Proffered Abstracts

PR01 Oncolytic adenoviruses expressing metabolic targets can improve viro-immunotherapy with bispecific T cell engagers through reducing acidosis in an *in vitro* model. Arthur Dyer[1], Sally Frost[1], Flurin Caviezel[1], Len Seymour[1]. [1]University of Oxford, Oxford, United Kingdom.

Oncolytic viro-immunotherapy holds huge promise for cancer treatment. While oncolytic viruses can robustly trigger immune activation, negative feedback is often upregulated in the tumor microenvironment (TME), an issue that is often neglected in early evaluation *in vitro*. Lactate accumulation and the resulting acidosis are commonly associated with viral infections and has been reported to be a key factor in shaping the immunosuppressive TME through various mechanisms which favor suppressive Tregs and MDSCs whilst polarizing macrophages towards an immunosuppressive state Given that the success of oncolytic viro-immunotherapy is now widely agreed to rely upon the induction of an immune response either from a transgene or from the release of DAMPs and PAMPs as opposed to direct cell lysis, understanding the impact of the increased acidosis upon immune cells and generating oncolytic viruses capable of avoiding or correcting acidosis is very promising. We found that infection with an oncolytic adenovirus led to a significant increase in lactic acid accumulation and acidosis in keeping with prior reports. An immunotherapeutic that shows promising results is the bispecific antibodies known as bispecific T cell engagers (BiTEs). Although BiTEs have particular success in hematological cancers, they have often shown negligible effects in solid tumor models. Here we show that BiTEs can robustly activate T cells resulting in target cell lysis; however, both T cell activation and BiTE-directed cytotoxicity are reduced when lactate concentrations increase or when acid is added to the media *in vitro*. Using media from virally infected cells, we show that acidosis following infection of tumor cells with an oncolytic adenovirus also negatively impacted T cell stimulation with BiTEs. Building on previously published results which show that inhibiting glycolysis can improve oncolytic viral infection, we have created oncolytic adenoviruses which inhibit acidosis at various stages through the expression of transgenes targeting this pathway. These next-generation oncolytic viruses were able to replicate whilst inhibiting glycolysis, or acidosis, and were able to halt the production of excess lactic acid following infection of tumor cells, ultimately allowing a rescuing of BiTE-mediated T cell activation. These findings provide a rationale for arming oncolytic viruses with anti-acidity agents, alongside those with T cell-activating abilities, which may offer a novel approach to overcome metabolic barriers of the tumor microenvironment and combat immune evasion.

PR02 The co-expression of VISTA and TIGIT on cytotoxic T cells defines subpopulation with altered immunometabolism. Cassandra Gilmour[1], Seong-Keun (Steve) Yoo[1], Timothy Chan[1], Lily Wang[1]. [1]Cleveland Clinic Foundation, Cleveland, OH.

Cancer immunotherapies, specifically checkpoint blockade therapies, are extremely successful at treating subpopulations of specific cancers long term. The idea is to disrupt the negative regulation of T cells and restore cytotoxic abilities allowing killing of the cancer cells and formation of memory T cells. This provides immunity that has the potential to last a lifetime

against recurring cancer. One major limitation to checkpoint blockade is that it is has limited response rate, one theory as to why this is the case, is that there are multiple non-redundant pathways of regulation of T cell activation. In response to the heterogeneous and suppressive tumor microenvironment, the T cell may present many immune checkpoint proteins simultaneously. Thus, it is important to study the immune checkpoint proteins in context of one another with the goal of developing combinatorial therapies. Here we present the co-expression of two immune checkpoint proteins and how their co-expression results in altered metabolism in cytotoxic T cells. V-domain Immunoglobulin Suppressor of T-cell Activation (VISTA) is a protein with many roles that are still largely undefined across a variety of immune cells in which it is expressed. T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) is a bi-directional receptor that has been reported to have a suppressive intrinsic T cell role when probed with agonistic antibody, the mechanism is yet to be defined. Our data suggest that VISTA and TIGIT are co-expressed in varying amounts across a variety of murine and human cancers and that this co-expression may contribute to an altered metabolic phenotype. Our results give insight to the contribution of multiple checkpoint proteins to the state of the cytotoxic T cell and declare the importance of studying these proteins in context of one another. This data reaffirm the need to study non-redundant pathways of T cell suppression with the goal of developing inhibitors that are more broadly applicable to restore anti-tumor immunity and increase patient survival.

PR03 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.

This abstract is also being presented as Poster P002.

PR04 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*.

This abstract is also being presented as Poster P004.

PR05 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.

This abstract is also being presented as Poster P020.

PR06 Reprogramming of naïve B cells in pancreatic cancer subverts humoral immunity. Yuliya Pylayeva-Gupta[1], Bhalchandra Mirlekar[1]. [1]University of North Carolina at Chapel Hill, Chapel Hill, NC.

B cells frequently infiltrate human tumors, and the intra-tumoral abundance of plasma cells can correlate with improved patient prognosis. While substantial evidence documents the presence of both regulatory B cells (Breg) and effector B cells, there is a significant knowledge gap in our understanding of how pro- versus anti-tumor B cell responses are generated and whether such responses are interconnected and/or amenable to re-programming. We report the existence of a negative regulatory signaling network that reprograms naïve B cells in pancreatic cancer to antagonize anti-tumor plasma B cells. This network is driven by IL-35-mediated STAT3 activation, which directly stimulates upregulation of the pioneer transcription factors Pax5 and Bcl6 in naïve B cells and impedes plasma cell differentiation while simultaneously activating regulatory B cell phenotypes. Significantly, inhibition of Bcl6 reversed this tumor-associated reprogramming of naïve B cells, enabling intratumoral accumulation of plasma cells, and reduced tumor growth. Our data provide evidence that the balance between Breg and plasma cell is the key to tumor immunity. B cell dysfunction in cancer involves a potentially targetable suppression program that alters the differentiation potential of naïve B cells.

PR07 Quiescent cancer cells form immunotherapy resistant reservoirs by forming an immune suppressive niche. 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.

This abstract is also being presented as Poster P007.

PR08 Context is everything: Microbiota-specific T follicular helper cells in colorectal cancer. Abigail E. Overacre-Delgoffe[1], Hannah J. Bumgarner[1], Anthony R. Cillo[1], Ansen H. P.

Burr[1], Justin T. Tometich[2], Amrita Bhattacharjee[1], Tullia C. Bruno[1], Dario A. A. Vignali[1], Timothy W. Hand[1]. [1]University of Pittsburgh, Pittsburgh, PA, [2]Children's Hospital of Pittsburgh, Pittsburgh, PA.

Colorectal cancer (CRC) is the third most common and one of the deadliest cancers in the US, and the survival rate for advanced cases is poor. Immunotherapy, especially checkpoint blockade, has revolutionized the way cancer is treated and has resulted in longterm, durable responses for some epithelial cancers. Unfortunately, CRC remains largely unresponsive to current immunotherapies, with only ~6% of total patients responding to anti-PD1. Therefore, it is necessary to not only determine hurdles that are preventing checkpoint blockade response, but to also develop novel therapies to use in conjunction with current therapies. The microbiome has recently been associated with better anti-PD1 response in melanoma patients; however, the underlying mechanism by which select bacteria provide a benefit and whether this can be applied to other cancer types remains unclear. The majority of microbes live within the gut, and adherent bacteria can have direct interaction with the colonic epithelium, resulting in a bacteria-specific immune response. Therefore, we hypothesized that rational modification of the microbiome may support anti-tumor immunity through activation of microbiota-specific T cells. Using a carcinogen-induced mouse model of CRC, we found that addition of a single colonic-residing, adherent bacteria (*Helicobacter hepaticus, Hhep*) after tumors had formed led to a significant reduction in tumor burden and a 2-3 fold increase in overall survival. Interestingly, *Hhep* colonization led to the activation of *Hhep*-specific T follicular helper cells (TFHs) that supported formation of large, organized tertiary lymphoid structures (TLS) found adjacent to or within tumors themselves. The presence of TLS supported an increase in cytotoxic lymphocytes (T and NK cells) specifically within the tumor core. Somewhat strikingly, the anti-tumor response was dependent on $CD4^+$ T cells but not $CD8^+$ T cells. Using TFH KO mice, we found that *Hhep*-specific T cells were both necessary and sufficient to drive TLS maturation, anti-tumor immunity, and ultimately, longterm survival in CRC. Overall, these findings suggest that microbiome modulation and the subsequent microbiota-specific $CD4^+$ T cell response may represent a new immunotherapeutic target for cancer subtypes that remain resistant to checkpoint blockade.

PR09 Macrophage promotion of anti-androgen resistance in prostate cancer bone disease. Xue-Feng Li[1], Cigdem Selli[1], Asier Unciti-Broceta[2], Neil O. Carragher[2], Hai-Yan Hu[3], Charles L. Sawyers[4], Bin-Zhi Qian[5]. [1]MRC Centre for Reproductive Health, College of Medicine and Veterinary Medicine, Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, UK, [2]Edinburgh Cancer Research UK Centre, Institute of Genetics & Molecular Medicine, University of Edinburgh, Edinburgh, UK, [3]Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, (Mainland), [4]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, [5]MRC Centre for Reproductive Health, College of Medicine and Veterinary Medicine, The University of Edinburgh, Edinburgh, UK.

Metastatic castration resistant prostate cancer (mCRPC) is the final stage of prostate cancer (PC) that acquires resistance to androgen deprivation therapies (ADT). Despite many recent

progresses in the mechanistic understanding of ADT resistance, the specific contribution of the metastatic microenvironment in mCRPC remains largely unknown. A novel *in vivo* model of androgen dependent bone metastatic PC was developed in address this question. Our identified that macrophages are the major stromal cells in bone metastatic PC. Using multiple genetic models, we demonstrated that macrophages, both monocyte-derived and bone resident populations, were critical for bone metastatic PC to develop resistance to enzalutamide, a clinically used anti-androgen. Mechanistically, macrophages drove resistance through induction of a wound healing like response in prostate cancer cells, which was strongly supported by bioinformatics analysis of multiple patient mCRPC datasets. Furthermore, macrophage depletion or SRC inhibition using a novel specific inhibitor significantly inhibited resistant growth of mCRPC. Together, our findings elucidated a novel mechanism of macrophage-induced anti-androgen resistance of metastatic PC and a promising therapeutic approach to treat this deadly disease.

PR10 Inhibition of MEK1/2 overcomes resistance to aPD-1 blockade in pancreatic ductal adenocarcinoma through modulation of NETosis in tumor-associated neutrophils.
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Purpose: Pancreatic ductal adenocarcinoma (PDAC), defined by an immunosuppressive desmoplastic tumor microenvironment (TME) orchestrated by oncogenic KRAS signaling, is notoriously resistant to both conventional therapies and immune-checkpoint blockade. We overcame this resistance through inhibition of the downstream KRAS effector MEK1/2 in combination with aPD-1 blockade. **Methods:** Tumor cell lines derived from *LSL-$^{KrasG12D/+}$;LSL-$^{Trp53R172H/+}$;Pdx-1-Cre* (KPC) mice were orthotopically transplanted into C57BL/6J mice for efficacy, survival, and downstream analytical procedures. All mice were computationally randomized into treatment groups, tumor volume was tracked ultrasonically. Mice were treated with aPD-1 (BMS, 5 mg/kg) or IgG isotype control (BMS 5 mg/kg), the MEK1/2 inhibitor (GSK1120212, 0.5 mg/kg) or vehicle alone (DMSO). A CXCR2 blocking antibody (BMS 12.5 mg/kg) was used to assess effects of direct CXCR2 blockade. Immunophenotyping and functional study of tumor-infiltrating leukocytes (TILs) were performed on both orthotopic and hemi-spleen TILs. Pooled bulk-tumor RNA from orthotopic tumors was used for RT-qPCR and RNA-seq analysis. Immunofluorescent staining of neutrophil extracellular traps in murine tumors was performed on formalin-fixed paraffin-embedded (FFPE) orthotopic tumors. Sections were stained for Ly-6G (CD66b in human samples), myeloperoxidase (MPO), histone H2-B (H2B), and DNA. NET(+) areas were identified as extracellular co-localization of DNA/H2B and MPO or Ly-6G(+) cells with > 75% nuclear MPO co-localization with HALO software. **Results:** aPD-1+MEKi therapy routinely eradicated tumors in >80% of mice, with tumor-free survival and protection from tumor re-challenge extending as far as one-year post-treatment. Initial gene expression data showed a reduction in IL-8 homologs in aPD-1+MEKi tumors. Subsequent immunophenotyping data showed increased CXCR2(+) neutrophils in aPD-1+MEKi tumors compared to aPD-1+DMSO. Given the functional role of CXCR2 in IL-8-mediated NETosis, we compared tumors from aPD-1+MEKi sensitive (KPC_S) and resistant (KPC_R) KPC cell lines for NETosis signatures via multiplex IF. Within KPC_S groups, mean NET

scores w/ SEM fell from 2.29 +/- 0.12 to 0.51 +/- 0.10 between aPD-1+DMSO and aPD-1+MEKi groups ($p <= 0.0001$). No difference was found within KPC_R treatment groups (2.10 +/- 0.11 vs 2.07 +/- 0.12, p = n.s.). Furthermore, 23.41 +/- 3.04% of ex vivo stimulated CD8(+) TILs from aPD-1+MEKi KPC_S tumors were IFNy(+) vs 9.09 +/- 2.02% in aPD-1+DMSO KPC_S and 6.55 +/- 1.38% in aPD-1+MEKi KPC_R cells ($p = 0.016$ and 0.011 respectively).
Conclusions: We have demonstrated that resistance to aPD-1 blockade in PDAC can be annulled with combined MEK1/2 inhibition. The elimination of immunosuppressive NETs from sensitive KPC tumors but not resistant tumors suggests that MEKi works by modulating the TME rather than neutrophils directly. Work is ongoing to evaluate aCXCR2 and NETosis inhibition in these resistant tumors. Correlative IHC/IF analysis of our J1568 clinical trial cohort is also near completion.