Invited Speaker Abstracts

IA01 Unconventional ER stress response pro-tumorigenic polarization and survival in TAMs. Ping-Chih Ho[1]. [1]University of Lausanne, Epalinges, Switzerland.

Tumor-associated macrophages (TAMs) display pro-tumorigenic phenotypes for supporting tumor progression in response to microenvironmental cues imposed by tumor and stromal cells. However, the underlying mechanisms by which tumor cells instruct TAM behavior remain elusive. Here we uncover that tumor cell-derived glucosylceramide stimulated unconventional endoplasmic reticulum (ER) stress responses by inducing reshuffling of lipid composition and saturation on the ER membrane in macrophages, which induced IRE1-mediated spliced XBP1 production and STAT3 activation. The cooperation of spliced XBP1 and STAT3 reinforced the pro-tumorigenic phenotype and expression of immunosuppressive genes. Ablation of XBP-1 expression with genetic manipulation or ameliorating ER stress responses by facilitating LPCAT3-mediated incorporation of unsaturated lipids to the phosphatidylcholine hampered pro-tumorigenic phenotype and survival in TAMs. Together, our findings reveal the unexpected roles of tumor cell-produced lipids that simultaneously orchestrate macrophage polarization and survival in tumors via induction of ER stress responses and therapeutic targets for sustaining host anti-tumor immunity.

IA02 Tumor microenvironment metabolism in T cell differentiation and dysfunction. Greg M. Delgoffe[1]. [1]University of Pittsburgh, Pittsburgh.

Cancer thwarts immune destruction using several adaptations to suppress cancer immunity: upregulation of ligands for coinhibitory receptors like PD-1, recruitment of immunosuppressive populations like Treg cells, and withstanding cytolysis to generate a source of chronic antigen stimulation to drive T cell exhaustion. However, behind the veil of these molecular and cellular interactions is a unique metabolic landscape, driven by the energetic deregulation of the cancer cells themselves, that is toxic to infiltrating immune cells. Indeed, we and others have shown T cells infiltrating tumors succumb to severe metabolic insufficiency, characterized by a progressive loss of functional mitochondria. These metabolic states occur concomitant with the progression to terminal exhaustion. We hypothesized metabolic stress not only characterizes T cell exhaustion but plays a dominant role in driving the fate. We found that while T cells could withstand mitochondrial stressors like hypoxia in isolation, if T cells were experiencing persistent stimulation, they became extremely sensitive to oxygen tension. Indeed, continuous stimulation through the TCR under hypoxic conditions rapidly generated exhausted-like T cells in vitro. We used our in vitro system to dissect molecular mechanisms for this fate change, revealing mitochondrial stress produces intolerable levels of reactive oxygen species, which played a major role in driving exhaustion. Antioxidant chemicals and proteins as well as hypoxia mitigation could prevent T cell exhaustion in vivo and synergize with checkpoint blockade immunotherapy. Exhausted T cells are a major population within solid tumors, but what is their true function in cancer? Our metabolic and transcriptomic studies found the most terminally exhausted T cells bear striking similarity to tumor-infiltrating regulatory T cells. We thus asked whether exhausted CD8+ T cells harbored regulatory function using miniaturized suppression assays. Strikingly, when sorted from the same tumor, exhausted T cells carried similar suppressive capacity as CD4+ Treg cells. Unlike Foxp3+ Treg cells, which suppress through a

myriad of mechanisms, exhausted CD8+ T cells suppress solely using the ectonucleotidase CD39, previously shown to define the most terminally exhausted cells. We found hypoxia exposure and HIF1α expression drive CD39 expression, which generates an adenosinergic local environment in which CD73+ immune cells create immunosuppressive adenosine. Deletion of CD39 within the CD8 lineage resulted in slowed tumor growth and heightened sensitivity to immunotherapy, highlighting a significant contribution by exhausted T cells to the tolerogenic environment in cancer. Our data highlight an unappreciated function of this terminally exhausted subset and suggest exhausted T cells are not merely dysfunctional, but in cancer become actively anti-functional, working against the immune response. By targeting adenosine, CD39, or the hypoxic nature of the tumor microenvironment, these deleterious functions may be limited to enable immunotherapy for cancer.

IA05 CAR NK Cells: The future of cancer immunotherapy. Rafet Basar[1]. [1]UT MD Anderson Cancer Center, Houston, TX.

T cells engineered with chimeric antigen receptors (CARs) have revolutionized the field of cell therapy and changed the paradigm of treatment for many patients with relapsed or refractory B-cell malignancies. Despite this progress, there are limitations to CAR-T cell therapy in both the autologous and allogeneic settings, including practical, logistical and toxicity issues. Given these concerns, there is a rapidly growing interest in NK cells as alternative vehicles for CAR engineering, given their unique biological features and their established safety profile in the allogeneic setting. Other immune effector cells, such as iNKT cells, γδ T cells and macrophages, are attracting interest as well and eventually may be added to the repertoire of engineered cell therapies against cancer. The pace of these developments will undoubtedly benefit from multiple innovative technologies, such as the CRISPR-Cas gene editing system, which offers great potential to enhance the natural ability of immune effector cells to eliminate refractory cancers.

IA06 Chimeric antigen receptor macrophages for the treatment of solid tumors. Saar Gill[1]. [1]University of Pennsylvania, Philadelphia, PA.

Engineered adoptive cell therapies redirect and augment the ability of immune effector cells to mount an anti-tumor response by introducing novel capabilities and targeting moieties. A prominent example of this approach is the use of T cells engineered to express chimeric antigen receptors (CARs), which have demonstrated significant efficacy against some hematologic malignancies. However, despite sophisticated strategies to harness immune cell function, CAR T cell efficacy against solid tumors has remained disappointing. Macrophages have recently emerged as prominent candidate effector cells for the treatment of solid tumors. Macrophages are innate immune cells that are intrinsically equipped with broad therapeutic effector functions, including homing to tumor sites, phagocytosis, activation of the tumor microenvironment and antigen presentation. In this presentation we will discuss strategies for genetic manipulation of CAR macrophages, illustrated by preclinical results.

IA07 Impact of immunotherapy on COVID-19 immunity: Insights from checkpoint blockade in cancer. Paulina Coutifaris[1], Kevin Wang[1], Sokratis Apostolidis[1], Mark Painter[1], Ahron Flowers[1], Rishi Goel[1], Divij Mathew[1], Ajinkya Pattekar[1], Ravi Amaravadi[1], Tara Mitchell[1], Paul Bates[1], Scott Hensley[1], Giorgos Karakousis[1], Lynn Schuchter[1], Allie Greenplate[1],

E. John Wherry[1], Alexander C. Huang[1]. [1]University of Pennsylvania, Philadelphia, PA.

Over the last few years, we have gained insights into how immunotherapy, including checkpoint blockade, modulates key CD4 and CD8 T cell subsets in anti-tumor immunity. This information can now give us insights into how immunotherapy can impact immunity in the setting of COVID-19. Indeed, we recently demonstrated that cancer patients with T cell depletion have high COVID-19 mortality despite adequate B cell and antibody production, highlighting the importance of cellular immunity. Conversely, in the setting of B cell depletion by anti-CD20 therapy, CD8 T cells can compensate for impaired humoral immunity. PD-1 blockade increases the activation and proliferation of CD4 T follicular-helper cells, which plays a key role in promoting B cell responses and quality antibody production. Thus, it is possible that PD-1 blockade enhances the efficacy of SARS-CoV-2 vaccination. However, PD-1 blockade in melanoma patients was actually associated with at 2-fold decrease in SARS-CoV-2 specific antibodies and neutralizing antibodies, compared to a healthy donor cohort. PD-1 blockade was also associated with depletion of memory CD4 T cells, suggesting there may be consequences to prolonged PD-1 blockade.

IA08 Adaptive immune dysregulation in cancer patients with SARS-CoV-2 infection.

Santosha A. Vardhana[1]. [1]Memorial Sloan Kettering Cancer Center, New York, NY.

Coronavirus disease 2019 (COVID-19) infection results in high rates of both acute and long-term mortality patients with hematologic malignancies, but the immunologic mechanisms underlying poor outcomes in this population remain poorly understood. In this presentation, we discuss how immune dysregulation, resulting from either underlying hematologic malignancy or immune-directed therapies, affects COVID-19 morbidity and mortality. First, we evaluated both clinical and immunological parameters of hospitalized cancer patients with COVID-19 infection. To our surprise, neither receipt of anti-CD20 therapy nor diminished B-cell counts were predictive of mortality in this population; however total CD8+ T-cell counts and lack of functional T-cell responses to COVID-19-derived antigens were strongly predictive of mortality. These results indicate that T-cell immunity is the dominant predictor of COVID-19-infected hematologic malignancy patients, and that loss of B-cell immunity is not associated with increased mortality so long as T-cell immunity is sufficient. In addition to short-term mortality, persistent and/or recurrent COVID-19 infection has been exclusively described in hematologic malignancy patients, but the primary drivers of persistent infection are not well understood. We identified patients with B-cell lymphomas as having a particularly high risk for persistent SARS-CoV-2 positivity. Subsequent analysis of patients with lymphoid malignancies and COVID-19 identified discrete risk factors for severity of primary infection as compared to disease chronicity. Active therapy and diminished T-cell counts were key drivers of acute mortality in lymphoma patients with COVID-19 infection. Conversely, B-cell depleting therapy was the primary driver of re-hospitalization for COVID-19. In patients with persistent SARS-CoV-2 positivity, we observed high levels of viral entropy consistent with intrahost viral evolution, particularly in patients with impaired CD8+ T-cell immunity. These results suggest that persistent COVID-19 infection is likely to remain a risk in patients with impaired adaptive immunity and that additional therapeutic strategies are needed to enable viral clearance in this high-risk population. Finally, we discuss disease and therapy-specific predictors of humoral responses to COVID-19 vaccination in the hematologic cancer population. As expected, patients

with hematologic cancers had a blunted humoral response to vaccination when compared to healthy donors, and this defect was even more profound when considering the neutralizing capacity of these antibodies, suggesting both a qualitative and quantitative defect to the humoral immune response in patients with hematologic cancers. Second, we identified novel populations of patients with poor humoral responses to vaccination, such as patients receiving anti-CD38 antibodies and BH3 mimetics. Our current ongoing work is exploring the development of functional T-cell memory in these unique patient populations.

IA09 Mapping myeloid programs that control tumor immunity. Miriam Merad[1]. [1]Mount Sinai School of Medicine, New York, NY.

Immune checkpoints blockade established the proof of principle that activating T effector immune function can significantly improve cancer outcome. However, the majority of patients fail to respond to checkpoint blockade therapy, likely because in many patients, suppressive mechanisms cannot be overcome with checkpoint blockade alone. T cells effector programs are instructed and modulated by professional antigen presenting cells (APC) that populate tumor lesions. Yet very little is known about the molecular wiring of APC in the tumor microenvironment. Here we used CITE-Seq to profile 600,000 immune cells in tumor and adjacent tumor-free tissues from 35 patients with lung cancer lesions. We also used CITE-Seq to profile APC that reside in experimental lung cancer lesions. We identified the molecular profile of APC that accumulate in tumors compared to the adjacent tissues and explored the functional relevance of these programs in experimental lung cancers. We show that most APC co-express both immunogenic and suppressive pathways that reduce their functionality in tissues. Specifically, we found that Th2 cytokines produced by tumor cells significantly contribute to limiting APC immunogenicity and to promoting APC -driven immunoregulation of T cell and NK cell effector function in the tumor microenvironment. These data emphasize the benefit of blocking Th2 response in epithelial tumors to enhance APC functionality and promote antitumor immunity.

IA12 Cancer evolution: Chromosomal instability and immune evasion. Charles Swanton[1]. [1]Francis Crick Institute & UCL Cancer Institute, London, United Kingdom.

Evidence supports complex subclonal relationships in solid tumors, manifested as intratumor heterogeneity. Parallel evolution of subclones, with distinct somatic events occurring in the same gene, signal transduction pathway or protein complex, suggests constraints to tumor evolution that might be therapeutically exploitable. Data from TRACERx, a longitudinal lung cancer evolution study will be presented. Drivers of tumor heterogeneity change during the disease course and contribute to the temporally distinct origins of lung cancer driver events. APOBEC driven mutagenesis appears to be enriched in subclones in multiple tumor types. Oncogene, tumor suppressor gene and drug-induced DNA replication stress are found to drive APOBEC mutagenesis. On-going chromosomal instability, manifested as Mirrored Subclonal Allelic Imbalance (MSAI) is found to be a major driver of intratumor heterogeneity across cancer types, contributing to parallel evolution and selection. Subclonal driver events, evidence of ongoing selection within subclones, combined with genome instability driving cell-to-cell variation is likely to limit the efficacy of targeted monotherapies, suggesting a need for new approaches to drug development and integration of cancer immunotherapeutic approaches. Multiple adaptive

mechanisms to neo-antigen evolution have been found in TRACERx highlighting cancer chromosomal instability driving immune evasion and HLA loss and loss of clonal neo-antigens as well as epigenetic repression of neo-antigens. The clonal neo-antigenic architecture may act as a tumor vulnerability to mitigate resistance and treatment failure.

IA13 Predicting clonal evolution in pancreatic cancer survivors. Marta Luksza[1]. [1]Icahn School of Medicine, Mount Sinai, New York, NY.

Immune editing of neoantigens is crucial for the success of immunotherapies, but it is still unknown to what extent the immune system naturally edits evolving tumors and what is the fitness cost associated with the presence of neoantigens. Here we develop a biophysically grounded neoantigen quality model, which quantifies the immunogenicity of tumor neoantigens. We use the model to define the fitness of tumor clones as a combination of negative selection due to immune recognition and positive selection due to oncogenic mutations. We investigate how pancreatic cancers – a lowly mutated, poorly immunogenic cancer, largely presumed to not be subject to immunoediting – evolve over 10 years. Our patient cohort includes a set of long-term survivors, who are characterized by high levels of immune infiltration of their primary tumors. With the fitness model, we show that long-term survivors evolve new clones of markedly lower immune fitness cost, to indicate clones with high-quality neoantigens are negatively selected. Importantly, the fitness model predicts the clonal composition of recurrent tumors of the patients. Thus, we submit longitudinal evidence that the human immune system naturally edits neoantigens. Furthermore, we present a model that describes how tumor cell populations evolve under immune pressure over time, with implications for cancer biology and therapy.

IA15 Targeting tumor associated macrophages for anti-cancer therapy. Jennifer L. Guerriero[1]. [1]Brigham and Women's Hospital, Boston, MA.

Despite objective responses to poly(ADP-ribose) polymerase (PARP) inhibition and improvements in progression-free survival (PFS) compared to standard chemotherapy in patients with *BRCA*-associated triple-negative breast cancer (TNBC), benefits are transitory. Using high-dimensional single-cell profiling of human TNBC, here we demonstrate that macrophages are the predominant infiltrating immune cell type in breast cancer susceptibility (BRCA)-associated TNBC. Through multi-omics profiling, we show that PARP inhibitors enhance both anti- and pro-tumor features of macrophages through glucose and lipid metabolic reprogramming, driven by the sterol regulatory element-binding protein 1 (SREBF1, SREBP1) pathway. Combining PARP inhibitor therapy with colony-stimulating factor 1 receptor (CSF1R)-blocking antibodies significantly enhanced innate and adaptive antitumor immunity and extended survival in mice with BRCA-deficient tumors in vivo, and this was mediated by CD8+ T cells. Collectively, our results uncover macrophage-mediated immune suppression as a liability of PARP inhibitor treatment and demonstrate that combined PARP inhibition and macrophage-targeting therapy induces a durable reprogramming of the tumor microenvironment (TME), thus constituting a promising therapeutic strategy for TNBC. This work highlights the importance of a deep understanding the tumor microenvironment (TME) before and after therapy.

IA16 Immune suppression in cancer and strategies for its reversal. Rosandra N. Kaplan[1].
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The microenvironment is involved in multiple aspect of cancer progression. Tumor metastasis is a critical step in the progression of solid tumors that is associated with patient mortality, and the metastatic microenvironment is key regulator of this process. The pre-metastatic niche is the microenvironment important for metastatic initiation that is established at distant sites in response to primary tumor factors during cancer progression. We characterized this microenvironment which involves changes in both stromal and immune populations in the lungs of sarcoma-bearing mice and in the liver in pancreatic- bearing mice by flow cytometry and RNA sequencing approaches. We identified a gene signature in pre-metastatic niche formation that demonstrates upregulation of immune suppression genes that is consistent across different metastatic tissue including lung and liver as well as across species with commonalities in murine and human early metastatic microenvironments. Performing single cell RNA sequencing of the pre-metastatic niche revealed key immune suppressive genes were found in the myeloid cell clusters. In addition to the increase of myeloid cells and immunosuppressive pathways, we discovered that T cell populations are reduced in pre-metastatic lungs. We hypothesized that reversing this immunosuppressive environment would restore T cell function and antitumor immunity. We designed a novel approach in which we generated Genetically-Engineered Myeloid cells (GEMys) to deliver IL-12, a potent antitumor molecule, into the pre-metastatic microenvironment. We evaluated the lungs by flow cytometry and observed that IL12-GEMy-treated mice had increased numbers of T cells and enhanced expression of activation markers, resulting in reduced metastasis and increased survival. This model was effective in an aggressive experimental metastasis model of pancreatic liver metastasis and was not only able to limit metastatic progression but was able to cure a subset of mice compared to rapid metastatic progression in the liver within a month in the untreated pancreatic cancer mice. When combined with chemotherapy pre-conditioning, IL12-GEMys cured mice of established tumors and generated long-lived T cell memory, as these mice were immune to subsequent tumor challenge. These studies demonstrate that IL12-GEMys can functionally modulate the core program of immune suppression in the pre-metastatic niche to successfully rebalance the dysregulated metastatic microenvironment in cancer.

IA17 The power of ONE: Immunology in the age of single cell genomics. Ido Amit[1].
[1]Weizmann Institute of Science, Rehovot, Israel.

The immune system is a complex, dynamic and plastic network composed of various interacting cell types that are constantly sensing and responding to environmental cues. From very early on, the immunology field has invested great efforts to characterize the various immune cell types and elucidate their functions. However, accumulating evidence indicates that current technologies and classification schemes are limited in their ability to account for the functional heterogeneity of immune processes. Single cell genomics hold the potential to revolutionize the way we characterize complex immune cell assemblies and study their spatial organization, dynamics, clonal distribution, pathways, and crosstalk. This emerging field can greatly affect basic and translational research of the immune system. I will discuss how recent single cell genomic studies are changing our perspective of various immune related pathologies from cancer to autoimmune disease and neurodegeneration. Finally, I will consider recent and forthcoming

technological and analytical advances in single cell genomics and their huge potential impact on the future of immunology research and immunotherapy.

IA18 Cancer metabolism: Emerging insights from single cell analysis. Jason W. Locasale[1]. [1]Duke University, Durham, NC.

This presentation will focus on cancer metabolism and new insights that can be gained from emerging technologies – notably single cell analysis. I will first provide a brief overview of our laboratory's research program around metabolism, emphasizing the research questions we focus on as well as the technologies we've been developing and implementing. I will next discuss work on the development of computational tools using single cell expression data to investigate metabolic networks and pathway activity in single cells to study metabolism in vivo. I will show how this technology can be used to gain insights into metabolism within the tumor microenvironment in human tumors. I will next present an application of this technology in the study a therapeutic our lab has been studying, digoxin – a cardiac glycoside that is under consideration for repurposing in cancer and which we show to target glucose and central carbon metabolism. As a proof of concept, we demonstrate that remodeling of the immune compartment can be achieved upon introduction of this metabolic agent.

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IA20 What does not kill it makes it stronger: Acquired resistance to anti-MAPK targeted therapy confers an immune-evasive tumor microenvironment and cross-resistance to immunotherapy. Anna C. Obenauf[1]. [1]Research Institute of Molecular Pathology, Vienna, Austria.

Targeted therapies and immunotherapies represent main pillars of cancer treatment, yet how they shape tumours during treatment response and resistance and thereby influence subsequent therapeutic responses is poorly understood. Here, we show in melanoma patients and mouse models that when tumours relapse on targeted MAPK pathway inhibitors, they are cross-resistant to immunotherapies, despite their entirely different mode of action. We find that cross-resistance is mediated via a cancer cell-instructed, immune-suppressive tumour microenvironment that lacks functional $CD103^+$ dendritic cells, precluding an effective T cell response. Restoration of CD103+ dendritic cell numbers and functionality can re-sensitize cross-resistant tumours to immunotherapy. Using our lineage tracing method CaTCH, which allows the retrospective isolation of founding clones prior to evolutionary selection, we demonstrate that cross-resistance is acquired during MAPKi treatment. Cross-resistance does not arise from the selective pressure of an immune response during the evolution of resistance, paradoxically it results from the MAPK pathway, which is not only reactivated during the formation of targeted therapy resistance but has gained increased transcriptional output driving immune evasion. Our work

provides mechanistic evidence for cross-resistance between unrelated therapies and a scientific rationale for treating patients with immunotherapy before they acquire resistance to targeted therapy.

IA21 Understanding responses to cancer therapy: The tissue is the issue, the scoop is in the poop, and you are what you eat. Jennifer A. Wargo[1]. [1]The University of Texas MD Anderson Cancer Center, Houston, TX.

We have made major advances in cancer treatment using multi-modality therapy, with immunotherapy now playing a major role across cancer types. However, responses to treatment are not universal, and strategies to improve outcomes (and to ultimately prevent cancer altogether) are needed. Tissue-based analyses in neoadjuvant and other studies have yielded important insights into known and novel therapeutic targets, leading to rational combination strategies to improve outcomes to therapy. Additionally, next-generation sequencing approaches have allowed interrogation of the microbiome in the tumor, and also in the gut and other niches. Microbiota within the body have a profound impact on physiology, with emerging evidence that they may contribute to carcinogenesis and therapy response. Importantly, it is not only the microbes that matter, but also the substrate for energy use (i.e. our diet). All of these aspects will be discussed in this talk, and rational combination strategies to improve cancer treatment will be proposed.

IA22 Immunotherapy for melanoma: Checkpoint blockade combinations. Jedd D. Wolchok[1]. [1]Memorial Sloan Kettering Cancer Center, New York, NY.

Given the activity noted with both CTLA-4 or PD-1 blockade, clinical trials are now investigating combination checkpoint blockade. The most mature data with a combination of ipilimumab + nivolumab in melanoma showed a response rate of 60% in the context of increased, yet manageable toxicity. Such responses are generally durable, even when treatment was stopped early for toxicity. Unlike in studies of PD-1 blockade monotherapy, there was no significant difference in clinical activity based on tumor expression of PD-L1. This approach has gained US regulatory approval for metastatic melanoma and is in late stage clinical trials for other malignancies. Attention is being paid to the reasons underlying the efficacy of checkpoint blockade in certain malignancies. One hypothesis has been that cancers having a high mutational load may be more amenable to immune modulation by virtue of the larger number of potential neo-epitopes present, fostering baseline immune recognition that can then be potentiated by checkpoint blockade. We have found that melanoma patients having long term clinical activity with ipilimumab have a significantly greater median number of non-synonymous passenger mutations, compared with patients who do not respond or those who have only short-term regression. Strategies to enhance baseline immune reactivity are therefore necessary to investigate as means to improve the impact of checkpoint blockade on a broad spectrum of cancers. The presence of suppressive myeloid cells and regulatory T cells in the tumor microenvironment is emerging as a mechanism of resistance to the anti-tumor activity for checkpoint blockade. Strategies to overcome this include agonism of GITR and selective suppression of PI3K-g.