Regular Abstracts

Immunometabolism

P001 The gut microbiome-prostate tumor crosstalk is modulated by dietary polyunsaturated fatty acids. Jalal Laaraj[1,2], Gabriel Lachance[1,2,3], Nikunj Gevariya[1,2], Thibaut Varin[3], Andrei Feldiorean[4,5], Fanny Gaignier[1,2], Isabelle Boudreau Julien[6], Hui Wen Xu[7], Tarek Hallal[4,8], Jean-François Pelletier[1,2], Sidki Bouslama[9], Nadia Boufaied[4], Nicolas Derome[9,10], Yves Fradet[1,2], Leigh Ellis[11], Ciriaco A. Piccirillo[12,13], Frédéric Raymond[6], David P. Labbé[4,5,8], Alain Bergeron[1,2], André Marette[3], Karine Robitaille[1,2], Vincent Fradet[1,2,6]. [1]Laboratoire d'Uro-Oncologie Expérimentale, Oncology Axis, Centre de recherche du CHU de Québec-Université Laval, Québec, QC, Canada, [2]Centre de recherche sur le Cancer de l'Université Laval, Québec, QC, Canada, [3]Centre de recherche de l'IUCPQ, Québec, QC, Canada, [4]Cancer Research Program, Research Institute of the McGill University Health Centre, Montréal, QC, Canada, [5]Division of Urology, Department of Surgery, McGill University, Montréal, Québec, Canada, Montréal, QC, Canada, [6]Institute of Nutrition and Functional Foods (INAF) and NUTRISS Center - Nutrition, Health and Society of Université Laval, Québec, QC, Canada, [7]Department of Mathematics and Statistics, Université Laval, Québec, QC, Canada, [8]Department of Anatomy and Cell Biology, McGill University, Montréal, QC, Canada, [9]Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada, [10]Department of Biology, Université Laval, Québec, QC, Canada, [11]Division of Medical Oncology, Department of Medicine, Cedars-Sinai Medical Center and Cedars-Sinai Samuel Oschin Comprehensive Cancer Institute, Los Angeles, CA, USA, [12]Infectious Diseases and Immunity in Global Health Program, Research Institute of the McGill University Health Centre, Montréal, QC, Canada, [13]Department of Microbiology and Immunology, McGill University, Montréal, QC, Canada.

Introduction and Objective: Recently, gut microbiota emerged as an important factor for success of immunity-based cancer treatments. However, its steady-state interaction and contribution to developing tumors is largely unexplored in non-intestinal cancers. Our objective was to investigate the connection between prostate tumor and the gut microbiota independently of cancer therapies.

Methods: Human fecal samples were obtained from men participating into a phase IIb double-blind randomized controlled trial testing 3g/day of monoglyceride-eicosapentaenoic acid (MAG-EPA) versus placebo for a 4-10 week period before their radical prostatectomy (NCT02333435). A second set of samples were from men taking the same intervention of MAG-EPA or placebo after a PSA increase following their radical prostatectomy (NCT03753334). Short chain fatty acids (SCFA) analysis of patient stool samples between baseline and surgery was performed by gas chromatography coupled with flame ionization detection. 16srRNA libraries were amplified by targeting a fragment of the V3-V4 hypervariable region of the bacterial 16S rRNA gene. High-throughput sequencing of the bar-coded amplicons was performed on a MiSeq apparatus and the bioinformatics analysis was conducted using Mothur pipeline. In addition to human fecal samples, fully immunocompetent C57BL/6 mice were injected subcutaneously with TRAMP-C2 or PTEN$^{-/-}$ or PTEN$^{-/-}$ RB1$^{-/-}$ mouse prostate cancer cells to measure changes in the gut microbiota during tumor growth. We also recapitulated the MAG-EPA intervention in our TRAMP-C2 mice model and fed by gavage four different fatty acids (omega-9 (high oleic

sunflower oil), omega-6 (MAG-arachidonic acid) and two omega-3 (MAG-docosahexaenoic and MAG-EPA).

Results: In human fecal samples from prostate cancer patients, we observed a reduced gut microbiota diversity correlating with tumor stage. We also found that tumor growth was sufficient to modulate the microbiota in three independent prostate cancer syngeneic mouse models. We showed that transplanted human gut flora was sufficient to modulate ectopic prostate tumor growth, supporting the causal impact of gut microbiota for prostate cancer. The analysis of SCFA in patient stool samples between baseline and surgery showed that MAG-EPA prebiotic intervention was associated with a decrease of fecal butyric acid levels in prostate cancer patients with downgrade at surgery. We finally investigated this gut-tumor connection using purified polyunsaturated fatty acids prebiotics in patients and mice. We observed a reduction in the levels of *Ruminococcaceae* following dietary omega-3 supplementation that correlated with prostate cancer downgrade in patients and reduced tumor growth in mice.

Conclusion: Overall our findings suggest that diet-actionable components of the gut microbiome can regulate prostate cancer growth.

P002 Immuno-reactive cancer organoid models to examine microbiome metabolite effects on immune checkpoint blockade efficacy. Ethan Shelkey[1], Yong Lu[2], David Soto-Pantoja[2], Shay Soker[2]. [1]Wake Forest Graduate School of Arts and Sciences, Winston-Salem, NC, [2]Wake Forest School of Medicine, Winston-Salem, NC.

Introduction: As the number of available immunotherapies for solid tumors increase, their prevalence in the clinic continues to rise as well. While the results are promising and immunotherapies have benefits over traditional chemotherapeutics, a sizable percentage of patients are non-responders to all types of immunotherapy as a treatment option. These differences in sensitivity can be either innate or acquired. Yet, there has been limited 3D *in vitro* models to assess tumor immune-reactivity. These systems are ideal for isolating specific molecular mechanisms that dictate cell behavior and interactions. Our goal was to create an organoid model containing cancer cells paired with cytotoxic T-cells to model immune checkpoint blockade (ICB) efficacy. This model could then be used to examine novel microbiome-ICB interactions shown by recent research to alter therapeutic response levels in patients.

Methods: We created tumor organoids consisting of matched tumor and immune cells, embedded in extracellular matrix (ECM)-like hydrogels. Organoids were treated with therapeutic equivalent doses of anti-PD-1 and anti-CTLA-4 or single dose of anti-CD-47. The organoids were also exposed to physiologic concentrations of metabolites 3-indolepropionic acid derived from the bacterial species *Clostridium sporogenes*, hippurate derived from *Clostridiales*, *Faecalbacterium prausnitzii*, and *Eubacterium*, pyocyanin derived from *Pseudomonas aeruginosa*, butyrate derived from *Faecalbacterium prausnitzii*, and inosine derived from *Bifidobacterium pseudolongum*. Each of these bacterial species and the associated metabolite represent a likely effector of host immune function described in literature and therefore a potential effector of ICB response. Organoids were analyzed with cell viability assays, flow

cytometry, RT-qPCR, and immunohistochemistry staining to determine the effects of the metabolites on ICB response.

Results and Discussion: We showed that ICB therapy stimulated internally localizing T-cells, inducing T-cell-mediated tumor cell killing. ICB treated samples resulted significant loss of viability with corroborating readings from the other methods of characterization. RT-qPCR and flow cytometry demonstrated the cellular changes due to bacterial metabolite co-administration. These results include increased expression of CD-8 T-cell co-receptor, increased cytokine production, and increased effector T-cell viability.

Conclusion: We have created an *ex-vivo* tumor immune-reactive organoid model for studying immunotherapy. We are working to elucidate the effects of microenvironment factors, such as microbiome metabolites, and observe their impacts on immunotherapy efficacy to better understand what conditions are conducive or detrimental to successful ICB treatment.

Advances in Immune Cell Engineering

P003 Efficacy results of a novel vaccine composed of stimulated and haptenized tumors cells in Balbc mice grafted with murine colon adenocarcinoma CT26 cells. Céline Gongora[1], Jacqueline Taleb[2], Benoît Pinteur[3], Lionel Chalus[4], Fanny De Luca[4], Paul Bravetti[3], François Ghiringhelli[5]. [1]Institut de Recherche en Cancérologie de Montpellier, Montpellier, France, [2]Université Claude Bernard Lyon 1, Lyon, France, [3]Brenus Pharma, Issoire, France, [4]Bio Elpida, Lyon, France, [5]Centre Georges François Leclerc, Dijon, France.

BACKGROUND: Metastatic colorectal cancer (mCRC) is a major cause of death. Unmet medical need in immunotherapy is high for MSS patients and still present for MSI-H/dMMR patients. STC vaccine (Brenus Pharma) is composed of selected tumor cell lines, stimulated to overexpress TAA/TSA and neoantigens including resistant factors that are further haptenized to form immunogenic hapten-protein complex to educate the immune system to recognize and target the patient's tumor cells expressing the same resistance factors. We report results of two studies aiming to A) evaluate efficacy of a one cell line-based product (CT26) physical stimulated (S=irradiation and heat shock) and/or haptenized (H) w/o immunostimulant (IS=cyclophosphamide + mGM-CSF w/o BCG) and to B) investigate a potential increase of antitumoral effect of 3 cell lines vaccine (3CL-SH made of CT26, CMT-93, LTPA).

STUDY DESIGN and METHODS: Female BALB/c mice were subcutaneously grafted with 5.10^4 CT26-WT cells. In study A, 9 groups (10 mice per group) are allocated to: G1) Control group, G2) IS, G3) CT26-S, G4) CT26-H, G5) CT26-SH, G6) CT26-S+IS, G7) CT26-H+IS, G8) CT26-SH+IS, G9) CT26-SH+IS+BCG. In study B, 5 groups (20 mice per group) are allocated to: G1) Control group, G2) CT26-SH + IS, G3) 3CL-SH, G4) 3CL-SH + IS once a week for 3 weeks and G5) 3CL-SH + IS twice a week for 4 weeks. Treatments were administered subcutaneously. Overall survival (OS) and tumor growth (TG) were recorded until 1000 mm^3, safety endpoint or on D41 (study A) or D50 (study B).

RESULTS: Stimulated cell-based treatments with IS (CT26-SH + IS) significantly increases OS compared to control group (Study A: G1/G8 p=0.0046 & study B: G1/G2 p=0.0023). Best mOS among groups is observed with 3CL-SH+IS (Study B: G1/G4 p<0.0001 38d vs 27d, Log-rank test). A direct comparison of 3CL-SH+IS and CT26-SH+IS confirmed a highly significant added benefit in favour of the 3 cell lines vaccine (Study B: G2/G4 p=0.0475) compared to the one cell line treatment. In addition, IS reinforced the effect of cell-based treatment, with CT26-SH (Study A: G1/G8, p<0.0001 at D20, Study B: G1/G2 p<0.0001 at D24) and with 3CL-SH (Study B: G4/G3 p=0.0004). Addition of BCG to CT26-SH+IS does not improve efficacy (Study A: G8/G9 p=NS). Results showed a significant impact on TG when cells were both physically stimulated then haptenized, such as CT26-SH (study A: G1/G5, p=0.003 at D20) or 3CL-SH (study B G1/G3 P<0.0001 at D24) compared to the control group. 3CL-SH+IS exhibits a significant efficacy on TG (Study B: G1/G4 p=0.0053 or G1/G5 p=0.0018) and OS whatever the administration schedule No side effect or inflammatory reaction towards the vaccines have been evidenced.

CONCLUSION: Brenus STC vaccine based on physical stimulation and haptenization demonstrated a significant anticancer effect in mice with immunostimulant and confirmed a better efficacy of the 3 cell lines vaccine versus a single cell line vaccine. Further studies are ongoing to test the efficacy of STC vaccine in PD1 resistant preclinical model and in combination with SOC.

P004 CRISPR-mediated PTPN2 deletion in CAR T cells enhances anti-tumor efficacy. Xin Du[1], Florian Wiede[2], Phillip K. Darcy[1], Tony Tiganis[2]. [1]Peter MacCallum Cancer Centre, Melbourne, Victoria 3000, Australia, [2]Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria 3800, Australia.

Chimeric Antigen Receptor T cell (CAR T) immunotherapy has been remarkably successful in the treatment of B-Cell Acute Lymphoblastic Leukemia (B-ALL). However, beyond hematological malignancies, CAR T cells have been ineffective in treating solid tumors. Novel approaches for enhancing the ability of CAR T cells to combat solid tumors are urgently required. Protein tyrosine phosphatases (PTPs) are enzymes that regulate a wide range of physiological processes including metabolism, cellular growth, proliferation and differentiation by controlling tyrosine phosphorylation-dependent signaling. PTPs are key regulators of T cell signaling and contribute to the maintenance of immune tolerance. Studies from our group have shown that PTPN2 plays pivotal role in negatively regulating T cell receptor (TCR) signaling by dephosphorylating and inactivating the Src family protein tyrosine kinase LCK (Wiede, Shields et al. 2011). PTPN2 also attenuates cytokine signaling by dephosphorylating JAK-1, JAK-3 and their target substrates STAT-1, -3 and -5 in a cell context-dependent manner (Simoncic, Lee-Loy et al. 2002, ten Hoeve, de Jesus Ibarra-Sanchez et al. 2002, Wiede, Shields et al. 2011, Wiede, La Gruta et al. 2014). Since CARs signal via LCK, and cytokine signaling is critical for CAR T cell function, we postulated that inhibiting PTPN2 might bolster the anti-tumor activity of CAR T cells. Here we used CRISPR-Cas9-ribonucleoprotein (RNP)-mediated genome editing to delete PTPN2 in CAR T cells. Using this approach PTPN2 was efficiently deleted in CAR T cells and the deletion of PTPN2 significantly enhanced the anti-tumor efficacy of CAR T cells *in vitro* and *in vivo*.

P005 GEN-011: A neoantigen-targeted peripheral blood-derived T cell therapy that has broad neoantigen specificity and high T cell purity while avoiding pro-tumor T cells. James Perry[1], Pranay D. Khare[1], Mercay Reuter[1], Daniel B. DeOliveira[1], Manish Jain[1], Colleen Winstead[1], Hubert Lam[1], Thomas Davis[1], Ray Stapleton[1], Jessica B. Flechtner[1]. [1]Genocea Biosciences, Cambridge, MA.

Candidate adoptive T cell therapies (ACT), such as tumor infiltrating lymphocyte (TIL) treatments, have resulted in unprecedented and durable efficacy in clinical trials. Despite this, the manufacturing requires viable tumor resection, and TIL expansion conditions have the potential to promote T cell exhaustion. Moreover, a large proportion of patients do not respond to treatment, possibly due to exhaustion or to bystander T cells that are not tumor-specific present in the product candidates. In addition, we have shown that naturally occurring pro-tumor T cell responses to tumor-specific antigens we term Inhibigens™ are generated in nearly every subject with cancer; these T cells may be inadvertently expanded in the non-specific TIL manufacturing process. In animal models, Inhibigen-specific responses drive tumor hyperprogression. To avoid these pro-tumor T cells and improve upon ACT limitations, we are developing GEN-011, a neoantigen-targeted, peripheral T cell (NPT) therapy. GEN-011 is designed to contain primarily tumor-specific T cells with broad specificity and limited exhaustion, starting from easily accessible peripheral blood. Putatively beneficial neoantigen targets and deleterious pro-tumor Inhibigen targets are identified through measurement of cytokines in the assay supernatants of an *in vitro* ATLAS™ screen, in which each mutation identified in a patient's tumor is screened with the patient's own peripheral $CD4^+$ and $CD8^+$ T cells in a recall (overnight) assay, without algorithm prioritization. Next, the patient's peripheral T cells and monocyte-derived dendritic cells are incorporated into the PLANET™ manufacturing process where they are specifically stimulated with up to 30 ATLAS-verified neoantigens, avoiding Inhibigens, in a scalable, closed system. Development and engineering runs performed at scale show that the NPTs are up to 96% tumor-specific, with responses maintained for up to 89% of the intended neoantigen targets. They are non-exhausted effector and central memory T cells that express both proliferative and tissue homing markers. In addition to being highly polyfunctional, secreting multiple combinations of IFNγ, Granzyme B, TNFα, and MIP1α in response to specific neoantigens, they are also cytolytic *in vitro* and express memory-progenitor stem-like cell markers. The TITAN™ clinical trial evaluating GEN-011 NPTs is ongoing (NCT04596033). TiTAN is an open-label, multi-center Phase1/2a trial evaluating safety, tolerability, T cell persistence and proliferation, and clinical efficacy. The TiTAN clinical trial is testing two dosing regimens, a repeated lower dose regimen of GEN-011 without lymphodepletion and a single high dose administration of GEN-011 NPTs after lymphodepletion. Both groups will receive interleukin-2 after GEN-011 NPT dosing. By enriching healthy, broadly-specific neoantigen-targeted T cells and avoiding Inhibigens, the GEN-011 NPTs may represent an accessible and promising ACT for treating solid tumors.

COVID-19 Immunology in Cancer Patients

P006 Intratumoral electroporation of IL-12 and SARS-Cov-2 spike plasmids drives a coordinated vaccine response and elicits robust anti-tumor immunity. Mia Han[1], Jack Y.

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Despite extensive clinical evidence on the efficacy and safety of SARS-CoV-2 vaccines, there remains a paucity of data on their effectiveness in cancer patients who are actively receiving antineoplastic therapeutics. A recent study demonstrated only ~30% of cancer patients had positive serologic test following 2 doses of FDA-authorized SARS-CoV-2 vaccines, in contrast to ~80% positivity rate in healthy individuals, regardless of the age. Therefore, further investigation into novel approaches to boost immune response to SARS-CoV-2 vaccines in cancer patients is required. Our previous preclinical and clinical studies have established intratumoral IL-12 plasmid (TAVO) electroporation (EP) induces localized expression of IL-12p70, converting immune-excluded tumors into inflamed immunogenic lesions, thereby generating objective responses in both treated and untreated, distant tumors. Based on the enhancement of immunotherapy efficacy by IL-12, we leveraged the flexibility of our DNA plasmid-EP platform to express SARS-CoV-2 spike protein in addition to IL-12 (CORVax12) as an intratumoral vaccine candidate which we hypothesized could not only drive anti-SARS-CoV-2 immune responses but also generate a productive anti-tumor response. Naïve mice were vaccinated via intradermal injection of SARS-CoV-2 spike plasmid followed immediately by EP with or without plasmid-encoded mIL-12 on days 1 and 21. Longitudinal serum samples were collected to interrogate virus-specific cellular responses as well anti-spike IgG antibody. A surrogate viral neutralization test (sVNT) assessed serum blockade of soluble human ACE2 binding to immobilized SARS-CoV-2 spike. Our data demonstrated that intradermally electroporated CORVax12 elicits significantly higher anti-SARS-CoV-2 spike IgG antibodies and neutralization when compared with EP of SARS-CoV-2 spike alone. Next, we asked if improved SARS-CoV-2 immune response may be observed when CORVax12 is incorporated into intratumoral EP in single-tumor bearing mice. CORVax12 robustly inhibited tumor growth, induced high percentages of germinal-center B cells and class switched B cells in tumor draining lymph nodes, and generated high of anti-spike IgG and neutralization antibodies. To further investigate systemic effects of this combination, we continued with contralateral tumor mice models. In both CT26 and B16-F10 tumor models, CORVax12 intratumoral EP induced strong systemic anti-tumor responses similar to IL-12 EP alone while also producing high serum levels of anti-SARS-CoV-2 spike IgG and neutralization antibodies. Critically, this anti-viral immunity did not limit this IL-12-based intratumoral anti-tumor therapy. In summary, our preclinical data indicates that intratumoral EP of CORVax12 can induce IgG responses to SARS-CoV-2 spike as well as apparent viral neutralizing activity all while maintaining local and systemic anti-tumor effects expected from TAVO Treatment. This combined intratumoral therapy represents a novel strategy to address both tumor burden and anti-SARS-CoV-2 immunity in patients with cancer.

Interrogating the Immune Landscape of Cancer

P007 Quiescent cancer cells form immunotherapy resistant reservoirs by forming an immune suppressive niche. Pilar Baldominos[1], Judith Agudo[1]. [1]Dana-Farber Cancer Institute, Boston, MA.

Immunotherapy is a promising treatment for Triple-Negative Breast Cancer (TNBC), but patients recur, arising the need to understand mechanisms of resistance. We discovered that in primary breast cancer, tumor cells that survive T-cell attack while still expressing the targeted antigen are in a quiescent state. Quiescent Cancer Cells (QCCs) are found clustering together forming intra-tumor cold regions with reduced immune infiltration. QCCs display superior tumorigenic capacity and higher expression of stemness genes than their proliferative counterparts. We adapted single-cell-RNA-sequencing with precise spatial resolution to profile infiltrating cells inside and outside the QCC niches. This transcriptomic analysis revealed hypoxia-induced programs and identified more exhausted T-cells, tumor-protective fibroblasts, and suppressive dendritic cells inside clusters of QCCs. This uncovered differential phenotypes in infiltrating cells based on their specific intra-tumor location and their proximity to functionally disntinct sub-populations of tumor cells. Thus, QCCs constitute immunotherapy-resistant reservoirs by orchestrating a local hypoxic immune-suppressive milieu that alters fibroblasts and dendritic cells leading to T cell dysfunction. Eliminating QCCs holds the promise to counteract resistance to immunotherapy and prevent disease recurrence in TNBC.

P008 Effect of route of Bacillus Calmette Guérin administration on the tumor immune microenvironment in a mouse model of non-muscle invasive bladder cancer. Aline Atallah[1], Arielle Grossman[1], William Tran[1], Jean-Francois Paré[1], Tiziana Cotechini[1], Charles H. Graham[1]. [1]Queen's University, Kingston, ON, Canada.

Background: Bladder cancer is the fifth most common cancer in North America, with up to 80% of cases being non-muscle invasive bladder cancer (NMIBC). The standard of care for high-risk NMIBC involves intravesical immunotherapy with Bacillus Calmette Guérin (BCG), a live attenuated bacterium. Unfortunately, most patients do not respond fully to this therapy, resulting in recurrences that sometimes progress to invasive disease. Moreover, our understanding of how BCG exerts its immunotherapeutic effect is incomplete. Using a mouse model of NMIBC, we compared the bladder tumor immune microenvironment (TiME) following intravesical versus intravenous (IV) administration of BCG.

Methods: Female C57Bl/6 mice (6-8 weeks old) were catheterized and 2.5 x 10^5 MB49 bladder cancer cells were instilled into the bladders after poly-L-lysine treatment. On day 7 post cancer cell instillation, mice with equal tumor volumes were randomized to receive three weekly intravesical or IV administrations of saline or BCG (2 mg/50 µl). Similarly, following catheterization, 50 µl of poly-L-lysine was administered into the bladders of control non-tumor bearing mice, which was then followed by three weekly intravesical instillations of BCG (2 mg/50 µl). In both cohorts, mice were sacrificed on day 23 and bladders were harvested and enzymatically dispersed to generate single-cell suspensions for analysis by polychromatic flow cytometry.

Results: Compared with intravesical BCG treatment, the TiME of mice treated with BCG intravenously was associated with a significantly higher proportion of $CD11b^-$ $CD3^+$, $CD3^-$, and $CD4^-$ lymphoid cells, as well as a significantly lower proportion of immature myeloid cells. Furthermore, compared with saline IV treatment, BCG administered IV resulted in a significantly larger $CD4^-$ T cell population. There were no significant differences in the TiME of intravesically BCG-treated vs intravesically saline-treated tumor-bearing mice. However,

compared with control non-tumor-bearing mice treated with saline intravesically, bladders of non-tumor-bearing mice treated intravesically with BCG had a significantly higher proportion of leukocytes, which were predominantly immature myeloid cells.

Conclusion/Significance: These results provide evidence that the route of BCG administration is an important determinant of the composition of the TiME in bladder cancer. Understanding the link between the TiME and BCG therapy may facilitate the development of new approaches to improve outcomes and reduce recurrence rates in patients with NMIBC. This knowledge may also help optimize BCG treatment regimen to avoid unnecessary therapy and induce an optimal anti-tumor immune response.

P009 Correlation between immune modulation of macrophage recruitment and new blood vessel formation in a subcutaneous murine mouse model of colorectal cancer. Shelby N. Bess[1], Timothy J. Muldoon[1]. [1]University of Arkansas, Fayetteville, AR.

Purpose: Colorectal cancer is the fourth most common cancer in the United States, accounting for more than 50,000 deaths annually[1]. The standard treatment is 5-fluorouracil (5-FU)-based chemotherapy, but emerging methods of immunotherapy have gained the attention of investigators and clinicians. A specific cytokine-based strategy for immunotherapy is the blockade of CCL2/MCP-1 (monocyte chemoattractant protein-1)[2]. CCL2 is a chemotactic cytokine that recruits circulating monocytes to the tumor microenvironment[2]. These monocytes differentiate into tumor-associated macrophages (TAMs), which promote pro-tumor functions including tumor growth, immunosuppression, and angiogenesis. CCL2 blockade in certain mouse models of cancer has been shown to reduce tumor burden but had not been studied in colorectal cancer. In this study, we examine how cytokine-targeted anti-CCL2 immunotherapy affects tumor-associated macrophage recruitment as well as the promotion of angiogenesis as a standalone and in combination with 5-FU.

Methods: Nine-week-old Balb/c mice (n=13) were acclimated for a week in the Small Animal Facility at the University of Arkansas. CT26 cells, a murine colon carcinoma cell line, were cultured in RPMI-1640 media, supplemented with 10% bovine serum, 0.2% amphotericin B/gentamicin, and 1% antibiotic antimycotic solution. $1x10^6$ CT26 cells were subcutaneously injected into the left flank of Balb/c mice (10 weeks old). The tumors grew to 75 mm^3, upon which Balb/c mice were randomly divided into saline control, immunotherapy, chemotherapy, and combination (immunotherapy + chemotherapy) groups. The immunotherapy and chemotherapy groups were intraperitoneally injected with a 28G insulin syringe with anti-CCL2 and 5-FU at a concentration of 5.0 mg/kg/dose and 15mg/kg/dose, respectively. The control group was injected with an equal volume of sterile saline on days while the combination group received a dose of anti-CCL2 and 5-FU. Tumor-associated macrophages were quantified via immunohistochemistry using anti-CD68 and tumor vasculature was quantified using anti-CD31. Correlation plots were also created to determine a relationship between macrophage recruitment and new blood vessel formation.

Results: There was a decrease in the total number of tumor-associated macrophages from Day 3 to 7 (33% decrease) when tumors were treated with anti-CCL2 and 5-FU. The area of vasculature in the combination group showed a slight decrease from Day 3 to Day 7 (29%

decrease). There was a slight positive correlation between total macrophage recruitment and new blood vessel formation ($R^2 = 0.3041$).

Conclusion: Overall, we have demonstrated that anti-CCL2 in combination with 5-FU has modestly slowed tumor growth, reduced the area of vasculature, and decreased the number of recruited tumor-associated macrophages in a murine model of colorectal cancer.

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P010 Antigen dominance hierarchies shape CD8 T cell phenotypes and immunotherapy response in tumors. Megan L. Burger[1], Amanda M. Cruz[1], Grace E. Crossland[1], Giorgio Gaglia[2], Cecily C. Ritch[2], Sarah E. Blatt[1], Arjun Bhutkar[1], Sara Z. Tavana[1], Sandro Santagata[2], Tyler Jacks[1]. [1]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, [2]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

CD8 T cell responses against different tumor neoantigens occur simultaneously, yet it is unclear whether they interact to potentiate or antagonize the overall anti-tumor response. In a genetically engineered mouse model of lung adenocarcinoma, we find that antigen dominance hierarchies are established in tumors wherein the antigen that most stably binds MHC dominates the CD8 T cell response. This negatively impacts the response to subdominant antigens, suppressing T cell expansion, differentiation and effector function; a phenotype that is reversed when the dominant antigen is removed. Intriguingly, the subdominant response is also enriched for a TCF1+ progenitor cell phenotype that has been correlated with response to immune checkpoint blockade (ICB) therapy. However, we find that the subdominant response does not preferentially benefit from ICB due to predominance of a dysfunctional subset of TCF1+ cells marked by CCR6 expression and differentiation to a Tc17 phenotype. CCR6+ TCF1+ cells are also found broadly across human cancers and do not correlate with patient response to ICB. This subset appears to be derived from poor T cell receptor stimulation, due to competition of T cells for good interactions with antigen presenting cells. Vaccination eliminates CCR6+ TCF1+ cells and disrupts the antigen dominance hierarchy, preferentially expanding the subdominant CD8 T cell response in tumors. Overall enrichment of TCF1+ cells is maintained amongst the subdominant response post-vaccination and current studies are exploring whether this promotes more durable tumor control or better response to ICB. These findings provide strong rationale for evaluating the relative response to high versus low pMHC stability antigens in clinical trials of pooled neoantigen vaccines, where low stability, subdominant antigens may contribute more to tumor control than previously realized.

P011 B cell subsets that correlate with anti-PD-1 resistance in a preclinical model of HPV+ oropharyngeal cancer. Pamela A. Merheb[1], Daniel L. Castañón[2], Michael Rivera[3], Jorge Galán[4], Stephanie M. Dorta-Estremera[3]. [1]Universidad Central del Caribe, Bayamón, Puerto Rico, [2]San Juan Bautista School of Medicine, Caguas, Puerto Rico, [3]University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico, [4]Comprehensive Cancer Center University of Puerto Rico, San Juan, Puerto Rico.

A subset of head and neck squamous cell carcinomas (HNSCC) is associated with infection with oncogenic strains of human papillomavirus (HPV) and its prevalence continues to rise in the US. Cancer progression has been associated, in part, with an impaired immunity. Because of this, immunotherapies such as immune checkpoint blockade (ICB) have been FDA approved to treat different types of cancers, including HNSCC. The ICB targeting the molecule programmed death cell-1 (PD-1) is used as treatment for HPV-related HNSCC, however, only around 30% of patients respond. Most studies have focused on characterizing T cell responses, however, B cells, are also present in the tumor microenvironment of HPV-positive HNSCC. Interestingly, different B cell subsets with differential functions that may promote or prevent an effective anti-tumor response are present in tumors and tumor-draining lymph nodes. Therefore, *fine regulation of anti-tumor and tumor-promoting B cell subsets may be necessary to promote an effective anti-tumor response and therefore prevent tumor progression*. However, the B cells that may be associated with responsiveness to anti-PD-1 in HPV+ oropharyngeal cancer remain undefined. By using the preclinical model for HPV+ oropharyngeal cancer (named mEER) we have determined that **tongue-implanted tumors are sensitive to anti-PD-1,** where around 50% of the mice clear the tumors, whereas **flank-implanted tumors are completely resistant to these treatments**. By using this in vivo model, we were able to characterize B cell subsets in anti-PD-1 sensitive and anti-PD-1 resistant tumors. First, we compared B cell subsets in untreated mice between tongue (anti-PD-1 sensitive) and flank-implanted (anti-PD-1 resistant) tumors by flow cytometry. Interestingly, flank-implanted tumors contained more PD-1-expressing B cells, which have been identified as an immunosuppressive population in hepatocellular carcinoma. The presence of immunosuppressive B cells on flank tumors was also supported by our finding that flank implanted tumors grew at a slower rate in B cell-deficient mice compared to wild-type mice. When we compared tongue-bearing tumor mice that responded or not responded to anti-PD-1, we observed that mice that did not respond to anti-PD-1 had a higher frequency of antibody-secreting cells in tumor-draining lymph nodes compared to responders. Lastly, when we cultured naïve B cells with tumor cell supernatant from mEER cells, B cells upregulated PD-1 and were able to produce the immunosuppressive cytokine IL-10. Our data suggest that HPV+ oropharyngeal tumors modulate B cell functions and that a differential infiltration of B cell subsets into tumors and tumor-draining lymph nodes correlates with responsiveness to anti-PD-1 therapy in a preclinical model of HPV+ oropharyngeal cancer. These results will aid in our long-term goal to elucidate novel mechanisms for the modulation of anti-tumor responses by B cells and the development of novel B cell-specific therapies for HPV-related cancers and, therefore, improve clinical outcomes in these patients.

P012 Investigation of interleukin-34 dependent regulation of renal carcinoma tumor microenvironment. Andrea Emanuelli[1], Wilfried Souleyreau[1], Lindsay Cooley[1], Tiffanie Chouleur[1], Marie-Alix Derieppe[2], Jean-Christophe Bernhard[3], Andreas Bikfalvi[1]. [1]University of Bordeaux, INSERM 1029, Bordeaux, France, [2]University of Bordeaux, Bordeaux, France, [3]Centre Hospitalier Universitaire (CHU) de Bordeaux, Bordeaux, France.

In 2020, Renal Carcinoma (RC) accounted for around 179,000 worldwide deaths and its mortality is predicted to double in the next 20 years. The major issue for RC patients is the absence of an efficient therapeutic option, especially for metastatic forms of the disease where the 5-years survival rate is close to 10%. The therapy of RC mainly targets the angiogenic and

immunosuppressive tumor microenvironment (TME), but it is rarely curative and drug resistance is almost inevitable. Lack of validated biomarkers and scarce knowledge of the biological processes occurring during RC progression are main reasons of therapy failure. In our unpublished work, using a syngeneic murine model of RC, we identified interleukin-34 (IL34) as potential biomarker of RC progression. In particular, we observed that increased IL34 expression was associated with enhanced cancer cell aggressiveness and reduced survival both in mice and in RC patients from two different cohorts (i.e. KIRC-TCGA, and UroCCR local cohort). IL34 exerts pleiotropic functions in different biological processes, including immunity regulation, cell proliferation and monocytes survival and differentiation into macrophages. The expression of IL34 in the TME is heterogeneous between cancer types, and high IL34 levels can represent either a poor (i.e. in brain and lung cancers) or a favorable (i.e. in neck and breast cancers) prognostic factor. Regarding to RC, IL34 role in the TME still remains elusive and has never been described. For this reason, using the KIRC-TCGA database, we performed Gene Ontology enrichment analysis using a list of IL34 co-expressed genes to predict potential IL34 regulatory functions in the TME of RC patients. This analysis revealed that such genes are involved in different immune system related processes, including positive regulation of leukocyte activation, IL-10 production and adaptive immune response. Subsequently, using IL34-overexpressing murine renal carcinoma RENCA cells implanted in BALB/c mice, we investigated the IL34-dependent alteration of the tumor immune microenvironment. In particular, we observed that, both in generated primary tumors and lung metastases, IL34 increased the density of tumor associated macrophages (TAM), which expressed M2-type markers (e.g. Cd206 and Cd163). Furthermore, in these samples, qPCR analysis showed an increase of interleukin-10 gene expression suggesting that IL34 could induce an immunosuppressive microenvironment. Conversely, when we blocked IL34 activity in lung metastases using an inhibitor of CSF1R, the main receptor of IL34, we observed a significant reduction in TAM accumulation and Il10 expression. The major aim of this project is to investigate whether IL34 can sustain immunosuppression and, consequently, chemoresistance by accumulating TAM in the TME. Furthermore, the IL34-dependent regulation of other immune cell populations (e.g. T-reg or myeloid-derived suppressor cells) is under investigation. The study of IL34 role in the TME of RC can be fundamental to improve the current therapy.

P013 Germinal center hypoxia in tumor-draining lymph nodes negatively regulates humoral immune responses and affects the activation of tumor-infiltrating T cells. Natalie Firmino[1], Kevin Bennewith[1]. [1]BC Cancer, Vancouver, BC, Canada.

Efforts to understand intrinsic and acquired resistance to anti-tumor immunotherapies have largely focused on characterizing factors within the primary tumor. However, lymph nodes are specialized hubs of immune response development, and an improved understanding of the microenvironmental conditions impacting immune cell activation in tumor-draining lymph nodes (TDLNs) may yield novel approaches for enhancing responses to anti-tumor immunotherapies. Using mouse models of breast cancer, we identified hypoxic regions amongst the B cell germinal center reactions of TDLNs. Injection of lethally irradiated tumor cells was sufficient to induce GC hypoxia, and hypoxia in TDLNs associated with the frequency of antibody- secreting cells. In vitro, hypoxia impaired the proliferation of activated B cells, and inhibited class-switching to IgG1 and IgA immunoglobulin isotypes. To assess the role of the hypoxic response in tumor-reactive GCs in vivo, we deleted von Hippel- Lindau factor (VHL) in class-switched B cells and

found decreased GC B cells in TDLNs, and reduced class-switched and tumor-specific antibodies in the circulation, indicating that the hypoxic response negatively-regulates tumor-induced humoral immune responses. pVHL deletion in class-switched B cells also reduced the expression of activation markers amongst tumor-infiltrating T cells and increased the presence of M2-like macrophages within the primary tumor. We detected the hypoxia marker carbonic anhydrase IX in the GCs of LNs from breast cancer patients, providing the first line of evidence that hypoxia develops within human GCs and validating our observations of hypoxia in TDLNs of pre-clinical mammary tumor models. We conclude that GC hypoxia develops in TDLNs, and that the hypoxic response negatively regulates tumor-induced humoral immune responses in pre-clinical models, with consequent impacts on the phenotype tumor-infiltrating T cells and macrophages.

P014 Driving immune-dependent metabolic vulnerabilities in the breast tumor microenvironment. John Heath[1], Stephanie Totten[1], Young Kyuen Im[1], Valerie Sabourin[2], Kathryn Hunt[1], Josie Ursini-Siegel[1]. [1]McGill University, Montréal, QC, Canada, [2]Lady Davis Institute for Medical Research, Montreal, QC, Canada.

The tumor microenvironment (TME) is a complex arms race composed of host stroma and rapidly adapting cancer cells. This symbiosis is further complicated when dissecting the influence of inflammation, due to its multifaceted function within the TME as a driver of both pro- and anti-tumor responses. Comprising a major component of the TME, targeting or exploiting tumor-associated inflammation has long been sought for therapeutic purposes. However, the optimal amount and composition of inflammation for such purposes remains elusive. We have recently identified an interferon (IFN)-dependent transcriptional response in breast cancer cells that renders a unique sensitivity to oxidative stress produced by the biguanide-class complex I inhibitor, phenformin. Using syngeneic murine models representing luminal B (PyMT) and basal (4T1) breast cancer, we identified a modest sensitivity to phenformin administration *in vivo*. However, phenformin effectiveness is dramatically enhanced when used in combination with the toll-like receptor 3 (TLR3) agonist, polyinosinic:polycytidylic acid (poly (I:C)). Critically, the effectiveness of this combination treatment was lost when performed in immune-deficient mice (SCID-beige), indicating that an immune component was essential. These findings highlight a novel role for inflammation and the immune system to promote sensitivity to oxidative stress in the TME. By identifying cell types responsible for cultivating a microenvironment conducive to biguanide sensitivity in breast cancer, we can identify a novel immune-biomarker of complex I sensitivity. Therefore, using high-parameter flow cytometry, we are analyzing systemic and tumor-infiltrating leukocyte diversity and enumeration in both our genetically and phenotypically distinct breast cancer models. Combination-therapy-induced immune populations will be further characterized in their molecular and cellular response to TLR agonism and complex I inhibition. Functional responses such as inflammatory-mediator production, immunomodulatory activity, and direct and indirect tumor cytotoxicity will be assessed, *ex vivo*. Furthermore, monoclonal antibody-mediated depletion of candidate cell types will be used to validate their requirement and contribution to the observed synergy to the combination therapy. Examples of tumoricidal synergy between biguanides and the inflammatory TLR agonists have not yet been described, establishing these findings as novel additions to the field of tumor biology. Furthermore, identifying how

sensitivity to oxidative stress can become situationally immune-dependent can greatly advance our understanding of how inflammation and metabolism intersect within the TME.

P015 Characterization and prognostic impact of DC-HIL in advanced colorectal cancer. Jude Khatib[1], Jin-Sung Chung[1], Sarah Reddy[1], Nivan Chowattukunnel[1], Allante Milsap[1], Yasin Goksu[1], Kiyoshi Ariizumi[1], Syed Kazmi[1]. [1]University of Texas Southwestern, Dallas, TX.

Introduction: DC-HIL is the T cell-inhibitory receptor that is expressed by exponentially expanding $CD14^{+}HLA\text{-}DR^{no/low}$ myeloid-derived suppressor cells (MDSC) and critically mediates the immunosuppressive function. We previously reported that this DC-HIL expression is associated with colorectal cancer (CRC) progression. The purpose of this investigation was to determine the correlation of DC-HIL-expressing MDSC subpopulation in blood with clinical outcomes in advanced CRC.

Methods: Patients with metastatic CRC (n = 63) were analyzed by flow cytometry for DC-HIL or PDL1 expression on blood $CD14^{+}HLA\text{-}DR^{no/low}$ MDSC. A retrospective chart review was performed to obtain data on demographics, tumor, and clinical characteristics including tumor laterality, histology, mutational and microsatellite stability status, stage, metastatic sites, CEA level, and white blood cell count, and differential and treatment outcomes including response to therapy and survival. Baseline characteristics were compared using the two-tailed Student's *t*-test, ANOVA test, or Pearson correlation test as appropriate. Survival analysis was estimated using the Kaplan-Meier method and Cox regression model, and groups were compared using the log-rank test.

Results: Median age at diagnosis was 56 years (range 30-78) and patients were predominantly male (59%) and white (76%). Forty pts had left-sided tumors (63%), the majority had moderately differentiated adenocarcinoma (66%) and stage IV colorectal cancer at diagnosis (68%). At the time of DC-HIL collection, 95% of pts had stage IV disease. Most (95%) pts had microsatellite stable disease, 24% of pts had a KRAS mutation and 22% had a BRAF mutation, 69% of patients underwent surgical resection of the primary malignancy or metastatic site or both. Median CEA level was 40 ng/ml, median white blood cell (WBC) count was 5.9 $x10^{9}$, median absolute monocyte count (AMC) was $0.64x10^{9}$, median absolute neutrophil count (ANC) was $3.6x10^{9}$, median monocyte to lymphocyte ratio (M/L) was 0.53 and median neutrophil to lymphocyte ratio (N/L) was 2.7. Most pts (90%) received FOLFOX chemotherapy as first-line and 84% of pts received an anti-VEGF agent throughout the course of their treatment. Median CD14+/PBMC for pts was 14.4, median %MDSC/PBMC was 7.0, median %DC-HIL+/MDSC was 66, median %DC-HIL+/MDSC/PBMC was 4.1 and median sDC-HIL was 20.0 ng/ml. Our results showed that there was a statically significant correlation between % MDSC/PBMC, %DCHIL+MDSC/PBMC, and the monocyte/lymphocyte (M/L) ratio with $p=0.047$ and $p=0.03$ respectively. There was no statistically significant correlation between DCHIL+/MDSC levels and response to therapy, time-to-next therapy (p=0.11), or overall survival (p=0.67).

Conclusion: $DC\text{-}HIL^{+}$ MDSC is positively correlated with M/L ratio, a possible prognostic marker in CRC patients. DC-HIL correlates poorly with other clinical outcomes; chemotherapy response, time-to-next therapy, or overall survival.

P016 Circulation immune landscape in canonical pathogenesis of colorectal cancer by CyTOF analysis. Ke-Feng Ding[1], Xiang-Xing Kong[1], Jia-Sheng Xu[1], Yu-Rong Jiao[1], Ye-Ting Hu[1], Qian Xiao[1], Xu-Ran Hao[2], Zong-Bao Gao[2], Jun Li[1]. [1]Zhejiang University, Hangzhou, China (Mainland), [2]Zhejiang Puluoting Health Tech CO. LTD, Hangzhou, China (Mainland).

Objective: The present study aims to firstly describe the circulation immune landscape in the canonical pathogenesis of colorectal cancer (CRC) by detecting the peripheral white blood cell using mass cytometry by time-of-flight (CyTOF) technology.

Methods: A total of 42 healthy controls (HC), 47 colorectal adenoma (CRA) patients, and 102 CRC patients were enrolled and their pathological information were also collected. A panel of 42 cell surface antigen markers were detected by mass spectrometry flow cytometry after peripheral blood mononuclear cells (PBMC) separated. The expression differences of various cell subsets among the three groups were further compared, and the cell subsets with different expressions were screened out for in-depth analysis.

Results: We annotated the PBMC as T cells, B cells, NK cells and myeloid cells, and performed identification and component comparison of cell subgroups respectively. Compared with HC and CRA, NKT cell subsets were significantly reduced in CRC, while Naïve CD4+ T cells, effector CD8+ T cells and central memory CD8+ T cells were increased. Compared with HC, Treg cells increased in CRA, and central memory CD4+ T increased in CRC; but Naïve CD8+ T cells and Naïve double-negative T (DNT) cells decreased in both CRA and CRC. Compared with HC and CRA, Naïve B cells were significantly decreased in CRC; but switched memory B cells were increased. CD16- NK cells were significantly higher in CRC while CD16+ NK cells were decreased compared with HC. CD16- monocytes were increased, but CD16+ monocytes were decreased in CRC when compared to HC. Basophils and monocytes were significantly increased in CRC compared to CRA. Furthermore, we also found Effector CD4+ T cells and Naïve B cells increased during lymph node metastasis, while unswitched B cells, plasmablast and basophils decreased.

Conclusion: We had successfully described the immune landscape of the canonical pathogenesis of CRC, and we further analyzed the changes in cell subsets related to the occurrence of lymphatic metastasis.

P017 Intra-tumoral infiltration of $GZMK_{high}$ $CD8^+$ T effector memory cells is associated with poor clinical outcome in early-stage colo-rectal cancer. Silvia Tiberti[1], Carlotta Catozzi[1], Caterina Scirgolea[2], Ottavio Croci[1], Danilo Cagnina[1], Stefano Campaner[1], Martin Shaefer[1], Nicola Fazio[1], Uberto Fumagalli-Romario[1], Guangwen Ren[3], Enrico Lugli[2], Luigi O. Nezi[1], Teresa Manzo[1]. [1]IEO-European Institute of Oncology, Milan, Italy, [2]Humanitas Clinical and Research Center, Milan, Italy, [3]The Jackson Laboratory Cancer Center, Bar Harbor, ME.

Background. A comprehensive understanding of the role of immune reactions in the progression of early-stage colorectal cancer (CRC) is currently lacking. Tumor infiltration by cytotoxic $CD8^+$ T cells has been associated with a better prognosis in several solid tumors, including CRC, and high levels of memory $CD8^+$ T cells prevent early metastatic invasion and are associated with better survival. However, the tumor immune infiltrate is highly heterogeneous and, although the

influence of various myeloid cell populations on T cell activity has been reported, our understanding of the interplay between different cell compartments at the tumor site and their impact on the clinical outcome is still in its infancy.

Methods. We sought to gain deeper mechanistic insights into the crosstalk between the TME and immune cells to elucidate their contribution to relapse and to implement patient stratification for immune-based therapeutics. Heterogeneity of immune cells within and across a novel prospective cohort of CRC patients (Stage I-III, treatment-naïve, n=60) was estimated by multiparametric flow cytometry, single-cell transcriptomics and multiplexed cytokine array while also obtaining functional information. Findings were validated on a larger independent cohort of CRC patients and a similar analysis extended to lung cancer (TCGA). Thus, we focused our attention on the crosstalk between $CD8^+$ T cells and neutrophils, of which the contribution in inhibiting or promoting tumor progression in humans remains marginally explored and often contradictory. Experiments were conducted in a pre-clinical mouse model and the mechanistic details dissected *in vitro*, providing initial evidence for future therapeutic applications.

Results. We defined an unique immune cell signature in which CRC tumors highly infiltrated by neutrophils contained a population of TILs with a peculiar activation and memory pattern, low levels of PD1 expression and characterized by high levels of Granzyme K ($GZMK^{high}$ $CD8^+$ T_{EM} cells). Remarkably, $GZMK^{high}$ $CD8^+$ T_{EM} cells were prospectively correlated with tumor relapse and their gene signature was found to be significantly associated with worse prognosis also in TCGA datasets. Here, we will present molecular details characterizing these interactions.

Conclusions. Our study highlighted the emergence of a crosstalk between $GZMK^{high}$ $CD8^+$ T_{EM} and neutrophils as one of the most important hallmarks in the CRC immune-landscape, crucial to implement current stratification, develop new targets of intervention and guide next steps toward personalized therapeutics.

P018 Gut microbiota-driven alterations in tumor immunity can modulate the growth of metastatic brain tumors. Golnaz Morad[1], Sarah B. Johnson[1], Jennifer A. Wargo[1]. [1]The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Metastatic brain tumors are associated with significant morbidity and mortality. The current limited understanding of the mechanisms underlying brain metastasis has hindered the development of efficient diagnostics and therapeutics for this disease. Microbiota has emerged as a novel hallmark of cancer, with a prominent role in tumorigenesis, tumor immunity, and response to treatment. However, the role of the microbiota 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 modulate the different steps of brain metastasis formation.

Methods: To explore the role of gut microbiota in brain metastasis, we depleted gut microbiota in conventionally raised mice using a broad-spectrum non-absorbable antibiotic regimen. Subsequently, melanoma tumor cells were 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: 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.

Conclusion: 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. Further studies, currently in progress, will determine the mechanistic role of dysbiotic microbiota and distinct microbial communities in this process.

P019 Intra-tumoral nerves regulate the local immune response at the tumor bed.

Anthony C. Restaino[1], Christopher T. Lucido[1], Jeffrey Barr[1], Paola D. Vermeer[1]. [1]Sanford Research, Sioux Falls, SD.

Patients with highly innervated tumors have a worse prognosis than those with less innervated disease. However, the process by which intra-tumoral nerves contribute to poor outcomes remains unclear. Previously, we identified that head and neck squamous cell carcinomas (HNSCCs) are infiltrated by sensory (TRPV1+) nerves. To map the source of these tumor infiltrating nerves, we intra-tumorally injected the fluorescently tagged nerve tracer lectin, wheat germ agglutinin (WGA), in our hind limb placed HNSCC tumors. Similar to other nerve tracers, WGA is taken up at nerve terminals and retrogradely transported to the neural somas. WGA labeled the somas of dorsal root ganglia (DRG), identifying them as the source of intra-tumoral nerves. Traced nerves were further characterized by immunostaining and identified as TRPV1+ (sensory), consistent with our initial findings. To test the contributions of tumor innervation to tumor growth and survival, wildtype and TRPV1-DTA (genetically deleted of TRPV1+ neurons) mice were implanted with a mouse model of human papillomavirus-induced (HPV+) HNSCC, mEERL cells. The absence of TRPV1 nerves results in slower tumor growth and improves survival. To mechanistically define how depletion of intra-tumoral nerves reduce tumor growth, we first collected tumors 25 days after injection in the hind limb and stained with a 12-color antibody panel. Stained tumors were then analyzed with flow cytometry to identify differences in the infiltrative immune cell populations between tumors grown in C57Bl/6 control mice and our TRPV1-DTA mouse model. Flow cytometry analysis indicated a decrease in the infiltrative myeloid derived suppressor cell (MDSC) population following ablation of TRPV1+ nerves. To understand how loss of TRPV1+ nerves mediate changes in the infiltrative immune cell population we conducted cytokine array analysis of condition media from cancer cells alone and in co-culture with DRG. Results indicate a shift in secreted cytokines following co-culture with DRG. These data indicate that intra-tumoral sensory nerves are recruited from loco-regional DRG to tumors injected in the hind limb. Our data also suggest that ablation of TRPV1+ nerves alter the intra-tumoral MDSC population and, in this way, potentially contributes to tumor growth. Finally, co-culture of mEERL cells with DRG in vitro results in changes in secreted cytokines, potentially explaining the change in the infiltrative immune cell populations.

Together, these data indicate a potential role for sensory nerves to regulate the local immune response in developing tumors.

P020 Lymph node colonization promotes distant tumor metastasis through the induction of tumor-specific immune tolerance. Nathan E. Reticker-Flynn[1], Weiruo Zhang[1], Julia A. Belk[1], Andrew J. Gentles[1], Ansuman Satpathy[1], Sylvia K. Plevritis[1], Edgar G. Engleman[1]. [1]Stanford University, Stanford, CA.

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. LNs are major sites of anti-tumor lymphocyte education, including in the context of immunotherapy, yet LN metastasis frequently correlates with further disease progression. Here, we find that LN metastasis represents a critical step in tumor progression through the capacity of such metastases to induce tumor-specific immune tolerance in a manner that promotes further dissemination of tumors to distant organs. 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 show that the presence of these LN metastases enables distant organ seeding of metastases in a manner that the parental tumor cannot, and this effect is eliminated in mice lacking an adaptive immune response. Furthermore, this promotion of distant seeding by LN metastases is tumor specific. Using flow cytometry and single-cell sequencing to perform organism-wide immune profiling, we identify multiple cellular mediators of tolerance. In particular, we find that LN metastases have the capacity to both resist NK cell cytotoxicity and induce regulatory T cells (Tregs) *in vitro*. Furthermore, depletion of NK cells *in vivo* enables non-metastatic tumors to disseminate to LNs, and ablation of Tregs using FoxP3-DTR mice eliminates the occurrence of lymphatic metastases. Adoptive transfer of Tregs from the LNs of mice bearing LN metastasis 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. Using genetic mouse models and photoconvertible tracking technologies, we show that Tregs induced within involved LNs preferentially traffic to distant sites compared to other CD4 populations. Through the use of whole exome sequencing, we show 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 regulation of chromatin accessibility. 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 (HNSCC), along with RNA-seq analysis of malignant populations sorted from HNSCC patients. Together, these findings demonstrate a critical role for LN metastasis in promoting tumor-specific immunosuppression.

P021 Highly multiplexed spatial analysis of the HCC tumor immune microenvironment using CODEX imaging. Benjamin Ruf[1], Noemi Kedei[2], Matthias Bruhns[3], Sepideh Babaei[3], Vanessa V. Catania[1], Simon Wabitsch[1], Chi Ma[1], Bernd Heinrich[1], Varun Subramanyam[1],

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Introduction: Hepatocellular Carcinoma (HCC) is a leading cause of cancer-related death and can be considered a prototype of inflammation-derived cancer arising from chronic liver injury. The cell composition of the HCC tumor immune microenvironment (TiME) has a major impact on cancer biology as the TME can have divergent capacities on tumor initiation, progress, and response to therapy. Recent development of multi-omics and single-cell technologies help us to comprehensively quantify the cellular heterogeneity and spatial organization of the TiME and to further our understanding of antitumor immunity.

Methods: Multiplexed immunofluorescence microscopy and high-dimensional flow cytometry was used to analyze immune cell infiltration in primary human liver cancer samples. We developed and validated a comprehensive 37-plex antibody panel for immunofluorescence imaging of human fresh frozen HCC samples. We applied highly multiplexed co-detection by indexing (CODEX) technology to simultaneously profile in situ expression of 37 proteins at sub-cellular resolution in 15 HCC patient samples using whole slide scanning. We established an image analysis pipeline to quantify all major cell populations in the human liver using supervised manual gating and unsupervised clustering algorithms. Proximity and nearest neighbor calculations as well as infiltration analysis was performed using HALO quantitative image analysis software.

Results: Using high-dimensional flow cytometry and parallel spatially resolved quantitative analysis of multiplexed immunofluorescence microscopy images, we profiled the single-cell pathology landscape for human HCC. The translation from raw images to numerical output was successfully established. *In situ* phenotyping of 4,500,000 single cells (including 1,500,000 $CD45^+$ immune cells) allowed for the quantification of cell phenotype clusters, differential analysis of activation markers and spatial features of each individual cell. CODEX imaging revealed detailed composition of the immune cell niche in human liver cancer tissue allowing for further distinct spatial analysis including infiltration analysis and nearest-neighbor analysis. We found impaired infiltration of $CD163^+$ macrophages, granulocytes, $CD8^+$ T cells, NK cells and MAIT cells into human HCC tumors compared to unaffected liver tissue. whereas regulatory T cells accumulated in tumor tissue. Flow cytometry data correlated well with image-based immune phenotyping. Beyond that, whole slide imaging allowed for the identification of the tumor-to-liver interface as a unique site of immune cell inhibition.

Conclusion: Here, we demonstrate that spatially resolved, single-cell analysis of human liver cancer tissue allows for the in-depth characterization of the immune cell composition of HCC. This tool can be used for biomarker research, to determine cellular functional states in intact

tissue and to spatially and functionally quantify interactions between immune cells in the context of hepatocarcinogenesis.

P022 Reciprocal influence of immune response and tumor hypoxia during glioblastoma progression. Anirudh Sattiraju[1], Valerie Marallano[1], Zhihong Chen[1], Sangjo Kang[1], Concetta Brusco[1], Aarthi Ramakrishnan[1], Li Shen[1], Dolores Hambardzumyan[1], Roland H. Friedel[1], Hongyan Zou[1]. [1]Icahn School of Medicine at Mount Sinai, New York, NY.

Tumor hypoxia is linked to poor outcome for glioblastoma (GBM), a highly malignant brain cancer, but underlying mechanisms and instigators that initiate tumor hypoxia remain unclear. We tracked tumor hypoxia in GBM in mice using a sensitive fluorescent reporter. We revealed that tumor hypoxia functions as a critical link between immune cells and tumor cells that drives malignant potency and immunosuppression in GBM. Single-cell RNA sequencing analysis revealed that hypoxic GBM cells are quiescent, display a mesenchymal transition, are more represented in recurrent GBM and predict worse patient outcome. Interestingly, the in vivo GBM hypoxia gene signatures surprisingly showed an enrichment for immune pathways. We unveiled two potential mechanisms of hypoxia-induced immunosuppression: by sequestrating activated immune cells in hypoxia zones, thus limiting inflammatory spread and cutting off immune cell communication, and by reprograming entrapped immune cells towards an immunotolerant state. Reciprocally, entrapped TAMs release CCL8 and IL1β as hypoxic niche factors that not only reinforce immune cell retainment in hypoxic cores, but also shape the transcriptional response of hypoxic GBM cells. Contrary to the conventional viewpoint that hypoxia arises from rapid tumor expansion outstripping vascular supply, we discovered anticancer immunity as an important driving force of tumor hypoxia; attenuating immune responses by implanting GBM in host mice with immunodeficiency or IL1β deletion greatly decreased GBM hypoxia. Altogether, our study revealed a reciprocal influence of anticancer immunity and tumor hypoxia, which has significant ramifications for prognosis and immunotherapy for GBM.

P023 Mismatch repair deficiency is not sufficient to increase tumor immunogenicity Peter M. K. Westcott[1], Francesc M. Remolar[2], Olivia Smith[1], Haley Hauck[1], Nathan J. Sacks[1], Zackery A. Ely[1], Alex M. Jaeger[1], William M. Rideout[1], Arjun Bhutkar[1], Daniel Zhang[1], Mary C. Beytagh[1], Roderick T. Bronson[3], David A. Canner[1], Santiago Naranjo[1], Abbey Jin[1], J. J. Patten[1], Amanda M. Cruz[1], Isidro Cortes-Ciriano[2], Tyler Jacks[1]. [1]MIT, Cambridge, MA, [2]EMBL-EBI, Cambridge, United Kingdom, [3]Harvard Medical School, Boston, MA.

Deficient DNA mismatch repair (dMMR) in human cancer is associated with high tumor mutation burden (TMB), frameshift mutation-derived neoantigens, increased T cell infiltration, and remarkable responsiveness to immune checkpoint blockade (ICB) therapy. Nevertheless, about half of these tumors do not respond to ICB for unclear reasons. While tumor cell line transplant models of dMMR have helped solidify the importance of TMB in immune response, critical questions remain regarding the role of immune surveillance in the evolution of dMMR tumors induced *in vivo*. Here, we developed autochthonous mouse models of lung and colon cancer with highly efficient ablation of MMR genes via *in vivo* CRISPR/Cas9 targeting. Surprisingly, dMMR in these models did not result in increased immunogenicity or response to ICB, which we showed is driven by profound intratumoral heterogeneity. Studies in animals depleted of T cells further demonstrated that immune surveillance in dMMR tumors has no

impact on TMB but shapes the clonal architecture of neoantigens. These results provide important context for understanding immune evasion in cancers with high TMB and have major implications for therapies aimed at increasing TMB.

Therapeutic Targeting of the Tumor Microenvironment

P024 Using innate immune ligands to activate adaptive immune cells for glioblastoma therapy. Richard T. Baugh[1], Hena Khalique[1], Janet Lei-Rossmann[1], Len Seymour[1]. [1]University of Oxford, Oxford, United Kingdom.

Glioblastoma (GBM) is one of the most aggressive and immunosuppressive brain tumors in adults. Despite conventional therapy of surgical resection, radiotherapy and temozolomide (TMZ) chemotherapy, tumors invariably recur and become resistant. The Natural Killer Group 2 Member D (NKG2D) receptor plays a key role in the innate immune control of cancers. The ligands for this receptor (NKG2DLs) are minimally expressed or absent on healthy cells. Stressed or malignant cells upregulate NKG2DLs, resulting in immune activation. We have developed a strategy targeting upregulated NKG2DLs on GBM cells with bi-specific T cell engagers (BiTEs). NKG2D BiTE contains a recombinant NKG2D receptor linked to an anti-CD3 domain. Simultaneous binding of the NKG2D BiTE to NKG2DLs on GBM cells and CD3 on T cells causes antigen-independent activation, pro-inflammatory cytokine release, and tumor cell death. Pre-treating GBM cells with radiation and TMZ causes further NKG2DL upregulation, increased NKG2D BiTE-mediated T cell activation, and sensitises GBM cells to T cell lysis. Meanwhile, healthy non-cancer cells exposed to the same pre-treatment do not trigger T cell activation with NKG2D BiTE. To deliver the BiTE directly into the tumor, the NKG2D BiTE has been encoded into the genome of an oncolytic herpes simplex virus 1 (oHSV-1). This oHSV is capable of selective infection, replication and cell lysis within GBM cells, but not healthy cells. Alongside the direct oncolytic capacity of the oHSV-1, it can also act as a gene therapy vector to express the NKG2D BiTE *in situ* to activate endogenous tumor infiltrating lymphocytes and direct cytotoxicity towards the remaining cancer cells. Combining conventional radio/chemotherapy with NKG2D BiTE immunotherapy presents an opportunity for synergy by enhancing NKG2DL expression on GBM cells and directing T cell lysis specifically towards these tumor cells, whilst avoiding responses towards non-cancerous cells. Meanwhile, local delivery of the NKG2D BiTE using an oncolytic virus endows tumor-specific expression directly within the tumor.

P025 Uncovering molecular actors of IDO-mediated T cell dysfunction with genome-wide CRISPR/Cas9 knockout screens. Raphaële Bombart[1], Jingjing Zhu[1], Benoit J. Van den Eynde[1]. [1]Ludwig Institute for Cancer Research, De Duve Institute, Université Catholique de Louvain, Brussels, Belgium.

Introduction: Despite tremendous progress in cancer immunotherapy, most patients fail to benefit because of poorly characterized immune resistance mechanisms. Among these, expression of the tryptophan-catabolizing enzyme Indoleamine 2,3-dioxygenase (IDO) has been found in several tumors and associated with local immune suppression, notably by inhibiting T

cell functions. However, the exact molecular pathways making T cells sensitive to IDO are still unclear. We propose to exploit the power of whole-genome single-guide RNA (sgRNA) CRISPR screens to uncover novel mechanisms of IDO-mediated T cell dysfunction.

Methods: Cas9-expressing transgenic mice were crossed with mice expressing a transgenic T cell receptor (TCR) specifically recognizing a tumor antigen called P1A. Primary Cas9xTCRP1A $CD8^+$ T cells were isolated from these mice and stimulated with P1A-expressing tumor cells. Cells were then transduced with the Teichmann retroviral genome wide CRISPR knockout library. Seven days after the first stimulation, $CD8^+$ T cells were re-stimulated in a control ($Tryptophan^{high}$, $kynurenine^{low}$) or selective ($Tryptophan^{low}$, $kynurenine^{high}$) medium, mimicking the function of IDO *in vitro*. After four days of screening selection, genomic DNA was extracted from remaining living cells and sequencing libraries were prepared. sgRNA representation was then assessed by next-generation sequencing.

Results: *In vitro* CRISPR knockout screening pipeline was successfully set up and validated. This includes: (1.) Successful transduction of the CRISPR knockout library, as well as efficient gene knockout in Cas9xTCRP1A primary $CD8^+$ T cells. (2.) Confirming the strong inhibition of T cell proliferation and survival in the selective medium as compared to the control medium, thereby validating the screening selection strategy. (3.) Optimizing genomic DNA extraction procedure, as well as sequencing library preparation. Finally, the proposed *in vitro* CRISPR knockout screening was successfully launched, and analysis of potential candidate genes is ongoing.

Conclusions and perspectives: Our study proposes to perform genome wide CRISPR knockout screens in T cells exposed to conditions mimicking IDO activity *in vitro*. Enriched sgRNA at the end of the selection should reveal genes whose inhibition improved T cell survival in $Tryptophan^{low}$/$kynurenine^{high}$ medium. Resulting potential candidate targets will then be validated by knocking them out in T cells and assessing T cell functions under conditions mimicking IDO-mediated immune suppression *in vitro.* Similar validation of top candidate genes will also be performed *in vivo* in a model of adoptive cell transfer of target-knockout T cells in mice bearing IDO-expressing tumors. The identified genes/pathways involved in T cell sensitivity to Tryptophan shortage / Kynurenine enrichment should reveal new relevant mechanisms of IDO-mediated immune suppression in the tumor microenvironment.

P026 Uncovering mechanisms of immune evasion in a novel immunogenic model of KRAS-mutant lung cancer. Jesse Boumelha[1], Sophie de Carné[1], Pablo Romero[1], Miriam Moliona[1], Julian Downward[1]. [1]Francis Crick Institute, London, United Kingdom.

Oncogenic KRAS mutations drive tumorigenesis in 30% of non-small cell lung cancer (NSCLC). Despite much effort, targeted therapies that aim to directly inhibit signaling pathways downstream of KRAS have limited clinical benefits for NSCLC patients, but the recent approval of PD-1/PD-L1 antibodies has led to striking durable responses. However, only a fraction of patients respond and therefore a deeper understanding of the mechanisms that drive immune evasion are required in order to broaden the clinical efficacy of immunotherapy. Increasing evidence suggests that oncogenic signaling pathways greatly influence the tumor immune landscape to impair anti-tumor immune responses. We therefore aim to understand the

mechanisms by which KRAS signaling mediates immune evasion in lung cancer. Current mouse models of KRAS-mutant lung cancer are poorly immunogenic, limiting investigations into tumor-immune interactions. To overcome this, we generated a novel transplantable KRAS-mutant lung cancer model, KPAR1.3, which triggers spontaneous anti-tumor immune responses and is sensitive to immune checkpoint blockade. To identify mechanisms of immune evasion we carried out an *in vivo* pooled CRISPR-Cas9 screen targeting 240 KRAS-regulated genes using this novel immunogenic model. This identified a number of genes that increased sensitivity or caused resistance to anti-tumor immune responses. As an alternative approach we utilized the recently developed class of mutant-specific KRAS-G12C inhibitors to assess the impact of inhibiting KRAS signaling on anti-tumor immune responses in KPAR1.3 tumors. KRAS-inhibition stimulated adaptive immunity *in vivo*, which contributed to the response of KPAR1.3 tumors in immune-competent mice. Together these data suggest that targeting KRAS, or KRAS-driven mechanisms of immune evasion, could broaden the clinical efficacy of immunotherapy in KRAS-mutant NSCLC.

P027 Extending intratumoral therapeutic durability using a multivalent immunotherapy platform. Livia W. Brier[1], Mavish Mahomed[1], Amy A. Twite[1], Adam Barnebey[1], Wesley M. Jackson[1]. [1]Valitor, Inc, Berkeley, CA.

Purpose: The risk of severe side effects has limited the development of immune cell activating immunotherapies. There is a critical need in immunotherapy drug development to enable focused and sustained immune cell activation within a tumor to induce a system-wide anti-tumor response. We have developed a novel immunotherapy platform that could be used to generate geographically focused cancer cell growth inhibition or immune cell activation, thereby stimulating an anti-tumor immune response against primary solid tumors that can also travel to secondary metastases.

Methods: Using published methods, we synthesized multivalent protein (MVP) conjugates by conjugating multiple copies (i.e. valency) of immune stimulating proteins (e.g. Interleukin-15) or anti-tumor antibodies (e.g. anti-Epidermal Growth Factor Receptor) to soluble, long-chain biopolymers (e.g. carboxymethylcellulose or hyaluronic acid, ~700 kDa). We verified that we can reproducibly generate MVP valencies ranging from 20-120 protein copies (±10%) per polymer backbone. We determined the binding affinity of these MVPs to their respective targets using biolayer interferometry and cell bioassays, and we measured the hydrodynamic radius of these immunotherapies using dynamic light scattering. Then, we injected flurosecently modified MVPs or their unconjugated counterparts directly into a variety of solid tumor models in mice. By taking longitudinal *in vivo* fuorescence measurments of the intratumoral (IT) drug signal over multiple days, we measured the IT half-life of each treatment.

Results: Based on binding affinity measurements, we found that MVP potency increased directly with their protein valency, and at high valency, the potency of MVPs were substantially greater than the unconjugated protein controls. Multivalent conjugation also increased the hydrodynamic radius of the MVPs to at least ten times larger than the unconjugated therapeutics. This large size was sufficient to slow the diffusion of MVP immunotherapies through dense tissues, such as solid tumors, as demonstrated by our *in vivo* studies. MVPs exhibited a higher IT

drug signal with a more durable gradient within the tumor compared to the unconjugated controls, resulting in an extension of their IT half-lives by >5X in mouse solid tumors.

Conclusions: The MVP platform can be used to modulate the potency and therapeutic durability for a wide range of immunotherapy targets. Further, the MVPs stay focused within the tumor after IT injection where they could generate a sustained anti-tumor immune response with minimal systemic exposure. Therefore, we expect MVP immunotherapies to have a better safety profile than IT or systemic delivery of an unconjugated therapeutic. We will continue to develop our internal MVP pipeline to finalize a candidate for IND-enabling studies. We are also seeking to collaborations for co-development of additional immunotherapies that could benefit from the extended IT exposure and potency modulation enabled by the MVP platform.

P028 Effects of histone deacetylase inhibition on major histocompatibility compatibility complex (MHC) class I expression, growth, and migration of cancer cells. Shelby M. Knoche[1], Gabrielle L. Brumfield[1], Benjamin T. Goetz[1], Bailee H. Sliker[1], Cecilia Barbosa[1], Svetlana Romanova[1], Tatiana Bronich[1], Donald W. Coulter[2], Joyce C. Solheim[1]. [1]University of Nebraska Medical Center, Omaha, NE, [2]University of Nebraska Medical Center, Children's Hospital & Medical Center, Omaha, NE.

Background: Cancer is a devastating scourge, causing morbidity and mortality in both adult and pediatric populations. In this study, we analyzed the effects of histone deacetylase (HDAC) inhibitors on the MHC class I expression, growth, and migration of cancer cells. Within cancer cells, MHC class I molecules bind to fragments of tumor-associated peptides, and then migrate to the cell surface to present the peptides to T lymphocytes and induce lysis of the tumor cells, thereby preventing further spread of the malignancy. Pancreatic cancer, a disease afflicting older adults, is now the third most common cause of cancer-related death overall in the U.S. Neuroblastoma is the third most common childhood cancer and results in 12% of cancer-associated deaths in children less than 15 years of age. In both of these cancers, HDAC expression has been shown to be dysregulated and abnormally high. Although several HDAC inhibitors have been extensively investigated in preclinical studies and have entered clinical trials, the two HDAC inhibitors that we are evaluating in our research (M344 and RGFP966) have been the focus of only a few prior studies, and much remains to be discovered about their therapeutic effects. Methods: We treated cancer cell lines with HDAC inhibitors over a range of concentrations for multiple timepoints. Our investigations included analysis of MHC class I expression by immunoblotting for total subunit protein levels and flow cytometric monitoring of MHC class I levels at the cell surface, immunoblotting for the co-inhibitory protein PD-L1, transwell assays for migration, and MTT assays for cell growth. New collaborative studies have been initiated to develop two varieties of nanoformulations for optimization of the delivery of these HDAC inhibitors to tumors. Results: Our flow cytometry analysis demonstrated that the expression of the cell-surface human MHC class I molecules detected by antibodies recognizing HLA-A and both HLA-B and –C was elevated on S2-013 cells following M344 treatment. Increased total MHC class I heavy chain protein expression was induced in S2-013 cells by both M344 and RGFP966, and RGFP966 also increased PD-L1 expression. In addition, M344 and RGFP966 reduced S2-013 pancreatic cancer cell growth, and M344 slowed S2-013 migration and Neuro2a neuroblastoma cell growth. Analysis of M344 efficacy in an S2-013 orthotopic xenograft mouse model showed significant reduction of the tumor growth rate. Novel

nanoparticles have been generated for M344 delivery using block copolymer and hyaluronic acid formulation strategies. Conclusions: Our evidence indicates that the HDAC inhibitors M344 and RGFP966 have anti-tumor potential via boosting MHC class I molecule expression to improve T cell recognition, as well as by tumor-intrinsic effects (i.e., down-regulation of proliferation and migration). Thus, both of these HDAC inhibitors warrant further analysis in adult and pediatric tumor mouse models, and may have potential for future clinical testing in patients.

P029 Expression of galectin-3 and pro-inflammatory cytokines in gliomas: Implications and therapeutic potential. John Caniglia[1]. [1]University of Illinois College of Medicine, Peoria, IL.

Galectin-3 (gal-3) is an integral protein in gliomagenesis that has been implicated in a myriad of oncogenic processes such as metabolic adaptation, angiogenesis, and tumor invasiveness. Gal-3 also has a well characterized role as a harbinger of inflammation, inducing the release of pro-inflammatory cytokines including interleukin-1 beta (IL-1B) and interleukin 6 (IL-6). However, these findings have not been well translated to oncology to date. Data from The Cancer Genome Atlas (TCGA) shows that gal-3 is highly overexpressed in low grade gliomas as well as glioblastoma ($n = 156$) compared to healthy brain tissue ($n = 5$). Further analysis of data from the Chinese Glioma Genome Atlas shows high expression of gal-3, IL-1B, and Il-6 are each associated with significantly ($p < 0.001$) worse survival in gliomas. Additionally, the expression of gal-3 is significantly correlated with both IL-1B and IL-6 expression across all malignant gliomas. In other cancers including neuroblastoma it has been shown that IL-6 expression is largely dependent upon gal-3 signaling pathways. This has been shown with regards to IL-1B as well, a molecule which along with IL-6 has been well described for promoting angiogenesis and metastasis. Given these cytokines' highly correlated expression to gal-3 in gliomas, it is likely that gal-3 is involved in inducing cytokine expression in glioma. Here we will discuss the myriad of signaling pathways through which gal-3 induces inflammatory cytokine release in cancer, the implications of these pathways on glioma progression, and the therapeutic potential of targeting this axis.

P030 Making cold tumors hot: Multi-faceted immune ignition by USP6. Ian C. Henrich[1], Kanika Jain[2], Laura Quick[1], Robert Young[1], Margaret M. Chou[2]. [1]Children's Hospital of Philadelphia, Philadelphia, PA, [2]Children's Hospital of Philadelphia/University of Pennsylvania School of Medicine, Philadelphia, PA.

Ewing sarcoma (ES) is the second most common bone cancer, affecting predominantly children and young adults. While patients with localized disease have a five-year survival rate of ~75%, this plummets to 20% for metastatic and recurrent patients. Immunotherapy has had limited success in ES, in large part because it is considered immunologically cold, with few tumor-infiltrating T lymphocytes (TILs). However, TILs actually vary significantly, and high levels portend greatly improved survival. In ES, TIL abundance correlates with the interferon (IFN)g-inducible chemokines CXCL9/CXCL10/CCL5. These immune features (i.e. IFNg response, CXCL9/10 production, and high $CD8^+$ TIL recruitment) define a hot TME, and predict both improved overall survival and response to immune checkpoint inhibitors (ICIs) across many malignancies. However, the tumor-intrinsic factors that confer this favorable

immune phenotype, in ES or any other cancer, are largely unknown, and their identification represents a window of opportunity for therapeutic intervention to improve patient outcomes.

We have discovered that high USP6 expression in ES patients is associated with dramatically improved overall survival. Our work has revealed that USP6 has potent and multi-faceted immunostimulatory properties: not only is it capable of triggering all hallmark features of a hot TME, but its expression in ES cells directly activates the cytolytic function of NK cells, and also induces secretion of a complex array of immune-activating, anti-tumorigenic chemokines that affect both innate and adaptive immune lineages. *In vivo*, USP6 inhibits growth of human ES cell xenografts in nude mice, and enhances intratumoral infiltration/activation of natural killer (NK) cells and myeloid lineages. We hypothesize that USP6 improves ES patient outcome due to these pleiotropic immunostimulatory functions, which engender a hot TME with resultant activation of multiple cytolytic immune lineages that effect tumor cell elimination.

We have sought to harness the multi-faceted immune-igniting properties of USP6 into a novel immunotherapeutic by delivering USP6 mRNA using lipid nanoparticles (LNPs) customized for *in vivo* delivery into ES cells. Preliminary data validate the efficacy of intratumorally administered USP6 LNPs in stimulating immune cell recruitment and activation, and suppressing xenograft growth, with a fraction of tumors undergoing complete regression. Furthermore, we have observed that intratumoral expression of USP6 can trigger systemic activation of the immune system, including increased levels and activation NK cells. Notably, increased circulating levels of NK cells have been associated with improved patient prognosis.

Despite the success of CAR-T cells and ICIs, there are significant hurdles limiting their efficacy, and it is essential to pursue therapeutics that engender an immunologically hospitable TME, as well as activate other cytolytic immune lineages, such as NK cells, both of which are encompassed by USP6 LNP therapy.

P031 Enrichment of photodynamically-primed anti-tumor immune infiltrates in pancreatic cancer: Enabling enhanced immunotherapy. Pushpamali De Silva[1], Mohammad Ahsan Saad[1], Zhiming Mai[1], Shazia Bano[1], Assiris P. Camargo[1], Tayyaba Hasan[1]. [1]Wellman Center for Photomedicine, Department of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, MA.

Pancreatic ductal adenocarcinoma (PDAC) has a dismal 5-year survival rate of 10%. It poorly responds to conventional cancer treatments. Immune checkpoint blockade (ICB) has revolutionized cancer therapeutics. However, in PDAC, even the modest success of ICB is limited to ~1-3% of patients as the majority of patients' tumors are considered as immunologically ''cold'' due to their highly immunosuppressive tumor microenvironment (TME). Photodynamic therapy (PDT) is an FDA approved anti-cancer therapy that utilizes light, a photoresponsive non-toxic chemical called a photosensitizer, and oxygen to generate reactive molecular species that confer direct cytotoxicity or vascular shutdown. PDT alters the TME transiently in a process termed photodynamic priming (*PDP*), making it more receptive to subsequent therapies, including chemo- and immunotherapy. Previous studies have demonstrated that *PDP* is capable of affecting both the innate and adaptive immune systems. These immune-stimulatory effects occur through its ability to induce immunogenic cell death via the release of

damage-associated molecules (DAMPs) and tumor-associated antigens. In this study, we investigated *PDP*-induced immunogenicity in PDAC. In an immunocompetent mouse model of PDAC, we evaluated tumor-infiltrating lymphocyte (TIL) enrichment in tumors and ongoing immune responses in mouse spleens/blood from 1h to 120h post-*PDP* treatment comparing the responses in untreated controls. We observed gradual increases in T and B cell infiltration from 1h to 120h post-*PDP* where the T cell subset analysis showed an enrichment of $CD8^+$ T cells in *PDP*-treated tumors. These $CD8^+$ T cells showed temporal increases in PD1, CTLA4 and TIM3 immune checkpoints suggesting *PDP*-induced immune priming in the TME. This was further evidenced by the upregulation of DAMPs, including high mobility group box protein-1 and calreticulin in *PDP* treated tumors. Analyzing spleens of mice, we detected a significant increase in $CD11C^+MHC11^{hi}$ dendritic cells from 1h to 24h post-*PDP*. Also, activation of an adaptive immune response in splenic B cell follicles was noted by the presence of proliferating germinal centers by 120h post-*PDP*. In addition, evaluating the blood of *PDP* treated mice, we detected expansion of $CD8^+$ effector memory T and natural killer cell populations, signifying *PDP*-induced systemic immune responses compared to untreated mice. We further investigated how immune cells infiltrate *PDP*-treated tumors. A reduced formation of blood ($CD31^+$) and lymphatic (Lyve-1^+) vessels post-*PDP* at 120h was observed. However, decreases in PDL1, collagen and fibroblast activation proteins were observed in *PDP*-treated tumors at 120h, suggesting mitigation of immunosuppressive mechanisms and enhanced tumor permeability, allowing TIL migration. Our data shows converting immunologically silent PDAC tumors into inflamed ‘’hot’’ tumors by triggering not only a local immune infiltration but also enhanced systemic immune responses, ultimately enhances the immunogenicity of pancreatic tumors.

P032 Expression of an IL-15 receptor fusion protein enhances the persistence of TRuC-T cells. Michelle Fleury[1], Courtney Anderson[1], Amy Watt[1], Holly Horton[1], Adam Zieba[1], Jian Ding[1], Robert Tighe[1], Robert Hofmeister[1], Derrick McCarthy[1], Dario Gutierrez[1]. [1]TCR2, Cambridge, MA.

Adoptive cell therapies have shown great promise in hematological malignancies. To realize the potential of T cell therapies in solid tumors, we have developed T cell receptor fusion construct (TRuC®) T cells, which are equipped with an engineered T cell receptor that utilizes all TCR signaling subunits and recognizes tumor-associated antigens independent of HLA. In clinical trials, mesothelin-targeting TRuC-T cells (aka TC-210 or gavo-cel) have shown unprecedented results in patients suffering from advanced mesothelioma and ovarian cancer. To potentially increase the effector function and persistence of TRuC-T cells in the hostile tumor microenvironment, we generated TC-210 T cells that express a membrane-tethered IL15Rα-IL15 fusion protein. IL-15 is a common γ chain cytokine that promotes the differentiation, maintenance, and effector function of memory CD8+ T cell subsets and confers resistance to IL-2-mediated activation induced cell death (AICD). In vitro, the co-expression of the IL-15 fusion protein enhances T cell proliferation and persistence upon repeated stimulation with MSLN+ cancer cell lines, while exhaustion marker expression is decreased. Furthermore, IL-15 enhanced TC-210 T cells sustain a significantly higher TCF-1+ population. When tested in a mesothelioma xenograft mouse model, the presence of the IL-15 fusion protein increased tumor infiltration and persistence of TC-210 T cells. Altogether, the presented data support clinical studies that explore the impact of IL-15 enhancement on the persistence of TC-210 T cells and depth of response in patients with MSLN+ malignancies.

P033 Bone microenvironment-suppressed T cells increase osteoclast formation and the development of osteolytic bone metastases in mice. Danna L. Arellano[1], Patricia Juárez[1], Paloma S. Almeida-Luna[1], Felipe Olvera[2], Samanta Jiménez[1], Pierrick G. J. Fournier[1]. [1]Biomedical Innovation Department, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Mexico, [2]Departamento de Biología Molecular y Bioprocesos, Instituto de Biotecnología Universidad Nacional Autónoma de México, Cuernavaca, Mexico.

Bone metastases are a highly debilitating complication in more than 70% of patients with advanced breast and prostate cancer, causing fractures, nerve compression, hypercalcemia. Currently approved treatments fail to cure bone metastases or increase patient survival. Immunotherapies activating T cells to fight cancer cells are changing cancer treatment, causing a durable response in some patients. However, it remains unclear whether immunotherapy could benefit patients with bone metastases. The bone microenvironment combines various immunosuppressive factors that could limit its efficacy. Also, T cells could increase bone resorption releases pro-metastatic growth factors from the bone matrix that can increase cancer cell growth. Using syngeneic mouse models, our study revealed that bone metastases from 4T1 breast cancer contain tumor-infiltrating lymphocyte (TILs) and that their development is increased in normal mice compared to immunodeficient and T-cell depleted mice (x3.6 and x1.6, respectively, $p<0.05$). This effect seemed caused by the TILs in bone, as T-cell depletion did not affect bone metastases from RM-1 prostate cancer cells that lack TILs and increased the volume of 4T1 orthotopic tumors. T cells from bone metastases expressed the pro-osteoclastic genes *Rankl* and *Tnfa*, and increased osteoclast formation *ex vivo* and *in vivo*, at the tumor-bone interface, contributing to bone metastasis development. This pro-osteoclastic effect is specific to inactivated T cells, since activated T cells, secreting IFNγ and IL-4, actually suppressed osteoclastogenesis, which could benefit patients. We confirmed that T cells in bone metastases were not activated, as >85% of T cells lacked the activation marker CD69. In addition, T cells from 4T1 bone metastases could not be activated in *ex vivo* cultures confirming the presence of immunosuppressive factors in this microenvironment. 4T1 bone metastases were associated with an increase of polymorphonuclear- and monocytic-MDSCs, metabolically active as they produced elevated levels of ROS and NO, respectively. While effective in other models, the PDE-5 inhibitor sildenafil and the bisphosphonate zoledronic acid did not affect the levels of MDSCs in bone metastases or their production of ROS and NO. Seeking other therapeutic targets, we found that 80% of monocytic-MDSCs are PD-L1^{+} in bone, which could trigger T-cell suppression since 70% express its receptor, PD-1. Collectively, our findings identified a new mechanism by which suppressed T cells increase osteoclastogenesis and bone metastases, and also provide a rationale for using immune checkpoint inhibitors since T-cell activation would increase their anti-cancer and their anti-osteoclastic properties.

P034 Tumor-targeted interleukin-12 and Entinostat combination therapy improves cancer survival by reprogramming the tumor immune cell landscape. Kristin C. Hicks[1], Paul L. Chariou[1], Yohei Ozawa[1], Christine M. Minnar[1], Karin M. Knudson[1], Thomas J. Meyer[2], Jing Bian[2], Margaret Cam[2], Jeffrey Schlom[1], Sofia R. Gameiro[1]. [1]NCI/CCR/LTIB, Bethesda, MD, [2]NCI/CCR/CCBR, Bethesda, MD.

Clinical benefit remains elusive for most patients with poorly inflamed carcinomas treated

with immune checkpoint blockade. Converting the tumor microenvironment into a functionally inflamed immune hub would increase clinical benefit. By using comprehensive single-cell transcriptome, proteome, and immune cell analysis, we demonstrate here that Entinostat, a class I histone deacetylase inhibitor, facilitates accumulation of the necrosis-targeted recombinant murine immune-cytokine, NHS-rmIL12, in experimental mouse colon carcinomas and poorly immunogenic breast tumor in which the therapy was curative. Combination therapy reprogrammed the tumor innate and adaptive immune milieu to an inflamed landscape, where the concerted action of highly functional CD8+ T cells and activated neutrophils drove a dramatic macrophage M1-like polarization leading to complete tumor eradication in 41.7%-100% of cases. Biomarker signature of favourable overall survival in multiple human tumor types showed close resemblance to the immune pattern generated by Entinostat/NHS-rmIL12 combination therapy. Collectively, these findings provide a rationale for combining NHS-IL12 with Entinostat in the clinical setting.

P035 A promising cancer immunotherapy target: Novel fully human agonist antibodies against the human T-cell costimulatory receptor CD27. Yulia Ovechkina[1], Shaarwari Sridhar[1], David Jurchen[1], David Peckham[1], Eric Tarcha[1], Shawn Iadonato[1], Thierry Guillaudeux[1]. [1]Kineta Inc., Seattle, WA.

CD27 is a member of the TNF receptor superfamily and plays a critical role in T-cell activation by providing a costimulatory signal. CD27 signaling enhances T-cell proliferation, activation and differentiation of effector and memory T cells and therefore promotes cytotoxic T cell (CTL)-based anti-tumor immunity. Agonistic stimulation of CD27 is a promising cancer immunotherapy approach to boost specific T cell driven anti-tumor responses. In this study, we generated a series of 147 fully human monoclonal anti-CD27 antibodies and tested their agonist properties to stimulate T cell activation. Using a NF-κB reporter Jurkat cell line, we evaluated *in vitro* the ability of anti-CD27 antibodies to induce CD27 receptor activation. With this assay, five antibodies have been selected for their agonist properties. When combined with suboptimal T cell receptor (TCR) stimulation, agonist antibodies induced CD27 receptor activation with an EC50 of 1-5 ug/mL. We also used human peripheral blood T cells to characterize the CD27-mediated costimulatory effects of agonist antibodies in combination with TCR stimulation. Our anti-CD27 monoclonal antibodies boosted T cell proliferation and induced IL-2 and TNFalpha secretion only in a presence of TCR engagement. Moreover, CD27 agonists induce strong T cell proliferation in a Mixed Lymphocyte Reaction. CD27 antibodies were shown to bind human and cynomolgus monkey CD27 with a KD value of 5-20 nM as determined by BioLayer Interferometry, but do not bind to mouse CD27. In vivo experiments are currently ongoing to demonstrate the efficient anti-tumor activity of the selected CD27 agonist antibodies in different mice tumor models. In conclusion, we have developed and successfully selected efficient fully human immunostimulatory agonist CD27 mAbs as a promising cancer immunotherapy.

P036 A high-throughput customized cytokinome screen of colon cancer cell responses to small-molecule oncology drugs. Kelsey E. Huntington[1], Anna Louie[1], Lanlan Zhou[1], Wafik S. El-Deiry[1]. [1]Brown University, Providence, RI.

Inflammatory cytokines, immune stimulants, chemokines, and tumor growth/growth suppressing factors are molecular messengers that circulate and have the capability to modify the tumor

microenvironment and impact response to therapeutics. The characterization of soluble mediators as biomarkers for diagnosis and prognosis is of interest in oncology. We utilize the cytokinome to characterize the response of colorectal tumor cell lines to selected small-molecules in oncology as a proof-of-concept dataset with immune synergy heat map rankings of analytes for drug and cell line combinations. We observed overall trends in drug-class effects with MEK-, BRAF-, PARP-inhibitors, and Imipridones in cytokine, chemokine, and growth factor responses that may help guide therapy selection. MEK-inhibitor treatment downregulated analytes VEGF, CXCL9/MIG, and IL-8/CXCL8 and upregulated CXCL14/BRAK, Prolactin, and CCL5/RANTES. BRAF-inhibitor treatment downregulated VEGF and IL-8/CXCL8, while increasing sTRAIL-R2. Treatment with PARP-inhibitors decreased CXCL9/MIG, IL-8/CXCL8, CCL3/MIP-1 alpha, VEGF, and CXCL14/BRAK, while treatment increased sTRAIL-R2 and prolactin. Treatment with Imipridones decreased CCL3/MIP-1 alpha, VEGF, CXCL14/BRAK, IL-8/CXCL8, and Prolactin and increased CXCL5/ENA-78. We also observed differential responses to therapeutics depending on the mutational profile of the cell line. In the future, a similar but larger data-set may be utilized in the clinic to aid in immune synergy prediction of a patient's response to therapy based on tumor genotype.

P037 Interleukin-10 is a dominant and reversible mechanism of immune evasion in human colorectal cancer liver metastasis. Teresa S. Kim[1], Kevin Sullivan[1], Xiuyun Jiang[1], Cynthia Hsu[1], Kevin Labadie[1], Karan Kohli[1], Heidi Kenerson[1], Sara Daniel[1], Arezou Abbasi[1], Raymond Yeung[1], Venu Pillarisetty[1]. [1]University of Washington, Seattle, WA.

Background: Colorectal cancer liver metastasis (CRLM) causes major morbidity and mortality. Improved systemic therapies are crucial. We previously reported that macrophages infiltrated CRLM and expressed the immunosuppressive cytokine interleukin-10 (IL-10). In patient-derived CRLM tumor slice cultures, we found that a neutralizing antibody against IL-10 (anti-IL-10) caused tumor apoptosis. Here, we investigated the efficacy and immune-dependent mechanisms of IL-10 blockade in a larger cohort of CRLM patients.

Methods: Tumor specimens were obtained from consenting patients at the time of surgery and cut into 250mm-thick tumor slice cultures as previously described. Tumor slices were treated with control or neutralizing antibodies against programmed cell death protein 1 (PD-1, EH12.1), IL-10 (JES3-9D7), IL-10 receptor alpha (IL-10RA, 3F9), major histocompatibility complex (MHC) class I (G46-2.6) or class II (Tu39). After 4-6 days of treatment, tumor apoptosis was evaluated by cleaved caspase-3 (CC3) immunohistochemistry (IHC) or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Tumors were evaluated by IHC and *in situ* hybridization (ISH) for immune markers. Student's t-test, paired t-test, or 1-way ANOVA was used as indicated. $p < 0.05$ was defined as significant.

Results: We generated tumor slice cultures from 34 unique CRLM patients, 76% (26/34) of whom received preoperative chemotherapy, and all of whom had either microsatellite stable tumors (76%, 26/34) or unknown microsatellite status. Anti-PD-1 did not generate tumor apoptosis (n = 4 patients' tumors). In contrast, anti-IL-10 caused nearly 2-fold increased tumor apoptosis compared with control, in the majority of 34 CRLM patients' tumors evaluated (median 50.1% versus 27.4% apoptotic cells, $p < 0.0001$). IL-10 receptor blockade generated a similar but non-significant increase in apoptosis. Tumors treated with anti-IL-10 demonstrated

increased frequency of $CD8^+$ T cells (median 17.8% versus 7.0% of total cells, n = 5 patients' tumors, p = 0.02) and a non-significant increase in activated $PD\text{-}1^+CD3^+$ T cells. IL-10 blockade also increased tumor *IFNG* expression (median 2.9 versus 2.3 counts per total cell number, n = 6 patients' tumors, $p < 0.05$). Macrophage frequency did not increase, but MHC class II expression nearly doubled (median 19.5% versus 11.5% of total cells, n = 5 patients' tumors, p = 0.02). To test which immune cells were required for anti-IL-10 effect, we treated human CRLM slices with anti-IL-10 +/- blocking antibodies against MHC class I or II. In the 3 of 4 patients' tumors that responded to anti-IL-10, the effect was nearly completely reversed by aMHC-I or aMHC-II (p = 0.0005).

Conclusion: IL-10 is a dominant and reversible mechanism of immune evasion in human CRLM. IL-10 blockade nearly doubled tumor apoptosis in a heterogeneous cohort of CRLM patients' tumors, through a mechanism that required intact $CD8^+$ T cell and antigen-presenting cell function. IL-10 is a compelling immunotherapeutic target for CRLM.

P038 Identification and functional evaluation of monoclonal antibodies specifically targeting human Carbonic Anhydrase IX. Anne E. G. Lenferink[1], Jason Baardsnes[1], Traian Sulea[1], Cunle Wu[1], Maurizio Acchione[1], Maria L. Jaramillo[1], Paul C. McDonald[2], Francois Benard[2], Shoukat Dedhar[2]. [1]National Research Council Canada, Montreal, QC, Canada, [2]BC Cancer Research Institute, Vancouver, BC, Canada.

Poor vascularization of solid tumors leads to inadequate nutrient and oxygen supplies which forces tumor cells to reprogram their metabolism. Consequently, the tumor cell's environment becomes acidic and hypoxic. This triggers signaling cascades involving e.g. heterodimeric hypoxia-inducible factor (HIF). Activation of this hypoxia-induced transcriptional program is crucial for the tumor cell to survive its hostile microenvironment and its ability to metastasize. One of the genes HIF upregulated is carbonic anhydrase (CA)-IX (CAIX, gene G250/MN-encoded transmembrane protein). CA-IX catalyzes carbon dioxide (CO_2) thereby generating a proton (H^+) and bicarbonate (HCO_3^-), the latter of which is transported back into the cell and utilized to help safeguard intracellular pH (pHi) stability. Except for the stomach and the gallbladder, CA-IX expression is negligible in normal tissues. In contrast, a broad range of tumors express high levels of CA-IX, where the protein can serve as a biomarker for the early stages of tumor development but also as tumor marker of hypoxia associated with resistance to chemotherapy and radiotherapy. Preclinical and clinical studies have shown that CA-IX is a promising therapeutic target for detection and therapy for several cancer types. To date only a limited number of ant-CAIX monoclonal antibodies (mAbs) have been available for clinical testing as therapeutic and imaging agents. In the current study, we generated and functionally categorized a panel of 51 mouse mAbs that specifically bind to human CA-IX. Characterization of the mAbs revealed that of the mAbs with the best biophysical characteristics, three (3) mAbs are suitable as an antibody-drug conjugate (ADC), two (2) mAbs inhibit the CA-IX enzyme activity, and one (1) mAb that is suitable for CA-IX imaging purposes. These preliminary data presented here could thus form the basis for the development of novel CA-IX targeted immunotherapies and diagnostic tools for the treatment of cancer.

P039 The immune profile of colorectal and pancreas adenocarcinomas: Differences between central and peripheric tumor regions. Andreia Maia[1], Joana R. Lérias[2], Luis Miguel

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Introduction The tumor microenvironment(TME) is a complex system, where malignant cells co-exist and communicate with immune and non-immune cells(1). This interaction can induce an immune response by releasing cytokines that will regulate and recruit immune cells which may promote tumor shrinkage (2). High tumor-infiltrating lymphocytes (TILs) in the TME have been correlated with favorable prognosis in colorectal and pancreatic adenocarcinomas (CRC and PDAC) (3,4). Also, high number of NK cells has been associated with a better prognosis in different solid tumors (5). Therefore, a detailed analysis of immune cells' distribution in the TME could help understand which of these cells have higher tumor-infiltrating capacities and may be used as cell product of immunotherapy.

Experimental Procedure Fresh CRC (n=6) and PDAC (n=6) tumor specimens were obtained within 20 minutes after surgery. A specialized pathologist collected the central and peripheric regions (CR and PR, respectively) of the tumor. From each region, half of the specimen was used for immunophenotyping analysis and the other half was cultured with medium enriched in IL-2 (1000 IU/mL) for 12 days. Immunophenotyping was performed using CD45, CD19, CD3, CD4, CD8, CD56, CD16 and LiveDead marker through flow cytometry at days 0, 6 and 12 (% correspond to mean). Formalin-fixed paraffin-embedded blocks were generated with the 'mirror' sample of the fresh collected specimens and immunohistochemistry (IHC) for CD8 and CD56 was performed.

Results In the PR of CRC we identified 12% of B-cells, 66% of T-cells (31% $CD4^+$ and 23% $CD8^+$ T-cells) and 5% of NK cells (17% $CD56^{bright}CD16^-$ cells and 24% $CD56^+CD16^+$ cells). In the CR of CRC we observed 7% of B-cells, 70% of T-cells (40% $CD4^+$ and 23% $CD8^+$ T-cells), and 6% of NK cells (15% $CD56^{bright}CD16^-$ cells and 44% $CD56^+CD16^+$ cells). In the PR of PDAC we observed 13% of B-cells, 76% of T-cells (38% $CD4^+$ and 35% $CD8^+$ T-cells) and 3% of NK cells (5% $CD56^{bright}CD16^-$ cells and 40% $CD56^+CD16^+$ cells). In the CR of PDAC we identified 16% of B-cells, 66% of T-cells (31% $CD4^+$ and 32% $CD8^+$ T-cells) and 10% of NK cells (2% $CD56^{bright}CD16^-$ cells and 55% $CD56^+CD16^+$ cells). During culture, the % of B-cells decreased while the % of T-cells and NK cells increased. IHC results show that $CD8^+$ T-cells are more abundant in the TME and formed larger clusters than NK cells. Furthermore, although the majority of NK cells were found in the stroma, in some cases NK cells were located inside the malignant epithelium.

Conclusions Our data in this small set of specimens show that TME immune cells in PRs and CRs present different phenotypes for both CRC and PDAC. Indeed, the PRs show higher TIL and NK cells' infiltration than the central ones. Among different patients, the distribution and

quantity of $CD8^+$ T-cells and NK cells were different, which may have clinical significance. We also observed that immune cells may communicate with tumor cells both in CRC and PDAC since these cells were identified inside the malignant epithelium in some tumor specimens.

P040 Harness the immune-modulatory activities of Toxoplasma gondii to improve lymphocyte infiltration into brain tumors. Yen Nguyen[1], Xiaoyu Zhao[2], Sarah Ewald[2], Tajie Harris[2], Hui Zong[2]. [1]University of Virginia, School of Medicine, Charlottesville, VA, [2]University of Virginia, Charlottesville, VA.

While checkpoint inhibitors carry great promises for T-cells-based immunotherapy for brain tumors, the presence of abundant T cells supported by the tumor microenvironment (TME) is a prerequisite for it to be effective. Our lab and others' work using mouse models and patient samples demonstrated that, Shh-subtype medulloblastoma, a well-studied pediatric brain tumor, lacks both T cell infiltration and a supportive TME, posing a critical barrier for immunotherapy. I envision that the protozoans *Toxoplasma gondii* (*T.gondii*) as a biological agent could provide a unique solution, because *T.gondii* can naturally disseminate to the brain and induce significant infiltration and activation of T cells asymptomatically. We hypothesize that *T.gondii* could attract T cells into brain tumors while at the same time convert the non-supportive TME into one that is conducive for T cells. Using a mouse model for medulloblastoma based on a genetic system called Mosaic Analysis with Double Markers (MADM) that recapitulates the tumorigenic process in human by producing few and precisely labeled mutant cells, I was able to assess the immuno-modulatory potential of *T.gondii* in the context of an intact immune system and endogenous TME. Our preliminary data revealed three exciting facets about *T.gondii*'s immunotherapeutic potential. Via histological assessment and flow cytometry, we found that at 24 days post infection, abundant T cells are recruited into TME. Correspondingly, we detected significant elevation of IFNg and IFNg-driven genes in the tumors, suggesting Th-1 immunity presence in TME. Lastly, we found that *T.gondii* challenge is associated with reduced tumor incidence. These results indicates *T.gondi*i holds potent immuno-modulatory capability and potentially anti-tumoral functions. My follow-up studies with dive more deeply into three aspects: **1)** determine whether *T. gondii* can sustainably recruit functional T cells into the tumor mass; **2)** determine whether *T. gondii* can help shape a T cell-supportive TME, especially through activating Th1-immune response in innate immune cells; and **3)** identify the underlying mechanisms for *T.gondii* to exert tumor-suppressing activities.

P041 Simultaneous checkpoint inhibition and immune cell activation that is safely localized to solid tumors. Richard A. Richieri[1], Navneet Narula[2], Cynthia A. Loomis[2], Valeria Mezzano[2], John Billimek[3], Glenn T. Reynolds[1], Chris Reutelingsperger[4], Andries Zijlstra[5], Missag H. Parseghian[1]. [1]Rubicon Biotechnology, Irvine, CA, [2]NYU Langone Medical Center, New York, NY, [3]University of California, Irvine, Irvine, CA, [4]Cardiovascular Research Institute, Maastricht University, Maastricht, Netherlands, [5]Vanderbilt University Medical Center, Nashville, TN.

Unlike other checkpoint inhibitors, our targeted immunotherapeutic localizes to any solid tumor and simultaneously shields an agent of immunosuppression while presenting a signal for immunostimulation. Phosphatidylserine (PS) exposure on the extracellular surface of living tumor cells and their vasculatures provides one avenue by which the tumor microenvironment

promotes immunosuppression. Extracellular surface PS is inherent to a tumor and its vasculature, even for inoperable tumors, and its expression cannot be mutated nor affected by acquired drug resistance. Annexin A5 (AnxA5) is a direct, high-affinity PS-binding protein that localizes to cells with PS exposed on the outer plasma membrane. In our studies, we conjugated a proprietary modified AnxA5, lacking cellular internalization, to TNFα (AnxA5$_{MOD}$-TNFα) to convert the immunosuppresive environs of a murine 4T1 triple negative breast cancer (TNBC) into an immunostimulated one. This strategy localized the immune response to the tumor and minimized side effects, as evidenced by a lack of toxicity for up to 7 days in non-tumor bearing Balb/c female mice given up to 1 mg/kg. Proper assembly and functionality of AnxA5$_{MOD}$-TNFα was verified simultaneously by ellipsometry, an optical technique similar to plasmon resonance. Fully assembled constructs were tested for binding to PS coated slides. The degree of light polarization is proportional to the amount of PS bound by the AnxA5 complex. Samples could be further incubated with TNF receptors to verify TNFα activity. Based on dose escalation studies in 4T1 tumor-bearing mice where the TNBC tumors were grown in the mammary fat pads, optimal dosages were determined for AnxA5$_{MOD}$-TNFα (18 μg) and AnxA5$_{MOD}$ alone as a control (180 μg). These doses were further tested in a 4T1 growth inhibition study. Tumor size was tracked by caliper in two groups of mice (n=5/group) receiving drug treatment on days 12, 14 and 16 and a repeated measures ANOVA was conducted on measurements taken before, during and post-treatment. While median tumor size did not differ between control and drug treatment groups during the pre-treatment interval ($p=0.84$), there was a significant difference post-treatment ($p<0.001$) with mice receiving AnxA5$_{MOD}$-TNFα having much smaller TNBC tumors. Tumors from the study were embedded in paraffin, sectioned (5 μm) and the overall immune cell content determined by H&E staining. Once it was evident there was a greater quantity of immune cells in AnxA5$_{MOD}$-TNFα treated tumors vs. controls, sections were stained with validated antibodies to identify and count the immunoactivated T-cells, NK-cells and macrophages. There was a 3X greater mean percentage of CD8 and CD4 T-cells in mice receiving drug vs. control ($p=0.03$) along with 2.5X and 5X increases in NK-cells and M1 immunoactive macrophages, respectively. Conclusion: Our AnxA5$_{MOD}$-TNFα inhibits the PS inhibitor while simultaneously activating TNF activators!

P042 Chemokine dysregulation creates the immunosuppressive tumor microenvironment and promotes human papillomavirus-associated head and neck cancer.

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Human papillomavirus (HPV)-associated head and neck squamous cell carcinoma (HNSCC) is a fast-growing health problem in the United States. By analyzing the transcriptomes of HPV-positive (HPV+) and HPV-negative (HPV-) HNSCC patient tissues, we previously revealed that expression of CXCL14, constitutively expressed in basal epithelial cells, is downregulated in HPV-positive HNSCC. In contrast, the expression of proinflammatory chemokines, particularly CXCL1, CXCL2, and CXCL8, are significantly upregulated in both HPV+ and HPV- HNSCCs. We further showed that restored expression of CXCL14 in HPV+ HNSCC cells leads to tumor-free survival in vivo by increasing MHC-I antigen presentation on the cancer cell surface and $CD8^+$ T cell activation. Interestingly, high CXCL1, CXCL2, and CXCL8 expression levels

significantly correlate to poor HNSCC patient survival, showing an exact opposite pattern of the clinical outcomes associated with the CXCL14 expression levels. To determine the roles of these chemokines in HPV+ HNSCC development, CXCL1 and CXCL2 genes (no murine CXCL8 ortholog) were deleted using a CRISPR-Cas9 knockout system in HPV+ murine HNSCC cells. Using these stably engineered HNSCC cells, we investigated tumor growth, immune cell infiltration, and metastasis in immunocompetent syngeneic mice. Interestingly, the deletion of CXCL1 and CXCL2 significantly delays in vivo tumor growth, decreases myeloid-derived suppressor cell (MDSC) infiltration in the tumor, and decreases lung metastasis. However, the numbers of natural killer (NK), $CD4^+$, and $CD8^+$ T cells in the tumor microenvironment were not affected by CXCL1 and CXCL2 knockout. Next, mice injected with the murine HNSCC cells were treated twice daily with AZD5068, a selective inhibitor of CXCR2, which is a common receptor of CXCL1, CXCL2, and CXCL8 expressed on myeloid cells. Our result showed that the treatment of CXCR2 suppresses in vivo tumor growth in a similar pattern with CXCL1 and CXCL2 knockout. These results suggest that the dysregulation of chemokine expression in HPV-infected cells plays an important role in regulating tumor growth, antitumor immune responses, and metastasis of HPV+ HNSCC. Our findings may provide promising strategies to develop novel immunotherapies for HPV+ HNSCC patients which can likely be extended to other non-viral cancers with immunosuppressive phenotypes.

P043 Extracellular matrix modulates T cell clearance of malignant cells in vitro. Claire Robertson[1], Aimy Sebastian[1], Aubree Hinckley[1], Naiomy Rios-Arce[1], William Hynes[1], Wei He[1], Nicholas Hum[1], Elizabeth Wheeler[1], Gabriela Loots[1], Matthew Coleman[1], Monica Moya[1].
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Introduction: Emerging evidence suggests that tumor extracellular matrix (ECM) may play a role in tumor-immune interactions. Breast tumors with high immune infiltrates have a distinct ECM profile, and T cell exclusion has been linked to specific ECM signatures. Despite this evidence suggesting a link between immune infiltrates and tumor matrix, it remains unclear whether ECM can directly affect the ultimate step in tumor clearance by the immune system, T cell mediated cytotoxicity.

Methods: We compared clearance of 4T1 mammary gland carcinoma cells (MCC) seeded on ECM arrays by T cells isolated from spleens of MHC mismatched strain of mice. Briefly, 4T1 were seeded at 10k/ml for 1 hour, cultured for 24 hours then cocultured with T cells for 2 hours before fixing and staining. For RNA sequencing, ECM proteins (Collagen 1 -Col1, Collagen 4-Col4, Fibronectin -Fn or Vitronectin- Vtn) were coated onto plates at 250ug/ml, then 4t1 were added for 24 hours, then T cells were added for 24 hours followed by RNA isolation and sequencing.

Results: We compared number of cells per spot with and without T cells across all ECM combinations and found that co-culture with T cells reduced the average number of MCCs, but this difference did not reach statistical significance. Only in the following conditions did MCC number significantly decrease: Col1 alone, Col6 alone, Fn alone, Vtn alone and Col6+ Eln (**Fig 2B**). In Col4 containing conditions, MCC cell number increased in the presence of T cells. Intensity of CD274 (PD-L1) and the MHC class 1 protein H2-K^d varied with substrate ($p<10^{-19}$, $p<10^{-22}$ respectively) with significantly higher expression of PD-L1 in Col1 and Vtn conditions

vs. Col4 or Laminin, and higher H2-Kd in Vtn conditions. These findings demonstrate a defect in T cell mediated MCC clearance in some ECM conditions that is distinct from the PD-L1 checkpoint. Comparing transcriptomes across, we observed that all MCC+ T cell conditions separated from MCC alone conditions (**Fig. 3B**), largely due to expression of known T cell related genes (such as *Ptprc, Trbc2, Sell, Itk,* and *Il7r*). Differentially regulated gene counts between MCC+ T cells and MCC alone conditions were lowest in the Col4 condition (**Fig. 3BC-E**), and significance and number of genes from T cell associated ontologies were lowest in the Col4 conditions (**Fig. 3E**). We observed that MCC on Col4 upregulated cytokines including *Ccl2, Cxcl3, Cxcl10, and Tgfβ2*, compared to both Fn and Vtn conditions, suggesting that this condition could suppress immune activation through altered cytokine expression.

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P044 Real-time visualization of tumor cell phenotype and microenvironmental heterogeneity enabled by a hyperspectral fluorescence microendoscope. Mohammad A. Saad[1], Bryan Q. Spring[2], Akilan Palanisami[1], Eric M. Kercher[3], Ryan T. Lang[2], Jason Sutin[4], Zhiming Mai[1], Tayyaba Hasan[1]. [1]Massachusetts General Hospital, Boston, MA, [2]Northeastern University, Boston, MA, [3]UMass Medical School, Worcester, MA, [4]Boston Children's Hospital, Boston, MA.

Tumor microenvironmental heterogeneity is a major driver of treatment resistance and variability in clinical response to therapy. This heterogeneity arises from variations in cellular phenotypes populating the tumor microenvironment (TME), their spatio-temporal localization and expression of surface markers, often associated with specific biological function – stemness, metabolism, proliferation, immune activation and others. Such features are usually studied through *ex vivo* immunofluorescence and cytometry to identify cellular phenotypes in TME; however, they cannot be applied *in vivo* for real time TME analysis. Moreover, current *in vivo* imaging techniques fail to provide real-time high-resolution visualization of the TME. Routinely employed biopsy tissue sampling through fine needle aspirates is limited by its invasive nature and inability to provide a global and dynamic overview of the TME, thus limiting our ability to study spatiotemporal TME dynamics and identify possible features leading to potentially resistant tumor phenotypes. In this study, we demonstrate the use of a hyperspectral fluorescence microendoscope (HFME) to monitor cellular phenotypes in TME with real-time visualization and high video imaging capability (~17 fps). Current fluorescence video microscopy is limited to simultaneous imaging of no more than 2 molecular markers with potential to be expanded to ~4 markers using dichroic mirrors and point detectors; the HFME demonstrated in this study can currently resolve 6 different molecular markers, simultaneously, using a multichannel linear array detector with potential to expand to 10 or more markers. Using a cocktail of near infra-red fluorophore-antibody conjugates targeted against key molecular (surface) markers of different cells in the TME, we are able to capture real-time TME dynamics at cellular resolution in two pre-clinical models; **1)** a xenograft orthotopic mouse model of peritoneal carcinomatosis (disseminated metastases within the abdominal cavity) and imaging epidermal growth factor receptor (EGFR), CD44, CA125 (MUC16), transferrin receptor, Thomsen-Friedenreich carbohydrate antigen (T antigen), and CD45, **2)** A syngeneic immunocompetent KPC cell line

implanted mouse model of pancreatic ductal adenocarcinoma and imaging CD3, CD4, CD8a and CD45. Imaging on these tumor models was performed pre- and post-sub-therapeutic verteporfin (benzoporphyrin derivative monoacid A) photodynamic therapy (PDT, a cytotoxic light-based therapy). PDT treatment resulted in reduction of cancer cell burden, immune cell infiltration and alterations in their relative spatial localization. The results were confirmed by histopathological validation and *ex vivo* immunofluorescence staining of tumor tissue sections. Collectively, these results demonstrate the capability of HFME to image cancer cell phenotypes and the tumor microenvironment, in real time in live mice. With the ability to monitor cancer growth and treatment effects at a cellular level, HFME can potentially assist in customizing therapies in a patient-specific manner.

P045 Preclinical transcriptome-based evaluation of the translatable potential of new treatments in Triple-Negative Breast Cancer. Ammar Salkini[1]. [1]University of Ottawa, Ottawa, Canada.

Objective: This study will evaluate the potential of four recently proposed TNBC treatments—which all successfully reduced tumor viability *in vitro* and/or *in vivo*—to inhibit genes involved in CSC survival, metastatic metabolomic signature, and tumor immunosuppression.

Methods: TNBC cell lines and/or patient-derived xenografts were treated with five different treatments: DCC-2036, 9Gy proton irradiation, miR302b+cisplatin combination, and DFX+doxorubicin combination. Genome-wide mRNA profiling (via either RNA-seq or microarray) was performed on control and treated groups. Data was obtained from NCBI GEO datasets. We assessed the differential expression of genes associated with CSC growth and metastatic metabolomic signature in TNBC tumors. Limma statistical analysis was performed. GSEA was also used to complement results from individual gene expression analysis.

Results: DCC-2036 treatment significantly induced the expression of CSC TNBC biomarkers—such as *ALDH2*, *CD44*, *CCR5*, and *SNAI1*—and genes associated with TNBC metastatic metabolomic signature—such as *PPARGC1A.* DCC-2036 showed inconsistent effects on the expression of immunosuppressive markers. Gene expression profiles of the remaining treatment groups are currently being analyzed. 9Gy proton irradiation has mixed effects on the expression of our candidate genes, yet mostly induced the expression of stemness, metastatic, and immunosuppressive markers. miR302b+cisplatin and DFX+doxorubicin both failed to inhibit the candidate genes, yet without significantly inducing their expression. GSEA analysis confirmed the results obtained for all four treatments.

Conclusions: Observing cancer rebound in TNBC patients after treatment with traditional cancer drugs is common and often happens when treatments fail to inhibit CSC growth, metabolic pathways associated with metastasis, and oncogenic immunosuppressive pathways. Our analysis shows that all four treatments failed to significantly impact the expression of protein pathways associated with increased metastasis and immunosuppression. It is worth noting that the researchers did report a decrease in tumor viability due to treatment of their experimental models with all four treatments. However, these findings correspond to the viability of the whole cell culture or tumor, not the viability of specifically the CSCs; in TNBC, CSCs make up only a small proportion of the total mass or the tumor, so the reported antiproliferative effects of the

treatments do not necessarily suggest the treatment has effectively targeted the CSC population. Therefore, we hypothesize that these treatments will likely not show positive effects in clinical studies. Furthermore, none of the researchers performed any assays evaluating CSC growth—such as CSC-labelled flow cytometry—or metastasis—such as secondary tumor transplantation. Therefore, we encourage the researchers to perform more rigorous assays to evaluate the translatable potential of their treatments. Finally, the outline of this study provides a useful rationale for to evaluate emerging TNBC therapies.

P046 NKG2A and HLA-E define a novel alternative immune checkpoint axis in bladder cancer. Bérengère Salomé[1], John P. Sfakianos[2], Jorge Daza[2], Andrew Charap[1], Adam M. Farkas[3], Daniel Geanon[4], Geoffrey Kelly[4], Ronaldo M. De Real[4], Brian Lee[4], Kristin G. Beaumont[5], Sanjana Shroff[5], Ying-Chih Wang[5], Yuan-Shuo A. Wang[2], Li Wang[5,6], Robert P. Sebra[5,6,7], Alice O. Kamphorst[1], Karl J. Malmberg[8,9,10], Emanuela Marcenaro[11], Pedro Romero[12], Rachel Brody[13], Yuko Yuki[5,6], Maureen Martin[5,6], Mary Carrington[3,5,6*], Reza Mehrazin[2], Peter Wiklund[2], Jun Zhu[5,6], Matthew D. Galsky[3], Nina Bhardwaj[3*], Amir Horowitz[1*]. [1]Department of Oncological Sciences, Precision Immunology Institute, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, [2]Department of Urology, Icahn School of Medicine at Mount Sinai, New York, NY, [3]Division of Hematology and Medical Oncology, Precision Immunology Institute, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, [4]Human Immune Monitoring Center, Icahn School of Medicine at Mount Sinai, New York, NY, [5]Icahn Institute for Data Science and Genomics Technology, Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, [6]Sema4, A Mount Sinai Venture, Stamford, CT, [7]Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, [8]Department of Cancer Immunology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway, [9]Institute of Clinical Medicine, University of Oslo, Oslo, Norway, [10]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden, [11]Department of Experimental Medicine, University of Genoa, Genoa, Italy, [12]Department of Oncology UNIL CHUV, University of Lausanne, Lausanne, Switzerland, [13]Pathology, Molecular and Cell Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, [14]Basic Science Program, Frederick National Laboratory for Cancer Research, Frederick, MD, [15]Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, *These authors contributed equally.

Background: Bladder cancer is characterized by a poor prognosis, with muscle-invasive cases harboring a 34-76% 10-year recurrence-free survival rate [1]. Neoadjuvant PD-1/PD-L1 blockade strategies have recently been approved by the US Food and Drug Administration for bladder cancer treatment, yet only achieving a complete response rate of 31-37%, thereby suggesting additional mechanisms of resistance [2]. HLA-E is a known inhibitor of NKG2A+ CD8 T cells and NK cell responses. A monoclonal antibody binding to the NKG2A receptor has been developed and proven to restore CD8 T cell and NK cell responses in head and neck cancer, with ongoing clinical trials across multiple tumor indications [3,4]. We evaluated the potential role of the HLA-E/NKG2A inhibitory pathway in modulating T cell immunity in bladder cancer. **Methods:** CyTOF was performed on $CD8^+$ T cells from fresh bladder tumors (n=6), as well as on expanded $CD8^+$ T cells from bladder-draining lymph nodes (n=11) and tumors (n=8). Flow cytometry (n=25) and single-cell RNA-sequencing (scRNAseq) (n=13) were performed on cells from fresh bladder tumors. **Results:** Mechanisms of tumor escape from $CD8^+$ T cell

recognition include impairment of antigen presentation. Accordingly, we found a significant reduction of HLA class I expression on tumors. However, expression of DNAM-1-activating ligands (e.g. CD112,CD155) on bladder tumors was retained, indicating a possible role for TCR-independent activation pathways traditionally ascribed to natural killer (NK) cells. Using mass cytometry and scRNAseq, we observed that acquisition of NKG2A on tumor-derived PD-1^+ $CD8^+$ T cells promotes tissue-resident memory features alongside diminished CD28 expression and significantly weaker sensitivity to CD3/CD28-signaling. However, $NKG2A^+$ CD8 T cells possess a proliferative advantage with enhanced expression of DNAM-1 and cytolytic machinery. Strikingly, we found that $NKG2A^+PD\text{-}1^+$ CD8 T cells are strongly activated in response to HLA class I-deficient tumors compared to their $NKG2A^-$ $PD\text{-}1^+$ CD8 T cell counterparts. TCR-independent NK-like function by $NKG2A^+$ CD8 T cell is partly mediated by the DNAM-1 pathway and inhibited by HLA-E. $NKG2A^+$ CD8 T cell functions are restored upon NKG2A blockade, where efficiency positively correlates with HLA-E expression on bladder tumors. **Discussion:** Collectively, our data indicate that $NKG2A^+$ CD8 T cells display a strong capacity for TCR-independent activation that enables them to circumvent bladder tumor evasion mechanisms. $NKG2A^+$ CD8 T cells lack expression of CD28 suggesting a lower susceptibility to PD-1-mediated inhibition. Our data suggest a need for thorough reappraisal of current protocols that assess CD8 T cell exhaustion and for strategies to restore their antitumor functions. **References:** 1. Sanli, O. et al., Nat Rev Dis Primers, 2017 2. Rouanne, M. et al., Eur Urol Oncol, 2020 3. André, P. et al., Cell, 2018 4. Van Hall, T. et al., J Immunother Cancer, 2019

P047 Inactivation of IFN signaling drive immunosuppressive MDSC function and can be therapeutically targeted. Emilio Sanseviero[1], Kevin Alicea-Torres[2], Yulia Nefedova[3], Serge Y. Fuchs[4], Dmitry I. Gabrilovich[5]. [1]AstraZeneca, Gaithersburg, MD, [2]University of Puerto Rico at Humacao, Humacao, Puerto Rico, [3]The Wistar Institute, Philadelphia, PA, [4]University of Pennsylvania, Philadelphia, PA, [5]Astrazeneca, Gaithersurg, MD.

Myeloid-derived suppressor cells (MDSC) are pathologically activated neutrophils and monocytes with potent immune suppressive activity that promote tumor progression and impair the efficacy of anti-tumor therapeutics. Herein we show that type I interferons (IFN1) receptor dampen immune suppressive activity of MDSC. Downregulation of the IFNAR1 is found in MDSC from cancer patients and mouse tumor models. Downregulation of IFNAR1 depends on the activation of the p38 protein kinase and is required for activation of the immune suppressive phenotype moreover genetic stabilization of IFNAR1 in tumor bearing mice reduces suppressive activity of MDSC and promote antitumor effect. Stabilizing IFNAR1 using inhibitor of p38 combined with the interferon induction therapy elicits a robust anti-tumor effect that is completely dependent on IFNAR1 expression on MDSC. Thus, inhibition of MDSC immune suppressive function can be exploited therapeutically.

P048 Steatosis promote liver cancer development by inducing chemokine production from Kupffer cells. Bangyan L. Stiles[1], Taojian Tu[1], Lina He[1], Mario Alba[1]. [1]University of Southern California, Los Angeles, CA.

Liver cancer is hallmarked with chronic inflammation resulting from underlying liver diseases such as liver steatosis. Steatotic liver injury recruits inflammatory cells and establishes the

tumor immune environment that can promote the development of liver cancer. Previously, we showed that macrophages produce growth factors to promote liver cancer growth in a genetic model where steatosis and liver tumors were induced by the deletion of tumor suppressor *Pten (phosphatase and tensin homologue deleted on chromosome 10*). In this model, we showed that attenuating steatosis and depletion of macrophages leads to inhibition of liver cancer development. To investigate the recruitment and functions of immune cells in liver cancer, we analyzed the expression profiles of these tumors as well as tumors from other mouse models together with human liver samples. Our analysis of the inflammatory cytokines identified one chemokine that was commonly upregulated in all tumor model and attenuated in non-tumor models. CXCL5 is a member of the neutrophil-activating chemokines. In the *Pten* deleted liver tumors, CXCL5 is significantly upregulated whereas its expression is inhibited when steatosis is attenuated via caloric restriction or deletion of a metabolic kinase, Akt2. We explored the cell types that may produce CXCL5. To our surprise, the upregulation of CXCL5 is concurrent with increases in macrophage but not neutrophil populations. We further observed significant upregulation of CXCL5 mRNA expression in Kupffer cells, the liver resident macrophages isolated from the *Pten* deleted mice. We explored the hypothesis that macrophages secrets CXCL5 to establish the tumor environment and promote tumor growth. Our data showed that CXCL5 treatment increased mouse hepatocytes and human HepG2 cells proliferation. This effect is blocked by inhibition of CXCR2, the receptor for CXCL5. Additionally, we explored the mechanisms of CXCL5 upregulation in Kupffer cells and discovered that lipopolysaccharide (LPS) induces the expression of CXCL5 by nearly 20-fold in Kupffer cells. This is in contrast with the lack of induction in murine macrophage cell lines and primary peritoneal macrophages. This unique response of Kupffer cells suggests that the source of CXCL5 in the *Pten* deletion mouse model is likely Kupffer cells. This data is collaborated by bioinformatic analysis of datasets where steatotic liver injury is induced by diet feeding. Furthermore, analysis of patient proteomic data detected CXCL5 as the primary chemokine that is induced in tumors among other neutrophil-activating peptides. In summary, our data identified CXCL5 as a novel chemokine produced by Kupffer cells that plays key roles in steatosis driven liver cancer development. Chronic liver diseases such as steatohepatitis can establish tumorigenesis environment through accumulation of LPS stimulated CXCL5 release from Kupffer cells.

P049 ONM-501 — A synthetic polyvalent STING agonist for cancer immunotherapy.

Suxin Li[1], Min Luo[1], Zhaohui Wang[1], Qiang Feng[1], Jonathan Wilhelm[1], Xu Wang[1], Wei Li[1], Jian Wang[1], Qingtai Su[2], Gaurav Bharadwaj[2], Jason Miller[2], Katy Torres[1], Stephen Gutowski[2], Agnieszka Cholka[1], Yang-xin Fu[1], Tian Zhao[2], Baran Sumer[1], Hongtao Yu[1], Jinming Gao[1]. [1]University of Texas Southwestern Medical Center, Dallas, TX, [2]OncoNano Medicine, Inc., Southlake, TX.

Background: The stimulator of interferon genes (STING) plays a central role in innate immune response against infection and cancer. Naturally, the cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS)-STING pathway adopts polyvalent interactions to form higher-order assemblies to achieve a specific and rapid response while limiting unnecessary stimulation by host DNA. Several cyclic di-nucleotide (CDN) and non-CDN small molecule STING agonists have been studied in clinical trials showing limited therapeutic efficacy. ONM-501, a dual-activating STING agonist employs PC7A, a synthetic polymer that induces polyvalent STING condensation and prolongs innate immune activation. Mechanistically, PC7A

binds to STING through a non-competitive surface site that is distinct from the binding pocket of CDN and non-CDN small molecule STING agonists. ONM-501 encapsulates the endogenous STING agonist cGAMP with the PC7A micelles offering dual 'burst' and 'sustained' STING activation. Effectiveness of using ONM-501 for immunotherapy against solid tumors has been demonstrated.

Methods: Polyvalent interaction between PC7A and STING was studied and characterized by several different methods including ITC, a FRET assay between fluorophore labeled STING and PC7A and Nile-Red assay. The binding valency was investigated using a series of PC7A polymers with an increasing number of repeating units. The PC7A-STING binding site was elucidated using STING mutants produced by site-directed mutagenesis. STING activation was evaluated by measuring Ifnb1 and Cxcl10 expression using RT-qPCR. STING activation by PC7A and ONM-501 was also investigated using freshly resected human tissue. ONM-501 antitumor efficacy was evaluated in 6 different murine syngeneic tumor models.

Results: PC7A activates STING through a non-canonical biomolecular condensation. It binds to a non-competitive surface site on the α5 helix of STING which is different from the CDN binding pocket. This binding was also retained with cGAMP-resistant STING variants (e.g., R232H)**.** The formation of STING-PC7A condensate and the downstream activation were dependent on polymer repeating units. ONM-501 achieves a synergistic, rapid and sustained (6-24h) STING activation *in vivo* compared to cGAMP which peaked at 6h. Injection of ONM-501 in fresh human tissue resulted in >100-fold increase in cytokine expression while free CDN only showed marginal effect. Antitumor efficacy was demonstrated in MC38, CT26, B16F10, 4T1, A20 and TC-1 models after ONM-501 treatment. Complete response was observed when combined with anti-PD1 checkpoint blockade therapy.

Conclusions: PC7A polymer achieves prolonged innate immune activation by polyvalent STING condensation through a distinct binding site. ONM-501 combines endogenous cGAMP with PC7A that potentially offers a synergistic strategy in spatiotemporal orchestration of immune environment for a highly effective immunotherapy against cancer.

P050 Oncogenic kinase therapy restricts CD8 T cell differentiation and clonal expansion. Andrew Tieniber[1], Andrew Hanna[1], Benjamin Medina[2], Kevin Do[1], Lillian Levin[1], Ferdiando Rossi[1], Ronald DeMatteo[1]. [1]University of Pennsylvania, Philadelphia, PA, [2]Memorial Sloan Kettering Cancer Center, New York, NY.

Introduction: Targeted therapy with tyrosine kinase inhibitors (TKI) including imatinib is effective in treating gastrointestinal stromal tumor (GIST), but resistance often develops within 18 months. Despite a robust immune T cell infiltrate, combining TKIs with immune checkpoint inhibitors has largely been unsuccessful in treating advanced GIST. The superagonist IL15/1L15Rα has shown promise in a number of cancer types. The objective of this study was to determine the effects of TKI therapy on intratumoral T cells in GIST and evaluate IL15/1L15Rα as an adjunctive therapy. Methods: $Kit^{V558\Delta/+}$ mice were treated with imatinib (600mg/L in water) and/or IL15/IL15Rα (2ug/12ug/dose, 3x/week), for 1-4 weeks and evaluated by TCRB sequencing (Immunoseq), RNA-seq, and flow cytometry (n=4-5/group). Intratumoral immune cells from $Kit^{V558\Delta/+}$ mice were evaluated by single cell RNA sequencing (scRNAseq)

(n=3). Results: RNA-seq of sorted CD8 T cells following 1 week of imatinib treatment suggested intratumoral CD8 T cells gain antigen experience but lose their effector profile, as *cd44*, *sell*, and *tcf7* increased while *gzmb* and *tnfrsf9* decreased ($p<0.05$). At 1 and 4 weeks of imatinib therapy, flow cytometry of intratumoral CD8 T cells demonstrated increased CCR7 and CD127 and decreased CD69, Tbet, Granzyme B, and Ki67 ($p<0.05$), confirming an intratumoral phenotypic transition from an activated/effector subtype. Imatinib therapy reduced p-AKT and p-mTOR MFI in intratumoral CD8 T cells by flow cytometry ($p<0.05$), signifying a deficiency in TCR signaling and/or co-stimulation. TCRB sequencing after 1 week of imatinib treatment showed no difference in T cell clonality, indicating T cells failed to undergo clonal expansion. scRNAseq in tumors from $Kit^{V558\Delta/+}$ mice established that IL-15R and IL-15 were primarily expressed on Batf3 dendritic cells and macrophages, while both IL-2R beta and gamma were expressed on NK and T cells. Although IL15/1L15Rα alone had no anti-tumor efficacy, combining IL15/IL15Rα with imatinib improved the anti-tumor response as measured by tumor weight ($p<0.05$). After combination treatment, CD8 T cells evaluated by flow cytometry exhibited a significant increase in granzyme B, Tbet, CD69, and Ki67, and PI3K signaling was restored ($p<0.05$). Conclusions: Imatinib therapy in GIST fails to induce T cell clonal expansion and results in a reduced CD8 activation/effector profile. IL15/IL15Rα therapy improves upon the effects of imatinib by rescuing CD8 T cell PI3K signaling and effector response.

P051 Function of shp-1 and shp-2 phosphatases in T cell-mediated anti-tumor response.

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After exposure to chronic inflammatory stimuli, the immune system can switch from a functional state where it acts to reestablish homeostasis to a dysfunctional state. In the context of cancer, T cells that become exposed to continuous stimulation eventually reach a state of exhaustion, characterized by poor effector function and expression of inhibitory receptors, such as PD-1. Although PD-1 signaling inhibition leads to T cell reinvigoration and has been applied as an effective treatment versus a wide range of tumors, the signaling pathway downstream of this receptor is still poorly understood. Recent work from others and us challenged the notion that the phosphatase shp-2 is essential for activation of the molecular cascade downstream PD-1 receptor engagement. The shp-2 homologue (shp-1) has also been associated with PD-1 signaling in T cells and functional redundancy between these phosphatases might occur downstream of this receptor. Therefore, we investigated the effect of shp-1 and the combination of both (shp-1/2) downstream of PD-1 by knocking out these phosphatases in T cells in a mouse model. *In vivo* results after tumor engraftment suggest that shp-1 as well as shp-1/2 deletion in T cells are not sufficient to ameliorate tumor control. Furthermore, ablation of shp-1 and shp-1/2 impair the beneficial effects of the anti-PD1 treatment. In fact, deletion of both phosphatases leads to decrease $CD8^+$ T cell presence in the tumor microenvironment and *in vitro* results show that these cells have impaired survival. This data implies that elimination or inhibition of shp-1/2 is not a suitable strategy for effective immunotherapeutic approaches as well as highlights the importance of further elucidating the mechanisms behind this important inhibitory pathway.

P052 Functional ADORA2A antibodies demonstrates the antagonistic and tumor suppression activities. Linya Wang[1], Ana Lujan[1], Eric Kwan[1], Crystal Safavi[1], Mouna Villalta[1], Tom Yuan[1], Melina Mathur[1], Joyce Lai[1], Hoa Giang[1], Qiang Liu[1], Fumiko Axelrod[1], Aaron Sato[1]. [1]Twist Bioscience, South San Francisco, CA.

G protein-coupled receptors (GPCRs), a family of seven-transmembrane receptor proteins function as sensors transmitting extracellular signals into the cell and mediate a diverse range of ligand signaling, are proven to be successful drug targets. GPCRs and the associated downstream signaling pathways have been linked to a wide range of disease types such as cancer, inflammatory and immune disorders, as well as metabolic and neurological diseases. Antibodies are becoming an increasingly promising modality to target these receptors due to their unique properties, such as exquisite specificity, long half-life, and fewer side effects, and their improved pharmacokinetic and pharmacodynamic profiles compared to peptides and small molecules, which results from their more favorable biodistribution. To date, there are only two US Food and Drug Administration-approved GPCR antibody drugs, namely erenumab and mogamulizumab, and this highlights the challenges encountered in identifying functional antibodies against GPCRs. ADORA2A operates as an adenosine receptor and immune checkpoint protein that prevents the inappropriate activation of T-cells and is an immuno-oncology target. Immune checkpoint blockades are one of the newest pillars of cancer therapeutic development. Current examples for targeting ADORA2A involve small molecule antagonists. Selective antibody binders of ADORA2A are therefore a largely untapped immunotherapeutic candidate. Using our proprietary biologics discovery and optimization platform, Twist Biopharma identified a potent high-affinity antibody, TB206-001, amongst other promising leads. Initial *in vitro* assays showed that TB206-001 binds with high affinity to both human and mouse ADORA2A. TB206-001 showed antagonistic activity by suppressing cAMP level stimulated by NECA, a high affinity adenosine receptor agonist. Primary T cell assays demonstrated that TB206-001 restored T cell activity which was inhibited by NECA which suppresses T cell activation. Subsequent *in vivo* testing also showed the efficacy in animal models of cancer, indicating its activity in tumor suppression. TB206-001 is a high-affinity antagonistic anti-ADORA2A antibody demonstrating preclinical activity.

P053 Ablation of CCR1 relieves immunosuppression in pancreatic cancer. Yaqing Zhang[1], Kristee L. Brown[1], Wei Yan[1], Zeribe C. Nwosu[1], Eileen K. Carpenter[1], Katelyn L. Donahue[1], Ashley Velez-Delgado[1], Sion Yang[1], Marina Pasca di Magliano[1]. [1]University of Michigan, Ann Arbor, MI.

Pancreatic Cancer is one of the deadliest malignancies, with 5-year survival rate of 10%. The tumor microenvironment of pancreatic ductal adenocarcinoma (PDA) includes abundant fibroblasts and infiltrating immune cells, the latter largely immunosuppressive. Mono-immunotherapy or combination immunotherapy approaches has been ineffective in pancreatic cancer, pointing to the need for additional avenues to target in pancreatic cancer microenvironment. We previously showed that targeting regulatory T cell (Treg), a prevalent T cell population in pancreatic cancer, failed to relieve immunosuppression and led to accelerated tumor progression. We discovered that Treg depletion reprogrammed tumor associated fibroblasts and increased immunosuppressive myeloid cell recruitment, an effect that was partially mediated by CCLs/CCR1signaling. We found that tumor educated macrophages express

the highest levels of *Ccr1* compared to non-activated (M0), pro-inflammatory (M1), or anti-inflammatory (M2) bone marrow derived macrophage subsets. Thus, we sought to investigate the functional role of CCR1 in pancreatic cancer. By single cell RNA sequencing, we found CCR1 to be mainly expressed by tumor associated macrophages (TAMs) and neutrophils (or granulocytes) in both human and mouse PDA. We then orthotopically transplanted syngeneic mouse pancreatic cancer cells in CCR1 knockout hosts and observed reduced tumor growth which was rescued by CD8 T cell depletion. Histological analysis showed elevated Granzyme B expression in infiltrating T cells, as well as an increase in apoptotic cells in tumors implanted in $Ccr1^{-/-}$ mice. Through cytometry by time of flight (CyTOF) and co-immunofluorescence we also discovered that TAMs in tumors implanted in $Ccr1^{-/-}$ mice expressed less Arginase 1 and CD206 -both markers of immunosuppressive macrophages- compared to TAMs in wild type tumors. Thus, our data is consistent with the notion that tumor associated macrophages lacking CCR1 expression are less immunosuppressive, consequently allowing increased CD8 T cell-mediated anti-tumor immunity. We are currently exploring combination approaches targeting CCR1 in pancreatic cancer.

P054 Immune determinants of the association between tumor mutational burden and immunotherapy response across cancer types. Neelam Sinha[1], Sanju Sinha[1], Christina Valero[2], Alejandro A. Schaffer[3], Kenneth Aldape[3], Kevin Litchfield[4], Timothy A. Chan[5], Luc G. T. Morris[6], Eytan Ruppin[3]. [1]Cancer Data Science Lab, National Cancer Institute, Bethesda, MD, [2]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, [3]National Cancer Institute, Bethesda, MD, [4]The Francis Crick Institute, London, United Kingdom, [5]Lerner Research Institute, Cleveland Clinic, Cleveland, OH, [6]Memorial Sloan Kettering Cancer Center, New York, NY.

The FDA has recently approved a high tumor mutational burden (TMB-High, defined by ≥10 mutations/Mb) as a biomarker for the treatment of advanced solid tumors with pembrolizumab, an immune checkpoint inhibitor (ICI) that targets PD1. However, recent studies have shown that this TMB-high biomarker is only able to stratify ICI responders in a subset of cancer types, where the mechanisms underlying this observation have remained unknown. We hypothesized that the tumor immune microenvironment (TME) may determine the ability of high-TMB to stratify responders of ICI (termed *TMB power*) in each cancer type. To systematically study this hypothesis, we first inferred the levels of 31 immune-related factors characteristic of the TME of different cancer types in the TCGA. We next integrated this information with a cohort of 2,277 ICI-treated patients with TMB and response measures, to identify the key immune factors that can determine *TMB power* across 14 different cancer types. This cohort was created by collating the largest publicly available cohort comprising 1959 patients together with a new cohort of 318 patients, where TMB has been quantified using the MSK-IMPACT panel. We find that high levels of M1 macrophages and low levels of resting dendritic cells in the TME characterize cancer types with high *TMB power.* These findings are aligned with prior reports that M1 macrophages could be anti-tumorigenic by fostering an inflammation response and activating CD8 T cells against tumor, and that resting dendritic cells may induce tolerance to tumor antigens via inducing T cell death or their anergic state. A model based on these two immune factors strongly predicts *TMB power* across cancer types (Spearman Rho=0.76, P<3.6E-04). Using this model, we provide predictions of the *TMB power* in nine additional cancer types, including rare cancers, for which TMB and ICI response data are not yet publicly available on a

large scale. These predictions can be used to prioritize the clinical trials testing the usage of TMB-high biomarker in new cancer types. In this line, our analysis indicates that TMB-High may be strongly predictive of ICI response in cervical squamous cell carcinoma, suggesting that such a study should be prioritized.

Technology and Systems Biology

P055 Evaluation of an immunoPET tracer for IL-12 in a preclinical model of inflammatory immune responses. James E. Glassbrook[1], Joshua Mandella[1], Nerissa T. Viola-Villegas[2], Heather M. Gibson[2]. [1]Wayne State University, School of Medicine, Detroit, MI, [2]Wayne State University, Department of Oncology/Karmanos Cancer Institute, Detroit, MI.

IL-12 is an attractive target for imaging active antitumor immunity, inflammation, and infection due to its function in innate and adaptive immune signaling. It has been widely reported to be involved in cancer initiation and progression, autoimmunity, as well as graft vs host disease. Here, we report the development and preclinical evaluation of an IL-12-specific positron emission tomography (PET) tracer. Biodistribution of tracer was evaluated in BALB/c mice, using lipopolysaccharide (LPS) administered intramuscularly to mimic infection and stimulate IL-12 production (40μg LPS in 50μL PBS). One hour post administration each participant received intraveinous administration of [^{89}Zr]- αIL12 tracer, and PET images were taken 5, 24, 48, and 72 hours post injection (hpi). Representative planar images show significantly higher uptake in LPS-treated mice as compared to controls. In a separate cohort of LPS-treated BALB/c mice, all tissues identified as significantly different via tracer were collected and evaluated via qPCR to verify IL-12 transcription in each tissue. To evaluate the utility of [^{89}Zr]- αIL12 as a metric of cancer immunotherapy response where antigen presenting cells are activated, we tested intratumoral delivery of Adv/GM-CSF, which we has been shown to promote anti-tumor immunity. BALB/c mice were seeded orthotopically with TUBO (murine mammary carcinoma) in the mammary fat pad. Once TUBO tumors reached a volume of ~50 mm^3, mice were divided into treated and untreated groups. Treated mice received three intratumoral injections of 10^8 PFU Adv/GM-CSF, once every two days, while control mice received vehicle control. On the day of the last dose, each subject received tracer injection and 72 hours later all mice were imaged via PET. Representative planar images demonstrate high uptake of [^{89}Zr]- αIL12 in treated vs. untreated mice. Targeting of soluble cytokines such as IL-12 by PET imaging may serve as a noninvasive way to evaluate the function of the immune milieu *in situ*.

P056 Rapid serial immunoprofiling of the tumor immune microenvironment by fine needle sampling. Juhyun Oh[1], Jonathan C. T. Carlson[1], Christian Landeros[1], Hakho Lee[1], Scott Ferguson[1], William C. Faquin[1], John R. Clark[1], Mikael J. Pittet[1], Sara I. Pai[1], Ralph Weissleder[1]. [1]Massachusetts General Hospital, Boston, MA.

As the field of cancer immunotherapy brings forth novel and combinatorial agents, there is increasing effort to discover and integrate predictive and/or prognostic biomarkers into treatment algorithms in order to optimize cancer care. While tissue-based methods can elucidate tumor-immune cell compositions at a single time point, serial assessment of the tumor immune

microenvironment (TME) can provide unique insight into how various therapies may modulate target tumor and/or immune cell populations over time. We propose that single-cell sampling via fine needle aspirates (FNA) can facilitate such analyses with a favorable risk-benefit profile. Thus, we developed and optimized a multiplexed bioorthogonal approach (FAST-FNA) which has been coupled with a deep learning algorithm that allows for comprehensive cellular analyses of FNA samples. We demonstrate that the FAST-FNA assay reproducibly captures the TME profile as compared to standard labor-intensive flow cytometry and immunohistochemical assays, and, furthermore, allows for time course analysis of the evolving TME in mouse and human cancers in vivo. The translational significance of the FNA-based technology is highlighted in the ability to rapidly assess PD-L1 expression within the TME and is further extended through the serial quantitation of both tumor and immune cell markers in cancer patients treated with immunotherapy. Collectively, these data indicate that FAST-FNA can serve as a robust and versatile clinical tool to monitor the evolving TME and has the potential to provide early insight into treatment response.

Combinatorial Studies to Overcome Resistance to Immunotherapy

P057 Targeting Treg cells with GITR activation alleviates resistance to immunotherapy in murine glioblastomas. Zohreh Amoozgar[1], Jonas Kloepper[1], Jun Ren[1], Rong En Tay[2,3], Samuel W. Kazer[4], Evgeny Kiner[3], Shanmugarajan Krishnan[1], Jessica M. Posada[1], Mitrajit Ghosh[1], Emilie Mamessier[1], Christina Wong[1], Gino B. Ferraro[1], Ana Batista[1], Nancy Wang[1], Mark Badeaux[1], Sylvie Roberge[1], Lei Xu[1], Peigen Huang[1], Alex K. Shalek[1], Dai Fukumura[1], Hye-Jung Kim[5], Rakesh K. Jain[1]. [1]Massachusetts General Hospital, Boston, MA, [2]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, [3]Harvard Medical School, Boston, MA, [4]MIT, Boston, MA, [5]Dana-Farber Cancer Institute, Boston, MA.

Glioblastoma (GBM) shows high level of resistance to currently available treatments including the standard of care and immunotherapy, representing the most fatal cancer type. Our study revealed that immune suppression by regulatory T cells (Treg) secondary to therapy with immune checkpoint blocker (anti-PD1) confers this resistance. In the GBM tumor microenvironment, Treg cells with increased suppressive phenotype were found of which frequency and anergic phenotype increase after ICB therapy, potentially contributing to the resistance. Targeting Treg has a dual-barreled effect on enhancing anti-tumor immunity: GBM is highly infiltrated with Treg while CD8 T cells are excluded. In view of Treg's intrinsic reactivity to self-antigens, mobilizing converted Treg as effector T cells can be an effective strategy to a tumor type that expresses a low level of neoantigens including GBM. Our study revealed that GITR (glucocorticoid induced TNFR family related protein) is a desirable therapeutic target based on its increased expression on GBM Tregs as compared to peripheral Tregs. Engagement of GITR with agonistic antibody led to conversion of Treg to Th1-like effector T cells, which is accompanied with downregulation of Helios and IL-10 expression that are associated with Treg suppressive function. Through combining anti-GITR with anti-PD1 therapy, tumor recognition by converted Treg and CD8 T cells could be enhanced via IFNg induced promotion of MHC class I and II expression by GBM cells, which also resulted in T cell memory formation in the long-term survivors. To obtain clinically relevant information, we established a standard of

care regimen consisting of surgery, radiation, and chemotherapy for orthotopic mouse GBM. We found that the anti-GITR +anti-PD1 therapy tailored to the GBM specific TME synergizes with the standard of care, suggesting a translational potential in patients.

P058 Tumor-targeted IL-2 by engineered mesenchymal stem cells reinvigorates CD8$^+$ T cells. Joonbeom Bae[1], Longchao Liu[1], Casey Timmerman[1], Eric Hsu[1], Anli Zhang[1], Jiankun Zhu[1], Yang-Xin Fu[1]. [1]UT Southwestern Medical Center, Dallas, TX.

Tumor microenvironment (TME) generates immunosuppressive niche to induce CD8$^+$ tumor infiltrating lymphocytes (TILs) exhaustion. Therapeutic targeting to functionally reinvigorate CD8$^+$ T cells is a promising strategy to enhance antitumor immunity. While interleukin-2 (IL-2) based therapies cause potent T cell activation and proliferation, the clinical application remains challenging due to short half-life and severe toxicity at therapeutic doses. To address this, we engineered mesenchymal stem cells (MSCs) to successfully proliferate and turn on or off CD8 T cell-preferential IL-2 mutein/Fc fusion protein (SIL2-EMSC) to target cytotoxic T cells in the TME. Peritumoral administration of SIL2-EMSCs permits local production of sufficient SIL2 inside the TME and induces complete tumor regression without adverse toxicity. Mechanistically, SIL2-EMSC remodels the TME that activates and expands preexisting CD8$^+$ TILs. Furthermore, local treatment of SIL2-EMSC elicits systemic antitumor responses for the clearance of distal tumor and metastasis. In advanced tumors, SIL2-EMSCs can overcome resistance to immune check blockade (ICB) and β-lapachone (β-lap) chemotherapy. The therapeutic benefits of SIL2-EMSC were also observed in humanized mouse models. Overall, tumor-targeted delivery of cytokines by next generation of MSCs reverses immunosuppressive environment, improves antitumor effects, and synergize with various therapies without adverse toxicity.

P059 An increase in eosinophils is associate with response to chemo-immunotherapy in metastatic triple negative breast cancer. Hazem Ghebeh[1], Mahmoud A. Elshenawy[1], Adher Al-Sayed[1], Taher Al-Tweigeri[1]. [1]King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Background: Several trials have reported on eosinophilia in cancer patients receiving immunotherapy therapy of cancer especially checkpoint inhibitors. There is limited reports on eosinophilia and immunotherapy in triple-negative breast cancer (TNBC).

Patients: We are reporting on eosinophilia and the increase in eosinophil count in a trial testing the combination of Durvalumab band paclitaxel in metastatic TNBC NCT02628132. In this trial 19 patients were recruited, however, only 14 received the combination therapy while the 5 drop out due to progression on paclitaxel alone priming cycle.

Results: One of the patients developed eosinophilia after having a complete response status. The developed eosinophilia lasted for a year and subsided with temporary holding treatment. Among all patients enrolled who received the combination therapy, there was a statistically significant correlation between an increase in eosinophil count (>300/mm^3) during treatment and a having partial or complete response to the combination therapy (p 0.028). In agreement, survival analysis showed a significant association between disease-free survival (DFS) and higher PCE

count (*p* 0.005). A similar trend existed with overall survival (OS), although it did not reach significance (*p* 0.167). The increase in eosinophil count correlated significantly with skin rash/pruritus (p 0.028). On the other hand, the association between baseline PEC before treatment and response to treatment was not statistically significant despite the positive trend.

Conclusions: An increase in circulating eosinophil count correlates with response to therapy with chemo-immunotherapy combination in metastatic TNBC. This study supports the role eosinophils are playing in the treatment of cancer.

P060 Identification of host-intrinsic resistance mechanisms to immune checkpoint inhibitors (ICI) in Diversity Outbred mice. Justin Hackett[1], James Glassbrook[1], Maria Muniz[1], Heather M. Gibson[2]. [1]Wayne State University, Detroit, MI, [2]Wayne State University/Karmanos Cancer Institute, Detroit, MI.

The advent of immune checkpoint inhibitors (ICI) for cancer therapy has improved outcomes for a variety of malignancies, however many a significant proportion of patients fail to benefit. While tumor-intrinsic mechanisms are likely involved in therapy resistance, it is unclear to what extent genetic heterogeneity of the host plays in response. To investigate this in a pre-clinical setting, we utilize the Diversity Outbred (DO) and Collaborative Cross (CC) mouse models. DO mice are genetically distinct animals generated by cross-breeding 8 inbred founder strains, and CC mice are recombinant inbred mice generated from the same 8 founders as DO. We find a wide variation in response to anti-PD-1/anti-CTLA-4 ICI in B16F0-bearing (C57BL/6xDO)F1 mice (n=142) with ~30% showing partial response with delayed tumor onset and ~13% showing complete tumor elimination with protection against subsequent contralateral rechallenge. Genetic linkage analysis using the R/qtl2 package identifies genomic loci associated with therapeutic outcomes. Importantly, the MHC locus was not significantly associated with response. Of the strongly associated loci, the region within chromosome 13 was further validated in CC mice bearing the positive (NZO) versus negative (C57BL/6) driver genotypes at this locus. ICI response was detected in 2/3 (C57BL/6xCC)F1 predicted responder models, with no response in predicted non-responders. This locus solely contains the murine prolactin family. Prolactin is a known immunomodulating cytokine with association to various autoimmune disorders. To directly test whether prolactin influences ICI response rates, we implanted inbred C57BL/6 mice, which notoriously fail to respond to ICI alone against B16F0 tumors, with subcutaneous slow-release prolactin pellets to induce mild hyperprolactinemia. Combined ICI with prolactin shows an interaction effect against B16F0, with 5/8 mice exhibiting slowed tumor growth relative to controls. To test whether this host-derived phenomenon applies to additional tumor models, we tested ICI response to Lewis Lung Carcinoma (LLC) in a (C57BL/6xCC)F1 model where response to B16F0 was observed. Relative to inbred C57BL/6 mice, where no response to ICI is observed, this (C57BL/6xCC)F1 model shows a similar response to ICI against LLC as was detected for B16F0. Collectively, this study shows that host genetics play a role in ICI response and that prolactin may be a positive regulator of ICI efficacy.

P061 Dendritic cell paucity in mismatch repair-proficient colorectal cancer liver metastases limits the efficacy of immune checkpoint blockade. William W. Ho[1], Igor L. Gomes-Santos[1], Shuichi Aoki[1], Meenal Datta[1], Kosuke Kawaguchi[1], Nilesh P Talele[1], Sylvie Roberge[1], Jun Ren[1], Hao Liu[1], Ivy X Chen[1], Patrik Andersson[1], Sampurna Chatterjee[1], Ashwin

S. Kumar[1], Zohreh Amoozgar[1], Qixian Zhang[1], Peigen Huang[1], Mei Rosa Ng[1], Vikash P Chauhan[1], Lei Xu[1], Dan G. Duda[1], Jeffrey W. Clark[1], Mikael J. Pittet[1], Dai Fukumura[1], Rakesh K Jain[1]. [1]Massachusetts General Hospital, Boston, MA.

Liver metastasis is a major cause of mortality for patients with colorectal cancer (CRC). Mismatch repair-proficient (pMMR) CRCs make up about 95% of metastatic CRCs, and are unresponsive to immune checkpoint blockade (ICB) therapy. Here we show that mouse models of orthotopic pMMR CRC liver metastasis accurately recapitulate the inefficacy of ICB therapy in patients, whereas the same pMMR CRC tumors are sensitive to ICB therapy when grown subcutaneously. To reveal local, nonmalignant components that determine CRC sensitivity to treatment, we compared the microenvironments of pMMR CRC cells grown as liver metastases and subcutaneous tumors. We found a paucity of both activated T cells and dendritic cells in ICB-treated orthotopic liver metastases, when compared to their subcutaneous tumor counterparts. Furthermore, treatment with FMS-like tyrosine kinase 3 ligand (Flt3L) plus ICB therapy increased dendritic cell infiltration into pMMR CRC liver metastases and improved mouse survival. Lastly, we show that human CRC liver metastases and microsatellite stable (MSS) primary CRC have a similar paucity of T cells and dendritic cells. These studies indicate that orthotopic tumor models, but not subcutaneous models, should be used to guide human clinical trials. Our findings also posit dendritic cells as antitumor components that can increase the efficacy of immunotherapies against pMMR CRC.

P062 PI3Kγδ inhibitor plus radiation enhances the antitumor immune effect of PD-1 blockade in syngenic murine breast cancer and humanized patient-derived xenograft model. In Ah Kim[1], Min Guk Han[1], Bum Sup Jang[2], Mi Hyun Kang[2]. [1]Seoul National University, Seoul, Republic of Korea, [2]Seoul National University Bundang Hospital, Seongnam, Republic of Korea.

Purpose: Breast cancer is generally viewed as immunologically 'cold', imposing an immune-suppressive tumor microenvironment (TME) and responding poorly to lone immune checkpoint blockade (ICB). As an adjunct to ICB, radiation therapy (RT) holds promise in terms of *in situ* tumor vaccination effect, although it is known to promote immune suppression, increasing regulatory T cells (T_{reg}), myeloid-derived suppressor cells (MDSCs), and M2 tumor-associated macrophages (TAMs). It was our contention that combined use of RT and a PI3Kγδ inhibitor to combat immune suppression might enhance the efficacy of ICB.

Methods: Murine breast cancer cells (4T1) were grown in both immune-competent and -deficient BALB/c mice, and tumors were irradiated by 3 fractions of 24 Gy. A PD-1 blockade and a PI3Kγδ inhibitor were then administered every other day for 2 weeks. Tumors from humanized patient-derived breast cancer xenograft (PDX) model was sequenced to identify immune-related pathways and to profile infiltrated immune cells. Transcriptomic and clinical data were acquired from The Cancer Genome Atlas (TCGA) pan-cancer cohort, and the deconvolution algorithm was used to profile immune cell repertoire.

Results: In the immune-competent syngenic 4T1 murine tumor model, PD-1 blockade alone led to tumor hyperprogression, whereas a three-pronged strategy of PI3Kγδ inhibitor, RT, and PD-1 blockade significantly delayed primary tumor growth, boosted abscopal effect, and improved

animal survival by comparison. The immune-deficient syngenic 4T1 murine tumor model failed to show this synergism in delaying tumor growth and the abscopal effect. According to FACS analysis, RT significantly increased not only CD8+cytotoxic T-cell fractions but also immune-suppressive T_{reg}cells, MDSCs, and M2 TAMs. However, PI3Kγδ inhibitor significantly lowered proportions of T_{reg}, MDSCs, and M2 TAMs, achieving dramatic gains in splenic, nodal, and tumor CD8+ T-cell populations after triple combination therapy. There were significantly decreased tumor expressions of p-AKT, PD-L1, and HIF1α by PI3Kγδ inhibition. Triple combination therapy significantly delayed primary tumor growth in humanized PDX model as well and analyses of RNA sequencing data of humanized PDX samples showed decreased immune suppressive pathways with decreased and M2 macrophage and increased CD8+ T-cell by triple combination therapy. In the TCGA pan-cancer cohort, high T_{reg}/CD8+T-cell and M2/M1 TAM ratios and poor overall patient survival was associated with high *PIK3CG* (PI3Kγ) or *PIK3CD* (PI3Kδ) gene expression.

Conclusion: These findings collectively indicate that PI3Kγ and PI3Kδ are clinically relevant targets in an immunosuppressive TME. Combining PI3Kγδ inhibitor, RT, and PD-1 blockade may thus be a viable approach, helping to overcome the therapeutic resistance of immunologically cold tumors such as breast cancer.

P063 Tumor cell-derived lactic acid inhibit anti-tumor immunity in the immune checkpoint blockade resistant tumor. Wonkyung Oh[1], Alyssa Min Jung Kim[1], Ruoxuan Sun[1], Seung-Oe Lim[1]. [1]Purdue University, West Lafayette, IN.

Immune checkpoint blockade therapy targeting the PD-1/PD-L1 axis, one of the most promising cancer immunotherapies, has shown remarkable clinical impact in multiple cancer types. Despite the recent success of PD-1/PD-L1 blockade therapy, acquired resistance, emerging as late relapses or recurrences, has been reported in the long-term follow-up of clinical trials. However, the resistance mechanisms of PD-1/PD-L1 blockade therapy are still unclear. Here we found that tumor cell-derived lactate rendered the immunosuppressive tumor microenvironment in the PD-1/PD-L1 blockade resistant tumors. Interestingly, increased lactate in the PD-1/PD-L1 blockade resistant tumors enhanced PD-1/PD-L1 interaction. Furthermore, combining a PD-L1-antibody-drug conjugate (ADC) with AZD3965, a MCT inhibitor eradicated the resistant tumor cells synergistically in 4T1 syngeneic murine models. Together, our results suggest a new combination treatment strategy to improve the therapeutic efficacy of immune checkpoint blockade therapies.

P064 Dual effect of epigenetic inhibitor and CAR-NK cell therapy in bladder cancer. Lucia Morales[1,2], Sandra Pinto Nunes[1,2,3], Ester Munera-Maravilla[1,2,4], Jose Antonio Casado[1,5], Paula Río[1,6], Antonio Valeri[7], Edurne San José-Enériz[4,8], Xavier Agirre[4,8], Felipe Prosper[4,8,9], Jesús María Paramio[1,2,4]. [1]Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain, [2]Biomedical Research Institute I+12, Hospital Universitario "12 de Octubre", Madrid, Spain, [3]Cancer Biology & Epigenetics Group-Research Center, Portuguese Oncology Institute of Porto (CI-IPOP), Madrid, Spain, [4]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain, [5]IIS-Fundación Jimenez Diaz and Autónoma University of Madrid, Madrid, Spain, [6]Centro de Investigación Biomédica en Red de Enfermedades Raras, Instituto de Investigación Sanitaria Fundación

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Bladder cancer (BC) causes 220,000 deaths per year worldwide since current therapies cannot be used in a large proportion of patients due to comorbidities. Recently, new treatments have been developed, including immunotherapies aimed to induce an antitumor response by activating the patients' immune system. However, since those treatments are only effective in a small subset of patients, new therapies are needed. Epigenetic modifications are drivers of BC and their modulation by inhibitors is considered a promising approach for BC management. Moreover, these compounds also show immunomodulatory properties, including NK cells antitumor activities. We observed that novel inhibitor of epigenetic machinery CM-1758, against histone deacetylases, display growth inhibition in a variety of human BC cell lines (5637, RT112, 253J, J82 and TCCSUP) in the low micromolar range. In addition, the compound induced the surface expression of NK cell ligands (NKG2DLs) in four of these tumor cells as measured by flow cytometry. Furthermore, we developed chimeric antigen receptor (CAR)-NK cells that express high levels of CAR composed by a fusion of NKG2D receptor ectodomain with 4-1BB and CD3z. Cytotoxic effects of NKG2D CAR-NK cells in co-culture with BC tumor cells were significantly potentiated as compared with parental NK-92 cells. In particular, 253J cells treated with CM-1758, that augmented its NKG2DLs levels, increased their sensitivity to NKG2D CAR-NK cells in co-culture and this effect was dependent on NKG2D receptor. Our results support that treatment with epigenetic inhibitors may facilitate the use of CAR-NK-based cell therapies as a promising strategy for BC management.

P065 Effects of targeted radiotherapy on tumor immune landscape in diverse murine tumor models. Tristan Wirtz[1], Catherine Lee[1], Tao Xie[1], Lisa Manzuk[1], Manfred Kraus[1], Christopher Dillon[1], Timothy Affolter[1], Anand Giddabasappa[1]. [1]Pfizer Inc, San Diego, CA.

Radiotherapy (RT) has traditionally been seen as a means to induce targeted tumor cell death, and more than 50% of all cancer patients receive RT. RT is also known to induce immune cell activation, and the advent of immunotherapeutic treatments such as checkpoint inhibition has sparked increased interest in using RT to enhance an anti-tumor immune response. However, to take advantage of the immunological effects of RT, a deeper understanding of the effects of RT on tumor-infiltrating leukocytes (TILs) is essential. Therefore, we systematically analyzed the effects of RT on tumor growth and the tumor-infiltrating immune cells in five syngeneic tumor models with diverse immune cell-infiltration profiles. These tumor types show strong differences in the overall immune cell infiltration, as well as in the composition of the infiltrating immune cell populations. After RT, tumors that were characterized as hot (e.g. CT26) showed increased sensitivity and dose-dependent tumor growth inhibition compared to cold tumor models (e.g. B16F10). Immune cell profiling indicated that RT led to strong changes in TILs of some tumor types, such as MC38 and CT26. These changes included reduced fractions of macrophages and increases in NK cells and also CD8 T cells. Single cell RNA sequencing also revealed an increase in CD8 T cells expressing proliferation-related genes. Furthermore, macrophage clusters

expressing markers of proliferation were specifically eradicated by RT, while monocytes and neutrophils were less affected. The monocytes and neutrophils in the models that showed little changes in TILs after RT expressed marker genes of type-I interferon response. These findings predict that tumors that are highly infiltrated by neutrophils and monocytes, with little intra-tumoral proliferation and type-I interferon response signature, are likely resistant to an RT-mediated anti-tumor immune response. The present work has laid a strong foundation to develop next-generation combinatorial treatments using RT and immunotherapy.

Other

P066 Prognostic and predictive value of pre-treatment T-Cell receptors (TCR) repertoire in non-small cell lung cancer (NSCLC) patients treated with single agent immunotherapy. Afaf Abed[1], Leslie Calapre[1], Samantha Bowyer[2], Michael Millward[3], Elin Gray[1]. [1]Edith Cowan University, Joondalup, WA, Australia, [2]Sir Charles Gairdner Hospital, Nedlands, WA, Australia, [3]Linear Clinical Research, Nedlands, WA, Australia.

Background: TCR repertoire plays a key role on the orchestration of the immune response. In particular, reduced pre-treatment Shannon diversity, increase clonality and increase convergence of TCRs have been suggested to reflect clonal expansion of antigen-specific T-cells in the tumor microenvironment. These are thought to be correlated with better response rate, improved progression free survival (PFS) and overall survival (OS). Here we aim to explore the above TCR repertoire features in peripheral blood of NSCLC patients (with PDL1≥50%) treated with single agent pembrolizumab in the first line setting; and correlate them with overall response rate (ORR), PFS and OS. Methods: We prospectively collected baseline blood from 48 NSCLC patients treated with first line pembrolizumab. High quality DNA was extracted from white blood cells and used for TCR sequencing using the Oncomine TCR Beta-SR Assay (Thermo Fisher). TCR clonality and convergence were calculated for each individual and correlated with survival using Kaplan-Meier curves and survival statistics. Multivariate analysis was carried out controlling for other variable that may influence the association of TCR repertoire and outcomes such as age, sex, ECOG, smoking status and pre-treatment neutrophil to lymphocyte ratio (NLR). Results: Our data matured for 29 patients only with a follow-up of at least 6 months. We observed a trend towards increased pre-treatment TCR clonality in patients with objective response to pembrolizumab and statistically significant reduced Shannon diversity (P = 0.042). Convergence did not seem to affect ORR in our cohort. Moreover, there was a significantly longer PFS in patients with reduced number of pre-treatment clones (HR = 0.54, 95%CI 0.21-1.43, P = 0.037), reduced Shannon diversity (HR = 0.52, 95%CI 0.20-1.38, P = 0.047), reduced Evenness (HR = 0.41, 95%CI 0.14-1.19, P = 0.044) and elevated clonality (HR = 2.45, 95%CI 0.84-7.11, P = 0.044). Reduced rather than increased convergence was correlated with a trend towards improved PFS. None of these parameters were statically significant in relation to OS. Conclusions: Increased pre-treatment TCR clonality and reduced diversity are associated with improved ORR and PFS, but not OS in NSCLC patients with high PD-L1 treated with pembrolizumab monotherapy. Further maturation of this cohort will demonstrate whether the circulating pre-treatment TCR repertoire is a prognostic factor for immunecheckpoint inhibition.

P067 Current status of regulatory-approved immunotherapies in Saudi Arabia. Reham Ajina[1]. [1]KSAU-HS, Riyadh, Saudi Arabia.

Immunotherapy has been shown to successfully turn aggressive malignancies in many patients into curable diseases. Thus, immunotherapy has received so much attention internationally, and Saudi Arabia was not an exception. Here, we evaluated the status of regulatory-approved immunotherapies in Saudi Arabia using the Saudi Food and Drug Administration (sFDA) database and the ClinicalTrials.gov website. We found that not all available immunotherapies in the clinic worldwide are approved by the sFDA, and the number of Saudi immunotherapy clinical trials are very limited. In fact, out of the eight regulatory-approved immune checkpoint inhibitors, 5 have received the sFDA approval. These immune check point inhibitors are Yervoy (ipilimumab), Nivolumab (Opdivo), Pembrolizumab (Keytruda), Atezolizumab (Tecentriq) and Avelumab (Bavencio). Also, out of the 5 regulatory approved chimeric antigen receptor (CAR) T cell therapy products, only KYMRIAH^{TM} (tisagenlecleucel) has received the sFDA approval**.** According to the ClinicalTrials.gov registry (accessed 27 July 2021), only 6 investigational clinical trials have been conducted in Saudi Arabia to evaluate immune check point inhibitors, and only 3 of these studies are currently ongoing. More surprisingly, no Saudi CAR clinical trials have yet been conducted. Taken together, there is an urgent need for a greater accessibility for immunotherapy in the country.

P068 Automated cell type specific PD-L1 quantification by artificial intelligence using high throughput bleach & stain 15-marker multiplex fluorescence immunohistochemistry in human cancers. Niclas C. Blessin[1], Elena Bady[1], Tim Mandelkow[1], Cheng Yang[1], Jonas B. Raedler[1,2], Ronald Simon[1], Christoph Fraune[1], Maximilian Lennartz[1], Sarah Minner[1], Eike Burandt[1], Doris Höflmayer[1], Guido Sauter[1], Katharina Möller[1], Sören A. Weidemann[1]. [1]Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]College of Arts and Sciences, Boston University, Boston, MA.

Introduction: The quantification of PD-L1 (programmed cell death ligand 1) has been used to predict patient's survival, to characterize the tumor immune microenvironment, and to predict response to immune checkpoint therapies. However, a framework to assess the PD-L1 status with a high interobserver reproducibility on tumor cells and different types of immune cells has yet to be established.

Methods: To study the impact of PD-L1 expression on the tumor immune microenvironment and patient outcome, a framework for fully automated PD-L1 quantification on tumor cells and immune cells was established and validated. Automated PD-L1 quantification was facilitated by incorporating three different deep learning steps for the analysis of more than 80 different neoplasms from more than 10'000 tumor specimens using a bleach & stain 15-marker multiplex fluorescence immunohistochemistry panel (i.e., PD-L1, PD-1, CTLA-4, panCK, CD68, CD163, CD11c, iNOS, CD3, CD8, CD4, FOXP3, CD20, Ki67, CD31). Clinicopathological parameter were available for more than 30 tumor entities and overall survival data were available for 1517 breast cancer specimens.

Results: Comparing the automated deep-learning based PD-L1 quantification with conventional brightfield PD-L1 data revealed a high concordance in tumor cells ($p<0.0001$) as well as immune

cells ($p<0.0001$) and an accuracy of the automated PD-L1 quantification ranging from 90% to 95.2%. Across all tumor entities, the PD-L1 expression level was significantly higher in distinct macrophage/ dendritic cell (DC) subsets (identified by CD68, CD163, CD11c, iNOS; $p<000.1$) and in macrophages/ DCs located in the Stroma ($p<0.0001$) as compared to intratumoral macrophages/ DC subsets. Across all different tumor entities, the PD-L1 expression was highly variable and distinct PD-L1 driven immune phenotypes were identified based on the PD-L1 intensity on both tumor and immune cells, the distance between non-exhausted T-cell subsets (i.e. PD-1 and CTLA-4 expression on $CD3^+CD8^+$ cytotoxic T-cells, $CD3^+CD4^+$ T-helper cells, $CD3^+CD4^+FOXP3^+$ regulatory T-cells) and tumor cells as well as macrophage/ (DC) subtypes. In breast cancer, the PD-L1 fluorescence intensity on tumor cells showed a significantly higher predictive performance for overall survival with an area under receiver operating curves (AUC) of 0.72 ($p<0.0001$) than the percentage of $PD\text{-}L1^+$ tumor cells (AUC: 0.54). In PD-L1 positive as well as negative breast cancers a close spatial relationship between T- cell subsets ($CD3^+CD4^{\pm}CD8^{\pm}FOXP3^{\pm}PD\text{-}1^{\pm}CTLA\text{-}4^{\pm}$) and Macrophage/ DC subsets ($CD68^{\pm}CD163^{\pm}CD11c^{\pm}iNOS$) was found prognostic relevant ($p<0.0001$).

Conclusion: In conclusion, multiplex immunofluorescence PD-L1 assessment provides cutoff-free/ continuous PD-L1 data which are superior to the conventional percentage of $PD\text{-}L1^+$ tumor cells and of high prognostic relevance. The combined analysis of spatial PD-L1/ PD-1 data and more than 20 different immune cell subtypes of the immune tumor microenvironment revealed distinct PD-L1 immune phenotypes.

P069 Semi-automated validation and quantification of CTLA-4 in 90 different Tumor entities using multiple antibodies and artificial intelligence. David Dum[1], Tjark L. C. Henke[1], Tim Mandelkow[1], Elena Bady[1], Jonas B. Raedler[1,2], Ronald Simon[1], Guido Sauter[1], Maximilian Lennartz[1], Waldemar Wilczak[1], Eike Burandt[1], Stefan Steurer[1], Niclas C. Blessin[1]. [1]Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]College of Arts and Sciences, Boston University, Boston, MA.

Introduction: CTLA-4 is an inhibitory immune checkpoint receptor and a negative regulator of anti-tumor T-cell function. This study aimed at a comparative analysis of $CTLA\text{-}4^+$ cells between different tumor entities.

Methods: To quantify $CTLA\text{-}4^+$ cells, 4,582 tumor samples from 90 different tumor entities as well as 608 samples of 76 different normal tissue types were analyzed by immunohistochemistry in a tissue microarray format. Two different antibody clones (MSVA-152R and CAL49) were validated and quantified using a deep learning framework for automated exclusion of unspecific immunostaining.

Results: Comparing both CTLA-4 antibodies revealed a clone dependent cytoplasmic cross-reactivity in adrenal cortical adenoma (63%) for MSVA-152R and in pheochromocytoma (67%) as well as hepatocellular carcinoma (36%) for CAL49. After automated exclusion of non-specific staining reaction (3.6%), a strong correlation was observed for the densities of $CTLA\text{-}4^+$ lymphocytes obtained by both antibodies ($r=0.87$; $p<0.0001$). The mean density of $CTLA\text{-}4^+$ cells was 674±1482 cells/ mm^2 and ranged from 71±175 cells/mm^2 in leiomyoma to 5916±3826 cells/mm^2 in Hodgkin's lymphoma. Within epithelial tumors, the density of $CTLA\text{-}4^+$

lymphocytes were higher in squamous cell (421±467 cells/ mm^2) and urothelial carcinomas (419±347 cells/ mm^2) than in adenocarcinomas (269±375 cells/ mm^2) and renal cell neoplasms (256±269 cells/ mm^2). A high CTLA-4^+ cell density was linked to low pT category ($p<0.0001$), absent lymph node metastases ($p=0.0354$), and PD-L1 expression in tumor cells or inflammatory cells ($p<0.0001$ each). A high CTLA-4/CD3-ratio was linked to absent lymph node metastases ($p=0.0295$) and to PD-L1 positivity on immune cells ($p<0.0026$).

Conclusion: Marked differences exist in the number of CTLA-4^+ lymphocytes between tumors. Analyzing two independent antibodies by a deep learning framework identifies clone-specific cross-reactivity and facilitates automated quantification of target proteins such as CTLA-4.

P070 The safety and efficacy of Durvalumab and Paclitaxel combination in metastatic triple-negative breast cancer: An open-label phase I/II trial with 2-years follow-up. Hazem Ghebeh[1], Adher Al-Sayed[1], Kauser Suleman[1], Riham Eiada Eiada[1], Asma Tulbah[1], Taher Al-Tweigeri[1]. [1]King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Weekly paclitaxel is an excellent therapeutic choice for metastatic triple-negative breast cancer, a type of cancer that typically has short survival. There is evidence that TNBC is relatively more immunogenic; THEREFORE, we tested the safety and efficacy of weekly paclitaxel at 80 mg/m^2 /IV given in combination with Durvalumab, (an anti-PD-L1). During a period of 24 MONTHS, 14 patients received the combination OF six cycles of concurrent weekly paclitaxel given on days 1, 8, and 15/IV and Durvalumab given on days 1 and 15 /IV of every 28 days' cycle. Upon completion of this combination, Durvalumab was given every 2 weeks until disease progression or unacceptable toxicity. The combination therapy had an excellent safety profile with no dose reduction, dose-limiting toxicity, OR death related to combination therapy. Adverse events (AEs) reported in 10 (71%) of patients, regardless of grade, while grade 3 or 4 AEs happening in 3 (21%) patients only. The most common AEs, were peripheral neuropathy and headache, which happened in 4 patients (29%) followed by fatigue and skin rash occurred in 3 patients (21%). Other less common AEs included: Anemia, leukopenia and/or neutropenia, diarrhea, alopecia, palpitation, and weight gain. The overall response rate for the combination was 36% (confirmed), with a median duration OF RESPONSE of 10 months. The median progression-free survival (PFS) and overall survival (OS) was 5.0 AND 20.7 months, respectively. In conclusion, WE ARE REPORTING an excellent safety profile for the weekly paclitaxel and Durvalumab combination. The data should be looked at with caution due to the small number of patients. Due to excellent safety profile, further trials involving additional, possibly synergistic, agents to the paclitaxel/Durvalumab combination are encouraged.

P071 Gemcitabine augments HLA class I expression in pancreatic cancer cells through alterations in transcript production and surface stability. Alaina C. Larson[1], Shelby M. Knoche[1], Joyce C. Solheim[1]. [1]University of Nebraska Medical Center, Omaha, NE.

Background: Pancreatic adenocarcinoma, or PDAC, is the fourth leading cause of cancer-related deaths in the United States. Gemcitabine, a nucleoside analog, is a primary standard of care in pancreatic cancer. In addition to its normative cytotoxic function, evidence suggests that this chemotherapy drug also harnesses immunomodulatory capabilities in the form of increasing human leukocyte antigen (HLA) class I expression in lung, breast, colon, and

cholangiocarcinoma cells. HLA class I is a complex (alpha heavy chain and beta 2-microglobulin light chain) located at the surface of nearly all cells where it presents peptides, including cancer-associated peptides, to cytotoxic T cells. Recognition of these peptides as atypical leads to T-cell mediated lysis of the presenting cell. Subsequently, understanding the ability of gemcitabine and the mechanisms by which it influences the HLA class I complex are of great importance.

Methods: To investigate the effect of gemcitabine treatment on HLA class I expression in pancreatic cancer, alterations in HLA class I protein levels were monitored via western blot analysis, flow cytometry, and quantitative polymerase chain reaction (qPCR) in three pancreatic cancer cell lines. Changes in surface stability of the HLA class I complex were evaluated through brefeldin A (BFA) assays at the 72-hour time point. For western blot and flow cytometry experiments, impacts on the alpha heavy chain were further assessed for all three types of alpha heavy chains (HLA-A, HLA-B/C) at 72 and 96 hours. In qPCR experiments, alterations were analyzed for the HLA-A, HLA-B, and beta 2-microglobulin transcripts after a 48-hour gemcitabine exposure period.

Results: Administration of gemcitabine to pancreatic cancer cell lines (S2-013, Capan-1, PANC-1) increased total protein levels of both HLA class I constituents (alpha heavy chain and beta-2-microglobulin light chain). All PDAC cell lines evaluated demonstrated enhanced surface expression of HLA-A2 and HLA-B/C with maximal increases of 3 and 2.5-fold respectively, as indicated by flow cytometry. Our qPCR analysis of the Capan-1 and S2-013 cell lines revealed increases in the levels of HLA-A, HLA-B, and beta 2-microglobulin transcripts (3-12-fold). BFA assays suggested that gemcitabine treatment also enhances stability of the surface HLA class I in the S2-013 and PANC-1 cell lines.

Conclusion: In summary, gemcitabine exhibits an immunomodulatory ability to stimulate expression of HLA class I in pancreatic cancer cells. We have demonstrated that this increase in HLA class I is seen at the mRNA, total protein, surface, and stability level. Characterizing the ability of gemcitabine to influence the HLA class I complex and defining the mechanisms by which it does so will increase the potential for identification of suitable immunotherapies, including novel peptide-based cancer vaccines with enhanced treatment efficacy for PDAC patients.

P072 Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) foster myeloid-derived suppressor cell-mediated tumor immune evasion in cholangiocarcinoma. Emilien Loeuillard[1], Juan Wang[1], Jingchun Yang[1], Haidong Dong[1], Gregory Gores[1], Sumera Ilyas[1]. [1]Mayo Clinic, Rochester, MN.

Background and Aims: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is predominantly expressed on immune cells. Although TRAIL biology has garnered considerable interest as a potential anti-cancer strategy, TRAIL agonists have had very limited anti-cancer activity in humans. TRAIL signaling in T cells may also potentially provide an immune checkpoint function as it can inhibit T cell activation and proliferation by interfering with T cell receptor signaling. However, this potential immune checkpoint function of TRAIL has not been examined in cancer biology. Cholangiocarcinoma (CCA), a highly lethal biliary tract cancer,

provides a model to examine the potential immune checkpoint function of TRAIL. **Methods:** Using a syngeneic, orthotopic murine model of CCA (PMID: 29464042), murine CCA cells (SB cells) that express both TRAIL and TRAIL receptor (TR) were implanted into livers of WT and *Tr*$^{-/-}$ mice. Hence, in this model the host immune cells express TRAIL but not the receptor; therefore, they would be capable of inducing TRAIL-mediated apoptosis in CCA cells but would be resistant to TRAIL-mediated immunosuppression. After 4 weeks of tumor growth, mice were sacrificed, and tumors were characterized using flow cytometry. **Results:** We observed that *Tr*$^{-/-}$ mice had a significant reduction in tumor burden compared to WT mice. Moreover, tumor bearing *Tr*$^{-/-}$ mice had a significant increase in cytotoxic T lymphocytes (CTLs) and enhanced CTL effector function. However, coculture of T cells with SB cells or SB cells deficient in Tr (SB-*Tr*$^{-/-}$) did not result in a significant difference in T cell apoptosis or function, implying that TRAIL-TR is not a direct T cell checkpoint. Myeloid derived suppressor cells (MDSCs) were significantly decreased in *Tr*$^{-/-}$ tumors compared to WT tumors. Furthermore, implantation of SB cells devoid of Trail (SB-*Trail*$^{-/-}$) into WT mice resulted in a significant reduction in tumor burden and MDSC infiltration. Coculture of SB cells with MDSCs from *Tr*$^{-/-}$ mice attenuated MDSC proliferation and immunosuppression compared to WT MDSCs. Moreover, treatment of MDSCs from *Tr*$^{-/-}$ mice with TRAIL recombinant protein resulted in a reduction in their proliferation and immunosuppressive function compare to MDSCs from WT mice implying that TRAIL-TR fosters MDSC growth and immunosuppressive function. **In conclusion,** we have demonstrated that *Tr*$^{-/-}$ mice have a significant reduction in CCA tumor burden and MDSC infiltration. Consequently, *Tr*$^{-/-}$ mice bearing tumors have enhanced CTL infiltration and function. These data suggest that the TRAIL-TR system mediates tumor immune evasion via MDSCs. Herein, we suggest that TRAIL-TR appears to function as an indirect T cell checkpoint by augmenting the immunosuppressive effects of MDSCs.

P073 Gut microbiota shift in melanoma patients undergoing immunotherapy is associated with clinical response. Angeli Dominique Macandog[1], Carlotta Catozzi[1], Ester Cassano[1], Sara Gandini[1], Pier Francesco Ferrucci[1], Emilia Cocorocchio[1], Teresa Manzo[1], Luigi Nezi[1]. [1]Istituto Europeo di Oncologia, Milan, Italy.

Background: The high resistance of melanoma to targeted therapy has made it a persistently lethal cancer type. Although the advent of immune checkpoint inhibition (ICI) therapy markedly improved outcome for melanoma in recent years, response remains heterogenous, with only 20-40% of patients achieving clinical response for anti-CTLA4 or anti-PD1 therapy. This variability in response has urged research towards host factors that drive response to immunotherapy, and studies have come out to show that the gut microbiota influences immunotherapy response. More importantly, mouse studies and more recent human clinical trials demonstrate that transplantation of responder (R) microbiota improves host immunity and alleviates tumor growth during immunotherapy. Although these findings confirm a close relationship between the gut microbiota and antitumor host immunity, FMT human clinical trials on melanoma patients report success in only 30-40% of patients, suggesting that current knowledge of underlying immunomodulatory mechanisms of the gut microbiota are still limited, and that analysis of data collected only before start of therapy is insufficient. Notably, there is a lack of published longitudinal studies that monitor gut microbiome changes during melanoma immunotherapy.

Methods: We performed longitudinal analysis of the gut microbiota in melanoma patients undergoing NEOAJUVANT immunotherapy, revealing distinct dynamics between R and non-responders (NR) over the course of treatment. Sequencing data were paralleled with a quantitative analysis of circulating inflammatory molecules, suggesting a dynamic interaction between the gut microbiota and immune system. Here, we will present key modulators of the immune cell compartment.

Conclusions: Overall, our results highlight the importance of longitudinal analysis to dissect the role of the gut microbiota on response to immunotherapy.

P074 MB097: A therapeutic consortium of bacteria clinically-defined by precision microbiome profiling of immune checkpoint inhibitor patients with potent anti-tumor efficacy in vitro and in vivo. Matthew J. Robinson[1], Kevin Vervier[1], Simon Harris[1], Amy Popple[1], Dominika Klisko[1], Robyne Hudson[1], Ghaith Bakdash[1], Laure Castan[1], Clelia Villemin[1], David J. Adams[2], Doreen Milne[3], Catherine Booth[3], Christine Parkinson[3], Roy Rabbie[2], Sarah J. Welsh[3], Emily Barker[3], Katie Dalchau[3], Pippa Corrie[3], Trevor Lawley[1]. [1]Microbiotica, Cambridge, United Kingdom, [2]Experimental Cancer Genetics, Sanger Centre, Cambridge, United Kingdom, [3]Cambridge University Hospitals, Cambridge, United Kingdom.

Independent groups have demonstrated that the pre-treatment gut microbiome of cancer patients impacts the subsequent response to Immune Checkpoint Inhibitor (ICIs) therapy [1-4]. However, each study identified different sets of bacteria linked to outcome, which has limited the development of drug response biomarkers and clinic-first design of novel microbiome-based therapeutics. The Cambridge (UK) MELRESIST study includes a cohort of advanced melanoma patients receiving approved ICIs. Pre-treatment stool samples from MELRESIST were analysed by Microbiotica using shotgun metagenomic sequencing. Microbiotica's platform comprises the leading Reference Genome Database to give the most comprehensive and precise mapping of the gut microbiome. A bioinformatic analysis identify a small discrete microbiome signature that was different between responders and non-responders. We extended this signature by reanalysing three published melanoma cohorts [1-3] using the Microbiotica platform. The resultant bacterial signature predicted whether or not a patient responded to anti-PD1-based therapy with an accuracy of 91% in all four studies combined and was also an effective biomarker for each cohort individually. We validated the signature using a NSCLC study [4] indicating that it has great potential as a clinical biomarker for a number of indications. The signature was strongly skewed towards species raised in abundance in responding patients, suggesting that the microbiome influences ICI treatment primarily through bacteria that enhance the efficacy of the drugs. At the core of the signature was nine species strongly associated a positive outcome, which we hypothesized to be a central driver of drug response. MB097 is a consortium comprised of all nine bacteria. In a syngeneic mouse model of cancer, MB097 was able inhibit tumor growth, but most strikingly was potently synergistic when dose with anti-PD1. To understand the mechanisms by which these bacteria drive an anti-tumor response, we have profiled the bacteria individually and as a consortium in multiple assays with primary human immune cells. The bacteria strongly activate dendritic cells with a number inducing high levels of IL-12 relative to IL-10. These bacteria-stimulated dendritic cells went on to trigger Cytotoxic T Lymphocytes (CTLs) to upregulate Granzyme B, Perforin and IFNg. Further, we have demonstrated that these primed CTLs are very effective at tumor cell killing in vitro. In

summary, Microbiotica's precision microbiome profiling and the MELRESIST study has allowed us to identify a consortium of bacteria, MB097, strongly linked to response in multiple melanoma cohorts and a NSCLC study. The consortium drives immune-mediated tumor killing in vivo and in vitro. MB097 is being scaled up for manufacture as a novel co-therapy with ICIs.

References1 Matson V et al *Science* (2018) 359:104 2 Gopalakrishnan V *Science* (2018) 359:97 3 Frankel AE et al *Neoplasia* (2017) 19:848 4 Routy B et al *Science* (2018) 359:91

P075 NTX-1088, a potent first-in-class, anti-PVR mAb, restores expression and function of DNAM1 for optimal DNAM1-mediated antitumor immunity. Pini Tsukerman[1], Anas Atieh[1], Akram Obeidat[1], Keren Paz[2], Guy Cinamon[3], Tihana Lenac Rovis[4], Paola Kucan Brlic[4], Lea Hirsl[4], Stipan Jonjić[4], Ofer Mandelboim[5]. [1]Nectin Therapeutics, Jerusalem, Israel, [2]Nectin Therapeutics, New York, NY, [3]Nectin Therapeutics, Tel Aviv, Israel, [4]MEDRI, Rijeka, Croatia, [5]HUJI, Jerusalem, Israel.

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

complemented by the efficient restoration of DNAM1. This is a step change in antitumor immune activation, which will soon be tested in the clinic.

P076 Humanized anti-αvβ3 antibody engineered to selectively promote macrophage-mediated cancer cell death. Hiromi I. Wettersten[1], Ryan M. Shepard[1], Sara M. Weis[1], David A. Cheresh[1]. [1]University of California, San Diego, San Diego, CA.

Integrin αvβ3, an epithelial-to-mesenchymal transition marker, promotes drug resistance and metastasis in a range of epithelial cancers. Hence, a humanized anti-αvβ3 antibody (Etaracizumab), developed in the 1990s, was designed to eliminate αvβ3+ cells via NK-mediated antibody-dependent cellular-cytotoxicity (ADCC). Etaracizumab has been tested in clinical trials and proven to be safe with some activity in a subset of patients. However, in a recent examination of the immune microenvironment of αvβ3+ human epithelial tumors, we observed a large number of tumor-associated macrophages (TAMs) but minimal accumulation of NK or T cells. This suggests the efficacy of Etaracizumab may have been limited based on the relative lack of NK cells in αvβ3+ tumors. Therefore, we re-engineered Etaracizumab by changing its Fc receptor binding properties to favor TAM engagement over NK cells. This newly designed anti-αvβ3 antibody (ABT-101) while binding to αvβ3 with the same affinity as Etaracizumab, not only eliminates tumor cells via TAM-mediated ADCC but promotes a two-fold increased anti-tumor activity in mice and accumulates to a higher degree in tumors compared to Etaracizumab. Based on these preclinical findings, ABT101 is currently being evaluated in IND enabling studies with the hope to initiate phase I clinical trials in mid-2022.

P077 Composition of CD4 T cell subsets and impact on tumor growth control across mouse syngeneic tumor models. Chunxiao Xu[1], Lindsay Webb[1], Sireesha Yalavarthi[1], Clotilde Bourin[1], Jacques Moisan[1]. [1]EMD Serono Research and Development Institute, Billerica, MA.

CD4 T cells include multiple sublineages orchestrating a broad range of effector activities during the initiation, promotion, and progression of carcinogenesis. Although regulatory T cells (Tregs) are well-characterized to promote tumor progression, the impact of effector CD4+ T cell subsets (Teff) in anti-tumor immunity is less well known. Further, the relative contribution of Teff and Treg cell subsets in different syngeneic tumor models has not been characterized. We observed that CD4 depletion had significantly different impact of tumor growth across different syngeneic tumor models: no effect on MC38 tumor growth; enhanced tumor growth in CT26.KSA tumor model; while almost complete tumor regression in the EMT-6 tumor model. We hypothesized that each tumor model has a vastly different composition in the CD4 T cell compartment. To test this, we characterized expression of activation and CD4 T cell differentiation markers as well as cytokine production from CD4 TILs in each of the syngeneic tumor models. We found that while effector CD4 TILs ($FoxP3^{-}CD4^{+}$) from MC38 and CT26.KSA tumors expressed high levels activation and differentiation markers, Teff from EMT-6 tumors expressed very low levels of T cell activation and differentiation markers, suggesting the Teff in EMT-6 tumors are largely dysfunctional. Ex vivo restimulation of tumor samples with anti-CD3/anti-CD28 promoted IFNY production in CD4 TILs from MC38 and CT26.KSA tumor models, but not from EMT-6 tumor models, further supporting that Teff from MC38 and CT26.KSA tumors remain highly active, while CD4 TILs from EMT-6 tumors are dysfunctional. When looking at the Treg compartment, the frequency of Tregs in CT26.KSA tumors was reduced compared to MC38 and

EMT-6 tumors. Further, Tregs in MC38 and EMT-6 tumors expressed very high levels of CD69, a marker of highly immunosuppressive Tregs, while Tregs in CT26.KSA tumors expressed lower levels of CD69, suggesting that these Tregs may be less immunosuppressive. In summary, the CD4 T cell compartment is vastly different across CT26.KSA, MC38 and EMT-6 syngeneic tumor models, resulting in very different impact of tumor growth control.

P078 Synergistic immunotherapy effects of anti-COVID-19 and anti-cancer vaccines in preventing tumors and infections. Narayana Garimella[1]. [1]NCAC SOT, Reston, VA.

To assure efficacy, safety and security of medicines and products including those of cancer and infection prevention ones, the concepts of immunology and immunotherapy, treatments, therapeutics, research, reviews, approvals and according agency, nation and world-wide managements are highly important. Often rationally planned combinations of U.S. Food and Drug Administration (USFDA) approved medicines in preventing or treating the same or similar or multiple diseases are realized to be pharmacologically and biologically effective in mechanisms and toxicologically safe in applications. Because, multiple mechanisms are simultaneously combined in enhancing systemic immunities within the permissible limits of the doses. In this work, the immunology and immunotherapy aspects of both anti-cancer vaccines and anti-COVID-19 vaccines are taken into account to prevent current and future occurrences of cancer and infectious diseases. Though the intended mechanisms of actions are only disease specific and are primarily different between anti-cancer and anti-COVID-19 vaccines, their combinations are worthwhile in providing synergistic effects against carcinogenesis, metastasis and infections. Based on the chemical, biological and pharmacological input and output information for both kinetics and dynamics of vaccines including mRNA-1273, BNT162b2, and JNJ-78436735 to prevent covid-19 are explored in combinations with anti-cancer vaccines including Human Papillomavirus (HPV) Vaccine and Hepatitis B Vaccine (HBV). In addition to safety and efficacy, mRNA, viral-vector, protein and antigen mechanisms, pH, vaccine-vaccine interactions, independent and combined dose regimens, toxicities, solubilities, intrinsic and extrinsic covariates were taken into account for careful planning of combinations. Simultaneous dual combinations from both groups were improved to three to five levels of combinations. It was realized that these combinations qualify for pre-clinical followed by clinical-investigations further. Also, these conclusions promised us to explore additional new and approved vaccines of these indications.