in melanoma patients treated with ICB.[2] The microbiome's impact on immunity may be important in healthy populations as well, as recent studies have described impaired influenza vaccination response secondary to antibiotic-induced microbiome perturbation.[3] Given the microbiota's role in shaping immunity, dietary intervention trials have emerged to manipulate the microbiome and elucidate immunomodulatory mechanisms. Therefore, in this two-part dietary study, we aimed to: 1) explore associations of gut microbiome profiles with vaccine response, 2) identify dietary determinants of the microbiome that can be modulated to enhance vaccine response, 3) evaluate effects of high-fiber, dietary intervention strategies on vaccine response, systemic immunity, and the gut microbiome.

Methods
In part one, volunteer employees undergoing seasonal influenza vaccination at our institution are enrolled into an observational cohort. Participants complete comprehensive dietary surveys, stool samples for 16S rRNA gene sequencing, and blood samples for post-vaccination hemagglutination-inhibition antibody (HAI) titers and SARS-CoV2 antibodies, to evaluate dietary and microbiome associations with vaccine seroconversion.
In part two, volunteer employees were enrolled into an interventional cohort and randomized to a 6-week dietary intervention: 1) isocaloric, high-fiber diet (HFD) provided as a full-feed or 2) prebiotic food enriched diet (PreFED) provided as weekly packouts. Blood and stool specimens are collected at baseline and post-vaccination (Figure 1).

Results
Overall, 55 participants enrolled into the observational study and 12 participants enrolled into the interventional study (HFD, n=5; PreFED, n=7). 16S-based fecal microbiome profiling is available for all baseline stool samples (n=67), thus, preliminary analyses focused on associations between baseline dietary factors and microbiome metrics. At baseline, median estimated fiber intake among all subjects was 16.5 g/d. Participants in the highest tertile for fiber intake demonstrated microbiomes with a trend towards higher alpha-diversity compared to lower tertiles (Figure 2A), though beta-diversity was similar between tertiles (Figure 2B). Further evaluation of diet-induced microbiome alteration and the effects on HAI titers is underway and will be reported at the conference.

Conclusions
Here, we describe a novel dietary intervention among healthy participants undergoing seasonal influenza vaccination. Work is underway to interrogate the influence of diet-induced microbiome alteration on systemic immune profile and seroconversion rates.

Trial Registration
NCT05239403

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Ethics Approval
This study was approved by the Institutional Review Board of the University of Texas MD Anderson Cancer Center, per protocols 2020-1054 and 2022-0450. All participants provided written, informed consent prior to taking part in this study.

Abstracts

Figure 1. Study schematic

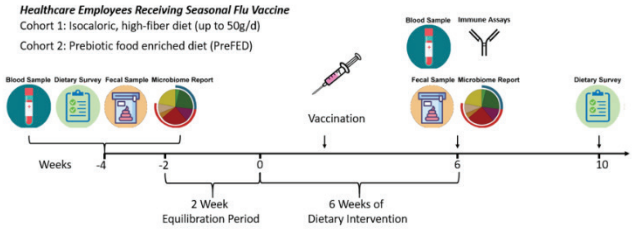
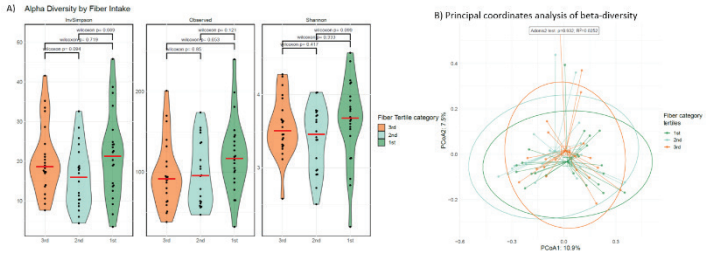


Figure 2. 16S sequencing data for all baseline stool samples



11

Bacteria specific IL-10 secreting T-cells derived from the gut are cross-reactive with tumor antigens and accelerate tumor growth in mouse models.

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Background
We developed a method of CD4 epitope identification that includes selecting Class II interacting sequences via a multi-algorithm followed by functional phenotyping. We have evaluated 152 epitopes from 17 non-mutated tumor antigens (TA) and demonstrated Class II restricted epitopes could be identified which elicit either a selective Type I (IFN-gamma) or Type II (IL-10) response across multiple human PBMC (n=40). IL-10 inducing TA epitopes often shared a >50% identity and cross reactivity with multiple gut bacterial species. We questioned how prevalent these bacteria-tumor antigen (BAC-TA) cross reactive T-cells were in humans and whether these cells had any effects in cancer.
Methods
IL-10 ELISPOT quantified BAC-TA T-cells in human PBMC, murine spleen, and tumor infiltrating lymphocytes (TIL). Human and murine T-cell lines were tested against bacteria, TA, and controls to show cross reactivity and specificity. Murine BAC-TA T-cells were used for adoptive transfer. T-cells were characterized by cytokine array, PCR, and flow cytometry. The C3(1)-Tag transgenic model of mammary cancer was used to assess effects of BAC-TA T-cells on tumor growth.
Results
Measurable BAC-TA T-cells occurred in up to 90% of PBMC. TA epitopes with the highest incidence of response shared significant sequence homologies with greater than 20 bacterial species. TA specific T-cell lines from multiple donors showed significant reactivity to homologous bacteria and recombinant TA protein, but not unrelated bacteria and protein. Human BAC-TA T-cells secreted IL-6 and IL-10, were memory T-cells, and expressed genes similar to intestinal intraepithelial lymphocytes. Similar BAC-TA T-cells were identified in mice. P. aeruginosa-specific T-cells generated from FVB mice secreted significantly more IL-10 when stimulated with the 70% homologous peptide YB1-p82-96 as compared to an irrelevant epitope ($p=0.0004$). We implanted a syngeneic tumor cell line into C3(1)-Tag. After tumor was established, fluorescently-labeled P. aeruginosa-YB1 specific T-cells were injected. Significantly increased florescence was seen in 100% of tumors and no fluorescence in mice injected with labeled naïve splenocytes ($p<0.0001$). Tumor volume 20 days after transfer was increased 55% in mice receiving P. aeruginosa/YB1-T-cells compared to splenocytes ($p<0.0001$). When C3(1)-Tag developed spontaneous tumors (500±75 mm^3) TIL analysis revealed numerous IL-10-secreting BAC-TA T-cells that had migrated from blood to tumor.
Conclusions
A select group of bacteria are associated with the majority of homologies driving generation of BAC-TA T-cells. We have also identified bacteria never associated with TA homologies. These data lay the foundation for precision probiotics designed to reduce the BAC-TA memory T-cell pool.

12

Metagenomic sequencing reveals unique gut microbial features associated with tertiary lymphoid structures in response to immune checkpoint blockade in solid cancers

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Background
Immune checkpoint blockade (ICB) therapy has significantly improved clinical outcomes; however, a significant proportion of patients fail to maintain durable responses [1]. Therefore, novel targetable biomarkers are needed. Our group has previously shown that the composition of the gut microbiome and presence of intratumoral tertiary lymphoid structures (TLS) can influence response to ICB [2-4]. Here, we study three randomized phase II neoadjuvant ICB trials [melanoma (MEL; NCT02519322; n=23), non-small-cell lung cancer (NSCLC; NCT03158129; n=31)[5] and sarcoma (SARC; NCT02301039; n=17)] to investigate the relationship and interplay between these two established determinants of response.
Methods
Patients were defined as responders (R) or non-responders (NR) based on major pathologic response (MPR; MEL and NSCLC-viable ≤ 10%; SARC-hyalinization ≥ 30%). Transcriptional profiles of tumor specimen collected pre-ICB (MEL and SARC) and post-ICB (MEL, SARC and NSCLC) were used to score and dichotomize patients (by median) based on a TLS gene signature (*LTA, LTB, TNFSF14, CXCL13, CCL18, CCL19, CCL21, CD79B, CR2, PAX5, MS4A1, MZB1*). Paired baseline stool samples were profiled via metagenomic sequencing to characterize the composition and the molecular and metabolic function using MetaPhlAn 3.0 and HUMAnN 3.0, respectively.
Results
There were 21 R overall (NSCLC n=9; MEL n=9; SARC n=3). TLS signature was significantly higher in R vs NR on-treatment with ICB (p=0.032; unpaired t-test). Longitudinal evaluation of transcriptional profiles showed increased expression of the TLS signature with treatment in R (p=0.032; paired t-test) but not in NR (p>0.99; paired t-test). Following patient dichotomization by TLS score, analysis of baseline gut microbiome profiles revealed a unified enrichment of specific taxa such as *Clostridium* and *Bacteroides* (ANCOM-BC; p<0.0001) in TLS^{high} patients. Further, imputed metabolic analysis of baseline gut metagenomes using MetaCyc revealed enhanced biosynthesis of L-Rhamnose (MetaCyc; q≤0.1) in TLS^{high} patients. Finally, network analysis of the baseline gut microbiome communities using SparCC identified *Ruminococcus torques* as a central hub species that characterized TLS^{high} patients.
Conclusions
Our data suggest that unique gut microbiome features are associated with TLS in the context of treatment with ICB in solid cancers. Future mechanistic and translational studies will seek to validate and parlay these novel findings into microbiome-based treatments to induce TLS and/or augment the efficacy of ICB in solid tumors.
Trial Registration
NCT02519322, NCT03158129, NCT02301039
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Ethics Approval
Patients were treated at the University of Texas MD Anderson Cancer Center and had tumor samples collected and analyzed under Institutional Review Board (IRB)-approved protocols (Melanoma: 2015-0041, 2012-0846, LAB00-063 and PA17-0261; NSCLC: 2016-0982; Sarcoma: 2017-0143)

13

Estrogen signaling via estrogen receptors alpha and beta impacts the function of Foxp3+ T-regulatory cells

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Background
While estrogen signaling is thought to play a role in nearly every cell type throughout the body, the biological significance of estrogen signaling via estrogen receptors within the immune system remains unclear.
Methods
We evaluated the immune effects of tamoxifen-induced deletion of estrogen receptor-a (ERa) and estrogen receptor-b (ERb) in addition to the conditional deletion of ERa and ERb from Foxp3+ T-regulatory (Treg) cells.
Results
In the absence of ERb, the ratios of T-effector (Teff) to Treg cells, and follicular Teff (Tfh) to follicular Treg (Tfreg) cells were drastically increased within the spleens ($p=0.0029$), superficial lymph nodes (sLNs) ($p=0.0049$ and $p<0.0001$, respectively), and mesenteric lymph nodes (mLNs) ($p=0.04$ and $p=0.0002$, respectively) of female mice. ERa KO mice also had decreased proportions of Tregs and Tfregs most prominently in the mLNs ($p<0.0001$ and $p=0.0014$, respectively).
Importantly, female CD4+CD25+ Tregs lacking ERa, ERb, or both ERa/b displayed an enhanced ability to suppress the proliferation of CD4+ ($p<0.05$, 0.001, and 0.05, respectively) and CD8+ ($p<0.05$, 0.001, and 0.01, respectively) T cells *in vitro*. Moreover, when C57BL/6 Rag-1-/- mice bearing BALB/c cardiac allografts underwent adoptive transfer with ERa or ERb KO Tregs at a 1:4 ratio with WT Teff cells, the allografts survived for more than 100 days ($p<0.01$ vs. acute rejection at <10 days using a corresponding ratio of WT Treg to Teff cells). Histopathology of cardiac allografts harvested at >100 days after adoptive transfer showed that the grafts from mice receiving ERb KO Tregs had undergone significant immune cell infiltration, focal necrosis, and vascular injury, whereas those receiving ERa KO Tregs showed only minor cellular infiltrates and well-preserved myocardium and vessels.
Conversely, male Tregs lacking ERa displayed reduced ability to suppress proliferation of CD4+ ($p<0.001$) T cells *in vitro*, and those lacking ERb showed no difference compared to WT male Tregs. Upon RNAseq analysis of FoxP3+ Tregs with ERa or ERb deletion in both females and males there were many notable differentially expressed genes. One of particular interest was SOSTDC1 (aka Sostdc1/ectodin/USAG1) which displayed an inverse relationship with Treg suppressive function.
Conclusions
These data show that estrogen signaling via ERa and ERb influences the function of T-regulatory cells. While studies are ongoing the current data suggest potential roles for estrogen receptor targeting to promote a tolerogenic immune environment suitable for prolongation of transplant survival, but also suggest that such targeting might favor the growth of tumors by promoting the suppression functions of Foxp3+ Treg cells.
Ethics Approval
Animal studies were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia (protocols 17-001047 and 19-000561).

14

Oncolytic vaccinia requires infection of tumor infiltrating lymphocytes for treatment efficacy

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Background

Oncolytic viruses can induce immune infiltrate by generating a tumor specific immune response. This is especially important for patients with immune desert or excluded tumors or no tumor specific T cells. However, the mechanisms of resistance to oncolytic virotherapy are not well understood and there has been minimal success in the clinic. To study resistance mechanisms to oncolytic therapy we developed a derivative of the head and neck cancer line MEER, which is resistant to αPD1 therapy but sensitive to oncolytic vaccinia (VV) termed MEER^{vvS}. While oncolytic viruses are engineered to preferentially infect and replicate in tumors, many viruses are capable of infecting other cell types as well. Using the MEER^{vvS} tumor model, we uncovered a novel role of the infection of lymphocytes by VV in the anti-tumor response.

Methods

For flow cytometric tumor infiltrating lymphocyte analysis, MEER^{vvS} tumors were implanted in mice and treated with a single intratumoral (IT) dose of VV, or PBS and harvested at 1- or 4-days post-treatment.

Results

Using a GFP reporter of VV replication, we observed infection of lymphocytes in MEER^{vvS} tumors at 1 and 4 days post-IT treatment. The highest percentage of infected cells was observed in regulatory T cells and exhausted CD8+ T cells. *In vitro* we confirmed that T cells were permissive to productive infection by VV. Using *in vitro* assays we showed that hypoxia was a main driver of infection of these cells which was then confirmed *in vivo*. Infection of T cells by VV lead to their eventual death *in vitro*. We also observed significant decreases in cell numbers of infected populations *in vivo* at 4 days post-treatment, suggesting death occurred after infection in the tumor as well. Using a mouse model where the anti-apoptotic protein bcl2 was overexpressed in all T cells, we tested the effects of sustaining T cell survival through VV infection. In these mice we found significantly decreased survival and increased tumor growth compared to control mice treated with VV.

Conclusions

Oncolytic vaccinia can infect lymphocytes in the tumor microenvironment and this infection may be a crucial part of its mechanism. We observed infection of suppressive T cell populations (regulatory T cells, exhausted T cells) early after treatment in a HNSCC mouse model. This infection leads to the death of these populations, which if prevented, results in worse survival.

16

Sensitizing quasi-mesenchymal breast tumors to immune checkpoint blockade therapy by targeting CD73

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Background

Although Immune checkpoint blockade (ICB) has generated dramatic responses in certain cancers, the response of breast tumors has been largely limited. We have previously demonstrated that the cellular plasticity of breast cancer cells, specifically, their residence in the epithelial or quasi-mesenchymal phenotypic states, can itself be an important determinant of responsiveness to ICB [1]. By establishing novel, pre-clinical models of epithelial and quasi-mesenchymal breast tumors, we have shown that epithelial tumors recruit $CD8^+$ T-cells to their tumors and are sensitive to anti-CTLA4. In sharp contrast, their quasi-mesenchymal counterparts assemble an immunosuppressive tumor microenvironment and are highly resistant to ICB [2]. To determine the mechanisms by which quasi-mesenchymal carcinoma cells resist elimination by ICB, we used multiple transcriptomic approaches and observed that these two cell types differ in the expression of cell-intrinsic immunomodulatory factors. Of these various factors, abrogation of quasi-mesenchymal cancer cell-derived CD73 resulted in recruitment of T-cells and dendritic cells to the tumor microenvironment and led to complete sensitization to anti-CTLA4 ICB [3]. While our previous studies have demonstrated that targeting CD73 can potentiate the efficacy of anti-CTLA4 in quasi-mesenchymal tumors, the underlying mechanism(s) of such sensitization remains elusive. Additionally, the regulatory networks that control the expression of CD73 itself in quasi-mesenchymal but not epithelial breast cancer cells are not well defined.

Methods

To determine the mechanisms of sensitization to ICB, we used novel, preclinical models of quasi-mesenchymal control tumors as well as those knocked out for CD73 in conjunction with CRISPR/Cas9 approaches, antibody based-depletion methods, and adoptive transfer of T-cells. To determine whether altering the phenotypic plasticity of cancer cells results in elevated levels of CD73, we activated the EMT program in human breast cancer cells by doxycycline induced expression of EMT- inducing transcription factors.

Results

Quasi-mesenchymal tumors lacking the expression of CD73 were infiltrated by both $CD8^+$ as well as $CD4^+$ T-cells. Both these cell types were functionally important for sensitization to anti-CTLA4 as adoptive transfer of either $CD8^+$ or $CD4^+$ T-cells from cured mice led to effective tumor regression in recipient tumor-bearing mice. Additionally, altering the cellular plasticity of cancer cells by activating the EMT program in human breast cancer cell lines led to elevated expression of CD73.

Conclusions

Taken together, our work suggests that the EMT program directly regulates the expression of immunosuppressive CD73. Importantly, targeting the adenosinergic signaling pathway in quasi-mesenchymal carcinoma cells can potentiate the efficacy of ICB in a $CD8^+$ and $CD4^+$ T-cell dependent manner.

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17

Potentiating immunotherapy and attenuating tumor cell growth through combination of MEK and HDAC inhibition in microsatellite stable (MSS) colorectal cancer (CRC)

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Background
Several studies have demonstrated that MEK inhibition (MEKi) augments tumor immunogenicity through drug-induced intrinsic tumor changes and the simultaneous enhancement of immune cell recognition of the tumor. Similarly, HDAC inhibition (HDACi) has been shown to increase tumor immunogenicity by altering cytokine production, reduce myeloid-derived suppressor cells (MDSCs) or Tregs (regulatory T cells) function, enhance cytotoxic T cell or NK cell activity, and increase expression of tumor associated neo-antigens. Taken together, these findings led to the hypothesis that MEKi in combination with HDACi and immunotherapy could increase immune recognition of tumors with low mutational burden. The aim of this project was to assess the effect of the triple combination of MEKi+HDACi+PD1 inhibitor through *in vivo* and *in vitro* assays.
Methods
The effects of the MEKi trametinib and the HDACi entinostat were assessed in mouse syngeneic CRC cell line models (MC38 and CT26). Treatment with each drug were administered separately with a maximum concentration of 10μM. Cellular proliferation was analyzed after 72 hours using Cell Titer Glo. Doses for the combination treatment were selected based on the single agent data collected. Moreover, to assess in vivo drug activity, MC38 or CT26 cell lines were implanted on the right flank of C57BL/6 or BALB/c mice and treatment was started once tumors reached a tumor volume of approximately 75mm^3. Tumors were measured three times a week, and immune organs and tumors were collected at the end of study. Immunogenic and cytokine markers were analyzed in both primary cell line culture and *in vivo* samples via flow cytometry.
Results
The CRC syngeneic cell lines tested demonstrated sensitivity to trametinib, with little response to single agent entinostat via Cell Titer Glo. No synergy was observed in the cell lines when testing the combination in vitro. However, *in vivo* analysis of the triple combination found significant response with total tumor regression in several mice, across multiple in vivo experiments performed. These results were supported by flow analysis.
Conclusions
Although entinostat and PD1 has been tested in early phase clinical trials, this combination has not been as of yet approved for clinical practice in MSS CRC patients. Our data supports the role of this triple combination as a novel and effective treatment approach for metastatic MSS CRC patients.

18

Targeting wild-type IDH1 in the tumor microenvironment as a novel therapeutic intervention in glioblastoma

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Background
Tumor associated macrophages (TAMs) play a key role in negating the therapeutic activity of anti-PD-1. The immunosuppressive phenotype of macrophages is in part mediated by the metabolic and epigenetic effects of alpha-ketoglutarate (a-KG), the key product of isocitrate dehydrogenase 1 (IDH1). IDH1 is a cytosolic enzyme that produces NADPH to regulate redox homeostasis and α-KG to support anaploresis. The role of IDH1 in glioblastoma TAMs has yet to be elucidated.
Methods
To study the effects of IDH1 within the tumor microenvironment (TME), wild-type (WT) and IDH1 knockout (IDH1.KO) murine models were developed (n=8-11 per group per experiment). Over time, orthotopically implanted tumor growth was measured via live animal imaging of luciferase expressing murine glioblastoma cells and overall survival was determined via Kaplan-Meier analysis. Tumor infiltrating immune cells were analyzed using single cell RNA sequencing (scRNA-seq) and high dimensionality flow cytometry. Mechanistic regulation of IDH1 on macrophage inflammation and cell survival was assessed ex vivo using cytokine assays, western blot, qRT-PCR, Incucyte live cell imaging, and Seahorse analysis, on bone marrow derived macrophages (BMDMs).
Results
Systemic loss of IDH1 resulted in significantly impaired tumor growth and increased overall survival compared to WT (39.5 vs 30.5 days). scRNA-seq and flow cytometry revealed increased inflammation in IDH1.KO, specifically in TAMs. In a myeloid specific IDH1 conditional knockout murine model, ablation of IDH1 increased overall survival compared to WT (59 vs 40 days). scRNA-seq revealed an inflamed TME, characterized by inflammatory macrophage gene signatures, and flow cytometry confirmed elevated inflammatory cytokine production (IFNy, TNFa, and CCL3) in TAMs. In vitro genetic ablation, or pharmacological inhibition of IDH1 using a wild-type specific small molecule inhibitor, resulted in decreased production of IL10 and Arg1, and increased TNFa and nitric oxide in immunosuppressive IL-4 treated BMDMs. IDH1 inhibition of BMDMs increased reactive oxygen species production and decreased mitochondrial activity *ex vivo*. Pharmacological or genetic inactivation of IDH1 improved PD-1 blockade outcomes in a murine glioblastoma model resulting in 70% long term survival compared to PD-1 blockade alone.
Conclusions
Loss of IDH1 activity enhances inflammation in the tumor microenvironment in glioblastoma TAMs due to reduced α-KG metabolite levels. As such, limited oxidative phosphorylation, decreased mitochondrial turnover, and accumulation of reactive oxygen species, limits epigenetic and metabolic support for pro-tumor polarization. Thus, macrophages are more glycolytic and inflammatory, resulting in a robust response to PD-1 blockade in vivo, overall suggesting metabolism is a key mediator in PD-1 resitance in glioblastoma.

20

Fecal microbial transplantation in combination with first-line immune checkpoint inhibition in patients with melanoma and lung cancer: interim analysis from a phase II clinical trial

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Background
The development of resistance to immune checkpoint inhibition (ICI) in patients with melanoma and non-small cell lung cancer (NSCLC) represents a major challenge. We previously reported results from a phase I trial demonstrating that fecal microbial transplantation (FMT) in combination with single-agent ICI was safe [1] while the capacity to reverse resistance to single-agent ICI was previously demonstrated [2,3]. We present interim results from the first phase II trial evaluating the combination of first-line ICI+FMT in patients with melanoma and NSCLC (NCT04951583).

Methods
FMT formulated in oral capsules from single-donor healthy volunteers was administered in combination with first-line ICI as part of a multicentric phase II open-label trial in 3 cohorts: (1) patients with advanced/unresectable melanoma treated with Ipilimumab+Nivolumab (Ipi/Nivo); (2) metastatic uveal melanoma treated with Ipi/Nivo; (3) advanced NSCLC with PD-L1 $\geq$50% treated with Pembrolizumab. The primary objective was objective response rate by RECIST 1.1 defined by the rate of complete response (CR) and partial response (PR). Avatar antibiotic-treated, specific pathogen-free (SPF) mice were transplanted with stool samples obtained from participants on the trial before and after FMT. Mice were subsequently implanted with MCA-205 tumors and received anti-PD-1+anti-CTLA-4 antibodies.

Results
18 patients were included. Median age was 61 (range; 41-71). 14 (77%) were male. 11 (61%) had cutaneous melanoma; 2 (11%) had uveal melanoma; and 5 (28%) had NSCLC. Out of 9 patients with evaluable disease in the cutaneous melanoma cohort, 7 (78%) experienced an objective response (6 PRs and 1 CR) and one non-responder patient had stable disease. Out of the 2 patients in the uveal melanoma cohort, 1 (50%) experienced a PR. Out of the 3 patients in the NSCLC cohort, 2 (67%) experienced PRs. FMT was associated with grade 1 abdominal pain/bloating in 5.6% of participants with no grade $\geq$3 adverse events. Grade 1 diarrhea related to FMT occurred in 11.1%. Grade 1-2 immune-related diarrhea was observed in 44.4% of patients while grade 3 immune-related diarrhea occurred in 22.2% of patients. In SPF mice recolonization with feces obtained post-FMT was associated with significantly improved tumor control with anti-PD-1+anti-CTLA-4 compared to pre-FMT sample, indicating that FMT contributed to the anti-tumor response observed in the patient.

Conclusions
This is the first report of FMT in combination with first-line dual ICI in melanoma, and the first report of a phase II trial of FMT+ICI in NSCLC. This study demonstrates encouraging clinical responses, supporting the study of FMT+ICI in the randomized setting.

Acknowledgements
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Trial Registration
NCT04951583

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Ethics Approval
All patients gave informed consent prior to participating in this study. This study was approved by the Insitutional Review Board of each participating center, ID number of multi-institutional approval: MP-02-2022-10121.

21

Antagonism of regulatory ISGs enhances the anti-melanoma efficacy of STING agonist ADU-S100

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Background
STimulator of INterferon Genes (STING) is a dsDNA sensor that triggers type I inflammatory responses. Recently this pathway has been manipulated in cancer treatment with the use of STING agonists. Our lab has demonstrated that treating mice bearing established tumors with STING agonists leads to an inhibition in tumor growth in association with tumor vascular normalization and immune cell recruitment within the tumor microenvironment. However, STING agonism also results in the upregulated expression of compensatory immunoregulatory interferon-stimulated genes (ISGs), including ARG2, PTGS2/COX2, NOS2, PD-L1, and ISG15, yielding an overall sub-optimal therapeutic paradigm. We hypothesized that combined treatment of STING agonists along with pharmacologic inhibitors of ARG2, PTGES/COX2, NOS, PD-L1, and ISG15 will result in improved control of tumor growth and more effective anti-tumor immune responses *in vivo* in murine melanoma models.
Methods
C57Bl/6J mice were implanted s.c. with either B16 ($BRAF^{WT}PTEN^{WT}$) or BPR ($BRAF^{V600E}PTEN^{-/-}$) melanoma cells and treated with i.) PBS, ii.) ADU-S100 (i.t.), iii.) ADU-S100 (i.t.) + ARGi/COX2i/NOSi (oral gavage), iv.) ADU-S100 (i.t.) + anti-PD-L1 (i.p.), or v.) ADU-S100 (i.t.) + anti-ISG15 (i.t.) and monitored for tumor growth and time to required euthanasia as an index of overall survival. Tumors were harvested at various timepoints for analysis by single cell RNAseq, flow cytometry and IFM.
Results
In the B16 ($BRAF^{WT}PTEN^{WT}$) melanoma model, we observed an improvement in tumor growth control over treatment with ADU-S100 alone when combining this modality with neutralizing/blocking anti-PD-L1 or anti-ISG15 antibodies, but not with ARGi/COX2i/NOS2i. Conversely, in the BPR ($BRAF^{V600E}PTEN^{-/-}$) melanoma model, improved tumor growth control over ADU-S00 treatment alone was only observed in combination therapy integrating ARGi/COX2i/NOSi. Underlying changes in the tumor immune microenvironment (TIME) associated with the improved efficacy of combination regimens in each tumor model are currently being evaluated to delineate underlying mechanisms of therapeutic action.
Conclusions
Our data suggest that regulatory ISG expression in the melanoma TIME limits the anti-tumor efficacy of STING agonism in different ways based on the specific melanoma model investigated. These results support antagonism of one or more regulatory ISGs to improve STING agonist-based treatment outcomes in the melanoma setting.

23

Redirecting in vitro glucose flux improves the in vivo anti-tumor function of cellular immunotherapies

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Background
While adoptive T cell therapies have shown impressive results in cancer therapy, persistence of cells remains a key feature of therapeutic efficacy. In all forms of cellular therapies, large numbers of cells are transferred to the patient, yet comparatively few can be detected within the body. We hypothesize this lack of survival and persistence is in part due to the hypermetabolic conditions, especially hyperglycemia, used to generate large numbers of T cells in vitro. Within this study, we aim to preserve a more in vivo-like metabolic state during T cell expansion so that upon transfer they may more easily re-enter the immune system and persist as a living drug.

Methods
T cells were activated in culture with cognate peptide or *in vivo* with cognate antigen. The pyruvate dehydrogenase kinase (PDHK1) inhibitor dichloroacetate (DCA) was used to redirect glucose flux in vitro. Mouse tumor experiments were performed with gp100-specific pmel-1 TCR-Tg T cells transferred into B16-F10 -bearing mice. Mouse co-transfer experiments were performed with pmel-1 T cells on a congenically mismatched background. Human CAR-T experiments were performed using anti-hCD19 CAR-T cells transferred into immunodeficient mice bearing hCD19-A549 lung cancer cells.

Results
Identical T cells stimulated in vitro or in vivo do not differ in effector function but differ heavily in their glycolytic metabolism. As direct inhibition of glycolysis severely hinders T cell expansion, the PDHK1 inhibitor DCA redirects glucose away from lactate production into mitochondrial metabolism, maintaining a robust expansion rate. Expansion under DCA improves cytokine production and promotes features of stemness. However, the most striking effect of expansion under DCA is evident after cells are transferred: improved immediate and long term survival of the transferred T cell product. These immediate changes in survival result in striking therapeutic efficacy, including increased tumor clearance, immunologic memory, and long-term cellular persistence in both mouse and human tumor models.

Conclusions
We demonstrate that by redirecting glucose into mitochondria using DCA, T cells experience vastly reduced metabolic stress during expansion. This relatively simple energetic shift in vitro drastically improves the immediate survival and engraftment of T cells after infusion, resulting in enhanced anti-tumor efficacy and long-term memory. Our study not only suggests the current manufacturing process for cell therapies, utilizing hypermetabolic media, may hinder their ultimate therapeutic success, but provides potential solutions to bring the promise of cellular therapies to additional patients.

25

Early insights into clinical trials through single-cell RNA sequencing from biopsy cores

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Background
Single-cell RNA-sequencing (scRNA-seq) yields valuable insights into the molecular heterogeneity of multiple cell types in normal and cancer tissues. scRNA-seq approach has the potential to not only answer basic research questions but also enhance clinical studies and clinical trials and accelerate drug development and testing. However, a consistent and reproducible method for isolating viable single cells and generating high-quality single-cell data from needle biopsy cores has been challenging to develop and apply to clinical samples. Based on our experience isolating single cells from a large number of surgical tumor tissues, we set out to optimize the dissociation and sample handling protocol to generate high-quality single-cell suspensions from needle biopsy cores of multiple human tissue types.

Methods
18 gauge needle biopsies were kept in RPMI medium on ice and transferred to EMPIRI. Samples were dissociated using mechanical and enzymatic methods to achieve a single-cell suspension. Cells were washed and viability and cell counts were verified before capture via a 10x Chromium controller. Captured cells were processed through the 10x Genomics Chromium Next GEM Single Cell workflow before sequencing on Illumina NovaSeq 6000 sequencer. Raw sequencing data were processed through EMPIRI's computational pipeline that includes QC steps, doublet removal, and Seurat and other downstream analyses.

Results
A total of 16 de-identified 18-gauge needle biopsy core samples from matched pre- and post-treatment patients were collected and analyzed. Samples were from the liver, lymph node, pelvis, abdominal, neck, and lung tissues and included matched pre- and post-treatment samples from eight patients. Time to capture ranged from 3 to 6.5 hours after biopsy collection. Recovery of viable cells from individual 18-gauge biopsy cores ranged from 2,000-300,000 cells per core, depending on the tissue type and cellularity.

Conclusions
scRNA-seq analysis can be incorporated into clinical trials or clinical studies to obtain rapid insights into on-target drug effects and anticipated cellular responses to therapies, including immunotherapies, within 3-4 weeks of biopsy collection. We developed a robust protocol for isolating high-quality single cells for scRNA-seq from tissue samples as small as a single 18-gauge needle core. We showed successful isolation of highly viable cells from the lung, lymph node, pelvis, abdominal, neck, and lung tissues. We were able to generate scRNA-seq data for downstream analysis from all pre- and post-treatment matched samples from 8 patients. Personalized immunotherapy response may be gleaned through scRNA-seq via evaluation of immune checkpoint inhibitor expression and shifts in immune population post-treatment.

26

Distinct gut microbe metabolites differentially impact proliferative responses in human T cells: Potential mechanism for checkpoint inhibitor response heterogeneity

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Background
Checkpoint inhibitor (CPI) therapies have improved cancer responses[1] but cause problematic toxicity[2], termed immune related adverse events (IrAEs), which are linked to cancer response[3]. Modulation of the gut microbiome *in vivo* has improved cancer responses[4]. Responders to CPIs have higher gut microbial diversity, with multiple bacterial species recognised to be beneficial or detrimental for cancer response and/or toxicity[5]. *Bacteroides Caccae* and *Escherichia Coli* have been linked to poorer cancer outcomes[5], with *Bifidobacterium Breve* by contrast linked to improved cancer outcomes and lower risk for CPI colitis[6,7]. To investigate potential mechanisms, we probed the impact of metabolites from these gut microbes on T cell responses *in vitro*.

Methods
We evaluated fresh, healthy human T cell proliferation (using a cell tracking dye) with activation markers via flow cytometry, and cytokine production via ELISA in response to anti-CD3/anti-CD28 polyclonal stimulation in the presence of supernatants from bacteria (*B.Breve, B.Caccae, E.Coli* or bacterial culture medium only as a negative control). Bacteria were cultured in identical conditions, in a novel human T cell medium optimised with amino acids, yeast extract and dextrose. Growth curves of bacterial growth were used to determine the amount of supernatant to add to the T cell assay, to ensure equivalency between species.

Results
Supernatants from both *B.Caccae* and *E.Coli* (linked to poorer cancer outcomes[5]) inhibited proliferation of stimulated CD4+ and CD8+ T cells by 60-80% relative to bacterial culture media negative control in each case **(figure 1)**. In contrast, supernatant from *B.Breve* (linked to improved cancer outcomes and lower risk for CPI colitis[6,7]) had no significant effect on proliferation. Results were statistically significant for *B.Caccae* and *E.Coli* ($P=<0.01$ in both T cell subsets). Results of cytokine ELISAs will also be presented.

Conclusions
This data demonstrates a relatively strong anti-proliferative T cell effect of supernatants from distinct bacteria associated with unfavourable cancer outcome, compared to minimal effect from bacteria associated with favourable outcome. This could account for an underlying mechanism by which differences in CPI response are dependent upon gut microbiota present in an individual.

We are also recruiting a cohort of patients (the MEDALLION study) for gastrointestinal and dermatological microbial monitoring to evaluate novel microbiota or stool metabolites linked to CPI response. Our findings will contribute to understanding of the role of microbiota in the development of immune pathology and suggest microbial taxa or functionally relevant metabolites as potential targets for improving outcomes in CPI recipients.

Acknowledgements
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Ethics Approval

This study was approved by NHS East of England Ethics Board; IRAS number 252992.

Figure 1. T cell Responses

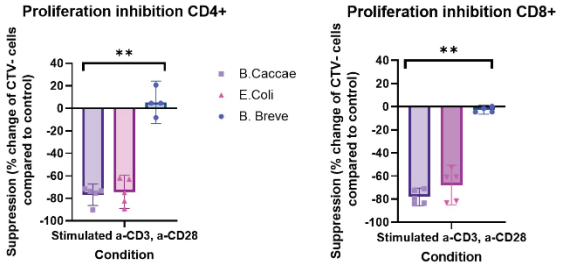

Figure 1. Proliferation inhibition demonstrated as suppression (percentage change of stimulated cell trace violet (CTV) negative cells) with addition of bacterial supernatants compared to bacterial culture medium negative control.

27

Pressure-enabled intravascular delivery of SD-101 into the liver with systemic or subcutaneous checkpoint inhibitor for control of liver metastases in a murine model

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Background
Myeloid-derived suppressive cells (MDSCs) blunt the activity of immunotherapy through the promotion of an immunosuppressive tumor microenvironment (TME) in the setting of liver metastases (LM). Ongoing clinical trials are evaluating intravenous checkpoint inhibitors (CPIs) in combination with the class C TLR9 agonist SD-101 via intravascular Pressure Enabled Drug Delivery™ (PEDD™) for multiple intrahepatic tumor indications (NCT04935229, NCT05220722). We delivered SD-101 in a murine model of PEDD in combination with an anti-PD-1 antibody administered either intraperitoneally (IP) or subcutaneously (SQ) to evaluate whether the route of CPI administration impacts the ability of intrahepatic TLR9 stimulation to control LM.

Methods
To develop LM, C57/BL6 mice were challenged with MC38-Luc tumor cells via the intra-splenic route followed by splenectomy. After a week (D0), mice were treated with 10µg SD-101 via PEDD and twice weekly anti-PD-1 antibody delivered either IP or SQ. Tumor burden was monitored by IVIS and on D10 liver was harvested to isolate $CD45^+$ cells. Flow cytometry (FC) analysis was performed to quantify MDSCs ($CD11b^+Gr1^+$), B cells ($B220^+$), T ($CD3^+$) cells, M1 ($F4/80^+CD38^+Egr2^-$), and M2 ($F4/80^+CD38^-Egr2^+$) macrophages in the TME.

Results
SD-101 delivered via PEDD in combination with anti-PD-1 antibody delivered either SQ or IP significantly reduced LM progression (expressed as fold over D0 tumor burden) compared to control (Veh: 87.46 vs. SD-101: 13.90 vs. SQ: 0.002 vs. IP: 0.04; $p< 0.0001$). FC analysis of $CD45^+$ cells isolated from the tumor-bearing livers revealed that CPI in combination with SD-101 significantly reduced liver MDSCs (Veh: 37.57% vs. SQ: 7.18% vs. IP: 10.18%; $p<0.05$). The percentage of B cells (Veh: 8.32% vs. SQ: 18.09% vs. IP: 15.65%; $p<0.05$); T cells (Veh: 7.14% vs. SD-101: 15.18% vs. SQ: 17.81% vs. IP: 19.13%; $p<0.05$) and the ratio of M1/M2 macrophages (Veh: 2.25 vs. SD-101: 12.8 vs. SQ: 12.99 vs. IP: 12.90; $p<0.05$) increased significantly as compared to Veh. There were no significant differences between SQ and IP delivery of CPI in controlling tumor progression or modulation of the TME.

Conclusions
SD-101 administered via PEDD in combination with CPI that was delivered IP or SQ provided control of LM with intrahepatic TLR9 stimulation enabling CPI via either route equally. PEDD mediated delivery of a class C TLR9 agonist has the potential to prime the TME to reduce immunosuppression in LM which may improve the anti-tumor efficacy of CPIs irrespective of the route of administration.

30

PD-1 combination therapy with IL-2 modifies CD8+ T cell exhaustion program

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Background
Enhancing the functions in exhausted CD8 T cells by blocking programmed cell death 1 (PD)-1 pathway now holds a great promise to treat cancer and chronic infections. There are a lot of interests in combining other immunomodulatory agents with PD-1 therapy to improve its efficacy and defining the underlying basis of effective PD-1 combination therapy is needed.
Methods
We examined how IL-2 synergizes with PD-1 immunotherapy during chronic lymphocytic choriomeningitis virus (LCMV) infection. Following questions were addressed.
1. Does depleting CD8+ T cells abrogate the efficacy of PD-1 + IL-2 combination therapy?
2. Is specific subpopulation in exhausted CD8+ T cells respond to PD-1 + IL-2 combination therapy? Adoptive transfer experiments were performed, where stem-like (PD-1+CXCR5+TIM3-) and exhausted (PD-1+CXCR5-TIM3+) CD8+ T cells isolated from LCMV chronically infected mice were transferred into infection matched mice, followed by the treatments.
3. Are LCMV-specific CD8+ T cells expanded after combination therapy transcriptionally and epigenetically different from those after PD-1 monotherapy? RNA-sequencing (RNA-seq), single cell RNA-seq (scRAN-seq), and the assay for transposase-accessible chromatin with sequencing (ATAC-seq) was performed for sorted LCMV-specific CD8+ T cells.
4. Is CD25 engagement with IL-2 has a role in synergy between PD-1 and IL-2? Anti-CD25 antibody was used to block IL-2 binding to CD25, or a mutated version of IL-2 (IL-2v) that does not bind to CD25 but still binds to CD122 and CD132 was administered.
Results
1. Depleting CD8+ T cells during PD-1 + IL-2 combination therapy almost completely abrogated the antiviral effect.
2. The response to PD-1 + IL-2 combination therapy came from the PD-1+ stem-like CD8+ T cells as seen to the response to PD-1 blockade alone.
3. PD-1 + IL-2 combination therapy, in contrast to PD-1 monotherapy, substantially changed the differentiation program of the PD-1+TCF1+ stem-like CD8+ T cells and results in the generation of transcriptionally and epigenetically distinct effector CD8+ T cells that resemble highly functional effector CD8+ T cells seen after an acute viral infection.
4. Either blocking CD25 with an antibody or using IL-2v almost completely abrogated the synergistic effects observed after PD-1 + IL-2 combination therapy.
Conclusions
The generation of the qualitatively superior CD8+ T cells that mediate viral control underlies the synergy between PD-1 and IL-2. Our results show that the PD-1+TCF1+ stem-like CD8+ T cells, also referred to as precursors of exhausted CD8+ T cells, are not fate-locked into the exhaustion program and their differentiation trajectory can be changed by IL-2 signals.

31

Tumor antigen expressing oncolytic HSV elicits temporally different anti-tumor effects in T cell subsets

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Background

Poor prognosis neural tumors, such as high-grade gliomas (HGGs) and malignant peripheral nerve sheath tumors (MPNSTs), have limited treatment options and are often resistant to conventional therapies (e.g. chemotherapy and radiotherapy) and immunotherapy. [1] These cancers possess a complex tumor microenvironment composed of both non-malignant and cancer cells. Infiltrating myeloid cells (e.g. tumor-associated macrophages and myeloid-derived suppressor cells) create a growth-factor rich and immunosuppressive environment that aids tumor growth and restricts the immune response. [2] We have developed an oncolytic Herpes Simplex Virus (oHSV) that expresses a shared tumor antigen, Ephrin A2 (EphA2), commonly expressed in many tumors (glioma, sarcomas, prostate, lung, colorectal) [3] in an effort to break immune tolerance to the antigen and improve anti-tumor immune recognition. Our results show that oHSV-EphA2 virus treatment increased CD8 T cell tumor infiltrates. Virus-expressed EphA2 improved EphA2 antigen recognition and survival. Of particular interest was that only the oHSV-EphA2 virus induced EphA2-specific CD8 populations despite abundant EphA2 expression in our CT2A glioma tumor model. [4] While phenotypic studies showed no CD4 differences between the oHSV and oHSV-EphA2-treated tumors, our **hypothesis is that both CD8 and CD4 T cell subsets are necessary for our OV anti-tumor response**.

Methods

To characterize how the CD4 or CD8 T cell subsets impact oHSV-EphA2 treatment response, we examined glioma (CT2A) and MPNST (67C4) tumor growth and animal survival after independent CD4 or CD8 depletion. Additionally, we analyzed the tumor infiltrating lymphocytes (TIL) of surviving mice to assess differences between the depleted cohorts. Finally, we also examined how MHCI restricted EphA2 expression in CT2A tumors impacts oHSV-EphA2 based survival using MHCI/EphA2 KO CT2A cell line.

Results

In the absence of CD4 T cells, CD8 T cells are unable to restrict tumor growth. In contrast, with CD8 T cell depletion and CD4 T cell presence, tumors initially grow rapidly then reduce their sizes 2-3 weeks post-treatment. Secondly, loss of MHCI on tumor cells did not affect initial oHSV-EphA2 anti-tumor effects, with delayed tumor growth in both CT2A and CT2A KO tumors. Our results demonstrate that CD8 and CD4 T cells both contribute to oHSV-EphA2 therapeutic effect but with temporally different activity.

Conclusions

Tumor therapy using an oHSV-EphA2 expressing virus induces antigen specific CD8 populations but also requires CD4 T cells for therapeutic activity. Our results suggest oHSV-EphA2 elicits a multicellular response different from the classical anti-viral immune responses.

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Ethics Approval

The experiments were conducted under our currently approved protocol (AR16-00057). No animal experiments were conducted without the approval of Nationwide Children's Hospital (NCH) Institutional Animal Care and Use Committee (IACUC).

32

STING agonism enhances anti-tumor immune responses and therapeutic efficacy of PARP inhibition in *BRCA*-associated breast cancer

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Background
PARP inhibitors (PARPi) have improved treatment outcomes of *BRCA*-associated breast cancer (BC) and of other homologous recombination (HR) repair-deficient cancers. In addition to mechanisms underlying the synthetic lethality of PARP inhibition and HR deficiency, PARPi induces innate immune responses via cGAS/STING pathway activation, resulting in cytotoxic T-cell infiltration, an event critical for maximal efficacy in BRCA-deficient models. Here, we have investigated an alternative approach to improving efficacy in *BRCA*-associated BC by combining PARP inhibition with STING agonism.
Methods
Immunoblot analysis and qPCR were performed in KB1P-G3 cells derived from the K14-Cre-*Brca1$^{f/f}$;Trp53$^{f/f}$* GEMM of triple-negative BC (TNBC) and the human *BRCA1*-mutant TNBC cell line MDA-MB-436 to determine pharmacological activation of the cGAS/STING pathway by combining olaparib and the STING agonist ADU-S100. To gain additional mechanistic insights into the anti-tumor immune response and enhance the therapeutic efficacy combination treatment, we performed nanoString immune gene expression analysis, immunophenotyping analysis and efficacy study in mice bearing K14-Cre-*Brca1$^{f/f}$;Tp53$^{f/f}$* tumors with vehicle, olaparib, ADU-S100 or the combination (n=3/8).
Results
Our data show that in cell lines activation of STING-TBK1 signaling in response to olaparib or ADU-S100 that was enhanced by combination treatment. The activation of STING signaling resulted in the production of β-interferon and T cell-attracting chemokines with greater effects of combination treatment. STING pathway activation did not occur in response to olaparib or the combination in MDA-MB-436 cells in which BRCA1 expression was repleted. Immunophenotyping analysis resulted in a superior immune response compared to monotherapies, characterized by increased cytotoxic T-cell recruitment and activation, and enhanced DC activation and antigen presentation at 3 days. Gene set analysis revealed that the top-most upregulated genes in response to the combination were involved in antigen processing, MHC, interferon and leukocyte pathways. Importantly efficacy study shows the combination treatment led to complete tumor clearance in all enrolled mice and 100% tumor-free survival across two experiments. Finally, mice that achieved tumor clearance after treatment were rechallenged with tumor implantation. While tumors were established in naïve mice, tumors could not be re-established in the mice that had been cured.
Conclusions
These findings demonstrate that STING agonism maximizes the anti-tumor efficacy of PARP inhibition, overcomes PARPi resistance and contributes to immunologic memory in BRCA-deficient TNBC models. The potent preclinical therapeutic efficacy of combined PARP inhibition and STING agonism warrants further development of this regimen as a treatment for *BRCA*-associated TNBC.
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Pantelidou, Constantia et al. “STING agonism enhances anti-tumor immune responses and therapeutic efficacy of PARP inhibition in BRCA-associated breast cancer.” *NPJ breast cancer* vol. 8,1 102. 6 Sep. 2022, doi:10.1038/s41523-022-00471-5

34

The impact of mesenchymal transition and macrophage phenotype switching in immunotherapy-resistant melanoma

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Background
Anti-PD-1 refractory melanoma remains a devastating clinical challenge. We have previously defined AXL tyrosine kinase is an emerging target of interest implicated in epithelial to mesenchymal type transition (EMT), tumor associated macrophage behavior, and immunotherapeutic resistance. Here, we aim to define the mechanisms by which AXL mediates tumor-immune crosstalk and the role of AXL inhibition strategies in mitigating resistance to modern immunotherapy.

Methods
For preclinical studies, YUMM1.7 (anti-PD-1 resistant) melanoma cells were used for in vitro proliferative and migratory assays. RAW macrophages were treated with GAS6 stimulation, GM-CSF stimulation, GAS6 inhibitor: warfarin, or AXL TK small molecule inhibitor bemcitinib. In vivo, subcutaneous tumors were treated with multiple AXL inhibition strategies as single agent or in combination with Invivomab (Anti-PD-1). For clinical studies, blood obtained from melanoma patients and evaluated for serum levels of sAXL by ELISA and TCGA-SKCM melanoma tumor mRNA expression and clinical data for metastatic melanoma patients were downloaded from the GDC legacy archive (https://portal.gdc.cancer.gov/legacy-archive) (n = 471). Differences in Kaplan-Meier survival curves based on level of expression were tested using G-rho family tests. Strength of relationships between biomarkers were measured using Pearson's correlation. All statistical analysis were performed using R package "survival".g.

Results
Serum detectable sAXL significantly increased by stage with highest levels noted in stage IV patients ($p=0.03$). AXL high expression was associated with PD-1 nonresponse in Stage IV patients ($p<0.01$). AXL expression diverged from T-cell related signatures but was associated with increase in immunosuppressive myeloid signatures ($p<0.001$). In vitro, Melanoma cell migration was significantly increased with exogenous Gas6 exposure and this effect was inhibited by warfarin ($P<0.05$). GAS6 sand GM-CSF stimulation increased macrophage proliferation however only GAS6 increased M2 expression markers of AXL and Arginase ($p<0.05$). Low dose warfarin or bemcitinib reduced Arginase expression in RAW macrophages, shifting toward anti-tumor M1 phenotype ($p<0.05$). In vivo, YUMM1.7 tumor growth was significantly inhibited by single agent warfarin, bemcitinib, or cabozantinib (RET/VEGFR2/cMET/AXL inhibitor). Tumor size was not significantly inhibited by single agent Invivomab, in line with known immunotherapeutic resistance. Combination with either warfarin, bemcetinib, or Cabozantanib significantly reduced tumor growth ($p<0.02$).

Conclusions
Our data indicate myeloid specific immunosuppressive effects of AXL activity in the tumor microenvironment are a viable target in PD-1 refractory melanoma. Analysis is ongoing to elucidate impact of AXL driven EMT on macrophage function in multiple preclinical models of melanoma. Investigation of specific mechanisms by which AXL directed therapy may improve immunotherapeutic response and is warranted.

Ethics Approval
All animal studies were approved by Stanford's IACUC committee.

36

Phagocytosis increases an oxidative metabolic and immune suppressive signature in tumor macrophages

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Background
Phagocytosis is a key macrophage function, but how it shapes tumor-associated macrophage (TAM) phenotypes and heterogeneity in solid tumors remains unclear.
Methods
We utilized both syngeneic and a novel autochthonous lung tumor model in which neoplastic cells express the fluorophore tdTomato (tdTom) to identify TAMs that have phagocytosed neoplastic cells *in vivo*.
Results
Phagocytic tdTom^{pos} TAMs upregulated antigen presentation and anti-inflammatory proteins, but downregulated classic proinflammatory effectors compared to tdTom^{neg} TAMs. Single-cell transcriptomic profiling identified TAM subset-specific and common gene expression changes associated with phagocytosis. We uncover a phagocytic signature that is predominated by oxidative phosphorylation (OXPHOS), ribosomal, and metabolic genes, and this signature is enriched in myeloid cells in human lung cancers. Expression of OXPHOS proteins, mitochondrial content, and functional utilization of OXPHOS were increased in TAMs.
Conclusions
Our identification of phagocytic TAMs as a distinct myeloid cell state links phagocytosis of neoplastic cells *in vivo* with OXPHOS metabolism and pro-tumor phenotypes.
Ethics Approval
The Stanford Institute of Medicine Animal Care and Use Committee approved all animal studies and procedures.

37

Immunotoxicology analysis of IL-12ns reveals biomarkers of beneficial and maladaptive immune responses in healthy BALB/c mice

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Background
Systemic administration of **interleukin (IL)-12**, an immunostimulatory cytokine, has been shown to induce anti-tumor immune responses in preclinical cancer models. While these data were promising, clinical trials using bolus IL-12 injection for treatment of solid tumors ultimately failed. Importantly, this failure was likely due to utilization of "tolerable" dosing strategies (previously developed for cytotoxic chemotherapies) as opposed to optimal biologic dosing. To address these clinical barriers, our group successfully developed **IL-12-loaded PLGA nanospheres (IL12ns)** that release their contents systemically in a slow, controlled manner. We also developed an **immune diagnostic platform (IDP)** capable of monitoring therapeutic response throughout therapy.
Methods
We compared the **systemic immune responses** from differential IL-12 treatment regimens in healthy 7-8-week male and female BALB/c mice via seven serial blood sampling timepoints. Five groups examined four IL-12 dosing strategies including 10,000 ng/kg daily [maximum tolerable dose (MTD)], 10 mg IL-12ns, 0.1 mg IL-12ns, and 0.001 mg IL-12ns, alongside a saline control. At each timepoint, peripheral blood was collected by cheek bleed for analysis with an IDP consisting of peripheral blood mononuclear cell (PBMC) spectral flow cytometric analysis, plasma cytokine/chemokine analysis, and bulk PBMC RNA sequencing. At euthanasia, peripheral blood was collected by cardiac puncture for PBMC metabolomic assessment followed by necropsy for histopathological analysis of various organs (**Figure 1**).
Results
Among numerous findings, **PBMC flow cytometric** analysis revealed that the MTD was associated with an increase in percent Ki67+ NK cells, PD-L1+ monocytes, as well as PD-L1+, CD66b+, and MHC-II+ PMN-MDSCs. **Cytokine** analysis depicted that while IFN-?? levels were increased upon initial stimulation within the MTD group, this increase was not sustained, evidenced by an over four-fold decrease in IFN-?? levels by euthanasia. However, the 10 mg IL12ns group maintained its peak IFN-?? levels with repeated dosing. **Chemokine** analysis also revealed that IL12ns dosing was associated with increased monocyte recruitment, as evidenced by increases in CCL2 and CCL4. **Bulk PBMC RNAseq** analysis supported aberrant peripheral immunostimulation with bolus MTD delivery, as demonstrated by increased expression of both IL-12 and IFNG reactome signatures within peripheral immune cells, signatures absent in IL12ns dosing groups. Finally, **PBMC metabolomic assessment** revealed that IL12ns was associated with increased cellular respiration within PBMCs, while the MTD was associated with minimal to no effect.
Conclusions
Markers of beneficial or maladaptive immune responses with IL-12-based therapies were identified by the IDP and further support a **potential therapeutic window of IL-12ns** in future clinical trials.
Acknowledgements
We acknowledge the use of the WVU Shared Research Facilities, University of Virginia Flow Cytometry Core (RRID: SCR_017829), and University of Virginia Genome Analysis and Technology Core (RRID:SCR_018883). This work was funded in part by Pittsburgh Cure Sarcoma and ICaPath Inc..

Abstracts

Figure 1. Overview of experimental design.

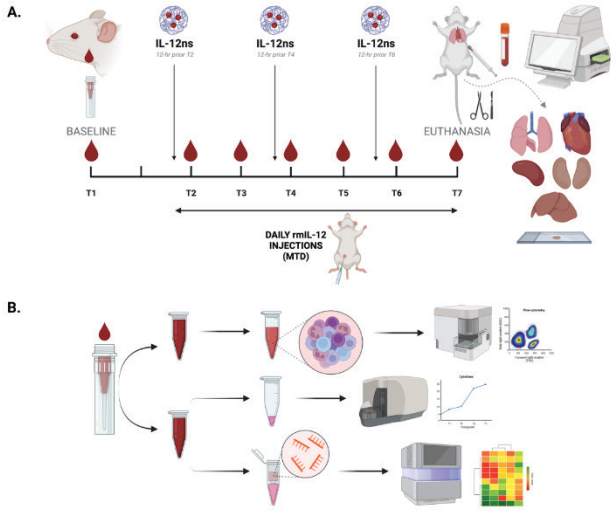

Figure 1. (A) The resulting systemic immune responses from differential IL-12 treatment regimens in healthy 7–8-week male/female BALB/c mice via 7 serial blood sampling timepoints were analyzed. Timepoints include baseline (T1), 12-hour (T2), day 4 (T3), day 8 (T4), day 11 (T5), day 15 (T6), and day 18 (T7), at which time mice were humanely euthanized following cardiac puncture for full necropsy. Five groups examined four IL-12 dosing strategies, 10,000 ng/kg/day daily [maximum tolerable dose (MTD)], 10 mg IL-12ns, 0.1 mg IL-12ns, and 0.001 mg IL-12ns weekly, alongside a non-treatment, saline control. At day 18 (T7, euthanasia), peripheral blood was collected via cardiac puncture for PBMC metabolomics through the Agilent Seahorse MitoStress assessment, alongside necropsy to harvest heart, liver, spleen, lungs, and kidneys for histopathological analysis by a board-certified pathologist. (B) At each sampling timepoint, peripheral blood was collected via cheek bleed (~ 80 uL) for analysis via an immune diagnostic platform (IDP) consisting of PBMC spectral flow cytometric analysis, plasma cytokine/chemokine analysis via LEGENDplex, and Bulk PBMC RNA sequencing [Figure by BioRender.com].

38

Impact & efficacy of a virtual streaming platform to provide equitable access for immuno-oncology education

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Background
Through its mass global reach, social media has emerged as a power outlet for medical information, especially in resource-limited regions. However, misinformation presents a widespread challenge and created a burden to individual and public health globally. To address this, *MedNews Week,* a free, virtual educational platform streamed on most social media platforms was developed to provide global health education through weekly immuno-oncology programming. The leading show, *Keynote Conference*, features live virtual presentations from immuno-oncology's premier global leaders discussing the latest developments in the field before a mainstream global audience. The aim of this study was to assess the worldwide reach and impact of this cost-free, virtual medical education platform.

Methods
From January-September 2022, *MedNews Week* hosted 20 immuno-oncology global leaders (h-index = 60). Viewership, impressions, and social media outreach data were collected from the accounts of *MedNews Week* and its respective members. In collaboration with Symplur, data was analyzed to measure the program's global reach, and a mixed-methods approach was employed to assess engagement.

Results
During this nine-month period, *MedNews Week* programming extended across 54 countries with over 11.9K tweets, 5.7K retweets, and 41 million Twitter impressions (Figure 1). A network analysis of *MedNews Week's* main accounts demonstrated that cross-community engagement was enhanced by the usage of hashtags, which successfully expanded global audience viewership. To assess the level of communication between *MedNews Week* and our audience, we focused our analysis on the *MedNews Week* Twitter chat organizers (@yleyfman and @CParkMD) and supporting researcher (@ShimaghavimiMD). We identified these usernames on the network map (Figure 2). Although not all other participants were identified, further analysis would reveal increased connections and heightened outreach. Additionally, a steady increase in the number of Keynote Conference attendees has been observed (Figure 3).

Conclusions
*MedNews Week'*s continued growth in viewership and global reach as a virtual and cost-free platform demonstrates its emergence as a viable outlet to combat medical misinformation, especially in lower socioeconomic regions. While studies have identified internet access and cost as barriers to high-quality medical information, *MedNews Week*'s growth has not stagnated and increased especially within these limited areas. The platform's ability to showcase global leaders to a mainstream audience at no cost offers a practical approach to combat educational inequity. Consequently, such a platform has demonstrated great potential to positively impact global immuno-oncology education.

Acknowledgements
We are grateful to the support of NSHSS for supporting our mission of contribution to global education and combatting healthcare inequity. We are grateful to Symplur for their help with data analytics.

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Abstracts

Figure 1. Global Reach of MedNews Week

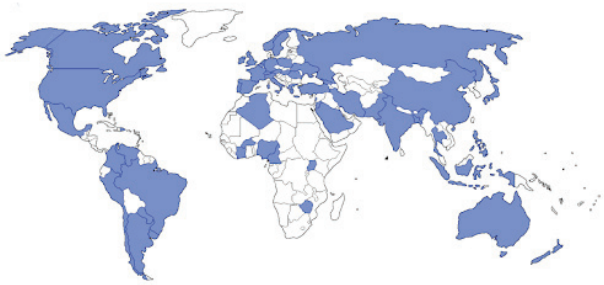

Figure 1. MedNews Week has reached a global audience across 54 countries from January to September 2022.

Figure 2. MedNews Week's Twitter Network

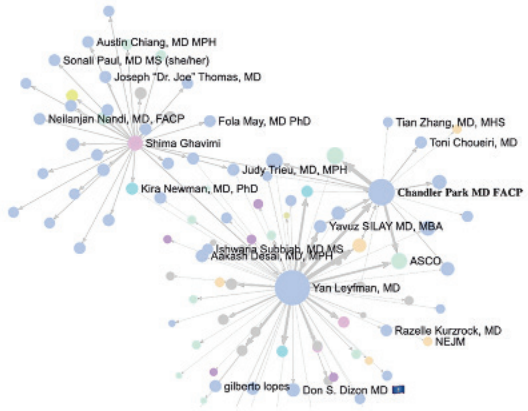

Figure 2. Network analysis of MedNews Week's cross-community engagement.

Figure 3. MedNews Week Keynote Conference Attendance

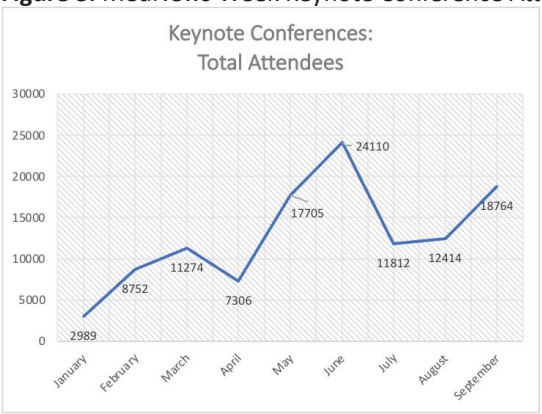

Figure 3. Attendance from January 2022 to September 2022 at MedNews Week Keynote Conference demonstrates a steady increase in viewers.

39

Impact of a global virtual platform providing immuno-oncology education to healthcare professionals

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Background
According to a 2021 Pew Research study, over 72% of U.S. adults use social media and its use has continued to increase where it has become an integral tool for the dissemination of healthcare information. Studies have shown greater disparities in healthcare outcomes exist in lower socioeconomic countries largely attributed to access and education, especially within immune-oncology. To rectify this, *MedNews Week*, a free, virtual education platform, was developed with the vision of providing attendees with the latest accurate medical information from the field's top global immune-oncology leaders through its bi-weekly programming. To reach healthcare professionals, *MedNews Week* has partnered with Vumedi, a global video education platform for doctors, in a joint effort to break down barriers within the healthcare community. The aim of this study was to assess the impact of *MedNews Week's* immune-oncology programming on Vumedi's platform on global oncology education.

Methods
From September 2022 to January 2023, 30 *MedNews Week* Keynote presentations from 22 immuno-oncology global leaders were featured on VuMedi's platform. Data including viewership, global reach, page views, and unique visits were collected. In collaboration with VuMedi, descriptive analysis was performed, and a mixed-methods approach was employed to assess engagement.

Results
During this five-month period, the *MedNews Week* channel demonstrated increased linear growth. Programming generated 4,387,213 total page views and 206 channel views with viewership from 22 countries (Figures 1, 2). Attendees were physicians from an allied field. During this time period, the channel had 1,750,161 total visits with 44,982 new registrations (Figures 3, 4).

Conclusions
MedNews Week has demonstrated an ability to attract and sustain a diverse, global, growing physician audience. The platform's virtual capabilities only require a stable internet connection and results demonstrate its successful ability to reach physicians globally, including those in lower socioeconomic areas. This study provides a proof-of-concept for a novel approach to disseminate medical information and due to its cost-effectiveness, will continue to demonstrate growth and increased international reach to effectively combat education inequity in immune-oncology globally.

Acknowledgements
We are grateful to the support of NSHSS for supporting our mission of contribution to global education and combatting healthcare inequity. We are grateful to VuMedi for their help with data acquisition and analytics.

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Figure 1. Global Reach of MedNews Week

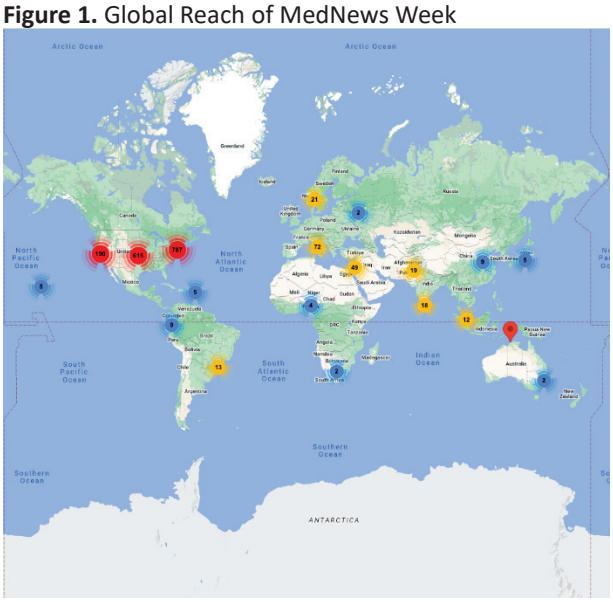

Figure 1. MedNews Week has reached a global audience across 2 countries from September 2022 to January 2023.

Figure 2. Channel Views

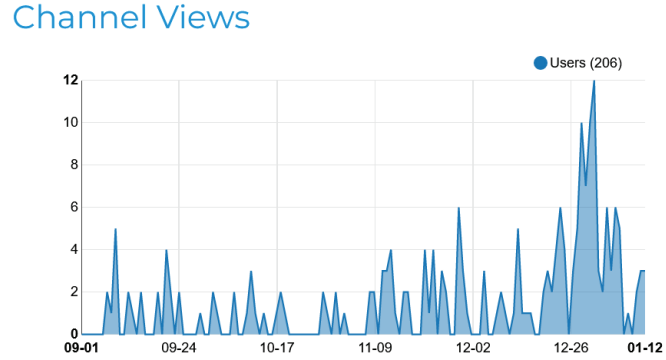

Figure 2. MedNews Week's channel on VuMedi had 206 views from September 2022 to January 2023.

Figure 3. Page Views

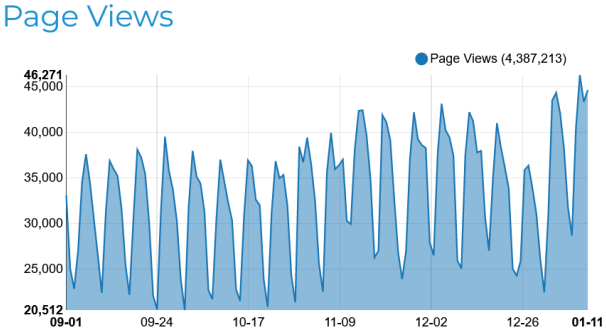

Figure 3. MedNews Week's page on VuMedi had 4,387,213 views from September 2022 to January 2023.

Figure 4. Visitors

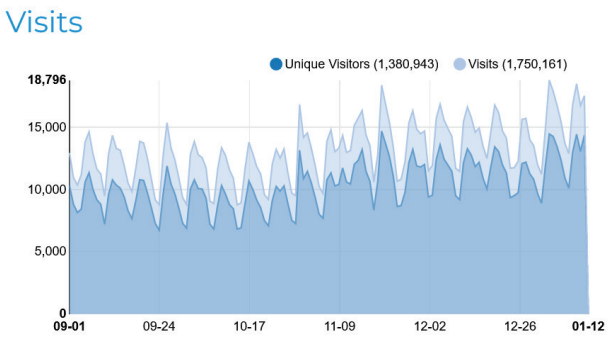

Figure 4. From September 2022 to January 2023, MedNews Week had 1,750,161 new visitors watching its content on VuMedi.

40

Severe SARS-CoV-2 & cancer: unraveling the immunological paradigm & a promising cellular anti-COVID-19 therapy for the immunocompromised

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Background
Cancer patients, due to their immunocompromised status, are at an increased risk of severe SARS-CoV-2 infection. According to the World Health Organization, 17.9% of cancer patients identified as positive for SARS-CoV-2 developed severe disease, had higher rates of hospitalization (55.2%), ICU admissions (25.7%) and 30-day mortality (13.4%). While patients with active malignancy had worse outcomes, those in remission also had an increased risk for more severe disease compared to COVID-19 patients without cancer. Given that limited information exists to explain the interplay between cancer and severe SARS-CoV-2, we (1) developed a model to explain the pathogenesis and interplay between the two conditions (2) explain the efficacy of a promising cell therapy that can treat severe SARS-CoV-2 based on clinical evidence.
Methods
Severe SARS-CoV-2 causes multi-organ injury and dysfunction through IL-6-mediated inflammation and hypoxic-induced metabolic changes leading to increased IL-6 production and apoptosis. Malignancy induces cell death through hypoxia-induced cellular metabolic alterations resulting in an upregulation of IL-6 release. Studies have shown that IL-6 is one of the major cytokines within the tumor microenvironment responsible for propagating inflammation and tumorigenesis including inhibiting apoptosis, promoting angiogenesis, invasion, and metastasis.
Results
In this model, infection with cancer and severe SARS-CoV-2 results in increased IL-6 production leading to enhanced systemic injury as compared to either alone due to an enhanced pro-inflammatory state, increased acute phase reactant production, complement activation and vascular permeability. Currently, there are limited effective therapeutic interventions against severe SARS-CoV-2 especially in patients with malignancy. Given the virus's complex nature, the most effective therapies will be ones that can control viral replication and systemic inflammation. One such approach uses mesenchymal stem cells (MSCs), which possess regenerative, antiviral and immunomodulatory properties that can inhibit viral replication, while dampening the cytokine response and resulting inflammatory systemic injury. Clinically, MSCs have demonstrated a 91% overall survival and 100% survival in patients younger than 85 years old within a month after treatment with results holding steady for 6 months.
Conclusions
In conclusion, MSCs have demonstrated clinical efficacy, safety, and tolerability in patients with severe SARS-CoV-2. Their ability to target disease pathogenesis at multiple steps within the pathway to hinder direct injury, suppress IL-6, and dampen systemic inflammation makes them a promising therapeutic approach that can thwart the virus's heterogeneity and mutational adaptations. Given their limited drug interactions and side-effects, MSCs are a viable approach to contain SARS-CoV-2 with limited interruptions to their treatment regimens.

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Figure 1. MSCs as a therapy against severe SARS-CoV-2 in Cancer

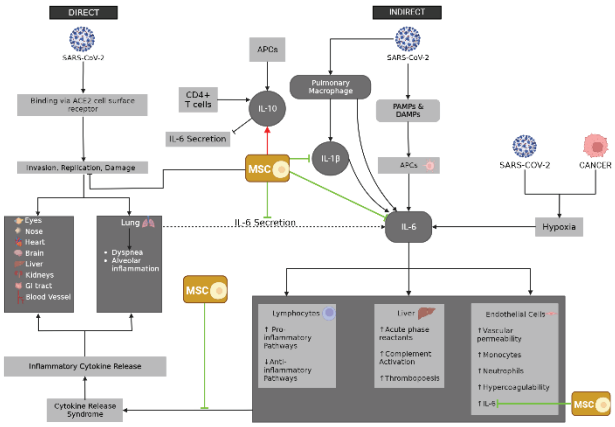

Figure 1. MSCs act to downregulate the pro-inflammatory pathways and upregulate the anti-inflammatory pathways as illustrated by the downregulation of IL-1 and IL-6 and an upregulation of IL-10 at multiple points and within multiple pathways simultaneously resulting in a net decrease in systemic inflammation.

41

Improving immunotherapeutic clinical trial access for BALT patients with COVID-19

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Background
Disparities within healthcare have been steadily rising over the past decade and further exacerbated during the COVID-19 pandemic. Studies have shown significant decline in cancer clinical trial enrollment that was further compounded by significant delays during the first year of the COVID-19 pandemic. Patients with rare malignancies were particularly vulnerable given the limited therapeutic options available and the global slowing down of drug approvals during the pandemic. Mucosal-associated lymphoid tissue (MALT) lymphomas are a rare form of non-Hodgkin lymphomas that develop from B cells and are often incidentally diagnosed, but who's pathogenesis remains unclear. Given their rarity and heterogeneity, a limited consensus exists on a standard treatment approach. Over the past decade, studies have identified an association between coronavirus infection and bronchial-associated lymphoid tissue (BALT) lymphoma, where mutations in serine proteases increase the risk of BALT. The aim of this study was to use a computational ontology-based platform improve immunotherapeutic clinical trial access for BALT patients stricken with COVID-19.

Methods
Ontologies for serine proteolytic homeostasis were constructed utilizing keywords and abstracts from PubMed, which were then used to generate search inputs for *ClinicalTrials.gov*, as a link between search terms and clinical trials. This method was utilized for and between input terms "BALT", "lymphoma", and "serine protease".

Results
Results showed that this platformed demonstrated a literary relationship between BALT lymphoma, serine proteases, and COVID-19. Additionally, it successfully found a clinical trial on *ClinicalTrials.gov* utilizing an Adaptive Cellular Therapy, which aims to develop NK cells to target SARS-CoV-2 to better control this virus with a goal of reducing BALT lymphoma onset.

Conclusions
This computational-ontology platform provides an effective approach to connect patients with active clinical trials. Given the difficulty of access for patients plagued with rare malignancies, this approach can help bridge the gap of access. Future studies will aim to expand this platform to detect clinical trials globally, while also expanding its utility to visualize biomolecular interactions between the search terms.

Acknowledgements
We would like to acknowledge the mentors who have played a profound role in our career development and to the patients who inspire us to strive for greatness daily. Yan Leyfman would also want to acknowledge MSSN for its support of scholarly endeavors.

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Figure 1. Search Query Results Connecting Patients to Clinical Trials

PubMed Abstract	EBI Accession Code	ClincialTrials.gov Summary
We used the dataset of the SWIFT-DIRECT trial, which randomized 408 patients to IVT+MT or MT alone. Potential interactions between assignment to IVT+MT and expected time from onset-to-needle (OTN) as well as expected time from door-to-needle (DTN) were included in regression models. The primary outcome was functional independence (modified Rankin Scale (mRS) 0-2) at 3 months. Secondary outcomes included mRS shift, mortality, recanalization rates, and (symptomatic) intracranial hemorrhage at 24 hours.	Q16553: Lymphocyte Antigen 6E	Immunotherapy based on Adoptive Cellular Transfer (ACT) uses several types of immune cells, including dendritic cells, cytotoxic T lymphocytes, lymphokine-activated killer cells, and NK cells. NK cell-based immunotherapies are an attractive approach for treating diseases because of their characteristic recognition and killing mechanisms; they are involved in the early defense against infectious pathogens and against MHC class-I-negative or -low-expressing targets without the requirement for prior immune sensitization of the host and are able to lyse target through the release of perforin and granzymes and using antibody-dependent cellular cytotoxicity pathways mediated by Fc receptor for IgG (CD16). The aim of this project is to evaluate the safety and immunogenicity of allogeneic NK cells from peripheral blood mononuclear cells (PBMCs) of healthy donors in patients infected with COVID-19 collected by apheresis. This allows us to collect cGMP PBMCs and immunomagnetic remove several types of undesirable cells including B, T and CD33+ cells with enrichment of NK cells that will be expanded in bioreactors with GMP culture media (AIM-V) supplemented with human AB serum and GMP grade IL-2, and IL-15. After quality control verification the final NK cell product will be resuspended in 300 mL saline solution for intravenous infusion. Initially, we will enroll in this study ten COVID-19 infected adult patients with moderate symptoms (NEWS 2 scale score>4). Consent forms will be signed by the patient before the therapy. Patients will be treated with three different infusions of NK cells 48 h apart with 1, 10, and 20 million cells/kg body weight. We will follow the patients for any adverse effect, clinical response and immune effects by flow cytometry including markers for NK cells expressing different markers (CD158b, NKG2A, and IFN-y). We anticipated that the release of IFN-y by exogenous NK cells could attract other immune cell populations to boost the immune response against COVID-19. *NCT04344548*

Figure 1. Results obtained from search query connecting patients with BALT Lymphoma to active clinical trials using clinicaltrials.gov.

42

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Background
Subpopulations of tumor cells that lack targeted antigen (Ag) expression can evade T cell-mediated immunotherapies—CAR-T, BiTEs, etc.—causing relapse in a significant proportion of patients (Ag escape)[1–3]. We have demonstrated a potential mechanism to prevent Ag escape: geographically localized "Bystander Killing" of Ag(-) cells[4]. T cells (and CAR-T) engage with Ag(+) cells and upregulate expression of death receptor ligands, such as Fas ligand (FasL), that kill neighboring tumor cells in a Fas-dependent, Ag-independent manner. Importantly, we show that increasing tumoral Fas signaling with inhibitors of Fas regulators or preventing FasL shedding from T cells can enhance Bystander Killing.
Methods
Anti-CD19 CAR-T cells were co-cultured with mouse A20 lymphoma cells in a 2:1 effector:target ratio for 24-72 hours. A20 targets consisted of CD19(+) cells, CD19(-) cells, and CD19(-) Fas(-) cells. Flow cytometry was conducted to identify each cell and live cells were normalized to counting beads in each well. In vivo, Balb/c mice were subcutaneously implantated with A20 tumors containing 95% CD19(+) and 5% CD19(-) Fas(+) Luciferase(+) or 5% CD19(-) Fas(-) Luciferase(+) tumors. Animals were given 6e6 CAR-T or no therapy and imaged by IVIS bioluminescent every week.
Results
Co-culturing aCD19 CAR-T cells with a 50:25:25 mixture of CD19(+)Fas(+):CD19(-)Fas(+):CD19(-)Fas(-) lymphoma cells yielded nearly complete clearance of CD19(+) populations and stark decreases in CD19(-)Fas(+) but not CD19(-)Fas(-) populations. In line with these findings, several other small molecules targeting regulators of apoptosis show greater Fas-dependent killing in tumor killing assays. Additionally, it has been shown that metalloproteinases (MMP) cleave T cell membrane-FasL (mFasL) into a soluble form[5,6], which reduces its apoptotic-inducing ability[7–9]. We therefore inhibited ADAM10[10], an MMP cleaving FasL, and observed increased mFasL on Ag-activated CAR-T cells and significantly greater Bystander Killing. To investigate this *in vivo,* mice were injected with mixed CD19(+):CD19(-)LUC(+) tumors and treated with aCD19 CAR-T, allowing for real-time tracking of Ag escape. While control mice exhibited large tumor growth and bioluminescence, CAR-T treated mice showed evidence of Bystander Killing that prevented Ag escape at early time points, but later grew CD19(-) tumors. Combining this imaging modality with Fas-potentiating therapies led to greater clearance of CD19(-) cells and prolonged tumor-free survival. In addition, these effects were seen only in tumors where Ag(-) cells express Fas.
Conclusions
Overall, we demonstrate that Fas-potentiating therapies, which increase signaling in tumor cells or reduce mFasL cleavage on T cells, can be combined with CAR-T therapy to mitigate Ag escape in heterogeneous tumor populations.
Acknowledgements
Figures were made with BioRender
Ethics Approval
IRB approval: IACUC-2017-0146
The protocol is approved from 1/9/2023 to 7/1/2023 effective as of 1/9/2023. Your next annual review is due 7/1/2023. Your next triennial review is due 7/1/2023.
Refer to your organization's standard operating procedures for conducting this protocol.
Funding Source:
Name: National Cancer Institute/NIH/DHHS, Grant Office ID: GCO 19-0176, Funding Source ID: 0244-6520 Name: Damon Runyon Cancer Research Foundation, Grant Office ID: 14-0345, Funding Source ID: 0267-0974 Name: Merck Sharp & Dohme Corp, Grant Office ID: GCO #20-0997, Funding Source ID: PD20-01208
Name: Gilead Sciences, Grant Office ID: 20-1282-00001- 01, Funding Source ID:
Name: NCI CIRB, Grant Office ID: GCO# 21-2113, Funding Source ID: 1F31CA275326 - 01

43

Spatial transcriptomic profilling reveals distinct dynamics in the tumor microenvironment relative to stress keratin 17 expression in immune check-point blockade treated head and neck carcinoma

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Background

Our group has previously demonstrated the correlation betweeen stress keratin 17 (K17) expression in head and neck squamous cell carcinoma (HNSCC) and lack of clinical benefit from immune check point blockade (ICB) [1]. Here, we performed single cell spatial transcriptome analysis to further elucidate the tumor microenvironment relative to K17 status and response to therapy in ICB-treated HNSCC.

Methods

Targeted single cell spatial transcriptomic profilling using the CosMx Spatial Molecular Imager 1000-plex Human Universal Cell Characterization RNA Panel (Nanostring Technologies) was performed on a tissue microarray consisting of archival FFPE pretreatment tissue samples from 22 HNSCC patients treated with pembrolizumab at UW.. PanCK, CD45, CD3, CD298 and DAPI were used for morphology visualization. Clinical outcomes were obtained from medical records. Available imaging data was evaluated following RECIST 1.1.

Results

Altogether, 38 fields of view (FOVs) were analysed. Using a semi-supervised clustering approach, we identified 4 distinct tumor subsets, including two subsets with intermediate or high K17 expression. Tumors with low K17 expression that received clinical benefit on ICB were enriched in CD8+ T cells and tumor-associated macrophages. We will present an in-depth comparative analysis into observed differences between the tumor microenvironment in K17 high versus low expressing tumors, and specifically, between K17 low tumors deriving clinical benefit from ICB compared to those with progressive disease on ICB.

Conclusions

Our results indicate that the expression of immune-related genes may further stratify patients with K17 low expressing tumors that may derive clinical benefit from ICB.

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Ethics Approval

The study was reviewed and approved by the institutional ethics committee (IRB 2018-1510, subproject number 2021-012).

46

Flt3L-primed *In Situ* Vaccination and pembrolizumab induce systemic tumor regressions of non-immunotherapy responsive tumors

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Background
The majority of patients with cancer fail to respond to checkpoint blockade, potentially due to the absence of preexisting anti-tumor immunity. Cancer vaccines aim to prime *de novo* anti-tumor immune responses against tumor neoantigens. We previously showed that an *in situ* vaccine approach combining intratumoral (IT) Flt3L, radiation (XRT), and IT polyICLC successfully 1)mobilized, 2)antigen-loaded, and 3)activated dendritic cells, which yielded partial and complete remissions in a subset of patients. Analysis of samples from these patients revealed this combination induced tumoral PD-L1 expression, potentially explaining resistance, and addition of PD-1 blockade to the vaccine improved cure rates in preclinical models.
Methods
This Phase 1/2 trial investigated a novel *in situ* vaccine in three tumor types not responsive to pembrolizumab: indolent non-Hodgkin's lymphoma (iNHL), metastatic breast cancer (MBC) or PD-1-refractory head and neck squamous cell carcinoma (HNSCC). Patients received local XRT to a single tumor on Days 1-2, 9 daily IT injections of Flt3L, followed by 8 IT injections of poly-ICLC over 6 weeks. On Day 23 patients received their first of 8 doses of intravenous (IV) pembrolizumab (Figure 1). Phase 1 enrolled 6 patients to assess safety, while in Phase 2, tumor-specific cohorts are enrolling, each with a parallel Simon's Two-Stage design (Figure 2). We report interim results from the first 10 patients.
Results
10 patients were enrolled between April 2019 and July 2022; 6 with MBC, 3 with iNHL and one patient with HNSCC. All patients experienced TRAEs, mostly low-grade injection-site reactions and inflammatory symptoms related to poly-ICLC. Grade 3 TRAE occurred in 2 patients, one was fever after poly-ICLC, while the other was grade 3 pembrolizumab-related colitis. One patient had a CR, two achieved partial response, one had SD, while six had PD. One patient with ER/PR$^+$ breast cancer had received 12 prior lines of therapy has achieved a near CR without evidence of new or recurrent disease 9 months from initiation of experimental therapy (Figure 3). Blood analysis of the responders demonstrated higher levels of key chemokines including CXCL5 and CCL8 compared with non-responders, and significantly higher Flt3L-induced DC1 expansion.
Conclusions
In situ vaccination with Flt3L, XRT, poly-ICLC and pembrolizumab is well tolerated, with early signs of efficacy in patients with tumor types that are non-responsive to standard checkpoint blockade, warranting expansion of this approach. Analysis of biopsies and blood from patients to define determinants of response to this *in situ* vaccine approach is ongoing.
Trial Registration
This trial is approved by the Mount Sinai Institutional Review Board. NCT03789097
Ethics Approval
This trial is approved by the Mount Sinai Institutional Review Board. NCT03789097
Consent
As part of the clinical trial the patients have consented to presenting deidentified images and data.

Figure 1.

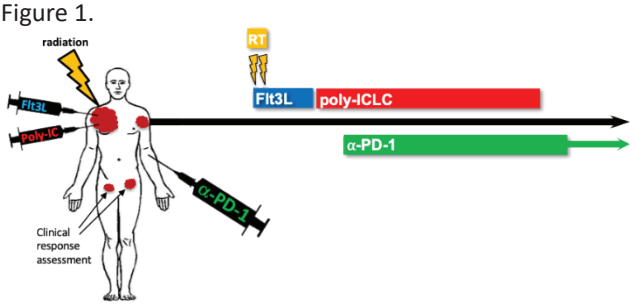

Figure 1. Trial Schema

Figure 2.

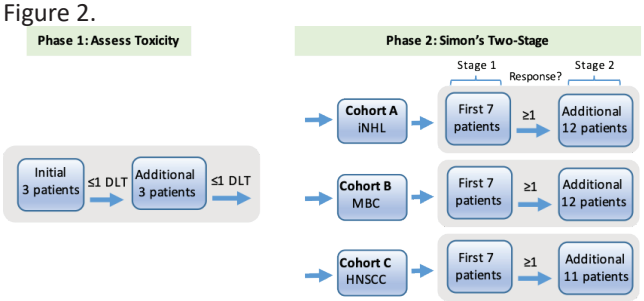

Figure 2. Statistical design

Figure 3.

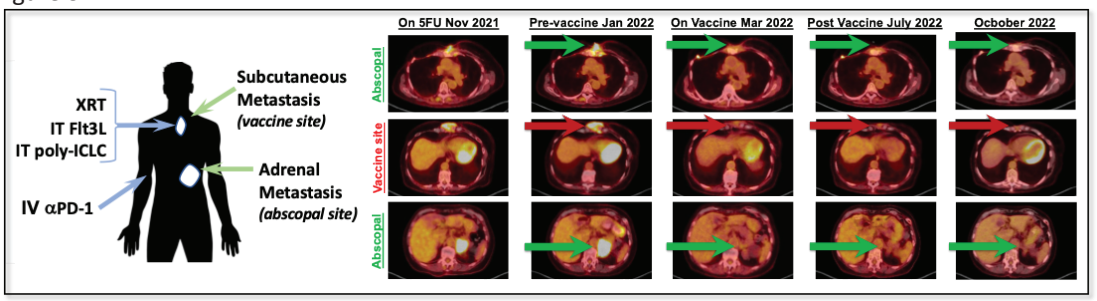

Figure 3. Patient with ER/PR+ MBC demonstrating resolution of superficial suprasternal lesion (vaccine site), as well as abscopal sites in the chest wall and left adrenal gland.

47

Use of immune checkpoint inhibitors in hospitalized patients

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Background

Pembrolizumab is approved by FDA in 2014 to treat metastatic melanoma. Use of immune check point inhibitors (ICI) has been expanding in multiple cancer types including non-small cell lung cancer since then. In this mini case series, we will evaluate the benefits and risks of immunotherapy in hospitalized patients. Use of immunotherapy agents in inpatient unit has been associated with poor clinical outcome and higher cost. Byrne et. al have recently shown that more than 45% of hospitalized patients receiving PD1 or PD-L1 antibodies have either died or discharged to hospice care. [1] A large retrospective chart review in Dana Farber Cancer Institute reveals 86% mortality rate in hospitalized immunotherapy recipients within the study period. [2] Understanding the safety and advantages of using immune checkpoint inhibitors in inpatient unit has a pivotal role in decreasing the healthcare associated costs and setting the appropriate goals of care with patients and families.

Methods

This is a retrospective observational cohort study conducted by review of electronic health records in last 5 years (from January 1st 2017- January 1st 2022) in Rutgers Health Community Medical Center. Adult patients admitted to inpatient unit who received Pembroluzimab, Atezolizumab, or Nivolumab were included.

Results

A total of 21 patients has received ICI therapy while in hospital. Mean age of study group was 69, with 57% males and 42% female. All patients had solid tumors, with most common malignancy being lung cancer(61%) followed by skin cancer (14%). Most common ICI given was pembrolizumab (76%) followed by atezolizumab (14.3%) and Nivolumab (9.5%). 38.1% of patients that were treated died in the hospital, 9.5% were discharged to hospice, 33.3% were transferred to another care facility and 19% were discharged home. No immune-related adverse events were reported. Age groups, cancer types were comparable with regard to discharge status.

Conclusions

Poor mortality and survival outcome from this case series should prompt clinicians to consider benefit and risks of ICI therapy when prescribed to patients who were hospitalized for non-oncological acute conditions.

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Ethics Approval

This study has been approved by Institutional Review Board at RWJBarnabas Community Medical Center with IRB number 22-001.

48

Loss of TGFβ signaling promotes genomically unstable cold cancers that are susceptible to immune checkpoint inhibition

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Background

The variable response to immune checkpoint inhibitors (ICI), even when T cells are present, suggests that cryptic interactions between immunity, tumor microenvironment and cancer cells are yet to be discovered. Transforming growth factor β (TGFβ) is a canonical tumor suppresser. Cancer cells evade TGFβ either by selectively overcoming the TGFβ proliferative barrier or completely shutting down TGFβ signaling. In several mouse models, loss of TGFβ signaling is accompanied by high TGFβ activity (1, 2), which is immunosuppressive. We have shown that loss of TGFβ is also critical to genomic integrity because it endorses classical DNA repair and inhibits error-prone repair (3). We described a score, βAlt, that reports the TGFβ/DNA repair functional relationship. Pan-cancer analysis showed that low βAlt cancers, which maintain TGFβ signaling, are resistant to genotoxic therapy whereas high βAlt cancers use error-prone DNA repair, are sensitive to chemoradiation, have a greater fraction of the genome altered and high frequency of a characteristic indel

Methods

We interrogated βAlt (4) in relationship to immune phenotype using recently described tumor educated immune cell gene signatures (TeIS) from human cancers (5).

Results

Unsupervised clustering of TeIS in TCGA specimens (Figure 1, n=3792) showed that βAlt was low (box a) in immune rich phenotypes (red bar). βAlt was highest in immune-poor cancers (blue bar). High βAlt reports gain of error-prone DNA repair; consistently, the fraction of the genome altered tracked with βAlt (box b) but tumor mutation burden did not (box c).
We hypothesized that cold tumors consist of TGFβ incompetent cancer cells within a TGFβ rich tumor microenvironment would respond to ICI. To test this, we analyzed IMvigor 210 metastatic bladder cancer patients treated with anti-PD-L1. As in TCGA, the TGFβ and DNA repair signatures were significantly anti-correlated (R= -0.42, p< 10-15) and TeIS signatures range from immune rich to poor (Figure 2). 25% of patients who were immune poor (blue box) responded. Within the immune poor, responders had higher bAlt (0.33$\pm$0.05) compared to non-responders (0.22$\pm$0.04)

Conclusions

We propose that overcoming the TGFβ neoplastic barrier promotes genomically unstable, cold cancers primed for ICI response. The integration of βAlt and TeIS identifies an unexpected patient population who may benefit from ICI.

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Abstracts

Figure 1. TeIS PanCancer Analysis

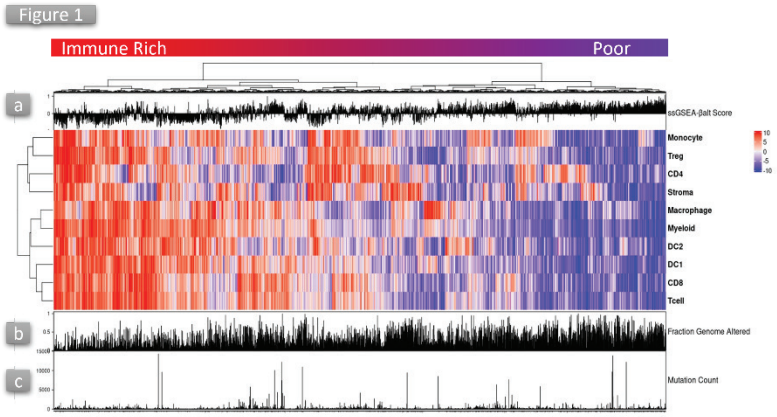
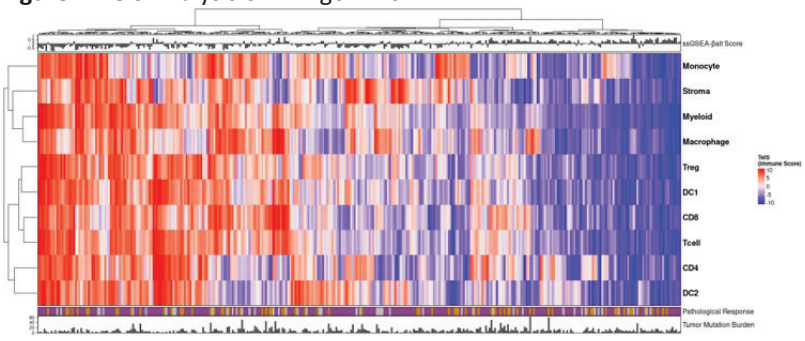


Figure 2. TeIS Analysis of IMvigor 210



49

Distinct microbial communities play a role in metastatic brain tumors

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Background
Metastatic brain tumors are associated with significant morbidity and mortality. The microbiota has emerged as a novel hallmark of cancer, with a prominent role in tumor immunity and response to treatment. However, the role of different microbial communities in tumor metastasis, and in particular brain metastasis, is poorly understood. We hypothesize that distinct microbial communities can alter the immune microenvironment in the brain and affect brain metastasis development.
Methods
To evaluate the role of different microbial communities in brain metastasis, matched stool, saliva, tumor, and plasma samples were collected prospectively from patients with metastatic brain tumors who underwent surgical tumor resection at the University of Texas MD Anderson Cancer Center. Stool and saliva samples from 30 patients and tumor and plasma from 25 patients were sequenced via metagenomic shotgun and 16S rRNA sequencing, respectively. Microbiome profiling was conducted through established computational pipelines reported previously by our group. To further explore the mechanistic role of the gut microbiota in brain metastasis, we depleted gut microbiota in conventionally raised mice using a broad-spectrum non-absorbable antibiotic regimen. Melanoma tumor cells were subsequently injected intracranially to evaluate the effect of gut microbiota depletion and associated immune changes on tumor growth. Tumor growth was measured through in vivo bioluminescent imaging and histology. Peripheral and tumor immune profiling was conducted through flow cytometry and immunohistochemistry.
Results
In all types of microbiota evaluated in this study, distinct signatures were identified to be associated with stable or progressive disease. Interestingly, we demonstrated an overlap between the tumor microbiome and the oral microbiome but not with the gut microbiome. These findings suggest a direct contribution of the oral microbiome and the potential indirect contribution of the gut microbiome to the development of brain metastasis. Our mechanistic studies on the role of gut microbiota in brain metastasis demonstrated that depletion of the gut microbiota in mice decreased tumor growth in the brain. Evaluation of the peripheral and tumor immune profiles suggested the underlying mechanisms to involve alterations in the circulating cytokine profiles and an increase in anti-tumor T cell activity.
Conclusions
Our clinical studies suggest the association of distinct microbial communities with brain metastasis. Our pre-clinical findings demonstrate that the absence of gut microbiota can modulate the regulation of T cell activity to induce an anti-tumor response in the brain. Future studies will determine the individual and collective role of different microbial communities in the development of brain metastasis.
Acknowledgements
This study was supported by the National Insitute of Health (F32CA60769).
Ethics Approval
The clinical study presented in this abstract was conducted under the approved Institutional Review Board protocols at the MD Anderson Cancer Center (2012-0441) and all participants gave informed consent to take part in this study.

Abstracts

50

DEKA-1 a dose-finding Phase 1 trial: observing safety and biomarkers using DK210 (EGFR) for inoperable locally advanced and/or metastatic EGFR+ tumors with progressive disease failing systemic therapy.

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Background
Both interleukin-2 (IL-2) and interleukin-10 (IL-10) have been extensively studied for their stimulatory function on T cells and their potential to obtain sustainable tumor control in RCC, melanoma, lung, and pancreatic cancer as monotherapy, as well as combination with PD-1 blockers, radiation, and chemotherapy. While approved, IL-2 retains significant toxicity, preventing its widespread use. The significant efforts undertaken to uncouple IL-2 toxicity from its anti-tumor function have been unsuccessful. The safety observed with PEGylated IL-10 in Phase I and II, was not repeated in a blinded Phase 3 trial.
Methods
Novel agent (Fig.1) DK2[10] (EGFR) will be evaluated in an open-label, dose-escalation (Phase 1) study at (0.025, 0.05, 0.10 and 0.20 mg/kg) monotherapy dose levels, and (expansion cohorts) in combination with PD-1 blockers, or radiation or chemotherapy in patients with advanced solid tumors overexpressing EGFR (Fig.3). Key eligibility criteria include: 1) confirmed progressive disease on at least one line of systemic treatment, 2) EGFR overexpression or amplification documented in histology reports, and 3) at least a 4-week window since last treatment or 5 half-lives, and 4) excluding subjects with multiple myeloma, multiple sclerosis, myasthenia gravis or uncontrolled infectious, psychiatric, neurologic, or cancer disease. Plasma and tissue samples will be investigated for pharmacodynamic, and predictive biomarkers and genetic signatures associated with IFNg secretion (Fig.2), aiming to select subjects for treatment in Phase 2.
Results
N/A
Conclusions
Coupling wild-type IL-2 with a high affinity IL-10 successfully decouples IL-2 toxicity from IL-2 potency, while concomitantly targeting accumulation in the tumor successfully and safely harnessing IL-2 and IL-10's known anti-cancer promise (Fig.4, 5).
Trial Registration
registration expected by 2/1/2023
Ethics Approval
approval is pending
Consent
Written informed consent will be 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

Figure 1.

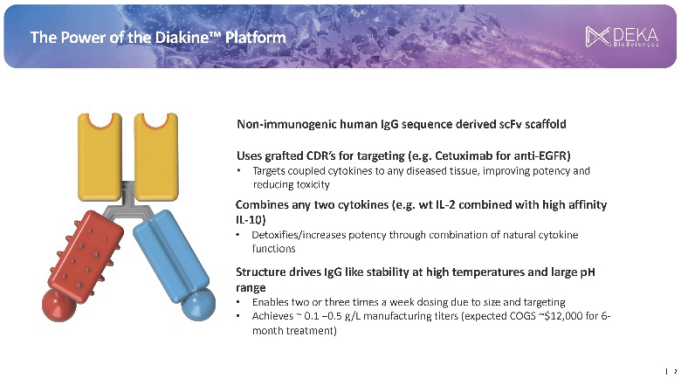

Figure 1. combining IL-2 with IL-10 using a targeted scaffold to EGFR

Figure 2.

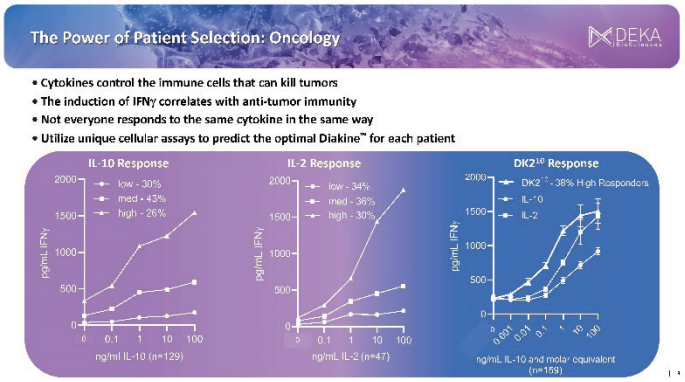

Figure 2. Patient selection based upon IFNg secretion, predictive for clinical result is expected to serve in validation of biomarker diagnostic test for Phase 2

Figure 3.

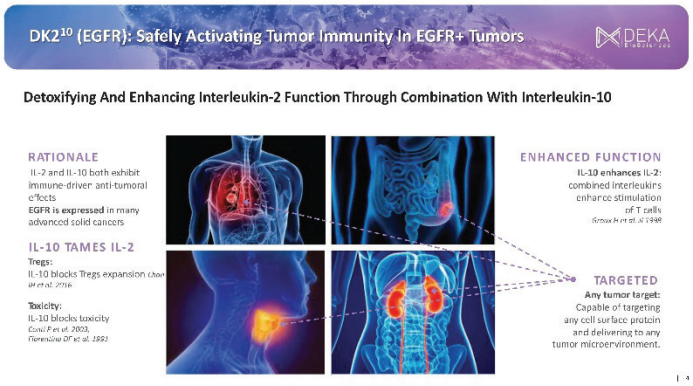

Figure 3. Bringing knowledge of clinical use of IL-2 and IL-10 together with targeted approach created new treatment strategy

Figure 4.

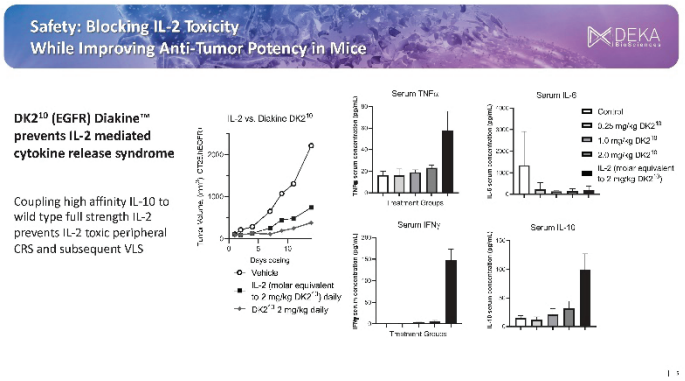

Figure 4. Combining IL-2 with IL-10 in the same molecule takes away the toxic inflammation while conserving anti-tumor effects

Figure 5.

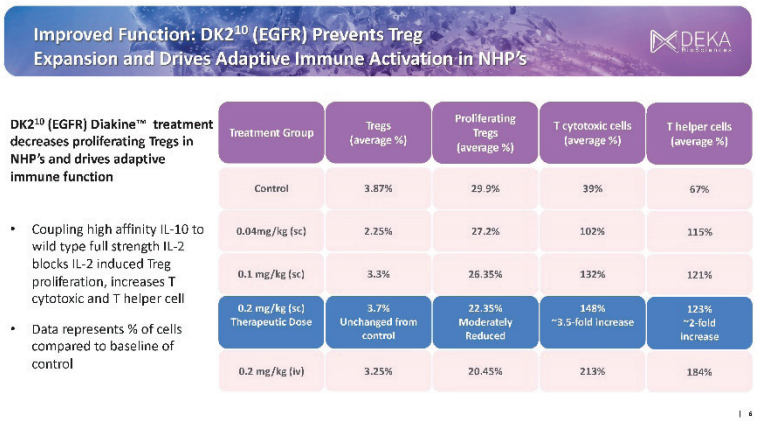

Figure 5. DK2-10(EGFR) does stimulate proliferation of cytotoxic T cells, without up regulation of regulator T cells, responsible for treatment resistance as observed in IL-2 mono therapy

51

The carry-over effect of immune-checkpoint inhibitors (ICIs) in microsatellite instability-high (MSI-H) metastatic colorectal cancer (mCRC): tumor response dynamics as predictive factors for post-progression survival

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[3]Istituto Oncologico Veneto, Padova, Italy
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Background
ICIs changed the therapeutic algorithm of MSI-H mCRC, often leading to durable responses and long-term survival. However, it is unknown where there is a "carry-over" effect on survival beyond progressive disease (PD). We therefore focused on patients experiencing PD on ICI, analyzing post-progression survival (PPS) and its association with clinical features including ICI response dynamics.
Methods
We collected real world data from 583 MSI-H mCRC patients from tertiary cancer centers worldwide. The Kaplan-Meier method and Cox proportional-hazards regression models were used for survival analyses. A maximally selected statistics method in a Cox regression model for progression-free survival (PFS) was used to determine the optimal cut-offs for and depth of response (DpR), while we considered any tumor reduction >0% at 8-9 weeks as early tumor shrinkage (ETS). Time to progression (TTP) was calculated as the time elapsed from ICI start to PD.
Results
After a median follow-up time of 31.9 months (IQR 29.0-56.3), 126 patients experienced PD during ICI. Notably, majority of patients (79.4%) had been treated with anti-PD1 monotherapy and generally received ICI(s) in later treatment lines (32,5% as 2nd line and 46% as ³3rd line), while only 21.4% received them as 1st line therapy. Regarding the association of RECIST1.1 best response and PPS, there was no significant difference in terms of PPS in patients with partial response (PR) vs stable disease (SD): HR 1.45, 95% CI 0.73-2.86, p=0.29), differently from early PD (HR 2.04, 95% CI 1.07-3.87, p=0.03 vs PR). We subsequently excluded this latest subset, and focused on patients with initial clinical benefit and acquired resistance to ICI (N=63). In this subgroup, PPS was significantly associated with both DpR ³50% (HR 0.26, 95% CI 0.09-0.73, p=0.01) and TTP ³8.0 months (HR 0.42, 95% CI 0.21-0.85, p=0.02) (Figure 1). The association was not significant for ETS (HR 0.53, 95% CI 0.25-1.11, p=0.09). No other baseline tumor- or patient-related factors - except for ECOG PS - showed a significant impact on PPS.
Conclusions
According to our proposed model, the extent of cytoreduction and duration of disease control may impact patients' survival after PD to ICIs. These intriguing results should be validated in RCTs with an ICI-free arm.
Ethics Approval
INT 117/15

Abstracts

Figure 1.

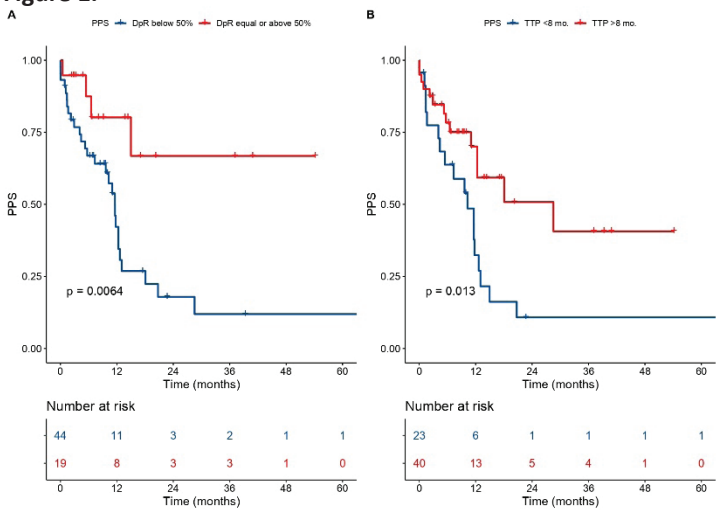

Figure 1. A: Association of depth of response (DpR) and post-progression survival (PPS) in previously ICI-treated patients. B: Association of time to progression (TTP) and PPS in previously ICI-treated patients.

52

Signal regulatory protein beta 2 is a novel positive regulator of innate anticancer immunity

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Background
In recent years, therapeutic (re)activation of innate anticancer immunity has gained prominence, with therapeutic blocking of Signal Regulatory Protein (SIRP)-α interaction with its ligand CD47 yielding complete responses in refractory and relapsed B cell lymphoma patients. SIRP-α has a crucial inhibitory role on phagocytes, thereby enabling the escape of cancer cells from immune surveillance. SIRP-α belongs to a family of paired receptors comprised of immune-inhibitory, but also putative immune-stimulatory receptors such as SIRP-beta 2 (SIRP-β2), an unreported family member. Here, we delineated the expression pattern, immunostimulatory signaling pathways and biological role of SIRP-β2 in innate immune responses against cancer cells *in vitro*.

Methods
Flow cytometry was used to determine expression of SIRP-β2 on innate immune cells. To study the function of SIRP-β2, SIRP-β2 or SIRP-β2.K202L (a mutant unable to interact with DAP12) were ectopically expressed in THP-1, HL-60 and cord-blood derived macrophages by lentiviral transduction. Functions investigated were: impact on cell adhesion, impact on CD11b expression (flow cytometry), impact of phagocytosis or trogocytosis of cancer cells (flow cytometry) and impact on T cell activation. The latter was studied by incubating a Jurkat.NFAT.luciferase reporter line that expressed a HPV-16 E7 T cell receptor with macrophages pulsed with cognate E7 peptide. Potential interaction partners of SIRP-β2 were investigated by GFP immunoprecipitation of GFP-tagged SIRP-β2 or control GFP-expressing THP-1 cells followed by mass spectrometry.

Results
SIRP-β2 was expressed at protein level under normal physiological conditions in a monocytes, macrophages and granulocytes. Furthermore, endogenous expression of SIRP-β2 on granulocytes correlated with trogocytosis of cancer cells. Ectopic expression of SIRP-β2 stimulated macrophage differentiation, adhesion, and cancer cell phagocytosis. These processes were regulated by immune activating adaptor protein DAP12, with DAP12 associating with SIRP-β2 upon immunoprecipitation. Furthermore, mutation of the charged lysine in SIRP-β2 responsible for DAP12 interaction abrogated pro-adhesive activity, differentiation and phagocytosis induction of cancer cells. Finally, ectopic expression of SIRP-β2 upregulated MHC-I and MHC-II expression and increased T cell activation, with SIRPB2 appearing to directly interact with MHC-I upon immunoprecipitation. Notably, the effect on T cell activation was independent of DAP12 signaling.

Conclusions
In conclusion, SIRP-β2 is a novel positive regulator of innate anticancer immunity and a potential costimulatory target for innate immunotherapy.

Ethics Approval
All blood donors gave informed consent (Sanquin Blood Supply, Groningen, the Netherlands)

53

Tumour microenvironment-mediated metabolic shifts in colorectal cancer regulate PD-L1 expression

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Background
Immune checkpoint blockade (ICB) for treatment of colorectal cancer (CRC) has been approved for clinical use since 2017. However, the approval of PD-L1 inhibitor for CRC has been encumbered by the lack of durable clinical benefits. Increasing evidence from other cancer types support that PD-L1 expression correlates to the efficacy of ICB [1, 2]. Concomitantly, many studies have also demonstrated the infrequent expression of PD-L1 in CRC cells [3, 4], thereby alluding to the possibility of low/absent PD-L1 expression as a primary obstacle to ICB efficacy. Given the integral role of tumour microenvironment (TME) in shaping the tumour's natural history, we sought to elucidate the role of tumour stroma in CRC PD-L1 expression.
Methods
To explore the role of different cell types in the TME on CRC PD-L1 expression, cocultures between major cell compartments of the TME and CRC cell lines were performed. Further various omics approaches were utilised to characterise the crosstalk between CRC and stromal cells that saw significant suppression of CRC PD-L1 expression.
Results
Coculture experiments preliminarily identified that CRC PD-L1 expression is regulated by: 1) CRC metabolic shift from glycolytic to oxidative phosphorylation in response to harsh metabolic competition and 2) macrophagic secretome.
Conclusions
We have identified potential cell populations and mechanisms which may explain, in part, low/absent CRC PD-L1 expression. This can thus serve to evaluate appropriate external manipulations that can raise CRC PD-L1 and go in tandem with anti-PD-L1 ICB to improve its clinical efficacy and durability.
Acknowledgements
Aik Seng Ng is supported by the National Science Scholarship from the Agency for Science, Technology, and Research, Singapore.

54

Therapeutic efficacy of a Listeria-based vaccine targeting interferon-stimulated gene 15 (ISG15) in colorectal cancer

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Background
Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in both men and women in the United States. While checkpoint inhibitor (ICI) is demonstrating remarkable clinical responses, resistance and immune-related toxicities associated with ICIs ignite the need to find other forms of immunotherapy for CRC patients. Cancer vaccines represent a safe and promising treatment approach for CRC. However, the current most mature tumor-associated antigen (TAA)-based cancer vaccines in CRC are not demonstrating satisfactory results. We proposed that interferon-stimulated gene 15 (ISG15) can be used as a novel TAA and therapeutic target for CRC. Our work demonstrated the anti-tumor efficacy of a Listeria-based vaccine targeting ISG15, designated Lm-LLO-ISG15, in the syngeneic tumor model of CRC.

Methods
For subcutaneous studies, 1×10^5 MC38 cells were implanted subcutaneously (s.c.) into the right hind flank, and mice were subsequently treated i.p. on days 5 and 12 with either PBS or Lm-LLO-ISG15. For orthotopic studies, 1×10^5 MC38 cells expressing luciferase (MC38-Luc) were implanted directly intra-cecally as previously described, and mice were subsequently treated i.p. on day 10, 17, and 24 with PBS or Lm-LLO-ISG15. All mouse experiments were performed in accordance with the regulations of the Institutional Animal Care and Use Committee (IACUC) at the TTUHSC. For flow cytometry analysis, tumor tissues were harvested to prepare single-cell suspensions and stained with appropriate fluorochrome-conjugated anti-mouse monoclonal antibodies. Data were acquired on BD Fortessa and analyzed with FlowJo software version 10.7.0. All statistical analysis was done with Prism 8 GraphPad software version 8.3.0., using the unpaired student t-test.

Results
ISG15 could be a novel TAA that potentially provided a therapeutic target in the treatment of CRC. We demonstrated for the first time the proof of concept that vaccination with Lm-LLO-ISG15 significantly controlled CRC tumor burden in both subcutaneous and orthotopic syngeneic CRC mouse models. The therapeutic efficacy of Lm-LLO-ISG15 was mediated by robust ISG15-specific anti-tumor immune responses. Further, we illustrated that the therapeutic efficacy of Lm-LLO-ISG15 was associated with an increased influx of functional T cells, higher production of multiple intracellular cytokines response, a lower number of regulatory T cells, and a greater ratio of effector to regulatory T cells (Teff/Treg) in the tumor microenvironment.

Conclusions
Vaccination with Lm-LLO-ISG15 significantly controlled CRC tumor burden in both subcutaneous and orthotopic models, as compared to that of the untreated group. Our results were consistent with previous studies evaluating the anti-tumor efficacy of Lm-LLO-ISG15 in breast cancer and renal cell carcinoma.

Abstracts

Figure 1. Expression and prognostic signature of ISG15 in human CRC

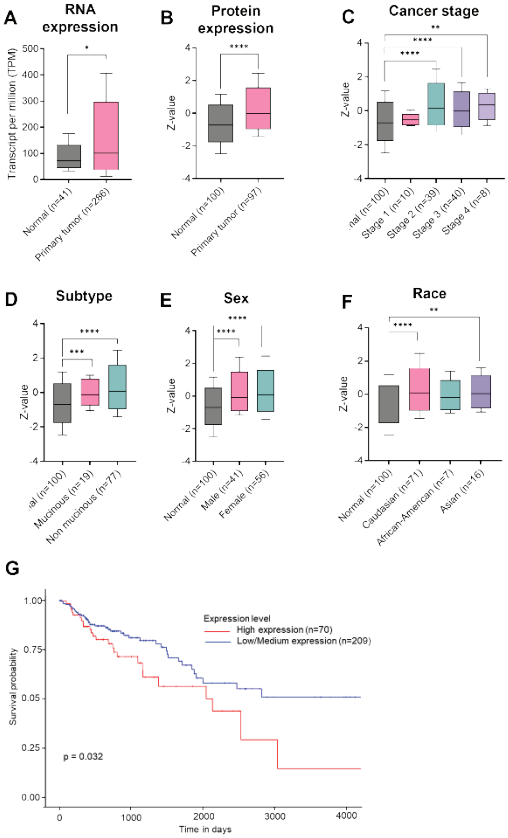

Figure 1. Gene expression (A) and protein expression (B) of ISG15 in normal colon tissues versus primary tumors in the overall population. The protein level of ISG15 in different cancer stages (C), CRC subtypes (D), genders (E), and races (F). (G) Survival probabilities in CRC patients with high versus low/medium ISG15 mRNA expression.

Abstracts

Figure 2. Therapeutic efficacy of Lm-LLO-ISG15 in CRC

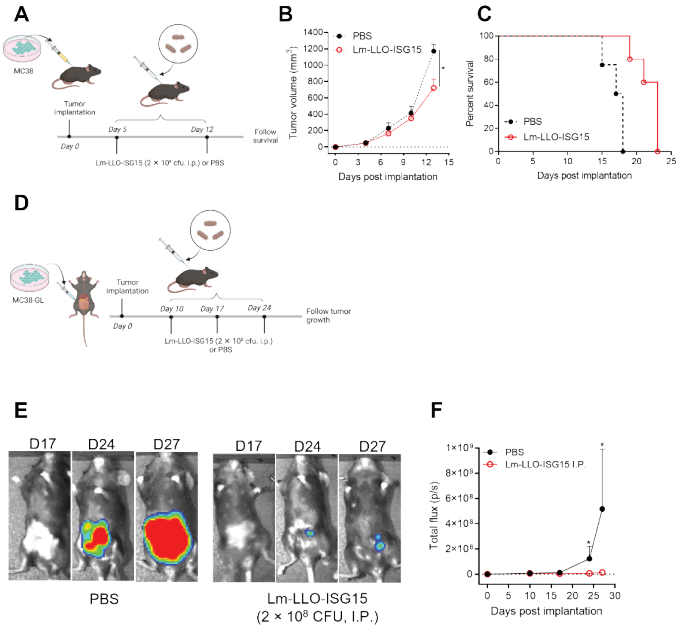

Figure 2. (A) Experimental schema in subcutaneous tumor model (made by BioRender). (B) Tumor growth kinetics and (C) survival probability of subcutaneous MC38 tumors treated with PBS vs. Lm-LLO-ISG15 (17.5 vs 23 days, respectively, $p<0.05$). (D) The experimental schema in the orthotopic tumor model (made by BioRender). (E) Representatives of each group on days 17, 24, and 27. (F) Tumor growth kinetics from D

Figure 3. Immune response followed Lm-LLO-ISG15 treatment

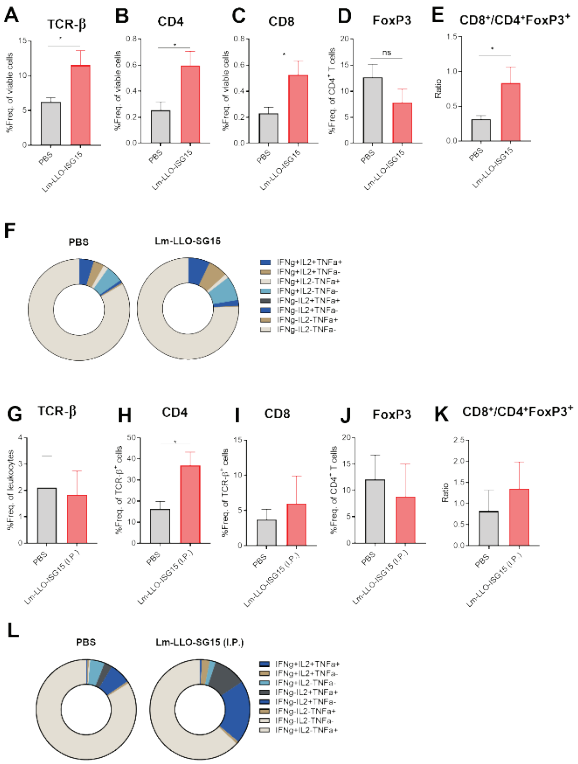

Figure 3. (A-F) Tumors from 2A were harvested, dissociated to single cells, and subjected to multicolor flow cytometry. Frequencies of live (D) TCR-β+, (E) CD4+, (F) CD8+, (G) CD4+FoxP3+, and (H) Ratio of CD8+/ CD4+FoxP3+. (I) Distribution of multi-cytokine produced by live CD8+ T cells. (G-L) Tumors from 2D were harvested, dissociated to single cells, and subjected to multicolor flow cytometry. Frequencies of live (G) TCR-β+, (H) CD4+, (I) CD8+, (J) CD4+FoxP3+, and (K) Ratio of CD8+/ CD4+-FoxP3+. (L) Distribution of multi-cytokine produced by live CD8+ T cells.

55

Low dose but not high dose RRx-001 repolarizes tumor associated macrophages from an M2 to M1 phenotype in colorectal cancer

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[2]EpicentRx Inc, La Jolla, CA, United States
[3]EpicentRx, Mountain View, CA, United States

Background
The presence of tumor associated macrophages (TAMs), one of the most abundant stromal cell types, is a poor prognostic indicator that correlates with tumor progression and therapeutic resistance. Macrophages are broadly classified into two subgroups: pro-inflammatory M1 and anti-inflammatory M2. In general, TAMs express an M2 phenotype. RRx-001 is a small molecule in Phase 3 for the treatment of small cell lung cancer, in Phase 2/3 for the treatment of mucositis in head and neck cancer, and under development in colorectal cancer, and as a medical countermeasure for a nuclear emergency. Its cytotoxic activity in cancer, which differs from its NLRP3-mediated protective effects on normal cells, is related to macrophage repolarization, vascular normalization, nitric oxide donation under hypoxia, and epigenetic modulation. The present study assessed whether RRx-001 reversed M2 macrophage polarization through epigenetic inhibition since HDAC inhibitors are associated with M1 macrophage polarization
Methods
The present study assessed whether RRx-001 reversed M2 macrophage polarization through histone deacetylase (HDAC) inhibition. Macrophages derived from the THP-1 monocytic cell line and peripheral blood mononuclear cell (PBMC)-derived macrophages were cultured with colorectal cancer cells and RRx-001, at a concentration that did not affect cell proliferation.
Results
Analysis of HDAC gene analysis by real time PCR in CACO2 and HT29 cells both in vitro and in vivo revealed that HDAC2 was significantly increased in M2 macrophages. RRx-001 at both low doses (1 mg/kg) and high doses (10 mg/kg) significantly reduced the tumor burden in two mouse models of colorectal cancer. Mechanistically, only low dose RRx-001 (1 mg/kg) suppressed HDAC2 and led to upregulation of M1 markers such as TNFa, IL8, IL6, and IL12 and downregulation of M2 markers such as IL10, and CD206. The anticancer effect of RRx-001 was abrogated with TMA depletion by clodronate liposomes.
Conclusions
RRx-001 is an NLRP3 inhibitor, which in tumors only, and at low doses only, repolarizes TAMs from an M2 to an M1 phenotype via epigenetic inhibition, suggesting that low-dose RRx-001 is preferable for the treatment of cancer

57

Treatment with metronidazole improves response and reduces toxicity to treatment with immune checkpoint blockade

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Background
Immune checkpoint blockade (ICB) therapy transformed clinical oncology by inducing durable responses in many types of cancers. However, only a subset of patients benefit from ICB. Recent studies suggest that the gut microbiome may act as a therapeutic target for ICB response, and that treatment with broad spectrum antibiotics abrogates response. However, targeted antibiotic approaches have not been well-studied, and could represent a tangible near-term treatment to improve outcomes to treatment with ICB for cancer.

Methods
We hypothesized that treatment with a targeted antibiotic would improve outcomes to ICB by selectively rebalancing deleterious and beneficial microbial taxa within the gut microbiome. To study this, we first assessed the impact of metronidazole on ICB treatment response in pre-clinical models. Mice were treated with oral gavage of metronidazole prior to MC38 tumor cell injection, with metronidazole maintained in the drinking water. Mice were then treated with ICB (aCTLA-4 or aPD-1), and tumor growth, immune responses and microbiome profiles were analyzed. Importantly, we also assessed survival in patients with solid tumors who received targeted (metronidazole) or beta lactam-based antibiotics within 60 days of treatment with ICB (aCTLA-4).

Results
In our pre-clinical studies, treatment with metronidazole in combination with ICB was associated with suppressed tumor growth compared to treatment with ICB alone (p=0.0013). Immune profiling via flow cytometry demonstrated an increase in the number of type 1 conventional dendritic cells (cDC1s) in the colon of metronidazole-treated mice, suggestive of enhanced antigen presentation. Additionally, we detected upregulation of CD80 and CD86 co-stimulatory molecule expression on cDC1s, implying superior T cell priming. Whole genome sequencing analysis of fecal samples revealed mice treated with metronidazole have increased abundance of bacterial taxa previously associated with positive ICB response, including *Bifidobacterium* ($\log_2$ fold-change 4.1, p=0.00001). To complement these studies, we assessed a cohort of patients at MD Anderson treated with metronidazole versus beta lactam-based antibiotics prior to ICB treatment. We found that patients who received metronidazole prior to ICB had improved survival compared to patients treated with ICB alone (median overall survival [OS] undefined vs. 20 months, p=0.0096), whereas patients who received beta lactam-based antibiotics had worse outcomes (9.7 months, p=0.0567).

Conclusions
Treatment with metronidazole prior to ICB is associated with improved survival in pre-clinical models and in an exploratory cohort of patients treated at MD Anderson. Further studies are underway to elucidate the mechanism behind these findings, with opportunities to translate these findings in clinical trials.

58

Lactic acid uptake through MCT11 enforces dysfunction in terminally exhausted T cells

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Background

Upon infiltrating tumors, $CD8^+$ T cells experiencing persistent antigen stimulation differentiate into a state of dysfunction, known as exhaustion. Terminally exhausted T cells (Tex) are characterized by the upregulation of co-inhibitory molecules and reduced effector cytokine production. Additionally, Tex cells exist in a state of metabolic dysfunction in the tumor microenvironment (TME), due to disrupted mitochondrial biogenesis, hypoxia and a lack of metabolites. Highly glycolytic tumor cells outcompete T cells for glucose, and secrete lactic acid into the TME, acidifying the extracellular space. Recent studies have shown that lactate can be incorporated into the TCA cycle by $CD8^+$ T cells and that it can be utilized in the TME as a fuel source by regulatory T cells and macrophages. We hypothesized that $CD8^+$ tumor-infiltrating lymphocytes (TIL) may take also up lactate as an alternative carbon source to meet their metabolic demands in the TME.

Methods

T cells differentiating to exhaustion in B16 melanoma were sequenced by low input deep RNAseq. For lactate uptake experiments, FACS sorted TILs were cultured in the presence of 14[C]-lactic acid for 6 hours, and then measured for their ability to oxidize lactic acid to CO_2.

Results

RNA sequencing and flow cytometry data of $CD8^+$ T cell from the TME revealed MCT11 (a monocarboxylate transporter encoded by *Slc16a11*), to be highly and uniquely expressed in Tex cells. As lactic acid is a tumor abundant monocarboxylate, we asked whether MCT11 supports lactate uptake into Tex cells. Culturing FACS sorted Tex cells in 14[C]-lactate revealed that these cells had increased capacity of oxidizing lactatic acid than draining lymph node $CD8^+$ T cells and progenitor exhausted T cells (Pex). Genetic and antibody blockade of MCT11 resulted in reduced 14[C]-lactate oxidation by Tex cells, but it remained unclear if lactic acid promoted or inhibited effector function. Tumor bearing mice with a conditional knockout of MCT11 in T cells ($Slc16a11^{f/f}$x$CD4^{cre}$) had an increase in CD8+ TIL in the tumor, increased production of TNFα and IFN?? production by $CD8^+$ TIL, and decreased tumor burden in mice. As MCT11's uptake function was blocked with an antibody, we used the antibody therapeutically in tumor bearing mice, revealing that single-agent MCT11 antibody therapy led to complete response (CR) in 40% of mice bearing MEER tumors.

Conclusions

Our data suggest MCT11 could be deleted on therapeutic T cells or blocked on endogenous T cells to render exhausted T cells impervious to lactic acid such they can mediate tumor eradication.

59

Androgen deprivation therapy allows for effective anti-TIGIT immunotherapy in murine model of castration resistant prostate cancer.

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Background
Prostate cancer is the second leading cause of cancer associated mortality in men, and for reasons that remain unclear, immunotherapy treatments have largely failed in patients with advanced prostate cancer. Standard of care therapy for these patients relies on androgen deprivation therapies (ADT) to block testosterone production combined with small molecules that inhibit androgen synthesis and/or the function of the androgen receptor (AR). Importantly, androgens are reported to be immunosuppressive; therefore, we postulated that androgen axis blockade might promote response to immunotherapy.
Methods
We performed single cell RNA sequencing (scRNA-seq) on cells isolated from eight individual metastatic tumor lesions from men who had biochemical or radiographic progression on enzalutamide prior to treatment with pembrolizumab. Biopsies were obtained and tumor infiltrating leukocytes (TIL) sorted. We merged cells from all patients and clustered the data into tumor cells and major lymphoid and myeloid immune cell subsets. We then looked at inhibitory receptor expression in the different clusters to identify which are expressed on TIL in these prostate cancer patients, and identified TIGIT as the most highly expressed inhibitory receptor.
We then used a mouse model of castration resistant prostate cancer, *PB-Cre*$^{+}$*;Pten*$^{L/L}$*;p53*$^{L/L}$*;Smad4*$^{L/L}$, referred to as PPSM, and confirmed the expression of TIGIT on TILs in this model. Finally, we treated PPSM tumor bearing animals with a combination of ADT (degarelix + enzalutamide) and anti-TIGIT and monitored tumor growth.
Results
We observed that TIGIT is highly expressed on tumor infiltrating T cells and NK cells in metastatic castration resistant prostate cancer patients treated with the AR antagonist enzalutamide. Likewise, in our mouse model of CRPC, we show that tumor infiltrating T cells express TIGIT. In this model, neither treatment with anti-TIGIT alone nor with ADT affects tumor growth. Remarkably, the combination of anti-TIGIT with ADT leads to delayed tumor growth and long-term cures in about 50% of the animals.
Conclusions
Taken together, our data suggest that androgen receptor blockade sensitizes immune cells in the tumor to respond to anti-TIGIT immunotherapy.
Ethics Approval
Metastatic castration resistant prostate cancer patients enrolled on clinical trial NCT02312557 underwent biopsy of a metastatic lesion at Oregon Health & Science University (Portland, OR). All patients had progressive disease on enzalutamide. All human investigations were carried out after approval by a local Human Investigations Committee and in accord with an assurance filed with and approved by the Department of Health and Human Services. Data has been anonymized to protect the privacy of the participants. Investigators obtained informed consent from each participant.
All animals were maintained under specific pathogen-free conditions in the Oregon Health & Science University (Portland, OR) animal facility. All animal experiments were approved by the Institutional Animal Care and Use Committee of OHSU.

60

A blood based qPCR assay is prognostic for immunotherapy in advanced stage non-small cell lung cancer

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Background
Immunotherapies have revolutionized the treatment options for a multitude of late-stage cancers, however their efficacy is notoriously unpredictable. Across a range of solid tumours, a positive response to immunotherapy is observed in an average of only 30% of patients [1]. There is widespread recognition and demand for better biomarkers to guide immunotherapy in NSCLC [2]. We have previously described the *miRisk* score that is predictive for immunotherapy efficacy in advanced stage NSCLC with PD-L1 >50% [3,4]. The *miRisk* score consists of the measurement of predominantly myeloid derived miRNAs from peripheral blood to capture host immune dynamics that influence the effect of immunotherapy. In order to translate this work into a clinically applicable test, we report results in the transition of this biomarker from an NGS to qPCR platform.
Methods
A pre-spotted qPCR plate was designed with custom miRCury LNA miRNA PCR assays (Qiagen), based upon the 44 OS associated miRNAs and 7 housekeeping miRNAs previously discovered via NGS [3]. Samples were measured in triplicate and normalized miRNA expression was calculated by subtracting the mean of the Ct values of housekeeping miRNAs. Elastic Net penalized, multivariable, Cox proportional hazards models were fit to miRNA expression data with the package scikit-survival (version 0.15.1). A previously described blood cell type, miRNA expression atlas was used to deconvolve the signature miRNAs into their originating cell types [3].
Results
miRNA expression from 64 patients with advanced stage NSCLC and high PD-L1 expression, treated with immunotherapy, in the training cohort were used to fit a Cox proportional hazards model predictive of OS. 14 miRNA expression features were selected with non-zero model coefficients. Patient stratification into low/high risk groups based on the median score in the training cohort revealed significantly shorter OS in the high-risk group (HR 4.15, 95% CI 2.00–8.61; $P < 0.01$) with generalized performance observed in the 52 patients in the validation cohort (HR 4.43, 95% CI 1.48–13.29; $P < 0.01$). Amongst the 14 selected features, we observed a significant over-representation of miRNAs from neutrophils and thrombocytes.
Conclusions
We have described a 14-miRNA, whole blood, qPCR signature that is prognostic in advanced stage NSCLC patients with high PD-L1 treated with immunotherapy, and further supports the growing evidence that information beyond the TME can predict the efficacy of immunotherapies.
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Ethics Approval
This study was approved by the Heidelberg University (S-296/2016, S-089/2019, S-916/2019) and Grosshansdorf Hospital ethics committee (AZ 12-238, AZ 19-286) and all patients provided written informed consent.

63

Multimodal single-cell analysis of human TILs across multiple tumor types reveals heterogeneity and potential opportunities for personalized immunotherapy

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Background
Immune checkpoint blockade (ICB) efficacy varies among tumor types likely due to differences in tumor infiltrating lymphocyte (TIL) composition and function within the tumor microenvironment (TME). To help understand these differences, we conducted multimodal single-cell analysis of TILs.
Methods
Primary human tumor specimens including non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), renal cell carcinoma (RCC) and breast cancer (BrCa) (n=48) were analyzed by flow cytometry, single-cell analysis including single-cell RNA sequencing (scRNA-seq), CITE-seq (oligo-tagged antibodies), and scTCR-seq (10x Genomics), bulk RNA-seq, and following in vitro restimulation to assess their functional status.
Results
We found that regulatory T cell (Treg) frequency was higher in HNSCC, whereas exhausted T cells (Tex) were higher in NSCLC and RCC. In contrast to other tumor types, Tex in RCC lacked the expression of CD103, a hallmark of tissue-resident T cells. On the other hand, expression of PD-1, TIM-3, and LAG-3 were more prominent in Tex in RCC. Interestingly, Tex in HNSCC showed higher expression of TIGIT than other tumor types. Previous work has demonstrated an increased presence of CD4+CD8+ double-positive T cells (DPT) in RCC, which was associated with better overall survival. Therefore, we used CITE-seq to identify DPT and then compared the composition of DPT among different tumor types. DPT were CD39+, a marker for tumor-reactive T cells, and the vast majority were transcriptionally categorized as CD8+ T cells in RCC, whereas DPT in other tumor types are mixture of CD4+ or CD8+ T cell subsets. We also found overlap of TCR profiles between DPT and CD8+ T cell subsets (Tex, ZNF683-CD8, and GZMK-CD8) in RCC.
Conclusions
Together, multimodal single-cell analysis of TILs highlighted heterogeneity among tumor types that may provide insight into novel strategies to treat cancer.
Ethics Approval
Fresh tumor samples were collected at Providence Cancer Institute (Portland, OR) under an IRB-approved protocol (#06-108) and with prior patient consent.

64

Trial in progress: a phase 1-2, first-in-human, open label, dose escalation and expansion study of AdAPT-001, an oncolytic adenovirus that expresses a TGF-β trap, which binds to TGF-β and inhibits TGF-β receptor binding, in patients with advanced solid tumors

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Background
Checkpoint blockade revolutionized cancer therapy for selected patients but often fails to benefit patients with cold, poorly immunogenic tumors. Oncolytic adenoviruses (OAVs) naturally induce inflammation and tumor specific immune responses through infection and oncolysis, which is especially important for patients with immune desert or excluded tumors. Data from preclinical models has demonstrated that blockade of TGF-β signaling yields additive or synergistic anti-tumor responses in combination with immune-checkpoint therapies. AdAPT-001 is an OAV, engineered to express a TGF-β trap that binds to TGF-β and prevents its activation of TGF-β receptors. Importantly, virally encoded TGF-β inhibition is local, and, therefore, unlikely to carry the toxicity associated with systemic routes. In a Phase 1/2 trial called BETA PRIME (NCT04673942), intratumorally injected AdAPT-001 with or without systemically administered checkpoint inhibitors is under study in patients with advanced treatment refractory cancers.
Methods
BETA PRIME is an open-label, non-randomized, dose-escalation three-part study where AdAPT-001 is evaluated in Phase 1 as A) single dose monotherapy B) multiple dose monotherapy until progression (or beyond progression if immune pseudoprogression is suspected) and C) in a Phase 2 expansion cohort with multiple AdAPT-001 doses or a combination of AdAPT-001 and a checkpoint inhibitor. The phase 1 explores ascending doses of intratumoral AdAPT-001 monotherapy in 28-day cycles to identify the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D). A preplanned extension (pilot expansion) study with checkpoint inhibitors has been initiated based on predefined positive efficacy signals in Parts 1 and 2. Patient inclusion criteria specifies any treatment-refractory solid tumor histology which is injectable with or without ultrasound and which is local or metastatic. Adverse events are graded by the Common Terminology Criteria for Adverse Events v5. Safety findings are reviewed by the DRC, which determined the RP2D and MTD. Efficacy is based on pharmacodynamic markers of immune stimulation, and objective responses according to RECIST v. 1.1 criteria. Paired pre / on-treatment biopsies are optional. For the Part 3 expansion cohort, patients may receive multiple doses of AdAPT-001 until progression if not checkpoint inhibitor eligible or multiple doses of AdAPT-001 with a checkpoint inhibitor if a checkpoint inhibitor is indicated for their tumor type.
Results
N/A
Conclusions
N/A
Trial Registration
NCT04673942

65

Lymph node colonization promotes distant tumor metastasis through the induction of tumor-specific immune tolerance

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Background
The majority of cancer-associated deaths result from distant organ metastasis, yet the mechanisms that enable this process remain poorly understood. For most solid tumors, colonization of regional or distant lymph nodes (LNs) typically precedes the formation of distant organ metastases, yet it remains unclear whether LN metastasis plays a functional role in disease progression.

Methods
Using an *in vivo* passaging approach of a non-metastatic syngeneic melanoma, we generated 300 unique cell lines exhibiting varying degrees of LN metastatic capacity. We subjected these lines to RNA-seq, ATAC-seq, and WES to identify key regulators of LN metastasis. Using *in vivo* experimental metastasis assays we evaluate their capacity to promote distant metastasis to the lungs. Using depletion studies, knockout mice, mice bearing conditional alleles, and adoptive transfer studies, we interrogated the cellular and molecular mechanisms of tolerance induction.

Results
We show that the presence of these LN metastases promotes distant organ seeding of metastases in a tumor specific manner, and this effect is eliminated in mice lacking an adaptive immune response. Using flow cytometry and single-cell sequencing, we identify multiple cellular mediators of tolerance. We find that LN metastases have the capacity to both resist NK cell cytotoxicity and induce regulatory T cells (Tregs). Furthermore, depletion of NK cells *in vivo* enables non-metastatic tumors to disseminate to LNs, and ablation of Tregs using FoxP3-DTR mice inhibits the promotion of distant metastases. Adoptive transfer of Tregs from the LNs of mice bearing LN metastases to naïve mice facilitates metastasis in a manner that Tregs from mice without LN metastases cannot, and we find that these Tregs are induced in an antigen-specific manner. Whole exome sequencing revealed that neither the metastatic proclivity nor immunosuppression evolve through the acquisition of driver mutations, loss of neoantigens, loss of MHC class I presentation, or decreases in melanoma antigen expression. Rather, by RNA-seq and ATAC-seq, we show that a conserved interferon signaling axis is upregulated in LN metastases and is rendered stable through epigenetic reprogramming of chromatin accessibility resulting from chronic exposure to interferons *in vivo*. Furthermore, using CRISPR/Cas9, we find that these pathways are required for LN metastatic seeding, and validate their conserved significance in additional mouse models of pancreatic ductal adenocarcinoma and head and neck squamous cell carcinoma and humans with LN metastatic disease.

Conclusions
These findings demonstrate that LN colonization is a critical step in metastatic progression due to its role in the induction of systemic tumor-specific immune tolerance.

66

Assessing toxicity and anti-tumor activity of CD40 agonist monoclonal antibody combined with mesothelin-directed immunotoxin in a murine model of pancreatic cancer

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Background
Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a median five-year survival of just 12%. The PDAC tumor microenvironment is immunosuppressive, and patients with PDAC have decreased number and function of dendritic cells. Agonistic CD40 antibodies (CD40 mAb) stimulate dendritic cells and improve antigen presentation. These therapeutics are currently being tested in PDAC patients. PDAC frequently expresses mesothelin (MSLN), a cell surface glycoprotein present on a small number of normal tissues. LMB-100 is a recombinant immunotoxin therapeutic that uses a bacterial toxin payload to kill MSLN-expressing cells. We hypothesize CD40 agonist will stimulate dendritic cells to upregulate the antigen presentation process and LMB100 will kill tumor cells in an immunogenic fashion, releasing tumor-associated antigens.

Methods
Here, we investigate the safety and efficacy of combining LMB-100 with CD40 agonist in a murine model of PDAC. We injected human MSLN-expressing KPC-derived PDAC cells orthotopically into the pancreas of syngeneic mice and analyzed toxicity and tumor growth with various treatment regimens. Syngeneic C57Bl/6J mice were engineered to express human MSLN in the native distribution.

Results
The CD40 mAb and LMB-100 combination is well-tolerated when both drugs are administered together on Day 1, and a second dose of LMB-100 is given on Day 4. Combination treatment did not significantly reduce tumor burden or increase survival compared to single agent treatments in studies using the orthotopic model.

Conclusions
Although the results demonstrate no statistically significant benefit to the combination therapy, we are investigating the immune response to the therapy within tumor tissue. Additionally, we are now assessing the efficacy of this combination in an autochthonous murine model of PDAC expressing humanized MSLN.

Ethics Approval
All animal experiments were performed in accordance with institutional guidelines and approved by the institution Animal Care and Use Committee.

Abstracts

67

Intratumoral bacteria influence immunotherapy efficacy

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Background

Microbes live in primary tumors and metastases, making the intratumoral microbiota an intrinsic and essential component of the tumor microenvironment (TME), yet how they regulate the TME and immunotherapy response are unknown. While we do not understand intratumoral microbiota contributions to immunotherapy efficacy, studies have implicated *intestinal* microbiota. Specifically, aPD-1 responding patients harbor specific intestinal microbiota that correlate with enhanced systemic immunity and intratumoral immune infiltration. Interestingly, gastrointestinal tract microbes traffic to distant tumors, potentially seeding the intratumoral microbiota. For example, *Bifidobacteria,* a genus associated with immunotherapy efficacy, translocates from the intestine to distant tumors. We hypothesized that one mechanism by which intestinal microbiota influences immunotherapy is through their dissemination to tumors, where microbes alter the TME and provide an essential source of microbial-associated molecular patterns that activate dendritic cells.

Methods

Tumor-bearing mice (MCA-205; MOC1; SQ) received an antibiotic cocktail (Abx; ampicillin, streptomycin, colistin[1]), doxycycline (Doxy; has minimal effects on intestinal microbiota and eliminates intratumor microbiota[2]), and/or aPD-1 (200ug, 3x). In separate experiments, tumors were measured over time or harvested to investigate the differentiation of tumor infiltrating lymphocytes (TIL) and myeloid cells 7 days post-treatment. Microbiota from stool, tumors, and skin swabs were monitored by 16s rRNA gene sequencing and qPCR. Data are representative of 1-2 independent experiments (n=6/group) and statistical significance was determined as indicated.

Results

MCA-205 tumor-bearing mice treated with Abx or Doxy did not respond to aPD-1, significantly reducing survival compared to aPD-1 alone (Log-rank test, $p<0.0001$ and 0.008, respectively). 16s rRNA gene qPCR of stool samples confirmed a minimal effect of Doxy on intestinal microbiota, as stool bacterial levels were not significantly different from control (average $6x10^9$ and $1x10^{10}$ bacteria/g, respectively) and a significant effect of Abx on intestinal microbiota, where bacteria levels were below qPCR detection limit (average 5 bacteria/g). Flow cytometry of TIL revealed a significant reduction in activated ($CD80^+$) dendritic cells and absolute abundance of $CD8^+$ T cells in the Doxy+aPD-1 group compared to aPD-1 alone (ANOVA, $p<0.05$). These phenotypes were present, but not as pronounced in the Abx+aPD-1 group. 16s rRNA gene sequencing from tumor samples revealed reduced species richness after both Abx and Doxy, although changes in bacterial phylum and class were not consistent, suggesting presence of microbiota diversity or abundance may be important components for promoting dendritic cell activation.

Conclusions

An abundant and diverse intratumoral microbiota promotes aPD-1 efficacy in MCA-205 tumors by supporting dendritic cell recruitment and activation.

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69

VirMAP for Cancer: Characterization of the intratumoral virome in virally-associated cancers and a resource for investigators

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Background
A major barrier to understanding the role of viruses in cancer progression has been the lack of sensitivity of current tools to identify and assemble viral genomes in an untargeted way. Here, we utilize our novel VirMAP algorithm to identify eukaryotic and prokaryotic viruses associated with cancers, using RNA sequencing data from The Cancer Genome Atlas (TCGA).
Methods
VirMAP was used to analyze TCGA RNA-Seq data derived from primary tumor tissues, with a focus on cancers with known viral associations (cervical, ovarian, head and neck, liver, stomach and esophagus). Previously published survival data were matched with viral signatures for exploratory analysis. ANCOM-BC, a differential abundance algorithm, was utilized to screen for viruses which associated with outcome. Overall survival (OS) was assessed using the Kaplan-Meier method, and p-values were calculated using log-rank test. Multivariate analysis (MVA) was done using the Cox proportional hazards model to control for age, sex, stage, and histology.
Results
Viral sequence recovery (among 723 billion sequencing reads from 2090 patients) was at least equal to previously published algorithms such as Kraken, but with higher taxonomic resolution. Cancers with high viral loads such as cervical cancer demonstrated high recovery rates (0.012% of all sequencing reads mapped to viral taxa, followed by 0.004% for liver, 0.0018% for head and neck squamous cell, 0.0016% for stomach, 0.0009% for esophageal, and 0.0002% for ovarian). There was a wide range of viral signature richness, with cervical cancer showing the highest viral read count predominated by HPV serotypes; in contrast, stomach cancer had lower abundance but contained over 300 unique viral taxa (Figure 1).
Exploratory clinical analysis revealed novel correlations between viral signatures and OS. In cervical cancer, presence of HPV45 predicted worse survival (median OS 837 days vs 4086, p=0.0043); MVA demonstrated hazard ratio (HR) of 5.7 [2.6-11.4, p<0.0001] (Figure 2). In stomach cancer, patients often had more than one viral taxon present (mean 3.7, range 0-18); presence of seven or more unique taxa predicted worse OS (median OS 403 days vs 1153, p=0.0001). MVA showed HR of 1.1 for every additional taxon (p=0.0004).
Conclusions
VirMAP performs deep characterization of the tumor-associated virome in both high and low abundance settings, with generation of partial genome reconstructions and strain level taxonomic classification. Utilizing publicly available platforms such as TCGA, clinical and correlative published data can be leveraged to better understand the nuanced pleotropic effects of the tumor virome on cancer progression.
Ethics Approval
All of the data analyzed and presented were obtained and derived from publicly available and ethically approved data from The Cancer Genome Atlas (TCGA) project Genomic Data Commons Data Portal from the National Cancer Institute (portal.gdc.cancer.gov).

Figure 1. Abundance and richness of virome

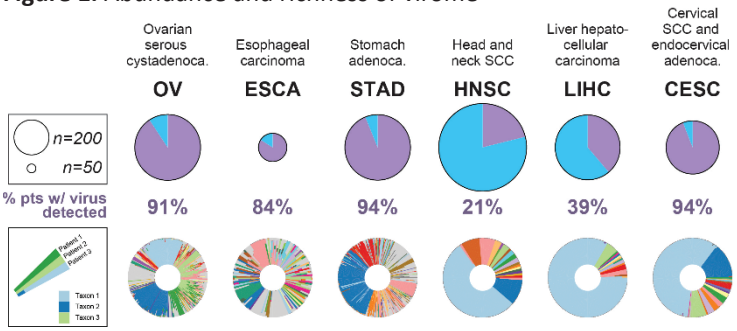

Figure 1. First row of pie charts denotes percentage of all analyzed patients who have any viral signature detected in their tumor samples; size of circle denotes number of patients in each data set. Second row denotes iris plots of viral taxa present in each patient with a viral signal; each slice represents one patient tumor, and each color denotes a specific species or serotype of virus.

Figure 2. Impact of intratumoral HPV45 in cervical cancer

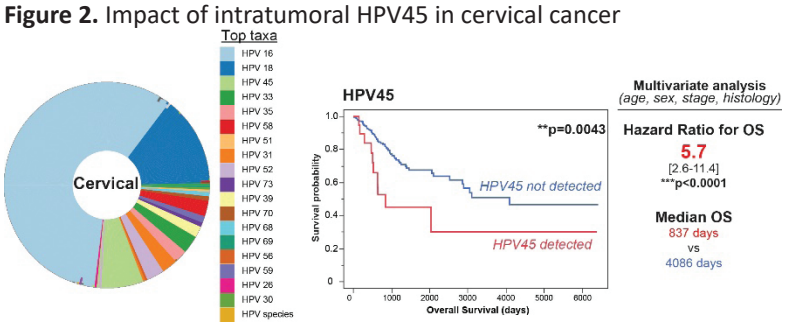

Figure 2. Iris plot denotes breakdown of intratumoral HPV serotypes present within patient tumors. Univariate analysis, denoted by Kaplan Meier curve, demonstrates significantly worse OS in patients who had HPV45 detected; correlation significant in multivariate analysis using proportional hazards model.

72

Sex-biased Yap1 function in immune suppression

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Background
Sex differences in cancer incidence and survival are well established, and recent studies have shown that this bias also extends to therapeutic responses, including immunotherapies. However, the underlying mechanisms remain poorly understood. Male sex is a well-recognized but understudied risk factor for medulloblastoma (MB), and MB incidence is consistently higher in males than in females across all subgroups and ages. MB is stratified into four distinct molecular subtypes: WNT, sonic hedgehog (SHH), Group 3, and Group 4. Genomic analyses of human MB have revealed that Yes-associated protein 1 (*YAP1)* is amplified or overexpressed in a subset of SHH MBs; however, the functional significance of this recurring genetic event in vivo is unclear. The Hippo/YAP pathway is one of the top ten most commonly dysregulated pathways in human cancers, and it is an essential signaling pathway for stem cell homeostasis and organ size control. Hence, we investigated the functional significance of *Yap1* amplification and overexpression in SHH-MB *in vivo*.

Methods
We genetically deleted *Yap1* in two different mouse models of SHH MB:*Fsmo;GFAP-cre* spontaneous model and *Ptch;p53* tumorsphere model. Mutli-dimensional in vivo and in vitro analyses including immune phenotyping, bulk RNA-sequencing, single-cell RNA-sequencing, and ChIP-seq were performed to identify direct targets of YAP1 in vivo and elucidate molecular mechanisms of YAP1 function, including cancer stem cell maintenance and immune suppression. To evaluate the clinical relevance of our findings, we also mined the TCGA datasets with our YAP1-target gene signature in multiple cancer types.

Results
Here, we report that the oncogenic function of *Yap1* is male-biased in MB progression and immune suppression. Mechanistically, *Yap1* promotes stemness and blocks the differentiation of cancer stem cells through at least two distinct but complementary molecular mechanisms that involve transcriptional regulation and modulation of protein functions of *Sox2, Atoh1, Neurod1, and Zic1/2*. While the stem cell-promoting function is equivalent in both sexes, *Yap1* deletion promotes inflammatory responses and extends survival significantly more in males than in females. YAP1 directly activates the transcription of *Csf1*, *Igfbp3, and Lsp1* and indirectly suppresses *Ccl5* transcription via *Tcf4* to generate immune suppressive microenvironment in MBs. Importantly, sex differences in *Yap1* function are evolutionarily conserved and evident in multiple human cancers.

Conclusions
We demonstrate a sex-biased function of YAP1 in mouse and human cancers and propose that oncogenic interactions with the immune system contribute to sex bias in human cancers.

73

Clinical and correlative results from a trial of in situ cancer vaccination in lymphoma

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Background
In situ vaccination aims to induce an immune response locally at one tumor site that propagates systemically to all tumor sites [1-3]. Based on prior preclinical work [4], we designed a novel clinical strategy combining *in situ* vaccination with intratumoral TLR9 agonist, systemic ibrutinib (a modulator of B and T cells), and local low-dose radiation, and here report the clinical and correlative results of this trial in adults with recurrent low-grade B cell lymphoma (NCT02927964).

Methods
Enrolled patients received intratumoral injections of CpG (SD-101, 3 mg) weekly for 5 doses and local radiation (4Gy in two fractions) to the same site. Daily oral ibrutinib (560mg) began after the second injection. Revised Lugano criteria [5] were used to assess overall radiographic responses to therapy. Distal responses were assessed by excluding the injected site. Fine needle aspirates (FNAs) were obtained from injected and non-injected nodal tumor sites pre- and post-treatment and analyzed by flow cytometry and single-cell RNA sequencing (scRNAseq). For patients with sufficient tumor tissue available, functional immune response assays were performed to measure tumor-reactive T cells.

Results
Twenty patients were evaluable for safety and efficacy. Most had follicular lymphoma, and all were previously treated. Adverse events were primarily low grade and included flu-like reactions, fatigue, diarrhea, and rash. The overall tumor response rate was 50%, including one patient with a complete response. All patients experienced tumor reduction at distant (non-injected, non-irradiated) index lesions (median reduction 45%, range 13-100%). In-depth single cell analyses revealed baseline predictors of response, treatment-induced effects, and treatment-induced correlates of clinical response. Baseline tumor cell expression of CD47, CD37, and IL4 and IL6 signaling-related transcripts portended lesser clinical responses, while treatment-induced antigen presentation by tumor cells, activation of NK and T cells, and expansion of T cell clones in the tumor associated with deeper clinical responses. Notably, treatment dampened immune suppressors such as TGFß signaling, inhibitory T regulatory 1 cells, and CD8 T cell PD1 expression. T follicular helper cells, frequently correlated with poor prognosis in B cell lymphomas, were also reduced. These reductions correlated positively with clinical outcomes. Greater increases in tumor-reactive T cells in a functional assay occured in patients with deeper clinical responses.

Conclusions
With the right combination of agents, *in situ* vaccination can generate all elements needed for *de novo* anti-tumor immune responses. Reversal of negative regulators may be as important as induction of immune effectors in producing robust immune responses and clinically meaningful tumor responses.

Trial Registration
Clinicaltrials.gov : NCT02927964

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Ethics Approval

The study protocol was approved and regulated by the Stanford Institutional Review Board and the Stanford Cancer Institute's Scientific Review and Data Safety and Monitoring committees. All investigations were performed in accordance with good clinical practice as defined in the International Conference on Harmonization guidelines and the US Code of Federal Regulations, which in turn are consistent with the Declaration of Helsinki.

Consent

All patients provided written informed consent.

74

Adrenergic receptor signaling regulates the CD40-receptor mediated anti-tumor.

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Background

Anti-CD40 agonistic antibody (αCD40), an activator of dendritic cells (DC) enhances antigen presentation and engagement with its cognate ligand (CD40L) on T cells. Although promising, systemic administration of aCD40 in patients is only moderately effective, and its efficacy depends upon the presence of an immunogenic environment and optimal immune priming. Prior research has shown that β2-adrenergic receptor (β2AR) expressed on DCs decline its functions. Herein, we determined the implications of β-adrenergic signaling on DCs activation and maturation mediated by αCD40, and whether propranolol, an FDA approved Pan-Beta blocker can improve local αCD40 *in-situ* immunotherapy of poorly immunogenic head and neck tumors (MOC2).

Methods

Unstimulated CD11c+ cells (nDCs) and MOC2 tumor cell lysate stimulated CD11c+ cells (iDCs) from C57BL/6 female mice were exposed to β2AR agonist (Isoproterenol, ISO; 1µM) and αCD40 (10ng/ml). Surface expression of MHC-II, CD86 & CD40 using flow cytometry, NFkB activation via western blot and expressed cytokine via RT-PCR & ELISA were determined. Mice bearing MOC2 xenograft in the flank region were randomized as follows: (1) Untreated control, (2) αCD40, (3) Propranolol (Prop; β-AR antagonist) & (4) αCD40+Prop. Prop (10mg/kg B.W.) was injected intraperitoneally 5 days post tumor inoculation. 30µg αCD40 was administrated intratumorally twice 8 days apart at an initial volume of ~50mm^3. 4-wks post-inoculation, tumors were profiled for immune cell dynamics using flow cytometry.

Results

β2AR signaling with ISO significantly decreased the expressions of MHC-II, CD86 and CD40 on nDCs and iDCs regardless of αCD40 treatment. In addition, αCD40+ISO decreased intracellular levels of pIkBα (NFkB activator) compared to αCD40 alone. The modification of intracellular signaling correlated with the decrease of pro-inflammatory cytokines (e.g., IL-6, IL-1b) and increase of anti-inflammatory cytokine (IL-10). In the mice model, combining αCD40 with Prop significantly suppress MOC2 tumor growth in the murine model compared to monotherapies, with the treated tumors demonstrating significantly higher infiltration of CD8+ T cells, CD8+ GZMB+ cytotoxic T cells, activated DCs (MHC-II CD86 and MHC-II CD40 double-positive DCs), and reduced populations of CD4+ FOXP+ Treg cells relative to monotherapies.

Conclusions

β-adrenergic signaling negatively impacts αCD40 induced DCs activation and maturation mediated. Combining αCD40 with propranolol reverses the β2AR signaling suppressed anti-tumor immunity, thereby providing the foundational basis for improving αCD40 immunotherapy outcomes in patients using β-blockers.

Acknowledgements

We acknowledge the National Cancer Institute of the National Institutes of Health under award number R01CA260974, and the Kerr Endowed Chair at Oklahoma State University for supporting this research.

Ethics Approval

All animal associated procedures were approved by Oklahoma State University Animal Care and Use Committee.
IACUC Protocol #VM:21-85

75

Initial assessment of utility of a serum proteomic test to predict outcomes of patients with advanced non-small cell lung cancer (NSCLC) treated in first line with immune checkpoint inhibitors (ICIs)

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Background
Tumor PD-L1 expression guides treatment for patients with advanced NSCLC without targetable genetic aberrations. However, studies have shown potential for improved outcome prediction when PD-L1 status is combined with proteomic testing characterizing host response to the tumor [1,2]. Also, criteria remain to be established for treatment selection in patients eligible for ICIs with or without platinum-based chemotherapy. The primary immune response (PIR) test was developed to stratify patients into groups with good ("Sensitive"), intermediate ("Intermediate") and poor outcomes ("Resistant") following ICI monotherapy in second-line advanced NSCLC based on mass spectrometry of pretreatment serum [1,2]. The test was associated with processes related to host response, including acute phase, acute inflammatory response, and complement activation [1].

Methods
Pretreatment serum from 105 patients with stage IV NSCLC treated in first-line with pembrolizumab alone or combined with carboplatin-based chemotherapy at the Netherlands Cancer Institute (Amsterdam, NL) or Erasmus University Medical Center (Rotterdam, NL) between 2016 and 2020 underwent testing using a version of the PIR test with improved mass spectral processing. All patients with low PD-L1 (<50%) received combination therapy (n=46). Association of outcomes with test classification was assessed using Cox models, and causal inference methods were applied to adjust for differences between patients with high PD-L1 (≥50%) assigned to mono- or combination therapy.

Results
41% of patients were classified Resistant, 42% Intermediate, and 18% Sensitive. Classification showed no association with PD-L1 status. When treated with monotherapy (n=41), patients classified as Sensitive showed a trend to better outcomes compared with those not classified as Sensitive (overall survival (OS) hazard ratio (HR) 0.28 (95% confidence interval (95%CI): 0.06-1.23)); early deaths and rapid progressions were mainly confined to the Resistant group. For patients receiving combination therapy, the test stratified OS, but not PFS for the low PD-L1 subgroup, but neither outcome for the high PD-L1 subgroup (OS HR Sensitive vs not Sensitive 1.07 (95%CI: 0.21-5.58)) (Figure 1). In multivariate and causal inference analysis adjusting for differences between treatment groups in the high PD-L1 subgroup, the benefit of addition of chemotherapy was concentrated in patients classified as Resistant (Figure 2).

Conclusions
Within the high PD-L1 subgroup, the PIR test showed potential for outcome stratification for first-line single-agent pembrolizumab treatment of advanced NSCLC, and benefit of addition of chemotherapy appeared to be concentrated in patients classified as Resistant when adjusted for differences between treatment groups in this observational study. Additional studies are necessary to validate this potential clinical utility.

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Ethics Approval
This study was approved by the Netherlands Cancer Institute Ethics Board (approval number PTC13.126) and Erasmus University Medical Center's Ethics Board (approval number NL72778.078.20).

Abstracts

Figure 1.

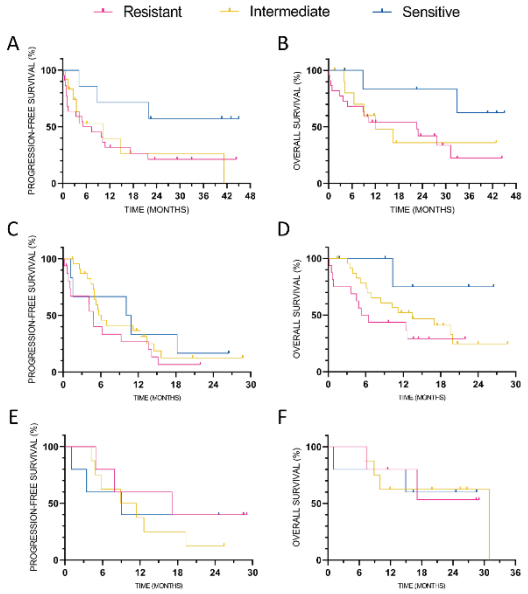

Figure 1. Kaplan-Meier plots of (A) PFS and (B) OS for patients receiving monotherapy (all high PD-L1 status; 22 Resistant, 12 Intermediate, 7 Sensitive), (C) PFS and (D) OS for low PD-L1 status patients receiving combination therapy (16 Resistant, 24, Intermediate, 6 Sensitive), (E) PFS and (F) OS for high PD-L1 status patients receiving combination therapy (n=5 Resistant, n=8 Intermediate, n=5 Sensitive).

Figure 2.

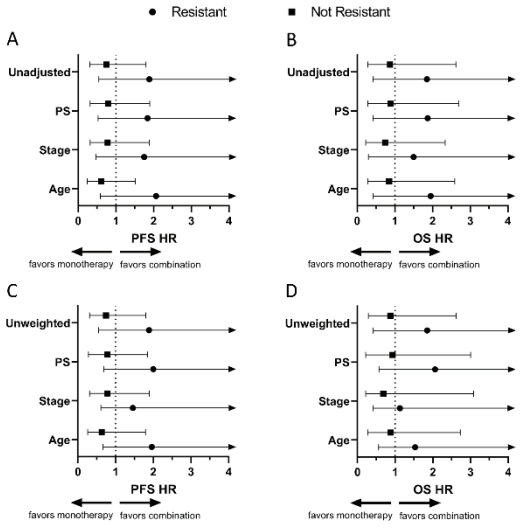

Figure 2. Forest plots of HRs between monotherapy and combination therapy in the high PD-L1 subgroup for Resistant and Not Resistant (Intermediate and Sensitive) subgroups adjusted for differences in population depending on treatment assignment. (A) PFS and (B) OS Cox HRs unadjusted and adjusted by covariate, (C) PFS and (D) OS HRs unweighted and weighted using inverse propensity score weighting (PS=performance status)

Abstracts

77

Mitochondrial citrate transport modulates functional exhaustion in CD8+ T cells

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Background
The efficacy of immunotherapy depends on the presence and persistence of functional immune cells within the tumor. While tumor-specific T cells can be activated and infiltrate the tumor microenvironment, they are quickly rendered dysfunctional by the combination of chronic antigen stimulation, hypoxia, and nutrient stress that creates an immune-suppressive environment. Thus, T cell dysfunction remains a significant hurdle for immunotherapeutic success. We have shown T cell exhaustion and metabolic dysfunction are driven by mitochondrial stress [1]. These features evoke an image of weak, starving T cells that are unable to sufficiently fuel their effector function. However, we and others have observed that CD8+ T cells accumulate large lipid stores as they progress towards exhaustion [2]. What remains unclear is whether lipid accumulation in these cells contributes to their dysfunction or represents an untapped source of carbon that may be the key to their reinvigoration.

Methods
Using an *in vitro* model of T cell exhaustion developed in our lab, we have evaluated the effect of inhibiting several steps in fatty acid synthesis, including mitochondrial citrate transport via SLC25A1 and malonyl-CoA production by acetyl-CoA carboxylase (ACC)[3]. We immunophenotyped CD8+ T cells for markers of terminal exhaustion, cytokine production, and lipid accumulation. We also employed ^{14}C-glucose to begin to examine changes in carbon distribution across the mitochondrial, cytosolic, and nuclear compartments upon SLC25A1 inhibition.

Results
Here we sought to define the role lipid metabolism plays in the progression of CD8+ T cell exhaustion. Inhibition of citrate transport from the mitochondria via SLC25A1 in CD8+ T cells resulted in reduced lipid accumulation and reduced expression of inhibitory receptors, PD-1 and Tim-3, which are known to be upregulated on exhausted T cells.

Conclusions
Taken together, our results suggest a role for mitochondrial citrate flux in the accumulation of cytosolic lipids and progression of CD8+ T cell exhaustion. We propose that as exhausted T cells experience mitochondrial stress and perform less oxidative phosphorylation, they shuttle excess citrate to the cytosol where it fuels production of acetyl-CoA and de novo fatty acid synthesis. This pathway may be targeted to delay exhaustion or reinvigorate exhausted T cells within tumors. These data provide new insight into the mechanisms of T cell exhaustion and may inform future immunotherapeutic development.

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78

TPST-1120, a first-in-human peroxisome-proliferator activated receptor-alpha (PPAR-α) antagonist: scientific rationale, clinical activity and biomarker assessments

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Background
TPST-1120 is a small molecule antagonist of peroxisome-proliferator activated receptor-alpha (PPAR-α), a regulator of fatty acid oxidation (FAO) and immune suppression. TPST-1120 inhibited proliferation of human tumors dependent on FAO, and, in combination with immune checkpoint or angiogenesis inhibitors, provided significant therapeutic efficacy and tumor-specific immunity in mice. TPST-1120 was well tolerated and showed signs of activity in a phase I trial as monotherapy and in combination with nivolumab (NCT03829436). As monotherapy, TPST-1120 conferred a 53% disease control rate. In combination with nivolumab, the objective response rate was 30% (3/10, all partial responses) in subjects treated at the two highest TPST-1120 doses and included two subjects previously refractory to anti-PD1 therapy [1]. We performed ctDNA mutational analysis at baseline and quantified lipid and gene expression changes in post-treatment whole blood to identify potential biomarkers of response.

Methods
Mutational analysis of ctDNA was assessed using the PredicineCARE™ assay, and lipid analysis was performed by tandem mass spectrometry. Gene expression changes were quantified using the nCounter® PanCancer Immune Profiling panel supplemented with 30 PPAR-α target genes. Putative clinical response biomarkers were identified as those differentially expressed by patients with partial response (PR) compared to those with progressive disease (p<0.05 by Mann-Whitney U Test). Lipid change magnitudes were assessed by Wilcoxon paired analysis (p<0.05).

Results
Baseline ctDNA mutational analysis revealed that patients with partial responses (PR) or stable disease were more likely to bear mutations in isocitrate dehydrogenase (*IDH*) and phosphatase and tensin homolog (*PTEN*) compared to patients with progressive disease. Patients with PR demonstrated significant elevations (p<0.05) in multiple genes including those associated with lipid transport (*APOE*), Th17 development (*RORC*) and down-regulation of CD155, a TIGIT ligand. Lipid analysis demonstrated acute changes in free fatty acids (FFA) four hours after first dose (p<0.05). Among patients receiving combination therapy, the highest post-dose elevations in FFA, lysophosphatidylcholine and lysophosphatidylethanolamine levels were observed in a PR patient exhibiting the longest duration of response.

Conclusions
TPST-1120 treated patients with PR demonstrated fatty acid oxidation perturbations and immune gene expression changes as potential biomarkers of clinical benefit. Increased frequencies of responding patients bearing PI3K pathway or *IDH* mutations may reveal populations likely to benefit from treatment with TPST-1120.

Trial Registration
Trial name: "A Phase 1/1b Open-label, Dose-escalation and Dose-expansion Study of TPST-1120 as a Single Agent or in Combination with Systemic Anti-Cancer Therapies in Subjects with Advanced Solid Tumors"
Registry No.: NCT03829436

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Ethics Approval
The study was conducted with adherence to the ethical principles based on the Declaration of Helsinki, International Council for Harmonisation guidelines for current Good Clinical Practice and the applicable national and local laws and regulatory requirements. Before the study began, the protocol, the informed consent form, other written materials provided to participants, and any other relevant study documentation was approved by institutional review boards associated with each clinical site participating in the study. All participants provided written informed consent before any study procedures were performed.

Consent
No identifiable information is included in this abstract.

79

Spatial analysis of local drug induced changes in tumor microenvironment predicts effective treatment combinations in breast cancer

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Background
Anticancer therapeutics primarily designed to target tumor intrinsic mechanisms may also affect components of tumor microenvironment (TME) - immune cells and non-immune stroma. Recent literature reenforces the concept that complex interactions between drugs, neoplastic cells and cells of the TME determine the efficacy of anticancer therapies. Systems understanding of these interactions can serve to predict effective treatment combinations simultaneously attacking tumor cell vulnerabilities, enhancing immune surveillance, and mitigating stromal mediators of resistance. The key challenge is to find such TME-modulating combinations in an efficient and informative process.

Methods
We have developed an integrated analytical platform termed Multiplex Implantable Microdevice Assay (MIMA) to rapidly decompose contributions to drug response and find biomarkers with predictive value for combination therapy efficacy including immunotherapy. The system deploys a (i) miniaturized implantable microdevice for localized intratumoral drug delivery and (ii) multiplex immunostaining to measure 30+ proteins in single cells at each drug well. Computational analyses of local drug-induced changes provide information about the composition, functional state and spatial cell organization of the tumor and associated TME providing new insights into drug mechanisms of action.

Results
We used MIMA in genetically engineered mouse models of breast cancer to evaluate effects of five targeted anticancer agents (olaparib, palbociclib, venetoclax, panobinostat, lenvatinib) and two chemotherapies (doxorubicin, paclitaxel) and predicted synergistic antitumor effects with anti-PD-1, anti-CD40, and anti-CSF1R immunotherapies and vasculature targeting agents (Tatarova et al., *Nature Biotechnology*, 2022). Some of the most effective combinations have not been previously reported. A pan-HDAC inhibitor, panobinostat synergized with anti-PD-1, induced immunogenic cell death and infiltration of antigen presenting neutrophils. Longitudinal spatial analyses revealed that mechanisms of resistance co-emerged with these response phenotypes and became prominent over time. We observed fibroblast, protumorigenic macrophage and cytotoxic B cell recruitment associated with heavy deposition of collagen VI, increased immune suppression and emergence of invasive and cancer stem cells. Combination with the stroma modulating agent, losartan, improved the efficacy of panobinostat/anti-PD-1 in systemic administration consistent with normalization of non-immune stroma favorably altering aspects of TME for immune and targeted therapy efficacy.

Conclusions
Thus, MIMA represents a new approach to predict effective combination regimens as well as biomarkers that could be applied to individual patients. Computational modeling of the spatial cell patterns could provide actionable information for development of effective drug doses and schedules.

References
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Figure 1. Multiplex Implantable Microdevice Assay (MIMA).

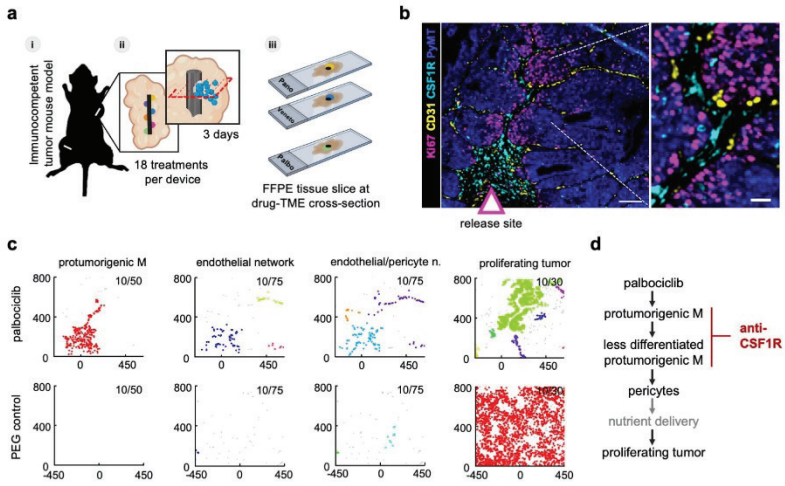

Figure 1. The MIMA system comprises of a miniaturized device for localized delivery of multiple drugs inside the tumor (a; Jonas et al., 2015) and an analytical system (b-d) to describe the responses of the tumor cell and tumor microenvironment to each of the drugs. Tissue sections are cut perpendicular at each drug well (a, right) and are prepared for multiplex immunostaining and imaging (b). Spatial analysis processing expression level of 30+ proteins in each single cell defines cell types and their associations in the tumor microenvironment (c) rapidly translating into models of drug response (d). Within these, we look for a therapeutic vulnerability to prevent acquired resistance. The figure summarizes results adapted from Tatarova et al., 2022.

80

TLT-1, a platelet released protein, suppresses CD8 T cell based systemic immunity, and exhibits prognostic and therapeutic significance in non-small cell lung cancer

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Background
Targeting tumor-associated immunosuppression has shown clinical benefit against several solid tumor malignancies, including non-small cell lung cancer (NSCLC). Platelet activation has been associated with poor prognosis of several cancer types including NSCLC. The emerging role for platelets in cancer immune response modulation has been largely unexplored. (TREM)-like transcript 1 (TLT-1) is a platelet expressed protein which is released from activated platelets in circulation.
Methods
We studied freshly isolated platelets from advanced stage NSCLC patients (n=42) and age matched controls by flow cytometry, electron & confocal microscopy. Clinical parameters of NSCLC patients were correlated with platelet TLT-1 levels. A humanized mice model was also used in which NSCLC patient derived tumors were xenografted. Using an immunocompetent syngeneic mouse tumor model and *in vivo* bioimaging, circulating T cells were evaluated for systemic immune response. Immunoprecipitation assays were used to identify the TLT-1 receptor on T cells. Patient derived T cells were cultured and CD8 T cell exhaustion response was studied.
Results
NSCLC patients exhibit a distinct platelet activation phenotype. A platelet specific protein TLT-1 (Trem like Transcript -1), was found to be highly expressed and released by NSCLC patient platelets. This NSCLC associated platelet phenotype was also observed in humanized mice model induced with patient derived NSCLC cells, confirming this to be independent of comorbidities or therapies. Administration of recombinant TLT1 in immunocompetent mice induced with subcutaneous syngeneic tumors suppressed IFNg producing CD8+ T cells in blood and increased tumor growth and metastasis *in vivo*. Study of transcription profiles of T cells in response to TLT-1 showed a distinct transcription profile supporting suppression of only CD8+ T cells. Through our mechanistic study, we identified T cell surface CD3 epsilon (CD3e) to be the receptor for platelet-released TLT-1, likely responsible for its immunosuppressive effect. Further, we observed that TLT-1 was able to induce an AICD like phenotype in circulating CD8 T cells. The clinical significance was highlighted by an inverse correlation between platelet TLT-1 levels and patient lymphocyte parameters, with high levels of TLT-1 being associated with reduced overall survival. Antibody mediated inhibition of platelet TLT-1 rescued NSCLC patient CD8+ T cells *in vitro*.
Conclusions
Taken together, we report platelet expressed TLT-1 as a novel immunomodulator which suppresses circulating T cells and promotes tumor progression. The effectiveness of antibody-based inhibition of the extracellular TLT-1 makes it an attractive anti-tumor therapeutic target in NSCLC, and possibly other solid tumors.
Acknowledgements
The studies were kindly supported by NIH NHLBI grants; R01HL115247, R01HL122815, R01 HL150515
Ethics Approval
All human studies were approved by the Yale Human Investigation Committee (#1005006865). Informed consent was obtained from each subject and conformed to the principles set out in the WMA Declaration of Helsinki and the Belmont Report. These are requirements for Yale HIC. All mouse procedures were approved under Institutional Animal Care and Use Committee at Yale and all guidelines duly followed (IACUC #2017-11413).

81

Distinct immune signatures in sentinel lymph nodes may provide insights to guide breast cancer immunotherapy

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Background
The effect of sentinel node (SN) tumor burden on outcome has been well established, however the significance of the immune gene signature(s) in the SN is unknown. We hypothesize that SN immune signatures will reflect host immunity and provide clinically meaningful information that can be used to guide immunotherapy approaches. Immunotherapy efficacy may thus not only be determined by the biology of the tumor microenvironment, but also by the biology of the SN microenvironment. Here we describe an exploratory retrospective breast cancer study to test the initial hypothesis.

Methods
We analyzed RNA specimens from a subset of women enrolled in a prior investigator-initiated multicenter breast cancer SN trial. Bulk RNA sequencing was performed on 47 primary breast cancers and 85 corresponding SN from 47 patients with a median follow up of 102 months and 15 recurrences. The initial cohort had a median age of 50 years, 68% white/28% Black, 60% Stage II/32% Stage I, 67% triple negative (TNBC). Hierarchical clustering supervised on clinical nodal status was performed. We subsequently focused on tumor-negative SN to remove confounding tumor contributions and allow scrutiny of early immune signatures, supervising the analysis on a calculated Risk of Recurrence (ROR-P) primary tumor score.

Results
On hierarchical clustering, most, but not all nodes, clustered according to their nodal status; >2500 differentially expressed genes were identified. Unsupervised RNASeq analysis of tumor-negative SN showed heterogeneous immune gene signatures likely reflective of diverse early immune responses. Directed analysis within a TNBC pilot cohort revealed 425 differentially expressed genes. GSEA supervised by ROR-P detected significantly different gene sets - 35 oncogenic and 2 immunologic (Th and Th2; Fig 1). Median Th2 and dendritic cell (DC) gene set scores differed markedly in TNBC primary tumors vs. corresponding tumor-negative SN (Fig 2,3). Th2 and DC gene sets in SN were associated with disease-free survival by Cox PH.

Conclusions
This exploratory study found that even tumor-negative SN express distinct immune signatures. The Th2 and DC findings suggest that SN immune signatures may have a role in prognosis and prediction of response to immunotherapy. These results are the basis for our current expanded study focusing on the transcriptome and IHC analysis of tumor-SLN pairs from 80 node-negative TNBC patients, the association of gene expression with outcome, and an understanding of the underlying cellular interactions. With immunotherapy showing efficacy in early TNBC, SN immune signatures may offer guidance for manipulation of the immune response and improved immunotherapy efficacy.

Acknowledgements
Study supported by ECU Oncology Research and Education Fund, Association of Women Surgeons, Brody Brothers Foundation, Lineberger Cancer Center UCRF, American Cancer Society. Authors acknowledge Dr. Joel Parker and Dr. Shannon Tsai for bioinformatics expertise.

Ethics Approval
This abstract involves specimens obtained from a University Medical Center IRB-approved study (UMCIRB 98-940). Participants gave informed consent to take part in the study.

Abstracts

Figure 1. GSEA of Tumor-negative SN Supervised by ROR-P

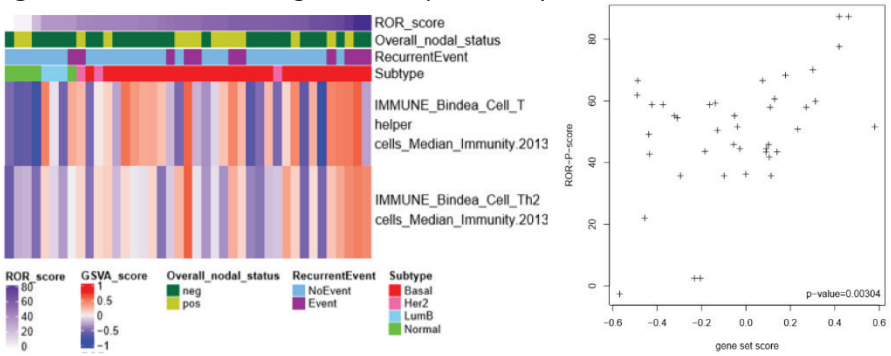

Figure 1. Gene Set Enrichment Analysis of Tumor-negative SLN Supervised by Risk of Recurrence-P (ROR-P) Continuous Scores using 39 Immunologic Gene sets in the Molecular Signatures Database

Left: Gene expression heat maps for Th2 cells (p=0.003) and T helper Cell (p=0.026) gene sets.

Right: Scatter plot showing association of nodal Th2 gene set score (Immunity 2013 PMID 24138885) and ROR-P scores of primary tumors

Figure 2. Comparison of median Th2 gene set scores of tumor-SLN pairs

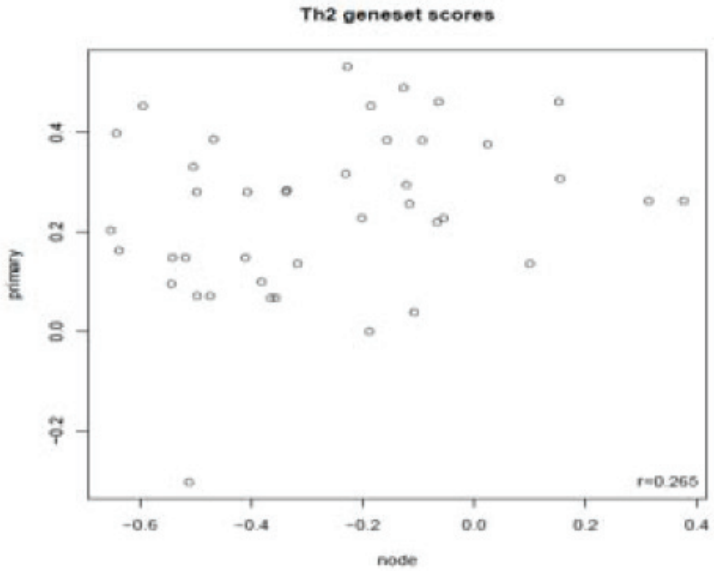

Figure 2. Figure 2, plots the median Th2 geneset scores in primary tumors (y axis) and corresponding SN (x axis) for each patient, depicting both the range of expression levels and the marked difference in the expression of the Th2 signature in the two tissues (r=0.265)

Figure 3. GSEA of Tumor-negative SN Supervised by outcome

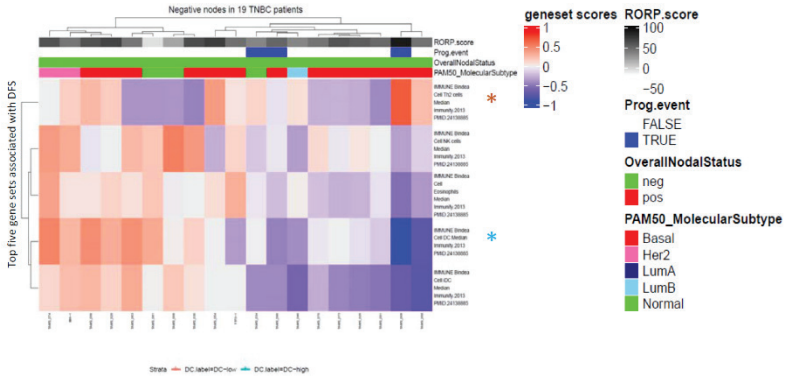

Figure 3. GSEA of Tumor-negative SLN Supervised by outcome using 39 Immunologic Gene sets in the MSigDB. Gene expression heat maps for the top 5 gene sets associated with DFS: Th2 cells = red asterisk and dendritic cells (DC) = blue asteris

82

Simultaneous assessment of the tumor microbiome and immune contexture in colorectal cancer using a metaproteomics approach in archival tissue

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Background
While the influence of the gut luminal microbiome on colorectal cancer (CRC) tumorigenesis is well established, the impact of the tumor-associated microbiome on the immune contexture in CRC is less clear. Delineating interactions between tumor microbiome and immune contexture holds great therapeutic potential, but has been hampered by the severe limitations of nucleic acid-based microbiome assessment techniques (e.g., 16s rRNA sequencing) in archival tissue. Here, we describe a metaproteomics approach in a pilot series of archival CRC specimens to simultaneously assess the host and microbial protein content, stratified by degree of lymphocytic infiltration.
Methods
Formalin-fixed, paraffin-embedded (FFPE) CRC specimens (n=11) were stratified into low- (<33rd percentile, n=5) and high- (>33rd percentile, n=6) CD3+/CD8+ cell density in a manner analogous to the Immunoscore™. Following deparaffinization and protease digestion, samples were analyzed using a LC-MS/M- based, label-free, quantitative proteomics platform with an Evosep-timsTOF Pro2 system. LC-MS/MS raw data files were processed using MaxQuant with the SEQUEST search algorithm, and matched with a Uniprot database containing libraries for *Homo sapiens* (20,594 entries, 2022) and also three bacterial taxa of interest in CRC (*Escherichia coli*, *Bacteroides fragilis*, and *Fusobacterium nucleatum)*.
Results
~3,000 human proteins were identified in each sample, among which 74 differed significantly in concentration based on CD3+/CD8+ cell density (Figure). High-CD3+/CD8+ cases were associated with greater amounts of IFN-g-inducible proteins (lysosomal thiol reductase, guanylate-binding protein 1), and other proteins relevant to the immune microenvironment including complement components (C9, C5), ICAM-1, STAT-6, and integrin a-M. CD3+/CD8+-rich tumors also contained higher levels of proteins with defined roles in neoplastic progression, including proliferation (MAPK1, a-enolase), neovascularization (LRG1) and anti-apoptotic factors (YWHAZ). Proteins from each of the targeted bacterial species were also identified in each CRC sample, including *72* unique proteins from *B. fragilis* (mean, 9/tumor), 38 from *E. coli* (mean 7.5/tumor), and 22 from *F. nucleatum* (mean, 5/tumor). Highly prevalent bacterial proteins included a *B. fragilis* predicted ATP-binding protein in >90% of samples, and the *E. coli c*arbonic anhydrase 2 protein and *F. nucleatum* chaperone protein DnaK detected in all tumors.
Conclusions
In this proof-of-concept study, we demonstrate simultaneous quantitative detection of human and bacterial peptides in archival CRC specimens. Validation studies underway in large retrospective series will assess critical relationships between the CRC microbiome, immune contexture, and patient outcomes. It is hoped that this approach will complement existing nucleic acid-based microbiome techniques, given their serious limitations in archival material, in developing innovative microbe-based immunotherapeutic interventions in CRC.
Ethics Approval
This study was approved by the Valley Health Institutional Review Board, Winchester, VA (Protocol # 20201201).

Figure 1.

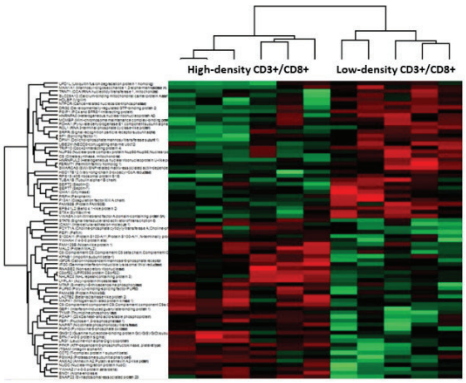

Figure 1. Figure. Heatmap and hierarchical clustering with average linkage of 74 proteins (out of a total of ~3,000 quantified proteins) differentially expressed based on CD3+/CD8+ lymphocyte density. DE analysis was performed by R package LIMMA, which used an empirical Bayes method. Log2 transformed and quantile normalized protein abundances (as assessed by signal intensity) were used for expression levels which are coded as shown in the color scale bar.

84

Exploring latent TGF-β as a tumor microenvironment target using MACSima™ imaging cyclic staining technology and Adapter CAR™ T-cells

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Background

The use of chimeric antigen receptor (CAR) modified T-cells has been highly effective in treating blood-related cancers, leading to FDA approval of six CAR T-cell products. However, applying this approach to solid tumors is the major challenge due to the complex and varied nature of the tumor microenvironment (TME). An element of this complex TME is TGF-β. Under benign conditions, TGF-β regulates cell growth and can suppress tumor formation. However, in solid cancers, TGF-β can promote the growth and spread of cancer cells by suppressing the anti-tumor response of the patient's immune system. The sources of TGF-β within the TMA are numerous. TGF-β can be secreted by tumor cells but also by stromal cells, e.g. cancer-associated fibroblasts. When TGF-β is secreted it binds to specialized binding proteins tethering TGF-β, e.g. to stromal components of the TME but also to potentially immunosuppressive cells like regulatory T-cells. Therefore, TGF-β is present in the TME of solid cancer either in its soluble form or membrane-bound.

Methods

In the study presented here, we investigated TGF-β as a potential target for CAR T-cell therapy. First, we analyzed tumor tissue of the ovaries with the MACSima™ imaging cyclic staining (MICS) technology[1]. By this, we could decode the spatial distribution of the membrane-bound form of TGF-β within the TME and its co-localization with >90 surface markers found in the TME. In addition, we have developed a new technique that allows Adapter CAR™ (AdCAR) T-cells to respond to soluble factors, e.g. soluble latent TGF-β. This is achieved by using adapter molecules specifically, tagged antibodies. This approach offers several advantages, including the ability to integrate multiple target moieties and maintain control over the AdCAR T-cell function.

Results

We have successfully demonstrated the ability of AdCAR T-cells to sense latent TGF-β using this adapter technology *in vitro* and *in vivo* in a subcutaneous pancreatic ductal adenocarcinoma (PDAC) mouse model. We were able to combine the targeting of a tumor-associated antigen and sensing of soluble latent TGF-β with the AdCAR system. We also tested this concept in a reconstituted PDAC mouse model to gain insight into the interplay of AdCAR T-cells and endogenous immune cells.

Conclusions

This technique has the potential to help us to better understand the role of soluble molecular cues in promoting immunosurveillance and could lead to the development of new AdCAR T-cell-based approaches for targeting solid tumors. This could ultimately improve the safety and effectiveness of solid tumor therapies.

References

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Ethics Approval

All studies were performed according to institutional guidelines and regulations (approval numbers: 81–02.04.2018.A096, 81–02.04.2020.A255, 84–02.04.2017.A021)

85

Short lived responses to anti-BCMA CAR-T cells are associated with expansion of activated cytotoxic CD4+ CAR-T cells

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Background
T cells engineered to express chimeric antigen receptor (CAR) targeting B cell maturation antigen (BCMA) have exhibited unprecedented efficacy with >70% response rate in relapsed/refractory multiple myeloma (MM). However, most patients experience relapse even following initial deep complete responses [1,2]. Understanding why some patients have only short-lived responses represents a significant unmet need.

Methods
We assessed longitudinally collected bone marrow (BM) and peripheral blood from 15 MM patients enrolled in 3 clinical trials, as well as from 5 healthy donors by multi-omic single cell sequencing. 690,939 T cells and 227,420 myeloid cells were profiled with single-cell RNA sequencing, including 5' gene expression profiling, surface protein sequencing (CITE-seq), and TCR sequencing. We developed methods to annotate CAR-T cells *in silico* and by targeted sequencing.

Results
Compared to healthy donors, MM patients had increased frequencies of GZMB CD8+ T effectors and lower frequencies of CD4+ naïve T cells. Disease progression at 6 months was used as a cut-off for transient responders (TR) and durable responders (DR). DR patients had more CAR-T cells at Day 90 and 180 with a significantly higher proportion of these CAR-T cells possessing CD8+ effector memory cell states. Furthermore, CD8+ CAR-T from TR group exhibited significantly higher expression levels of activation/exhaustion genes like *HAVCR2* and *PDCD1* while CD4+ CAR-T cells exhibited increased expression of *LAG3*, *TIGIT* as well as cytotoxicity genes like *NKG7*, *PRF1*. By calculating the cytotoxicity score and activation score, we discovered a dramatic enrichment of cytotoxic activated CD4+ CAR-T cells only in TR patients at early time points (Day14 and 30), that was not seen in CD8+ CAR-T cells. TCR sequencing indicated these cytotoxic activated CD4+ CAR-T cells were polyclonal. Analysis of the myeloid compartment in the BM niche revealed a unique population of non-classical monocytes in MM patients compared with healthy aged-matched controls. Interaction analysis between MM-associated non-classical monocytes and CD4+ CAR-T cells uncovered TGFbeta axis, which is more pronounced in TR group.

Conclusions
Our results show that while durable responders have persisting CAR-T cells, patients with transient response to treatment have increased frequencies of cytotoxic, activated CD4+ CAR-T cells with a polyclonal state. TGFbeta contributes to the dysfunction of CD4+ CAR-T cells.

References

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2. Usmani SZ, Berdeja JG, Madduri D, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen (BCMA)-directed chimeric antigen receptor T-cell (CAR-T) therapy, in relapsed/refractory multiple myeloma (R/R MM): Updated results from CARTITUDE-1. Journal of Clinical Oncology 39, no. 15_suppl (May 20, 2021) 8005-8005.

Ethics Approval
This study is approved by UCSF IRB and IRB protocol number is 10-00545. All participants gave informed consent before taking part in the study.

Figure 1. Dynamics of anti-BCMA CAR-T cells

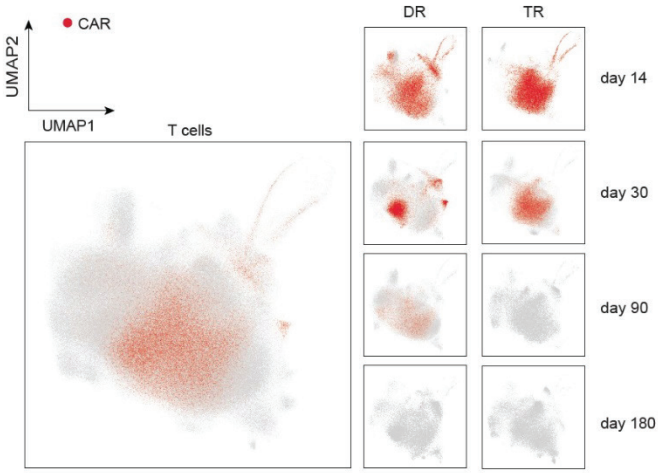

Figure 1. UMAP of CAR T cells in Transient Responders (TR) and Durable Responders (DR) at day14, day30, day90, day180 post-infusion.

Figure 2. Cytotoxicity and activation scores in CD4+ cells

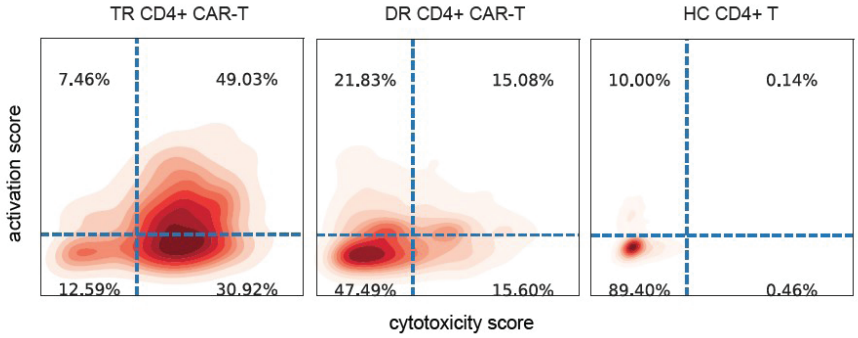

Figure 2. Density plot showing cytotoxicity score and activation score in CD4+ CAR T cells in TR and DR group and CD4+ T cells in healthy control (HC). Thresholds are determined based on HC.

87

The microbiome-derived metabolite TMAO drives immune activation and boosts responses to immune checkpoint blockade in pancreatic cancer

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Background
Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer with poor prognosis. Although pancreatic tumors exhibit prominent leukocyte infiltrates, immunotherapy has so far failed to improve clinical outcomes in patients with PDAC.The immunotherapy responses can be improved through strategies that shift the PDAC tumor microenvironment (TME) from an immunosuppressive state to a more immune-activated state. The composition of the gut microbiome controls innate and adaptive immunity and has emerged as a key regulator of tumor growth and the success of immune checkpoint blockade (ICB) therapy. However, the underlying mechanisms remain unclear.
Methods
The main objectives of this study were to elucidate how the gut microbe-derived metabolite trimethylamine N-oxide (TMAO) influences the host immune responses in PDAC TME, to determine if TMAO would synergize with immune checkpoint therapy to reduce tumor growth and to assess whether there is a clinical correlation between TMAO production and survival in cancer patients. The overall design did employ some in vitro approaches but relied on in vivo mouse model systems, including orthotopic tumor implantation, to achieve the first two objectives; to test for a clinical correlation, we assessed datasets of bacterial species generated by three other groups.
Results
Using a nontargeted, liquid chromatography–tandem mass spectrometry–based metabolomic screen, we identified the gut microbe–derived metabolite trimethylamine N-oxide (TMAO), which enhanced antitumor immunity to PDAC. Delivery of TMAO intraperitoneally or via a dietary choline supplement to orthotopic PDAC-bearing mice reduced tumor growth, associated with an immunostimulatory tumor-associated macrophage (TAM) phenotype, and activated effector T cell response in the tumor microenvironment. Mechanistically, TMAO potentiated the type I interferon (IFN) pathway and conferred antitumor effects in a type I IFN–dependent manner. Delivering TMAO-primed macrophages intravenously produced similar antitumor effects. Combining TMAO with ICB (anti-PD1 and/or anti-Tim3) in a mouse model of PDAC significantly reduced tumor burden and improved survival beyond TMAO or ICB alone. Last, the levels of bacteria containing CutC (an enzyme that generates trimethylamine, the TMAO precursor) correlated with long-term survival in patients with PDAC and improved response to anti-PD1 in patients with melanoma.
Conclusions
Our study demonstrated that the microbial metabolite TMAO enabled TAMs to become immunogenic and promote effector T cell activity, transformed the TME to an immune-activated state, and rendered PDAC sensitive to checkpoint immunotherapy, suggesting that strategies that alter levels of TMAO could be a promising clinical intervention to manage PDAC.

90

High pretreatment DHEA level is associated with shorter overall survival in newly diagnosed metastatic non-small cell lung cancer patients receiving immune checkpoint inhibitors

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[1]H. Lee Moffitt Cancer Center, Tampa, United States

Background

Sex is an important factor in determining response to immune checkpoint inhibitors (ICI) in cancer patients [1,2]. Sex hormones can modulate the immune response in preclinical studies. Our study aimed to determine if the pretreatment sex hormone level can predict outcomes in metastatic non-small cell lung cancer (mNSCLC) patients undergoing ICI therapies.

Methods

This study included 61 patients with newly diagnosed mNSCLC who received ICI as part of the upfront therapy. Pretreatment plasma and fecal samples were collected before the first ICI infusion, and we measured sex hormone levels using ultra-high-performance liquid chromatography high-resolution mass spectrometry. Sex hormone levels were compared between the clinical benefit and no clinical benefit group. Patients were then divided into high DHEA and low DHEA groups based on the sex-specific median of the cohort. Overall survival (OS) and progression-free survival (PFS) were compared between high DHEA and low DHEA using Kaplan Meier's methods. A similar analysis was based on the 5-androstenediol level. We used the univariate and multivariate Cox proportional hazards (PH) model to measure hazard ratios (HRs) for PFS and OS.

Results

Pretreatment plasma samples were collected from 61 patients, and 31 patients were female (table 1). Among them, 30 plasma samples had measurable DHEA levels, and 46 patients had measurable 5-androstenediol levels (table 2). Patients in the clinical benefit group had significantly higher plasma DHEA levels and 5-androstenediol levels than those in the no clinical benefit group (figure 1).

The high DHEA group had fewer patients with clinical benefits from ICI therapy (27% vs. 87% in the high DHEA and low DHEA groups, respectively) (figure 2). Patients with high DHEA also had shorter OS (mOS: 11.4mo vs. not reached for high DHEA and low DHEA group respectively, p=0.0001) and shorter PFS (mPFS: 4.1mo vs. 22mo for the high DHEA and low DHEA groups, respectively, p<0.0001). High 5-androstenediol also had fewer patients with clinical benefit (46% vs 72% for the high 5-androstenediol and low 5-androstenediol groups, respectively).

Univariate Cox PH analysis confirmed our observation. High DHEA level was associated with poor OS (HR-8.29, 95% CI:2.31-29.79) and PFS (HR=10.23, 95% CI:3.4-30.74). High 5-androstenediol level was associated with shorter PFS (HR=2.26, 95% CI: 1.07-4.75) (table 3).

Conclusions

Pretreatment DHEA level and 5-androstenediol level were significantly lower in patients with clinical benefit from ICI. Our study supports the use of pretreatment DHEA as a promising predictive biomarker in patients with metastatic NSCLC receiving ICI therapies.

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Ethics Approval

Informed consent was obtained from all patients. This study was approved by Advarra IRB (MCC 18611, PRO00017235).

Abstracts

Figure 1.

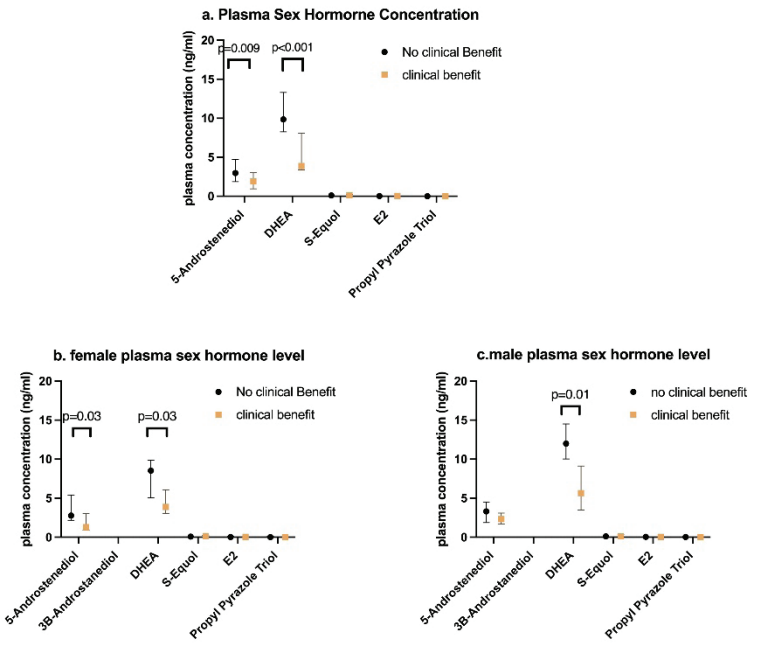

Figure 2.

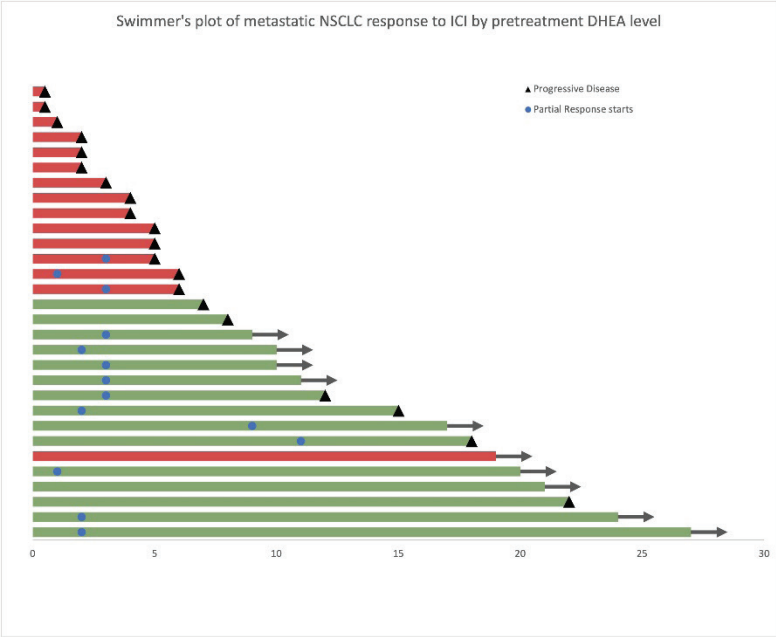

Table 1.

Table 1. Patient Demographics and Clinical Characteristics of 61 enrolled patients based on clinic benefit

	clinical benefit N=28	no clinical benefit N=33	P value
age at diagnosis median, range	68[49,84]	67 [52.8, 82.1]	0.89
Male Sex, %	15 (45.5)	15 (53.6)	0.527
Race			0.074
White	31 (94)	23 (82)	
Black	1 (3)	3 (11)	
BMI (median, 95% CI) at the time of treatment	26.1 [18,7, 36.9]	23.8 [16.9,46.9]	0.36
Smoking Status			0.803
Current	9 (32.1)	8 (24.2)	
former	15 (53.6)	21 (63.6)	
never	4 (14.3)	4 (12.1)	
ECOG performance status			0.353
0	5 (15.2)	2 (7.1)	
1	28 (84.8)	25 (89.3)	
histology:			0.529
non-squamous, %	28 (85)	24 (86)	
Squamous, %	5 (15)	4 (14)	
CNS involvement	4 (14.3)	2 (6.1)	0.282
Laboratory Evaluation			
Absolute Neutrophil Count, e3/DL, median, 95% CI	5.1 [2.3, 11.7]	5.1 [3.2,10.5]	0.34
Absolute Lymphocyte Count, e3/DL, median, 95% CI	1.2 [0.3,2.8]	1.05 [0.3, 2.7]	0.61
Absolute Eosinophil Count, e3/DL, median, 95% CI	0.17 [0,0.92]	0.15 [0.01,0.84]	0.32
Absolute Platelet Count, e6/DL, median, 95% CI	262 [109.1, 526.2]	251 [114.4, 469.4]	0.99
Neutrophil to Lymphocyte ratio (median, 95% CI)	4.2 [1.8, 14.8]	4.9 [1.6,13.7]	0.85
Platelet to Lymphocyte ratio (median, 95% CI)	245.6 [62.2, 1104.2]	229.9 [105.7, 796.2]	0.81
Co-morbidities			
COPD	12 (36)	16 (57)	0.11
HLD	22 (66.7)	17 (60.7)	0.63
OSA	6 (18.2)	5 (17.9)	0.97
MI/Heart Failure	3 (9.1)	6 (21.4)	0.18
DM	4 (12.1)	10 (36)	0.038
Prior therapy including neoadjuvant and adjuvant			
Prior chemotherapy	14 (42.9)	20 (61)	0.406
Prior Radiation Therapy	10 (36)	9 (27)	0.478
PD-L1 positive (>1%)	20/27 (74)	14/24 (58)	0.84
If positive, PD-L1 = 50 %, median, 95% CI	9 (32)	10 (30)	0.88
Other mutation			
ALK fusion	1/19	1/20	0.93
EGFR	5/24	2/31	0.16
KRAS	6/19	7/31	0.07
NRAS	0/14	0/15	0.72
TP53	6/11	6/15	0.95

Table 2.

Table 2 Plasma sex hormone level and stool phytoestrogen level of patients based on clinical benefit

	clinical benefit N=28	No clinical benefit N=33	P value
Plasma			
DHEA level (ng/ml), median, 95% CI	3.7 (2.1-8.3)	9.6 (3.8-16.6)	<0.001
5-androstenediol level (ng/ml), median, 95% CI	2.7 (0.66-5.0)	2.3 (0.87-5.5)	0.01
S-equol level (ng/ml), median, 95% CI	0.11 (0.006-0.32)	0.11 (0.027-3.7)	0.73
Propyl Pyrazole Triol level (ng/ml), median, 95% CI	0.015 (0.0016-0.056)	0.0080 (0.0017-0.079)	0.95
E2 level (ng/ml), median, 95% CI	0.043 (0.013, 0.079)	0.03 (0.009-0.12)	0.43
Stool phytoestrogen level normalized to Daidzein			
Quercetin level, median, 95% CI	0.89 (0.096-129)	0.86 (0.007-15.0)	0.43
Naringenin level, median, 95% CI	1.18 (0.1-48)	0.91 (0.03-29)	0.36
Genestein, median, 95% CI	2.1 (0.07-21)	1.46 (0.07-20)	0.68
S-equol, median, 95% CI	7.81 (0.70-703)	7.38 (0.1-747)	0.58

Table 3.

Table 3. Univariate and multivariate analysis of clinical factors affecting progress-free Survival and overall Survival in metastatic non-small cell lung cancer undergoing first line immune checkpoint inhibitor therapy

Clinical Factors		Progression Free Survival			Overall Survival		
		no of events/ no of cases	Univariable-HR (95% CI)	Multivariable HR (95% CI)	no of events/ no of cases	Univariable-HR (95% CI)	Multivariable HR (95% CI)
age		45/61	1.01 (0.98-1.05)	1.11 (1.01,1.23)	34/61	0.98 (0.94, 1.02)	1.03 (0.92, 1.16)
sex							
	male	21/30	1	1	17/30	1	1
	female	24/31	0.85 (0.47,1.53)	1.12 (0.32,3.89)	17/31	1.39 (0.70,2.73)	2.08 (0.44, 9.85)
	p for trend		0.58	0.86		0.35	0.36
BMI		45/61	1.00 (0.95,1.06)	1.09 (0.97, 1.22)	34/61	0.98 (0.93, 1.05)	1.03 (0.91, 1.16)
Smoking Status							
	current	13/17	1	1	12/17	1	1
	former	27/36	0.92 (0.47,1.79)	0.25 (0.037, 1.72)	18/36	0.58 (0.28, 1.20)	0.52 (0.047, 5.62)
	never	0.625	0.84 (0.30,2.36)	0.20 (0.018, 2.12)	4/8	0.67 (0.21, 2.07)	0.26 (0.017, 4.09)
	p for trend		0.94	0.29		0.33	0.63
Medical Comorbidities							
	DM present	10/14	1.74 (0.85,3.59)	1.14 (0.23,5.68)	10/14	2.63 (1.21, 5.71)	0.40 (0.07,2.32)
	DM absent	35/47	1	1	24/47	1	1
	p for trend		0.13	0.87		0.015	0.31
NLR		45/61	1.01 (0.93,1.09)	1.15 (0.88,1.50)	34/61	1.08 (0.99, 1.17)	1.13 (0.84, 1.53)
PLR		45/61	1.00 (0.99, 1.00)	1.00(0.99, 1.01)	34/61	1.00 (0.99, 1.00)	1.01(0.99, 1.01)
Systemic therapy within 1 year of Immunotherapy Initiation							
	yes	23/29	1.12 (0.62, 2.02)	1.39 (0.41, 4.7)	21/29	1.90 (0.95, 3.8)	2.51 (0.55, 11.4)
	no	22/32	1	1	13/32	1	1
	p for trend		0.72	0.59		0.071	0.23
DHEA							
	Higher than median	14/15	10.23(3.4-30.74)	45.3 (5.46, 376.60)	13/15	8.29 (2.31,29.79)	28.69 (3.40, 242.43)
	Lower than median	6/15	1	1	4/15	1	1
	p for trend		<0.001	<0.001		0.001	0.002
5-androstenediol							
	Higher than median	20/24	2.26 (1.07, 4.75)	2.65 (0.39-18.01)	16/24	2.15 (0.95, 4.88)	7.04 (0.61, 81.11)
	Lower than median	12/22	1	1	9/22	1	1
	p for trend		0.032	0.32		0.068	0.117

Abbreviation: NLR: neutrophils to lymphocytes ratio, PLR: platelet to lymphocyte ratio, HR: hazard ratio, DM: diabetes mellitus
*sex-specific median for DHEA is 8.7 ng/ml for male and 4.7 ng/ml for female. ᵃsex-specific median for 5-androstenediol is 2.71 ng/ml for male and 2.22 ng/ml for female

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