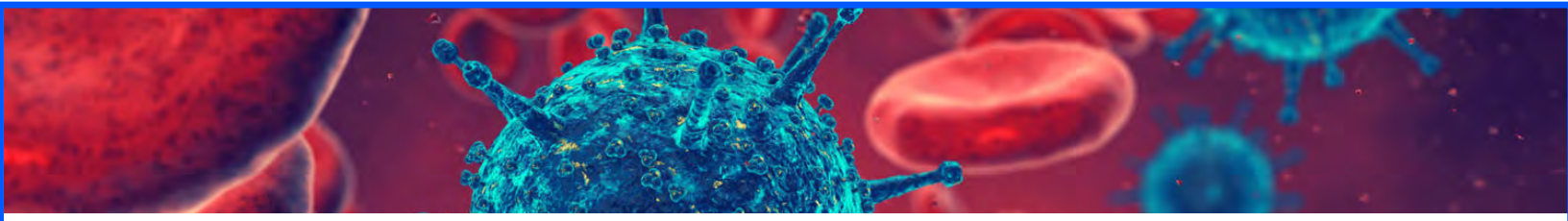

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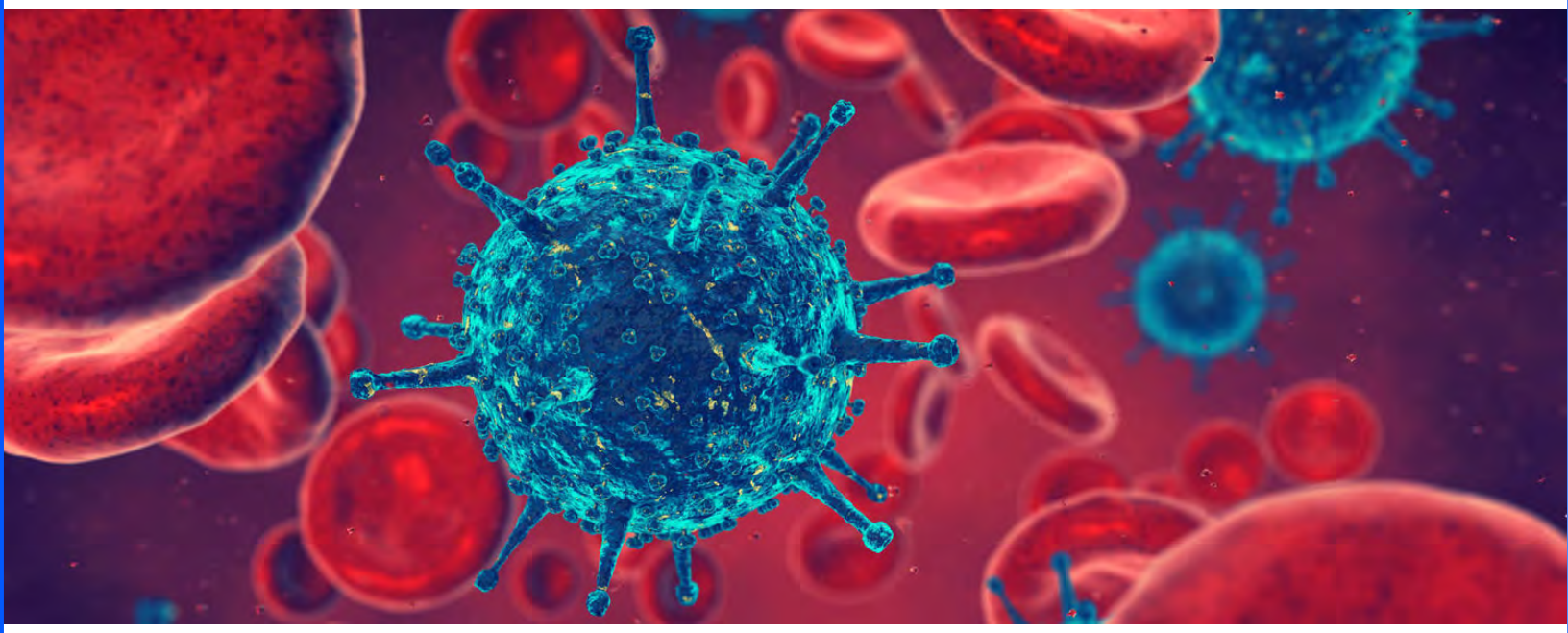
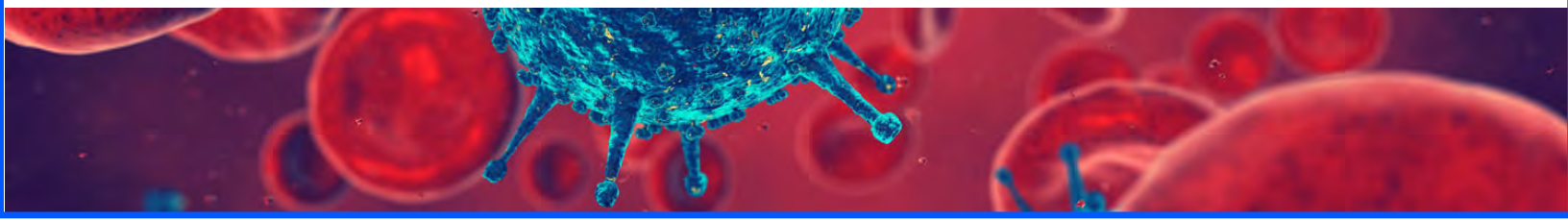


April 28-29, 2021



Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi- institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting-edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Pharmacological Sciences, Precision Environmental Health Sciences and Antimicrobial Resistance. GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include AI in Healthcare, Antimicrobial Resistance, Cellular and Molecular Biophysics, Innovative Drug Discovery and Development, Immunology, Mental Health, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, Translational Imaging and Translational Pain Research. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

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2021 Future of Immunology Research Symposium
April 28-29 2021

Wednesday, April 28, 2021

2:00-2:05 PM **Welcome and Symposium Introduction**
David Corry, Baylor College of Medicine

Keynote Introduction and Moderator:
Cynthia Ju, University of Texas Health Science Center at Houston

2:05-2:50 **Keynote Address**
COVID-19 Immunity and Vaccines: Unknowns and The Role of Mucosal Immunity
Linda Saif, Ohio State

Session 1: Coronavirus, Antiviral Immunity

2:50-3:15 *mRNA-1273 Efficacy in a Severe COVID-19 Model: Attenuated Activation of Pulmonary Immune Cells After Challenge*
Michelle Meyer, University of Texas Medical Branch

3:15-3:40 *Tackling Variability in RNA Viruses*
Catherine Schein, University of Texas Medical Branch

3:40-4:05 *Antiviral Immune Response in Alzheimer's Disease*
Wei Cao, Baylor College of Medicine

Trainee Presentations

4:05-4:20 *Engineering T-cell Therapies for Alloimmune Diseases*
Feiyan Mo, Baylor College of Medicine

4:20-4:35 *Human Norovirus Exhibits Strain-specific Sensitivity to Host Interferon Pathways in Human Intestinal Enteroids*
Shih-Ching Lin, Washington University School of Medicine

4:35-5:05 Poster session

Thursday, April 29, 2021

8:45-8:50 Welcome and Keynote introduction
David Corry, Baylor College of Medicine

8:50-9:35 **Keynote Address**
Inhibitory Mechanisms in the Tumor Microenvironment
Dario Vignali, University of Pittsburgh

**All times listed are Central Daylight Time*

Session 2: Tumor Immunobiology and Resistance
Moderator: **Max Mamonkin,** Baylor College of Medicine

9:35-10:00 *Microbes and NETs in Pancreatic Cancer*
Florencia McAllister, MD Anderson Cancer Center

10:00-10:25 *Regulation of T Cell Metabolism and Function in Cancer Immunity*
Shao-Cong Sun, MD Anderson Cancer Center

10:25-10:50 *Targeting Cytokine Network as an Immunotherapeutic Modality for Kras Mutant Lung Cancer*
Seyed Javad Moghaddam, MD Anderson Cancer Center

Trainee Presentations

10:50-11:05 *Irreversible Mitochondrial Metabolic Reprogramming in Alveolar Macrophages Accelerates Lung Cancer Progression*
Cheng-Yen Chang, Baylor College of Medicine

11:05-11:20 *Anti-CTLA-4 Generates Memory T-cells with Greater Expansion and Cytotoxicity Than Anti-PD-1*
Stephen Mok, MD Anderson Cancer Center

11:20-11:30 Break

Session 3: Pathogen Immunity

Moderator: **Momoko Yoshimoto**, University of Texas Health Science Center Houston

11:30-11:55 *T Helper Plasticity in Balancing Pathology and Protection in Malaria*
Robin Stephens, University of Texas Medical Branch

Trainee Presentations

11:55-12:10 *Candidalysin Drives C. albicans-Induced Allergic Airway Disease Through Platelets*
Yifan Wu, Baylor College of Medicine

12:10-12:25 *T Cell Antigen Identification Using Fas-reporter Cells*
Takahiko Miyama, MD Anderson Cancer Center

12:25-1:00 Lunch/Networking break

1:00-1:10 Keynote Introduction
Moderator: **Stephanie Watowich**, MD Anderson Cancer Center

1:10-1:55 Keynote Address
Systems Biological Analysis of Immunity to Infection and Vaccination
Bali Pulendran, Stanford University

**All times listed are Central Daylight Time*

1:55-2:20 *Gut Microbiota-derived Metabolites in the Regulation of Intestinal Homeostasis and Inflammation*
Yingzi Cong, University of Texas Medical Branch

2:20-2:45 *Infection Drives Dnmt3a-loss of function Clonal Hematopoiesis via IFNg Signaling*
Katherine King, Baylor College of Medicine

2:45-3:10 *Therapeutic Strategies for HPV Associated Cancers*
Jagan Sastry, MD Anderson Cancer Center

3:10-3:20 Break

Trainee Presentations
Moderator: **Nancy Huang**, Texas A&M Institute of Biosciences and Technology

3:20-3:35 *Heme Activates the Inflammatory Caspases to Induce Cytokine Release and Cell Death*
Beatriz Bolivar, Baylor College of Medicine

3:35-3:50 *Continuous Generation of Effector T Cells with Mouse Cytomegalovirus Vaccination to Prolong Malaria Immunity*
Komi Gbédandé, University of Texas Medical Branch

3:50-3:55 *GPR120 Suppresses Intestinal Inflammation Through Regulation of CD4+ T cell IL-10 Production*
Wenjing Yang, University of Texas Medical Branch

4:05 Closing Remarks
David Cory, Baylor College of Medicine
Stephanie Watowich, MD Anderson Cancer Center

Beatriz Bolívar, PhD
Postdoctoral Fellow
Pediatrics

Heme Activates the Inflammatory Caspases to Induce Cytokine Release and Cell Death

Beatriz Bolívar is an NIH NRSA F32-funded postdoctoral fellow in the laboratory of Dr. Lisa Bouchier-Hayes in the Hematology-Oncology section of the Department of Pediatrics, at Baylor College of Medicine. She graduated with a B.Sc. in Chemical Engineering and received her Ph. D. in Chemistry at the University at Albany. She is currently studying the mechanism of activation of the inflammatory caspases and their role in the heme-signaling pathway.

Abstract: Background: Increased levels of extracellular heme from red blood cell destruction underlie the pathophysiology of several disease states, including bacterial sepsis, malaria, and sickle cell disease (SCD). A consequence of this excessive extracellular heme is uncontrolled-inflammation. Heme has been shown to activate caspase-1, an enzyme required to ensure correct regulation of inflammatory signaling. Caspase-1 is recruited to inflammasomes in response to pathogenic and non-pathogenic insults, and stimulates maturation of the pro-inflammatory cytokines, interleukin (IL)-1β and IL-18. The other inflammatory caspases, -4 and -5 (and their murine homolog caspase-11) also promote IL-1β release by inducing an inflammatory form of cell death called pyroptosis.

Hypothesis: We hypothesize that heme activates caspase-4 and caspase-5 to differentially regulate caspase-1 activation, cytokine release and cell death, promoting inflammatory complications in hemolytic conditions such as Sickle Cell Disease (SCD).

Results: We show in primary human macrophages that heme induced IL-1β release, and this was increased in cells derived from patients with SCD. Heme also promoted the oligomerization of the inflammatory caspases, caspase-1, -4 and -5 in macrophages, a step required for their activation. While caspase-1 activation was inflammasome-dependent, silencing of inflammasome proteins did not prevent heme-induced activation of caspase-4 or -5. This indicates that caspase-4 and -5 are activated independent of inflammasomes. Loss of caspase-4 or -5 blocked heme-induced IL-1β release, suggesting a co-operative regulation between these two caspases and caspase-1, rather than a redundant function. Interestingly, cells lacking caspase-4 showed a reduction in heme-induced cell-death.

Conclusion : Altogether, our results provide evidence that both caspase-4 and caspase-5 are essential for heme-induced IL-1β release, while caspase-4 is the primary contributor to heme-induced cell death. In addition, we identified that extracellular heme acts as a damage associated molecular pattern (DAMP) that can promote canonical and non-canonical inflammasome activation as a key mediator of sterile inflammation in macrophages.

Acknowledgements: Funding for the project includes NIH/NIDDK T32DK060445 (BEB), NIH/NIDDK F32DK121479 (BEB), NIH/NIGMS T32GM008231 (ANBS), NIH/NHLBI R01-HL136415 (JMF), NIH/NIGMS R01GM121389 (LBH), and NIHRR024574 (Cytometry and Cell Sorting Core at Baylor College of Medicine)

Wei Cao, PhD
Associate Professor
Huffington Center on Aging, Molecular and Human Genetics

Antiviral Immune Response in Alzheimer's Disease

Wei Cao is an Associate Professor at the Huffington Center on Aging, Molecular and Human Genetics at Baylor College of Medicine in Houston. For many years, Dr. Cao has studied the fundamental mechanisms of innate immune activation and regulation, which govern type I interferon biology. Now Dr. Cao's research focuses on the neuroinflammatory responses imperative in neurodegenerative processes, especially Alzheimer's disease.

Abstract: With an aging population, Alzheimer's disease (AD) becomes an increasing public health challenge. Type I interferon (IFN) is a key cytokine known to curb viral infection and cell malignancy. We recently detected an IFN-stimulated gene (ISG) signature in the brains of multiple murine Alzheimer disease (AD) models, where activated ISG-expressing microglia exclusively surrounded nucleic acid (NA)-positive Aβ plaques and accumulated in an age-dependent manner. Selective IFN receptor blockade effectively diminished the ongoing microgliosis, synapse loss and improved cognitive functions in AD models. Furthermore, we observed activated ISG-expressing microglia enveloping neuritic plaques in post-mortem brains of AD patients and grossly upregulated IFN pathway expression in clinical AD. Interestingly, polymorphisms of several ISGs pose as genetic risk factor for AD and IFN signaling has been linked to brain aging. Therefore, IFN constitutes a pivotal element within the neuroinflammatory network of AD and critically contributes to neuropathogenic processes.

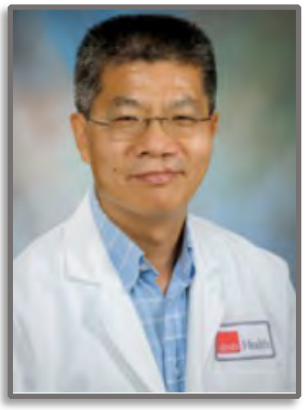
Cheng-Yen Chang
Predoctoral Student
Translational Biology and Molecular Medicine Graduate Program

Irreversible Mitochondrial Metabolic Reprogramming in Alveolar Macrophages Accelerates Lung Cancer Progression

Cheng-Yen had her bachelor's degree at National Tsing Hua University in Taiwan. She further trained herself and had her master's degree in microbiology at National Taiwan University. She came to the US for graduate training in The Translational Biology and Molecular Medicine Program at Baylor College of Medicine. In 2017, she joined Dr. Farrah Kheradmand's lab. During her time at Baylor, she attained meetings and obtained awards, including the NIOSH T41 training grant and CPRIT fellowships.

Abstract: Small airborne pollutants from incomplete combustion of tobacco and other organic matter drive inflammation and have been associated with chronic lung diseases. We have discovered that nano-sized (<50 nm) particulate matter (e.g., elemental nano-carbon black; nCB) is formed in cigarette smoke and accumulates in lung macrophages. Mice exposed to nCB develop pulmonary emphysema, supporting the clinical significance of nCB in causing lung inflammation. However, little is known about the role of nCB in lung cancer initiation and progression. Using two murine models of non-small cell lung cancer (NSCLC), we found nCB accelerates NSCLC progression. nCB-exposed lungs had increased lactate, an immunosuppressive metabolite, resulting in PD-1+ T lymphocytes, PD-L1+ myeloid cells, and FOXP3+ Tregs recruitment and tumor growth. The source of increased lactate is from alveolar macrophages, which condense nCB particles in their cytoplasm and mitochondria, impairing mitochondria energy homeostasis and promoting glycolysis. Due to the hydrophobic nature of nCB, this metabolic reprogramming is irreversible, permanently activating the mTOCR1-HIF1 pathway to drive glycolysis. Furthermore, nCB-induced expression of PD-L1, IL-10, Il4i1, and Arg1 expression in lung macrophages leads to impaired anti-tumor immunity. Our findings demonstrate that nCB metabolically reprograms lung macrophages and fosters immunosuppression to promote lung cancer.

Reference: You, R., et al. Nanoparticulate carbon black in cigarette smoke induces DNA cleavage and Th17-mediated emphysema. Elife (2015).



Yingzi Cong, PhD, PhD
Professor
Microbiology, Immunology, and Pathology

Trends in Microbiota Regulation of Immunity: What our Another Half is Doing?

The host and microbiota have evolved mechanisms for coexistence over millions of years. Accumulating evidence indicates that a dynamic mutualism between the host and the commensal microbiota has important implications for health, and microbial colonization contributes to the maintenance of intestinal immune homeostasis. However, alterations in communication between the mucosal immune system and gut microbial communities have been implicated as the core defect that leads to development of chronic intestinal inflammation and cancer as well as other diseases, such as diabetes, obesity etc. Dr. Yingzi Cong's basic research programs focus on investigating host immune system, microbiome interaction in the intestines, pathogenesis of inflammatory bowel disease, and development of mucosal vaccines, which are based on the analysis of unique murine models of inflammatory bowel disease using a battery of reagents that have been developed recently. A number of research projects are underway in his laboratory and these NIH funded studies involve a number of significant collaborations both at UTMB as well as with other Universities and Research Institutes. Specifically, individual projects include:

1. The role of T cells reactive to commensal bacterial antigens in mucosal immunity and pathogenesis of IBD.
2. Gut microbiome and its metabolites regulation of host immune responses and experimental colitis.
3. microRNA regulation of host response to commensal bacteria and pathogenesis of IBD.
4. Regulation of intestinal IgA response to microbiota and pathogens
5. Development of mucosal vaccines

Abstract: The intestinal mucosa establishes state of hypo-responsiveness against commensal bacteria and of active readiness against pathogens. Despite enormous challenges by the microbiota, the intestine lives in harmony with it, in part due to interactions of the microbiota with the host to maintain intestinal homeostasis. Multiple

host mechanisms have evolved to regulate this relationship, including both innate and adaptive immunity. Host regulates the microbiota, and gut bacteria, in turn, adapt to host by altering their gene expression patterns and immune responses. Different gut bacteria have different roles in regulation of host immune responses. Some bacteria preferably promote T effector cells while the others preferably promote Treg cells in the intestines. It has also been emerging that gut bacterial metabolites profoundly affect intestinal mucosal immune responses. I will present data from our current research projects on gut microbiota regulation of host immune responses at mucosal surface.

Komi Gbedande, PhD
Postdoctoral Researcher
Internal Medicine

Continuous Generation of Effector T cells with Mouse Cytomegalovirus Vaccination to Prolong Malaria Immunity

Dr. Gbedande is an immunologist with several years' research focusing on malaria. His academic and professional accomplishments begin with a BSc degree in Biochemistry and M.Sc degree in cell biology and immunology. Then he earned his PhD in Immunology from Paris Descartes University in France and conducted intensive research on pregnant women and neonatal immunology, with a particular focus on malaria in pregnancy and clinical development of a vaccine to prevent malaria during pregnancy. Presently, he is a postdoc researcher at the University of Texas Medical Branch, where he is conducting research on malaria immunology focusing on vaccinology aspects investigating immune mechanisms associated with protection. His current research project aims to investigate mechanisms of development of effective and long-lived T cell responses to malaria using *P. chabaudi* infection.

Abstract: Immunity to *Plasmodium* infection or vaccination is known to decay. In mouse models, this decay correlates with loss of malaria-specific T cells, not antibody. Whole parasite vaccines, such as "infection and drug cure", protect. However, sterile protection from *P. chabaudi* lasts less than 200 days. Effector (Teff) and effector memory (Tem) T cells can contribute to prolonged protection. We recently identified effector T cells making IFN-*γ* s the immune component likely responsible for the protection provided by persistent *P. chabaudi* infection. Therefore, we have reverse-engineered a vaccine strategy that first induces a long-lived protective antibody response with live parasite vaccination, and then boosts the IFN-γ $CD4^+$ Teff and Tem response to prolong protection. We chose CMV as it is an excellent T cell-inducing vaccine vector with early promise against SIV, tuberculosis and liver-stage malaria. Therefore, we used chronic vaccination with MCMV to test our hypothesis that promoting generation of Teff for longer would be protective if maintained over longer periods of time. We observed that mice with chronic MCMV infection have strongly reduced parasite growth upon *P. chabaudi* infection. Persistent MCMV infection also induces non-specific protection to *Leishmania* and gamma-herpesvirus, suggesting a useful adjuvant effect. To promote continuous induction of *P. chabaudi*-specific Teff/Tem, we expressed the *P. chabaudi* MSP-1 epitope B5 as an MCMV immediate

early gene (MCMV-B5). Upon infection of mice with MCMV-B5, adoptively transferred B5 TCR Tg T cells and MCMV-specific $CD4^+$ T cells proliferated and maintained over 2.5 months. Importantly, we found that MCMV promotes the highly-differentiated Teff and Tem phenotypes that we have previously shown to be protective. We found that a prime-boost strategy using the MCMV vector worked to prolong protection generated by the “infection and drug cure vaccine”. In investigating the mechanisms of protection, we found that IFN-γ induced by MCMV prolongs the protection, potentially through promotion of IL-12 producing. Our findings suggest that chronic vaccination can play an important role in malaria immunity to redress the problem of short-lived protection to malaria infection and vaccines, which is known to be due to decay of CD4 T cell memory.

Katherine Y. King, MD, PhD
Associate Professor
Pediatric Infectious Diseases

Infection Drives Dnmt3a-loss of function Clonal Hematopoiesis via IFNg Signaling

Katherine Y. King MD PhD is Associate Professor of Pediatric Infectious Diseases at Baylor College of Medicine, where she is part of the faculty for the Stem Cells and Regenerative Medicine Center and serves as a co-director of the BCM MSTP. A native Houstonian, Dr. King received her BA in Biochemical Sciences from Harvard University and her MD and PhD degrees from Washington University in St. Louis. Her research focuses on the molecular mechanisms by which inflammation affects blood and immune cell production by hematopoietic stem cells in the bone marrow. Dr. King has been the recipient of a NIH K08 mentored physician scientist training award, the March of Dimes Basil O'Connor Starter Scholar Award, and in 2019 she received the Presidential Early Career Award for Scientists and Engineers (PECASE). When not seeing patients at Texas Children's Hospital or conducting research in the lab, Dr. King enjoys running, yoga, spending time with her husband and 7-year-old daughter, and volunteering for health care advocacy.

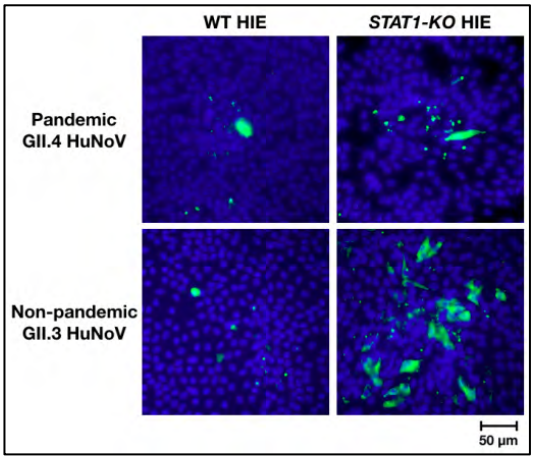
Abstract: Age-related clonal hematopoiesis (CH) is a risk factor for malignancy, cardiovascular disease and all-cause mortality. Somatic mutations in *DNMT3A* are drivers of CH, but decades may elapse between acquisition of a mutation and CH, suggesting that environmental factors contribute to clonal expansion. We used a murine model to investigate the prediction that infection provides selective pressure favoring expansion of Dnmt3a-mutant hematopoietic stem cells (HSCs). We created Dnmt3a mosaic mice by transplanting a mixed population of Dnmt3a-/- and WT HSCs into WT mice and observed substantial expansion of Dnmt3a-/- HSCs during chronic mycobacterial infection. Transcriptional profiling and functional studies indicate reduced differentiation and reduced secondary stress-induced apoptosis account for Dnmt3a-/- clonal expansion during infection. Both injection of recombinant IFNγ alone and infecting mice transplanted with HSCs lacking the differentiation factor Batf2 partially phenocopied CH by Dnmt3a-mutant HSCs upon infection. This is the first study demonstrating that IFNγ signaling induced during chronic infection can drive DNMT3A-loss of function CH.

Shih-Ching Lin, PhD
Postdoctoral Researcher

Human Norovirus Exhibits Strain-specific Sensitivity to Host Interferon Pathways in Human Intestinal Enteroids

Dr. Lin obtained his Ph.D. degree in the Program in Integrative Molecular and Biomedical Sciences (IMBS) at BCM with my thesis work in Dr. Mary K. Estes's lab. His primary research interest was to elucidate the molecular mechanisms that regulate productive infection of human norovirus (HuNoV) in the human intestinal enteroid (HIE) model, and to improve HuNoV cultivation efficiency. He investigated the following two projects: 1) Elucidation of the interferon responses in HIEs upon HuNoV infection, and 2) Identification of the host receptor(s) that make HIEs susceptible to HuNoV infection. His next step is to pursue advanced research training in the field of viral-host interaction and viral immunology as a postdoctoral researcher in the United States.

Abstract: Background: Human norovirus (HuNoV) infection of human intestinal epithelium is the predominant cause of viral gastroenteritis worldwide. Our laboratory reported successful cultivation of HuNoVs in stem cell-derived human intestinal enteroids (HIEs). Innate immune responses serve as the first immune barrier against viral infection, and previous studies with other enteric viruses have shown that the intestinal epithelium preferentially responds to infection by triggering a type III interferon (IFN) response rather than a type I IFN response.



Goals: To identify the role of IFN responses in HuNoV replication in HIEs.

Methods: Non-transformed, differentiated, multicellular HIE cultures were infected with two prevalent genogroup II (GII) HuNoV strains, genotype 4 (GII.4, a pandemic strain) and genotype 3 (GII.3, a non-pandemic strain). The kinetics of IFN responses to HuNoVs were evaluated by RNA-Seq, Luminex assays and RT-qPCR. Antiviral activity of either type I or III IFN was determined by exogenous administration of IFN before infection, by attenuating IFN activity with receptor antagonists and by knocking out the IFN receptor genes in HIEs via CRISPR/Cas9.

Results: We identified a predominant type III interferon (IFN)-mediated innate response to HuNoV infection. Replication of both strains is sensitive to exogenous addition of IFNs, suggesting the potential of IFNs as therapeutics. To obtain insight into IFN pathway genes that play a role in the antiviral response to HuNoVs, we developed knockout HIE lines for IFN alpha and lambda receptors and the signaling molecules, MAVS, STAT1 and STAT2. An unexpected differential response of enhanced replication and virus spread was observed for the GII.3, but not the globally dominant GII.4, HuNoV strain in STAT1-knockout HIEs compared to parental HIEs. GII.3 grew to higher titers and spread in the knockout cultures (Figure).

Conclusions: These results indicate cellular IFN responses restrict GII.3 but not GII.4 replication. The strain-specific sensitivities of innate responses against HuNoV replication provide one explanation for why GII.4 infections are more widespread and highlight strain-specificity as an important factor in HuNoV biology. Genetically modified HIEs for innate immune genes are useful new tools for studying immune responses to viral or microbial pathogens.

Florencia McAllister, MD
Associate Professor
Clinical Cancer Prevention

Microbes and NETs in Pancreatic Cancer

Dr. McAllister is a physician-scientist with basic science training in Host Defense and Tumor Immunology which she acquired during her postdoctoral training at the University of Pittsburgh and Johns Hopkins University where she trained in the laboratory of Dr. Steven Leach with co-mentorship from Dr. Drew Pardoll, leaders in Pancreatic Cancer and Tumor Immunology, respectively. She has completed 2 clinical fellowships at Johns Hopkins University: in Medical Oncology with focus on Gastrointestinal Medical Oncology and Clinical Pharmacology. She has been recruited to MD Anderson in 2014 and in the past 6 years she has established a translational research program focused on further understanding the fundamental microenvironmental mechanisms that influence pancreatic tumor initiation and progression with the ultimate goal of discovering novel immunopreventive and immunotherapeutic approaches for this disease. She has participated in key discoveries on T cell immunobiology, including unraveling the epithelial IL-17 signaling pathway, the characterization of IL-17-secreting immune cells in the initiation and progression of premalignant pancreatic and colorectal lesions and the key role of inflammation and bacteria -induced T cells responses in the initiation and promotion of pancreatic and colon cancer. Recently, they have published in Cell a study that implicates the gut-tumor microbial axis in modulating the tumor microenvironment, using the powerful approach of human-into-mice fecal microbial transplantation. Furthermore, she has developed a clinical platform, including a gastrointestinal cancer microbiome repository and pancreatic cancer high risk cohort, which will be very relevant to further validate the microbiome preclinical work in a clinically relevant population. Her ultimate goal is to better understand the immune and microbial microenvironment surrounding pancreatic cancer initiation, progression and responsible for therapies responses.

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is resistant to most therapies in part due to an immunosuppressive tumor microenvironment. Using several immunological analytical tools as well as genetic and pharmacological approaches, we assessed for immunosuppressive mechanisms.

We determined that IL-17 mediated neutrophils chemotaxis and induction of neutrophil extracellular traps (NETs), a host defense mechanism, can result in tumor progression and resistance to immunotherapeutics in the context of cancer.

Michelle Meyer, PhD
Research Scientist II
Pathology

mRNA-1273 Efficacy in a Severe COVID-19 Model: Attenuated Activation of Pulmonary Immune Cells After Challenge

Dr. Meyer obtained her PhD in Virology and Microbiology at the University of Queensland, Australia. While completing her degree, she joined Panbio as a R&D Scientist to successfully develop a Dengue virus early rapid diagnostic test. Overseeing its development through to market release sparked her interest in conducting translational research. Dr. Meyer went on to do her post-doctoral research at the GNL BSL4 high containment facility at the University of Texas Medical Branch, Galveston, in the laboratory of Alexander Bukreyev, PhD. Dr. Meyer studied the immune responses and protective efficacy of an aerosolized virus-vectored respiratory vaccine against Ebola virus. She is currently a Research Scientist II focusing on novel vaccine development for emerging pathogens and elucidating the correlates of vaccine-mediated protection. Overall, her expertise encompasses a broad spectrum of RNA viruses.

Abstract: The mRNA-1273 vaccine was recently shown to be highly efficacious against symptomatic COVID-19 disease in a large Phase 3 study and was authorized for emergency use by the Food and Drug Administration. Human studies, however, cannot provide the controlled response to infection and complex immunological insight that are possible with preclinical studies. The golden Syrian hamster is the only model that reliably exhibits severe COVID-19 disease similar to hospitalized patients, making it pertinent for vaccine evaluation. We demonstrate that prime-boost dose regimens of mRNA-1273 in hamsters provides more robust neutralizing antibody responses and effective SARS-CoV-2 clearance in the airways compared to a single dose. Among all regimens tested, the highest prime-boost dose conferred better protection against clinical disease and lung injury. Unlike results from previous preclinical studies on mRNA-1273, the infection-permissive immunity coincided with an anamnestic response. Single-cell RNA sequencing of lung tissue isolated from vaccinated hamsters during acute infection permitted high resolution analysis at the transcriptional level which is not possible in vaccinated humans and has not been performed in other challenge models. mRNA-1273-established immunity prevented the influx of inflammatory cells and the reduction of lymphocyte proportions. The transcriptional

programs of immune cells from vaccinated hamsters appeared to promote pulmonary homeostasis following infection while supporting virus clearance. Surprisingly, transcriptional programs activated in some myeloid and lymphoid cells after infection were shared in vaccinated and mock-vaccinated hamsters. These findings indicate core effector immunological responses are stimulated by transient viral replication in the lungs of vaccinees. Our results highlight the importance of a two-dose mRNA-1273 schedule to protect against severe disease and provides insight into the potential responses within the lungs of vaccinated humans who are exposed to SARS-CoV-2.

Takahiko Miyama, PhD
Postdoctoral Fellow
Genomic Medicine

Fas-Reporter Cells As A Tool For TCR Antigen Identification

Takahiko Miyama is a postdoctoral fellow in the Department of Genomic Medicine at MD Anderson Cancer Center. He earned his M.D. from Hiroshima University, Japan in 2006 and his Ph.D. in 2017. After many years of working as a hematologist specializing in hematopoietic cell transplantation, he has made researches that aim to elucidate the mechanism by which specific T-cell clonotypes are selected for effective immune responses against virus, tumor, and alloantigens. Recently, he reported that highly shared TCR chronotypes against cytomegalovirus prevailed in the T cell repertoire. Takahiko is currently working on the development of a TCR antigen screening platform.

Abstract: [Background] Recent advances in high-throughput sequencing technology have led to understanding of the landscape of T cell receptor (TCR) repertoire against life-threatening viral infections and malignant diseases. However, the identification of antigens for orphan TCRs remains a challenge. The purpose of this study is to establish a cell-based reporter system for screening TCR antigens. We developed reporter cells by engineering antigen-presenting cells (APCs) to express Fas-tumor necrosis factor receptor 2 (TNFR2) chimeric receptor that can be specifically activated by antigen-specific T cells. [Method] We constructed a chimeric receptor composed of the extracellular domain of Fas receptor and the cytoplasmic domain of TNFR2. 293T cells which are HLA-A2 positive were used as APCs and lentivirally transduced with the chimeric receptor gene followed by NF-kB-GFP reporter gene. To test antigen-specific activity, HLA-A2 restricted epitopes derived from human papillomavirus 16 (HPV16) and cytomegalovirus pp65 (CMV pp65) were chosen as model antigens and expressed on APCs by peptide or gene transfer. Cell-based reporter assays were performed by co-culturing reporter APCs with CD8 positive Jurkat cells expressing cognate TCR. Reporter activity was assessed by measuring GFP expression levels using flow cytometry. [Result] Treatment of reporter cells with anti-Fas monoclonal antibody increased GFP expression levels, verifying that NF-kB activation was induced by the extracellular Fas signal via the chimeric receptor. Both HPV16 and CMV pp65 antigen-specific Jurkat cells specifically activated cognate antigen-presenting reporter

cells with particularly high GFP expression in the antigen-mediated aggregate fraction. Antigen-specific activation was also achieved in different antigen-expressing cell mixtures, indicating cognate reporter cells were distinguishable from non-cognate cells by antigen-reactive T cells. [Conclusion] Our results indicate Fas-TNFR2 chimeric receptor can medicate activation of reporter cells by antigen-specific T cells. This technology may be applied to screening TCR antigens using peptide libraries as well as cDNA libraries from target cells.

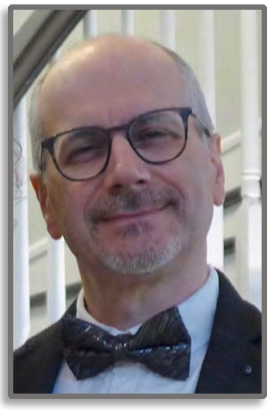
Feiyan Mo, PhD
Graduate Student
TBMM program

Engineering T-cell Therapies for Alloimmune Diseases

After obtaining her bachelor's degree in Biological Sciences at Shanghai Jiao Tong University (China), Feiyan Mo enrolled in the Translational Biology and Molecular Medicine (TBMM) graduate program at Baylor College of Medicine. She later joined Dr. Maksim Mamonkin's laboratory at Center for Cell and Gene Therapy to conduct her thesis research, co-mentored by Drs. Malcolm Brenner and Helen Heslop. Her research interest is to develop effective and affordable cancer immunotherapies using genetically engineered T cells, with the ultimate goal of translation to the bedside.

Abstract: Adoptive chimeric antigen receptor (CAR) T cell therapies produce remarkable clinical benefit in patients with certain tumors, yet their application to non-malignant diseases is less explored. We hypothesized targeted elimination of activated pathogenic T cells by engineered T cells would prevent or reverse the onset of alloimmune diseases, such as immune rejection. We then we developed a T-cell alloimmune defense receptor (ADR) that recognizes 4-1BB, a costimulatory molecule transiently expressed on activated T- and NK-cells, enabling targeted elimination of these pathogenic lymphocytes. Our proof-of-concept study has demonstrated that expression of a 4-1BB-specific ADR protects allogeneic CAR T cells from host rejection while preserving their anti-tumor activity in preclinical models of human cancer.

Robust and specific activity of ADR-armed T cells against pathogenic T cells enabled extending this platform to other alloimmune conditions. One of them is acute graft-versus-host disease (GvHD) – a devastating complication of hematopoietic stem cell transplantation (HSCT) produced by systemic activation of host-reactive donor T cells. Using a mouse model of xenogeneic GvHD, we demonstrated that T cells expressing an OX40-specific ADR prevented GvHD by eliminating pathogenic T cells that mediate tissue damage. T cells co-expressing both an ADR and a CAR protected from GvHD and cleared systemic leukemia in vivo. This strategy can be used clinically to prevent both GvHD and tumor relapse, which are the two major causes of morbidity and mortality following allogeneic HSCT.



Seyed Javad Moghaddam, MD
Associate Professor
Pulmonary Medicine

Targeting Cytokine Network as an Immunotherapeutic Modality for Kras Mutant Lung Cancer

Dr. Seyed Javad Moghaddam is a tenured Associate Professor at the Department of Pulmonary Medicine, UT MD Anderson Cancer Center (UT-MDACC), Houston, Texas. He received his medical degree from Shaheed Beheshti University of Medical Sciences, Tehran, Iran. He joined Baylor College of Medicine in 2004 for an NIH T32 fellowship program in lung diseases. Later in 2007, he accepted an Instructor position in the Department of Pulmonary Medicine, UT-MDACC where he has been a faculty since then. He is also a faculty member and lecturer at UT health graduate school of biomedical sciences, as well as other training programs such as the CPRIT-CURE Training Program, and CPRIT Postdoctoral Fellowship in Cancer Prevention Program.

Dr. Moghaddam has received numerous awards including Lung Cancer Discovery Award (American Lung Association), Research Scholar Award (American Cancer Society), and Cyrus Scholar Award in Basic/Translational Research (Cyrus Family Foundation). He is the 2017 recipient of the American Thoracic Society Early Career Achievement Award in Thoracic Oncology where he currently serves as an executive committee member on its Thoracic Oncology Assembly.

Dr. Moghaddam‘s research focuses on airway inflammation and its role in lung tumorigenesis. In his laboratory, they specifically study the cell type and sex-specific roles of oncogene-driven (intrinsic) and COPD-related (extrinsic) lung inflammation with a particular emphasis on cytokine signaling in the pathogenesis and promotion of lung cancer using genetic and pharmacologic approaches for the development of preventive and therapeutic modalities. He has actively published, been well-funded, and trained several postdoctoral fellows, medical students, as well as college, and graduate students in this field.

Abstract: Worldwide, lung adenocarcinoma (LUAD) is the leading cause of cancer mortality because of a high incidence, and a low cure rate. Unfortunately, patients harboring activating mutations of Kras, the most common type of oncogenic alteration in these patients which are heavily caused by tobacco exposure, are resistant to most forms of systemic or targeted therapies and are associated with poor prognosis.

Therefore, there is an urgent and unmet need for novel and alternative approaches to prevent and treat Kras-mutant LUAD. Recent data provide evidence that the inflammatory tumor microenvironment (TME) is one of the main players in lung tumorigenesis, not just a supporting tumor compartment. Our group and others have shown that numerous cytokines released during inflammation can reprogram the lung TME and promote carcinogenesis, introducing inflammation as a vulnerability factor for K-ras mutant LUAD. Accordingly, a better understanding of the lung TME cellular context and the complex bidirectional interplay between the TME and cytokine milieu in the pathogenesis of this deadly disease is needed. Specifically, the role of various immune cells, as well as cytokines and their downstream molecular pathways that can contribute to lung tumor initiation, progression, and metastasis should be explored. This will be fundamental in tailoring rationally directed preventive strategies for high-risk former and current smokers of whom there are more than 90 million in the United States. This could also help to improve the efficacy of currently available therapeutic modalities such as chemotherapy, immune checkpoint blockade, and targeted therapies (e.g. MEK inhibitors). These rationalized strategies that are based on reformatting the lung TME through targeting cytokine networks will be covered by this presentation.

Stephen Mok, PhD
Postdoctoral Fellow

Anti-CTLA-4 Generates Memory T-cells with Greater Expansion And Cytotoxicity Than Anti-PD-1

Prior to joining UT MD Anderson, Dr. Mok completed his graduate training in Dr. Antoni Ribas' laboratory at University of California, Los Angeles, where he developed murine melanoma model that simulated clinical conditions of patients with melanoma and optimized efficacy of immunotherapy treatments. He joined Dr. James Allison's laboratory in 2015 and has focused his work on memory T-cell formation mediated by immune checkpoint blockade therapy including anti-CTLA-4 and anti-PD-1. He has examined the effects of immunotherapy in murine models on the differentiation, expansion, and cytotoxic function of memory T-cells. His work provides insights on the long-term effects of these treatments on the immune system.

Abstract: Blocking either cytotoxic T-lymphocyte antigen-4 (CTLA-4) or programmed cell death-1 (PD-1) pathway relieves the negative regulation of T-cells resulting in durable tumor rejection in patients with cancer and improved survival rate. However, it remains unclear how these immunotherapies affect immunological memory response. Here we address whether anti-CTLA-4 and anti-PD-1 have different effects on memory T-cells. We used anti-CTLA-4 or anti-PD-1 therapy in combination with irradiated cancer vaccine in mice. After re-challenge, we observed that in murine tumor models, anti-CTLA-4 mediates a more robust memory antitumor response than anti-PD-1 as demonstrated by smaller tumor volumes at all time points. By tracing antigen-specific CD8 T-cells throughout priming, memory phase, and re-challenge, we demonstrated that during re-challenge, the memory T-cells generated by anti-CTLA-4 1) expand in greater frequency, 2) have greater cytotoxic function, 3) are more memory-like, and 4) more frequently differentiate into the population of KLRG1+ effector CD8 T-cells than those generated with anti-PD-1. We found each of these traits correlated with a more effective memory response.

Bali Pulendran, PhD

Violetta L. Horton Professor, Institute for Immunity, Transplantation and Infection, Department of Pathology, Department of Microbiology and Immunology, Fellow at ChEM-H (Chemistry, Engineering and Medicine for Human Health),

Systems Biological Analysis of Immunity to Infection and Vaccination

Bali Pulendran is the Violetta L. Horton Professor at the Stanford University School of Medicine, and a member of the Institute for Immunology, Transplantation and Infection, and the Departments of Pathology and Microbiology & Immunology at Stanford University. He is also an adjunct professor at Emory University and the Yerkes National Primate Center, and director of the NIH Center for Systems Vaccinology, at Emory University in Atlanta. He received his undergraduate degree in the Natural Sciences Tripos from Queens' College, Cambridge University, and his Ph.D., from the Walter & Eliza Hall Institute in Melbourne, Australia, under the supervision of Sir Gustav Nossal. He then did his post-doctoral work at Immunex Corporation in Seattle. Dr. Pulendran's research is focused on understanding the mechanisms by which the innate immune system regulates adaptive immunity and harnessing such mechanisms in the design of novel vaccines. More recently, his laboratory pioneered the use of systems biological approaches to predicting the efficacy of vaccines and deciphering new molecular correlates of protection against infectious diseases. Dr. Pulendran's research is published in front line journals such as Nature, Science, Cell, Nature Medicine, and Nature Immunology. Furthermore, Dr. Pulendran is the recipient of numerous grants from the National Institutes of Health, and from The Bill and Melinda Gates Foundation, serves on many editorial boards, and is the recipient of two concurrent MERIT awards from the National Institutes of Health. Dr. Pulendran serves on many advisory boards including that of Keystone Symposia and on the External Immunology Network of GSK. He is listed on Thomson Reuter's list of Highly Cited Researchers, which recognizes the world's most influential researchers of the past decade, demonstrated by the production of multiple highly-cited papers that rank in the top 1% by citations.

Abstract: Although the development of effective vaccines has saved countless lives from infectious diseases, the basic workings of the human immune system are complex and have required the development of animal models, such as inbred mice, to define mechanisms of immunity. More recently, systems biological approaches have been developed to directly explore the human immune system with unprecedented

precision. I will discuss how these approaches are advancing our mechanistic understanding of the human system and its response to infections and vaccines and facilitating the development of vaccines against infections diseases.

Linda J. Saif, PhD
Distinguished University Professor Food Animal Health Research Program (CFAES, OARDC) and the Veterinary Preventive Medicine Department

COVID-19 Immunity and Vaccines: Unknowns and The Role of Mucosal Immunity

Dr. Linda J. Saif is a Distinguished University Professor at The Ohio State University (OSU) in the Food Animal Health Research Program (CFAES, OARDC) and the Veterinary Preventive Medicine Department (CVM, OSU). She is a virologist and immunologist, whose research focuses on comparative aspects of enteric and respiratory viral infections (coronaviruses, rotaviruses and caliciviruses) of food animals and humans. Her lab studies mucosal immunity and vaccine development and is currently focusing on the impact of malnutrition and micronutrient deficiencies (vitamin A) on vaccines and interactions of probiotics and the gut microbiota with the neonatal immune system, vaccines and viral pathogenesis. Dr. Saif's coronavirus research spans 4 decades and includes her MS and PhD research on swine coronaviruses, immunity and vaccines. Dr. Saif is known nationally and internationally for her work on enteric and respiratory viruses (rotaviruses, caliciviruses and coronaviruses) that affect food producing animals, wildlife, and humans [Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS)] and their zoonotic potential and mechanisms of interspecies transmission. Dr. Saif was an advisor to the WHO and CDC during the 2003 SARS outbreak and her laboratory is a WHO International Reference Lab for Animal coronaviruses in the SARS network. She was an advisor to the Ministry of Agriculture in Saudi Arabia on MERS in camels and control strategies. She is a member of the US National Academy of Sciences since 2003. During the COVID-19 pandemic, she is providing One Health expertise about the SARS-CoV-2, including diagnostics, interspecies transmission, vaccines and control strategies. Dr. Saif holds 5 US/foreign patents and has authored or coauthored over 400 referred journal publications and 78 book chapters pertaining to her research.

Abstract: SARS-CoV-2 infects mucosal sites including the oropharynx, upper and lower respiratory tract and in some cases the gastrointestinal tract. Understanding the pathogenesis of COVID-19, including mucosal infection and shedding profiles, and the induction and longevity of mucosal immunity at the infection sites as related to disease severity, age, etc will aid in control measures and vaccine design.

Accordingly the objectives are:

•. Understand basic characteristics of CoVs

• Appreciate the diversity and ecology of CoVs, host reservoirs and interspecies transmission to humans and animals

•. Clarify unique aspects of mucosal immunity focusing on secretory IgA and mucosal B cell trafficking patterns

•. Provide a perspective on mucosal infection by SARS-CoV-2 and induction of mucosal immunity at the sites of infection, including memory responses

•. Identify unknowns/gaps in our understanding of immunity/mucosal immunity to COVID-19 and vaccines

Jagannadha Sastry, PhD
Professor
Thoracic Head & Neck Medical Oncology and Comparative Medicine

Therapeutic Strategies for HPV Associated Cancers

Dr. Sastry's research program is centered on understanding the molecular, immunological and biochemical aspects of viral infections, specifically HIV-AIDS and HPV-associated cancers. The overall goal of their research is to develop procedures and reagents for prediction, treatment as well as prevention. Over the past 25+ years, he has led and/or participated in investigator initiated as well as team-oriented research programs supported by federal, state, industry, and philanthropy funds. For the past several years the lab is focusing on designing effective and practical vaccine delivery strategies and specifically concentrating on mucosal routes of vaccine delivery and the selection of appropriate adjuvants for formulating mucosal vaccines against HIV-induced AIDS and HPV-associated genital and oral cancers. They have developed intranasal and oral/sublingual mucosal vaccination strategies employing diverse modes of harnessing innate immune modulators for use with protein/peptide as well as viral-vector encoded antigens/immunogens in murine and nonhuman primate (rhesus macaque) models to induce effector/memory cellular and humoral immunity at multiple systemic and mucosal tissues. In their studies related to HPV-associated cervical pre-neoplastic lesions, they discovered synthetic peptides specific to the E6 and E7 oncoproteins of the high-risk HPV, HPV-16 for which memory T cell responses correlated with recurrence-free survival of women post-ablative treatment for pre-cancerous cervical lesions. Using orthotopic preclinical HPV tumor models in mice they demonstrated protective efficacy of these peptides in combination with adjuvant(s) and/or immune checkpoint therapy for enhanced antigen-specific cytotoxic T cell immunity to afford sustained complete regression of oral and genital HPV tumors. Their group offers expertise in the areas of adjuvants for mucosal vaccine formulations, preclinical mouse models, and correlating treatment outcomes with the various immunological parameters within the tumor microenvironment employing sophisticated multiparametric flow cytometry analyses.

Abstract: High-risk type human papillomaviruses (HPV) cause most cervical cancers and majority of Head & Neck cancers. We are using preclinical immunocompetent syngeneic mouse models for testing of novel therapeutic strategies. We demonstrated

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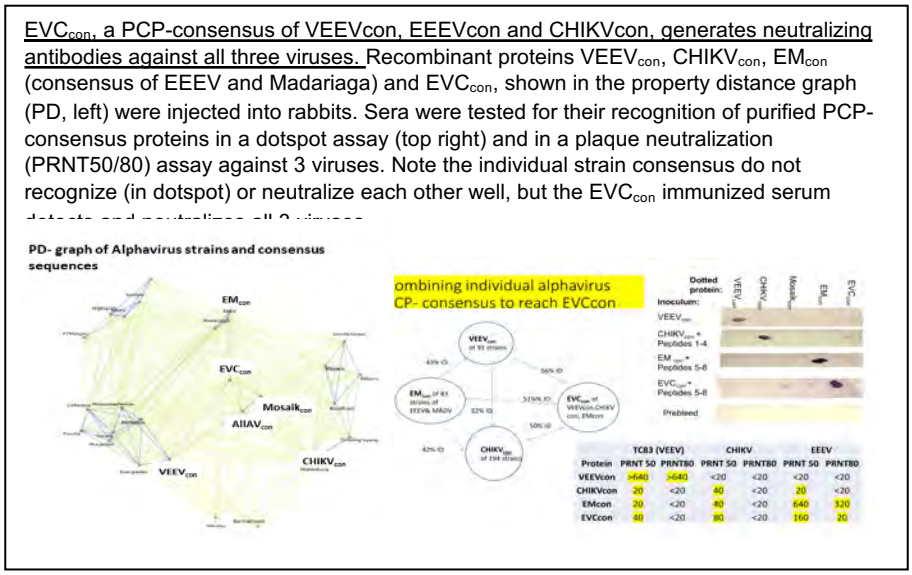
that immunotherapy targeting combinations of checkpoint receptors based on genetic and immunologic footprints at the anatomical location of the tumor is necessary for achieving sustained and significant tumor-free survival. We also showed that therapeutic HPV peptide vaccination employing combinations of diverse acting adjuvants is safer, relative to immune checkpoint therapy, and is necessary for significant induction of multiple antitumor cytotoxic mechanisms with concurrent reduction/ablation of immunosuppressive populations within the tumor as well as in the systemic tissues. Finally, we are testing chemotherapy targeting key cell cycle events to induce immunogenic cell death in tumors along with potential selective down modulating immune suppressive signals.

Catherine H. Schein, PhD
Adjunct Professor
Biochemistry and Molecular Biology

Tackling Variability in RNA viruses

Dr. Schein is a medicinal biochemist with experience in soluble protein production in bacteria, interferon and cytokine mechanisms, catabolic reactions of ribonucleases and autophagy, databases of allergens and viruses (SDAP, Flavitrack), bacterial toxin inhibitors, protein structure and computational drug design. All of this experience contributes to the current research in her lab group on broad spectrum antigens for vaccines designed with physicochemical property (PCP)- consensus and motif recognition, and synthetic conformational epitope mimetics (constrained and click chemistry peptides and proteins).

Abstract: The recent SARS-CoV-2 (COVID-19) epidemic has highlighted how the high variability of RNA viruses can present major obstacles to designing effective vaccines, therapies and diagnostics. However, long before coronaviruses became an existential threat, it was recognized that circulating RNA viruses, including poliovirus, influenza, dengue, West Nile, chikungunya etc. are not stable, but mixtures of evolving sequences differing from one another at a few positions. We have developed and validated, in 4 different virus groups, a suite of programs to identify regions of PCP-motifs and PCP-consensus sequences, as well as alignment-free methods to cluster sequences based on property distance (PD-Graph).



EVC_{con}, a PCP-consensus of VEEVcon, EEEVcon and CHIKVcon, generates neutralizing antibodies against all three viruses. Recombinant proteins $VEEV_{con}$, $CHIKV_{con}$, EM_{con} (consensus of EEEV and Madariaga) and EVC_{con}, shown in the property distance graph (PD, left) were injected into rabbits. Sera were tested for their recognition of purified PCP-consensus proteins in a dotspot assay (top right) and in a plaque neutralization (PRNT50/80) assay against 3 viruses. Note the individual strain consensus do not recognize (in dotspot) or neutralize each other well, but the EVC_{con} immunized serum

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A PCP-consensus, which is closest in its properties to all the sequences used to calculate it, provides a rational reference for a given virus type. More importantly, it can be combined with experimental data to design single antigens that generate protective antibodies against virus groups with up to 68% diversity.

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Robin Stephens, PhD
Associate Professor
Immunology and Pathology of Malaria

T Helper Plasticity in Balancing Pathology and Protection in Malaria

Dr. Stephens started her career as a parasite immunologist while an undergraduate at Cornell University. After earning a Master's at NYU, and a PhD in Immunology at Washington University, Dr. Stephens trained in malaria immunology at the National Institute for Medical Research in Mill Hill, London (now the Crick Institute). The Stephens lab at the University of Texas Medical Branch is dedicated to understanding protective adaptive immune responses to Plasmodium spp., including lethal cerebral pathology, in order to find novel solutions for vaccination and treatments for malaria.

Abstract: Complete protection from malaria requires both antibody and Th1 cells. Evolution has solved the competing nature of these two responses with the hybrid Th1/Tfh cell (IFN-g+IL-21+CXCR5+), which predominates in response to several persistent infections. In Plasmodium chabaudi infection, IFN-g+ T cells control parasitemia, whereas antibody and IL-21+Bcl6+ T cells effect final clearance, suggesting an evolutionary driver for the hybrid population. We found that CD4-intrinsic Bcl6, Blimp-1, and STAT3 coordinately regulate expression of the Th1 master regulator T-bet, supporting plasticity of CD4 T cells. Bcl6 and Blimp-1 regulate CXCR5 levels, and T-bet, IL-27Ra, and STAT3 modulate cytokines in hybrid Th1/Tfh cells. Infected mice with STAT3 knockout (KO) T cells produced less antibody and more Th1-like IFN-g+IL-21- CXCR5lo effector and memory cells, and were protected from re-infection. Conversely, T-bet KO mice had reduced Th1-bias upon re-infection and prolonged secondary parasitemia. Therefore, each feature of the CD4 T cell population phenotype is uniquely regulated in this persistent infection, and the cytokine profile of memory T cells can be modified to enhance the effectiveness of the secondary response. As some increase in pathology was seen in mice with a shifted Th1/Tfh balance, we are currently investigating the protective and functional capacity of hybrid Th1/Tfh cells to understand if this solution is ultimately maladaptive.

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Shao-Cong Sun, PhD
Professor
Immunology

Regulation of T Cell Metabolism and Function in Cancer Immunity

Dr. Shao-Cong Sun is a professor and Deputy Chair in the Department of Immunology, Director of Center for Inflammation and Cancer, and holder of the Moshe Talpaz Endowed Chair in Immunology at The University of Texas MD Anderson Cancer Center. He received PhD degree from Stockholm University and pursued postdoctoral training with Dr. Warner Greene at The Gladstone Institute of Virology and Immunology, University of California at San Francisco. His laboratory studies signal transduction in immune and inflammatory responses, with a focus on ubiquitin-dependent signaling and the NF-kB pathways. He has made a number of major contributions to the field, including pioneer work in NF-kB signaling and seminal findings regarding the role of ubiquitination in immune regulation. Dr. Sun was among the first to demonstrate IkB inducible degradation as the fundamental mechanism of NF-kB activation, and his laboratory pioneered the discovery of the noncanonical NF-kB pathway and demonstration of its regulatory mechanism. Dr. Sun's laboratory has also made seminal findings regarding how ubiquitination regulates T-cell function in autoimmunity and anticancer immunity. An important part of his ongoing research is to dissect the signaling pathways regulating T-cell activation, metabolism, and exhaustion in animal models of autoimmunity and antitumor immunity.

Abstract: T cells play a central role in immune responses against infections and cancer. Upon activation by a tumor antigen, CD8 T cells differentiate into antitumor effector T cells that migrate to the tumor to mediate tumor cell destruction. However, in tumor microenvironment, CD8 T cells are rendered hypofunctional due to immunosuppressive conditions, metabolic challenges, and functional exhaustion. How to induce potent and long-lasting CD8 T cell responses and to reinvigorate exhausted CD8 T cells has become a central issue in cancer immunotherapy. A major focus our research is to dissect the signaling network regulating T cell metabolism, effector function and exhaustion, hoping to characterize novel targets for cancer immunotherapy. We have recently shown that ubiquitination serves as a key mechanism of metabolic regulation in T cells and acts through controlling the AKT-mTORC1 signaling axis. Targeting specific E3 ligases and deubiquitinases can

promote antitumor immunity and improve the efficacy of cancer immunotherapy in animal models. Our recent work has also led to the discovery of a redox-dependent mechanism of metabolic regulation that is important for maintaining T cell effector function and preventing T cell exhaustion in tumor microenvironment. This mechanism is connected to the T cell receptor signal and costimulatory signals by the NF-kB-inducing kinase (NIK). While NIK is well known for its function in the regulation of noncanonical NF-kB signaling, the NIK-mediated regulation of redox generation and glycolytic metabolism in T cells is independent of noncanonical NF-kB. I will discuss the mechanism underlying this novel function of NIK and present evidence that ectopic NIK expression overcomes T cell exhaustion and improves the efficacy of adoptive T cell therapy in mouse models.

Dario Vignali, PhD
Vice Chair and Professor
Immunology

Inhibitory Mechanisms in the Tumor Microenvironment

Dario AA Vignali, PhD is the Frank Dixon Chair in Cancer Immunology, Vice Chair and Professor of Immunology in the Immunology Department, University of Pittsburgh School of Medicine. He is also Associate Director for Scientific Strategy, co-leader of the Cancer Immunology and Immunotherapy Program and co-director of the Tumor Microenvironment Center in the UPMC Hillman Cancer Center. He received his PhD in 1988 from the London School of Hygiene & Tropical Medicine, University of London, where he studied immunity to Schistosoma mansoni. He then held two post-doctoral positions from 1988-1993, first at the Institute for Immunology and Genetics, German Cancer Research Center, Heidelberg, Germany, with Prof. Gunter Hammerling, and then at the Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts, USA, with Prof. Jack Strominger. He then started his own independent research program in the Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee, rising to the rank of Vice Chair and Member (Full Professor equivalent). After nearly 21 years at St Jude he moved to Pittsburgh in July 2014. His research focuses on molecular and cellular aspects of negative regulatory immune mechanisms including regulatory T cells, inhibitory receptors, and inhibitory cytokines. His lab was instrumental in uncovering the role of LAG3 in mouse models of cancer, tolerance, autoimmunity and immune regulation. His lab discovered the inhibitory cytokine IL35 and the NRP1:SEMA4A axis, which are key regulators of intratumoral Treg stability and function. His current research extensively uses systems immunology approaches to understand transcriptional regulation of effector T cell exhaustion and regulatory T cell function and fate in murine models of cancer and autoimmunity, and numerous human tumors. He has been a Highly Cited Researcher (top 1% by citations; Clarivate Analytics) for the last five years (2016-2020) and has published over 195 papers with over 37 as senior or co-author in high impact journals (IF>10). He has a strong record of extramural funding, which currently includes an NIH P01 and four R01 grants. His innovative, discovery-based research has led to 15 patent awards (11 in the US) and 11 pending patent applications worldwide, and he is a co-founding scientist of several companies (Potenza Therapeutics [sold to Astellas], Tizona Therapeutics [sold to Gilead], Novasenta). Lastly, he is Director of the Cancer Immunology Training Program (NCI T32), and has trained, or currently training, 46

postdoctoral research or clinical fellows and 14 graduate students, with several successfully obtaining extramural fellowships (14 total), emphasizing his commitment to train the next generation of immunologists.

Abstract: Immunotherapies targeting the PD1/PDL1 pathway have had a major impact on cancer treatment. However, only a proportion of patients respond, and an even smaller proportion exhibit a long-term, durable cure. Several mechanisms of resistance and potential combinatorial approaches will be discussed. Many cancer patients do not develop a durable response to current standard of care immunotherapies despite substantial advances in targeting immune IRs (IRs). A potentially important and unappreciated compounding issue, which may serve as a dominant resistance mechanism, is the inherent systemic immune dysfunction that is often associated with advanced cancer. Although this has been described for decades, primary mechanisms and drivers remain unknown. Lack of response to IR blockade therapy and increased disease burden has been associated with circulating, peripheral $CD8^+$ T cell exhaustion, which is defined by poor T cell function linked to increased IR expression (eg: PD1, LAG3, neuropilin-1 [NRP1]). LAG3 is the third IR to be targeted in the clinic, consequently garnering considerable interest and scrutiny. However, persistent antigen exposure in the tumor microenvironment results in sustained PD1/LAG3 expression, contributing to a state of exhaustion manifest in impaired proliferation and cytokine production. However, the striking synergy between LAG3 and PD1 observed in multiple settings. There are now at least 10 LAG3-targeted therapies in the clinic with many more in preclinical development, emphasizing the broad interest in LAG3. Lastly, regulatory T cells (T_{regs}) inhibit beneficial anti-tumor responses. T_{reg} depletion enhances tumor rejection in animal models and the clinic but also leads to substantial adverse events. Thus, approaches have been sought to target Tregs in tumors while limiting systemic autoimmune and inflammatory manifestations. For instance, interleukin-35 (IL35) is a T_{reg}-secreted cytokine known to inhibit effector T cell proliferation and mediate infectious tolerance via induction of suppressive IL35-producing induced T_{regs}, iTr35. Using antibody-mediated neutralization, T_{reg}-restricted deletion of Ebi3 and novel reporter mice, we have shown that IL35 facilitated tumor growth by limiting anti-tumor immunity in transplantable and genetically-induced murine models of melanoma and lung carcinoma. These findings reveal the previously unappreciated importance of IL35 in limiting anti-tumor immunity and present IL35 as a potential therapeutic target in cancer. Alternatively, we have shown that the immune cell surface ligand semaphorin-4a (Sema4a) on conventional T cells and DCs, and the T_{reg}-restricted receptor NRP1 interact to potentiate T_{reg} function. Mice with a T_{reg}-restricted deletion of Nrp1 exhibit limited tumor-induced tolerance and thus substantial resistance to tumors, yet do not develop any autoimmune manifestations. Thus, NRP1 ligation maintains T_{reg} stability and function in highly inflammatory sites but is dispensable for the maintenance of immune homeostasis, highlighting NRP1 as a potential immunotherapeutic target in cancer.

Yifan Wu, MD, PhD
Postdoctoral Associate

Candidalysin Drives C. albicans-Induced Allergic Airway Disease Through Platelets

Yifan Wu is currently a postdoctoral associate dividing his time between the labs of Dr. David Corry and Dr. Jill Weatherhead. He completed his medical degree at Shanghai Jiao Tong University School of Medicine and obtained his PhD from Baylor College of Medicine. Dr. Wu's research focuses on the host immune response to the fungi Candida albicans, specifically on the response to cerebral and pulmonary infection. He has discovered novel infectious mechanisms, pathologies, and immune pathways involved in C. albicans mediated cerebritis and allergic airway diseases.

Abstract: Background: The commensal yeast *Candida albicans* promotes allergic responses and is implicated as a cause of asthma, with mechanisms remains unknown. Candidalysin is a cytolytic peptide secreted by *C. albicans* and is a potent immune activator. Dickkopf-1 (Dkk-1) is a platelet-derived WNT pathway antagonist peptide that drives allergen-induced TH2 responses.

Hypothesis: Candidalysin promotes TH2-predominant allergic airway disease by stimulating the secretion of Dkk-1 from platelets in mice.

Methods: C57B6 mice were challenged intranasally with either viable *C. albicans* or synthetic candidalysin every other day for 8 challenges and assessed for induced airway hyperresponsiveness (AHR). Recombinant Dkk-1 or DKK-1 inhibitors were administered i.p. in similarly challenged mice. For *in vitro* studies, human platelets were incubated with *C. albicans* or candidalysin and Dkk-1 release was quantified by ELISA. Flow cytometric analysis of platelets for activation markers was also performed.

Results: Wild type *C. albicans* strongly induced AHR and TH2 responses in wildtype mice, but both parameters were significantly reduced or abrogated when a Dkk-1 inhibitor was given. In contrast, candidalysin-deficient *C. albicans* failed to induce AHR, but AHR was restored by administering exogenous Dkk-1. Candidalysin alone was sufficient to induce AHR and mild eosinophilia and induced both Dkk-1 release and activation of human platelets.

Conclusion: Candidalysin is both necessary and sufficient for *C. albicans*-induced allergic airway diseases, suggesting the crucial role that candidalysin and platelets play in driving a fungus-dependent allergic disorder.

Wenjing Yang, MD, PhD
Postdoctoral Researcher

GPR120 Suppresses Intestinal Inflammation Through Regulation of CD4+ T cell IL-10 Production

Dr. Yang has several years' research training in the field of IBD and mucosal immunology. As a medical student, she not only mastered the clinical knowledge of clinical medicine, especially gastroenterology, but also attended several biological courses, including medical cell biology, molecular biology, physiology, pathophysiology, immunology, and research methods, from which she drew much of her interest in scientific research. She received her MD degree 2013. During her residency training from 2013-2016 at Shanghai 10th People's Hospital, Tongji University, she grasped the diagnosis and treatment of IBD, and gained interest in it. She was able to conduct research on IBD with Dr. Zhanju Liu, a renowned physician scientist specialized in IBD research and clinic care in Tongji University. In 2016, she was enrolled to the PhD graduate program of Tongji University in Dr. Liu's laboratory. Her research focused on the roles of Rho-associated kinase (ROCK)2 in CD4+ T cell differentiation and the development of inflammatory bowel disease (IBD). In addition, she also took part in other projects closely related with the basic research of mucosal immunology and the pathogenesis of IBD. These works helped her to gain key knowledge on mucosal immunology and related techniques. Since 2017, she chose to further her research as a predoctoral fellow with Dr. Yingzi Cong, a professor at UTMB as well as a renowned expert in the mucosal immunology and immune regulation of IBD, on the roles of microbiota metabolites in regulating innate and adaptive immune responses. This position has broadened her perspectives on research and helped to equip herself with important professional skills. After she received her PhD degree, she continues to work with Dr. Cong as a postdoctoral fellow. During these four years in UTMB, she was actively involved in several projects related with IBD and mucosal immunology, including intestinal barrier functions and have published ten articles. Aside from these experiences, she also has a passion for investigating the mechanisms involved in the pathogenesis of colitis, so she enjoys recent work.

University of Texas Medical Branch at Galveston

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Lele	Zhu	MD Anderson Cancer Center	*TBKBP1 and TBK1 Form a Growth Factor Signaling Axis Mediating Immunosuppression and Tumorigenesis*	33

Abstract: Background: $CD4^+$ T cell production of IL-10 plays a critical role in maintaining intestinal homeostasis. G protein-coupled receptor (GPR) 120, a receptor for omega 3 fatty acids, has been implicated in regulating metabolic syndromes with anti-inflammatory function. However, the role of GPR120 in intestinal inflammation is unknown. In this study, we investigated whether and how GPR120 regulates $CD4^+$ T cell production of IL-10 to the maintenance of intestinal homeostasis.

Methods: Dextran sodium sulfate (DSS) induced colitis model and *Citrobacter. rodentium* induced infection model were used for comparison of intestinal inflammation between wild-type (WT) and *Gpr120*$^{-/-}$ mice, or *Cd4*^{cre} *Gpr120*$^{fl/+}$ and *Cd4*^{cre} *Gpr120*$^{fl/fl}$ mice. GPR120 expression in different cell types was measured by Western blot. $CD4^+CD45RB^{hi}$ T cell adoptive transfer model was utilized to analyze the pathogenesis of WT and GPR120-deficient $CD4^+$ T cells in inducing colitis. Mouse splenic $CD4^+$ T cells were treated with or without GPR120 agonist, CpdA, and the gene differences were analyzed by RNA sequencing and qRT-PCR. The effect of GPR120 on T cell metabolism was measured by glucose uptake assay and Seahorse metabolic assay. Chemical inhibitors, including mTOR inhibitor and glycolysis inhibitor, and Blimp1-deficient $CD4^+$ T cells isolated from *Cd4*^{cre} *Prdm1*$^{fl/fl}$ mice were used for mechanistic studies. Mice were administered GPR120 agonist for investigating the potential treatment of GPR120 agonist in treating intestinal inflammation.

Results: We demonstrated that deficiency of GPR120 resulted in more severe colitis in mice upon inflammatory insults and enteric infection. Interestingly, $CD4^+$ T cells expressed GPR120 at a high level, and mice specifically lacking GPR120 in $CD4^+$ T cells were more susceptible to colitis development. Transfer of GPR120-deficient $CD4^+$ $CD45Rb^{hi}$ T cells induced more severe colitis in *Rag*$^{-/-}$ mice with increased IL-17 and IFNγ producing $CD4^+$ T cells and decreased IL-10 producing $CD4^+$ T cells in intestinal lamina propria than WT T cells. Treatment with GPR120 agonist, CpdA, promoted $CD4^+$ T cell production of IL-10 by upregulating Blimp1 and enhancing glycolysis, which were regulated by mTOR. Consistently, docosahexaenoic acid (DHA), a dietary long-chain fatty acid, also upregulated the IL-10 production in $CD4^+$ T cells. GPR120 agonist-treated WT but not Blimp1-deficient Th1 cells induced less severe colitis. Importantly, oral administration of CpdA protected mice against intestinal inflammation.

Conclusions: Our findings demonstrate the role of GPR120 in regulating intestinal $CD4^+$ T cell production of IL-10 to maintain intestinal homeostasis, which identifies GPR120 as a potential therapeutic target for IBD treatment.

Poster 1

HLA-Arena: Enabling Structure-based Virtual Screening of Tumor-associated Antigens

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Background: Cancer immunotherapy treatments aim at inducing an immune response in the patient, specifically targeting and eliminating tumor cells. Some of the most promising immunotherapies are based on the cellular immunity, which relies on the functions of T-cell lymphocytes. T-cells are "trained" to identify non-self peptides displayed at the surface of other cells by Human Leukocyte Antigen (HLA) receptors. In the context of a cancer cell, this mechanism allows circulating T-cells to recognize tumor-associated peptides (antigens/neoantigens) being displayed, which will in turn trigger T-cell activation and tumor elimination. Despite successful trials, many technical challenges continue to limit broader application of these treatments, especially regarding the specificity and safety of T-cell-based therapies. **Hypothesis/Goals**: Structural features are known to play a central role in both the binding of peptides by HLAs, and in the recognition of peptide-HLA complexes by T-cells. Therefore, our goal is to incorporate structural information into existing pipelines for personalized cancer immunotherapy, in order to improve these therapies. However, the variability of HLAs and the length of these peptide-ligands prevent the use of experimental methods for structural determination, and the use of conventional docking tools. **Methods**: We developed HLA-Arena, a computational environment integrating methods for structural modeling and analysis of pHLA complexes. HLA-Arena includes new geometric-based modeling methods that can be deployed at scale, enabling applications for personalized immunotherapy. As a proof-of-concept, we perform a structure-based virtual screening of HLA-binders against a full HLA diplotype (i.e., six classical class I HLA alleles). The peptide dataset was built by selecting 500 known binders and 1,000 decoys for each allele, for a total of 9,000 peptides. First, the whole dataset of peptides is screened for HLA binding with MHCflurry, using an affinity threshold specified by the user. Then, we proceed with the structural modeling and ranking of selected peptide-HLA complexes. **Results**: Our structure-based analysis can usually eliminate at least half of false positive predictions, and recover significant numbers of false negative predictions, although results vary depending on the studied HLA allele. **Conclusions**: HLA-Arena is a fast and customizable environment for structural analysis, available on Github (https://github.com/KavrakiLab/hla-arena). **Acknowledgements**: This work was funded in part by the Cancer Prevention & Research Institute of Texas (CPRIT), through Grant award RP170508, and through two Fellowships from the Computational Cancer Biology Training Program (RP170593). This work was also partially supported by a training fellowship from the National Library of Medicine Training Program in Biomedical Informatics (T15LM007093).

Poster 2

Elucidating the Tumorigenic and Immunogenic Potential of MAGE-A4 in the Bronchial Epithelium

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Background: Despite advances in treatment in recent years, lung cancer remains the leading cause of cancer death world-wide. The accumulation of multiple mutations in tumor suppressor genes (e.g., *Pten*, *p53)*, activation of oncogenes, and epigenetic changes including hypomethylation have been implicated in lung cancer in smokers. Such epigenetic alterations can cause the aberrant expression of cancer testis antigens, such as Melanoma Antigen A4 (MAGE-A4). The tumors of approximately 30% of NSCLC patients express MAGE-A4. However, evidence for its role in carcinogenesis has been restricted to *in vitro* work and the results have been conflicting. Notably, expression of *MAGE-A4* and loss of PTEN has been shown to confer genetic susceptibility to NSCLC.

Hypothesis/Goals: We hypothesized that MAGE-A4 is not a benignly expressed protein, but actively drives oncogenesis and recruits tolerogenic immune cells to the tumor microenvironment.

Methods: To address this hypothesis, we generated a novel model of NSCLC using constitutive expression of human MAGE-A4 in bronchial epithelial cells. Human MAGE-A4 under a floxed stop codon was inserted into the ROSA locus in C57/B6 mice, which were crossed to mice expressing *Cre* under the Club-cell secretory protein promoter, specific to bronchial epithelial cells (*MAG4*). These were crossed to floxed *Pten* mice, in order to conditionally express *MAGE-A4* and ablate *Pten* (*MAG4/Pt*$^{d/d}$) in airway epithelia, conferring genetic susceptibility as is seen in patients with NSCLC. The lungs of these mice were assessed by histology for tumor. Immune microenvironment was evaluated by flow cytometry of whole lung homogenate, immunohistochemistry of formalin-fixed paraffin-embedded lungs, and ELISAs of bronchoalveolar lavage fluid.

Results: *MAG4/Pt*$^{d/d}$ mice develop papillary adenocarcinoma as early as 2.5 months of age, whereas *Pten*$^{d/d}$ mice only develop airway hyperplasia and MAGE-A4 expression alone do not develop tumors. Importantly, a subset of these demonstrate an infiltration of IgA-producing, clonal plasma cells in the lung parenchyma.

Conclusions: This novel finding demonstrates that MAGE-A4 is an oncoprotein which promotes tumorigenesis. This confirms that its expression is not benign, and may be a driver of mutagenesis since it promotes tumor growth in the absence of a tumor suppressor. Here we also demonstrated that MAGE-A4 alters the immune microenvironment. Future work will include the molecular mechanisms by which MAGE-A4 promotes tumor growth and the role of plasma cells in MAGE-A4-driven NSCLC.

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Poster 3

Investigating the Impact of TLR Ligand Induced Id2 Expression in Dendritic Cell Development and Function

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Background: The inhibitor of DNA binding (Id) proteins and E proteins are basic helix-loop-helix proteins that act as mutual antagonists to direct immune cell development and function. In dendritic cells (DCs), Id2 is required for type 1 conventional DC (cDC1) development, but represses E2-2, an E protein and master regulator of plasmacytoid DC (pDC) development. DCs detect viruses, bacteria, and tumor cells via pattern recognition receptors, including Toll-like receptors (TLRs), to elicit immune responses. Previously, we found Id2 mRNA and protein was induced in TLR agonist-stimulated murine pDCs. These data suggest TLR signaling alters the balance of Id and E proteins in DCs, which may subsequently skew DC development and maturation.

Hypothesis/Goals: The study goals were to 1) determine effects of TLR signaling on *Id2* mRNA in common DC progenitors (CDPs) and DC lineage development, and 2) determine the role of Id2 in TLR-maturing pDCs and cDC1s.

Methods: CDPs from murine BM were purified, treated *in vitro* with the TLR4 ligand lipopolysaccharide (LPS), and evaluated for changes in *Id2* mRNA expression. To examine effects of TLR stimulation on CDP differentiation *in vivo*, mice were treated with LPS and analyzed for changes in CDPs, pre-DCs, and peripheral DC subset abundance and frequencies. To determine the role of Id2 in TLR agonist-matured DCs, conditional *Id2* knockout mice (CreER $Id2^{f/f}$) were used to generate *Id2*-sufficient and -deficient pDCs or cDC1s. Purified pDCs and cDC1s were challenged with TLR7 (Imiquimod) and TLR3 (Poly-I:C) agonists, respectively, and evaluated for changes in gene expression, co-stimulatory molecule expression, and soluble factor production.

Results: LPS-stimulated CDPs expressed higher amounts of *Id2* mRNA compared to non-treated controls. LPS-treated mice exhibited a rapid decrease in CDPs that recovered within 24-hours, consistent with prior work, as well as changes in peripheral DC subset frequencies. *Id2*-sufficient and *Id2*-deficient pDCs exhibited similar gene expression, phenotypic, and functional changes upon TLR agonist stimulation. Comparable data were obtained from *Id2*-sufficient and *Id2*-deficient cDC1s.

Conclusions: These data suggest LPS induces *Id2* mRNA in CDPs, and may modulate DC lineage development *in vivo*. Future work will test the requirement of Id2 in LPS-induced DC lineage differentiation. Finally, our data indicate Id2 is dispensable for pDC and cDC1 TLR-induced maturation.

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Poster 4

Longitudinal Dynamics of Single T Cell Molecular States Define Clinical Outcomes to Adoptive Cellular Therapy

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Background: Immune therapies have transformed the cancer therapeutic landscape but fail to benefit most patients.

Hypothesis/Goals: To elucidate the underlying mechanisms by which T cells mediate elimination of leukemia, we generated a high-resolution map of longitudinal T cell dynamics within the same tumor microenvironment (TME; bone marrow) during response or resistance to donor lymphocyte infusion (DLI), a widely used immunotherapy for relapsed leukemia.

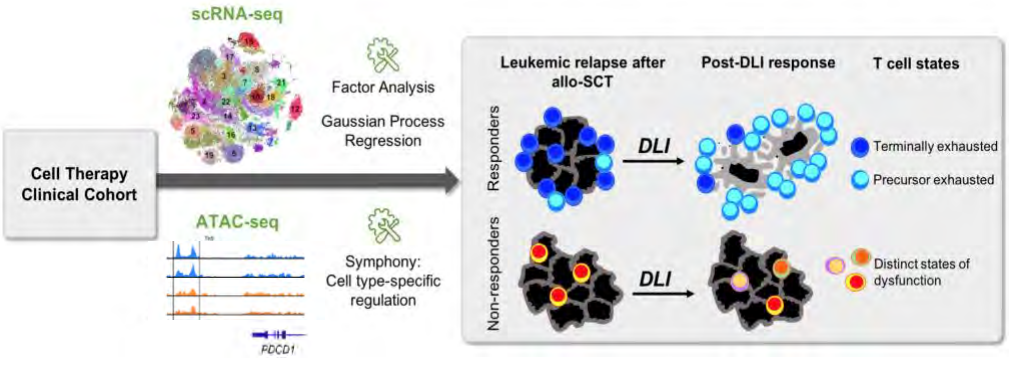
Methods: We analyzed 87,939 bone marrow-derived single T cell transcriptomes, along with chromatin accessibility and single T cell receptor clonality profiles, by developing novel machine learning tools for integrating longitudinal and multimodal data.

Results: We found that pre-treatment enrichment and post- treatment rapid, durable expansion of 'terminal' (T_{EX}) and 'precursor' (T_{PEX}) exhausted subsets, respectively, defined DLI response. In contrast to the common, shared pathways marking DLI response, a heterogeneous pattern of T cell dysfunction marked DLI resistance. Unexpectedly, T_{PEX} cells that expanded in responders did not arise from the infusion product but instead from both pre-existing and novel clonotypes recruited to the TME. Further, we introduce a Bayesian method, Symphony, to define the T cell regulatory circuitry and master regulators underlying T_{EX} and T_{PEX} subsets that may be broadly relevant to other exhaustion antagonists across cancers.

Conclusions: In conclusion, our data implicate the hierarchy of both T_{EX} and T_{PEX} subsets for immunotherapeutic responses in leukemia, extending the scope of their relevance beyond checkpoint blockade to adoptive cellular therapy. Moreover, our results provocatively suggest that immunologic 'help' from DLI, rather than direct transfer of anti-leukemic T cells, drove leukemic remission. Finally, we provide a general analysis paradigm for exploiting temporal single-cell genomic profiling for deep understanding of how immune therapies differentially shape the evolutionary trajectories of the TME in accordance with clinical outcome.

Acknowledgements: NCI (K08 to PB; R01 to CJW; K99 to EA); CPRIT to PB; patients, clinical staff

Summary model.



Poster 5

Developing a Chronic Murine Cerebral Mycosis through Gut Colonization

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Background: Alzheimer's Disease (AD) is the sixth leading cause of death in the United States and the only cause of death in the top ten that cannot be prevented, treated, or cured. Recent evidence suggests that AD may be linked to fungal brain infections. To study this possibility, we established a model of cerebral mycosis by intravenously (IV) injecting the pathogenic yeast Candida albicans. The resulting cerebral mycosis induces mild memory deficits and fungal induced glial granulomas (FIGGs) consisting of microglial aggregates, amyloid β (aβ) deposits, and amyloid precursor protein (APP) surrounding yeast. This structure resembles AD's characteristic senile plaques, but cerebral mycosis and memory loss do not persist beyond 10 days after a single intravenous infection. In contrast, AD involves numerous senile plaques and tauopathy that presumably accrue over many years resulting from chronic cerebral mycosis resulting in progressive, irreversible dementia.

Hypothesis/Goals: *C. albicans* might persist in a remote tissue site, such as the intestines, from which it might periodically mobilize to chronically re-infect the brain. As both *C. albicans* colonization of the GI tract and low-level candidemia deriving from the GI tract have been documented in humans, we hypothesize that chronic *C. albicans* enteritis leads to low-level transmission of fungal cells into the bloodstream and persistent cerebral mycosis.

Methods: To test this hypothesis and establish a more translationally relevant chronic model, we administered yeast from *C. albicans* to wildtype C57BL/6 mice via oral gavage. Brain, kidney, fecal matter, and gut tissue were isolated from infected mice at various timepoints. Organs were then processed and homogenized and cultured to determine fungal burden.

Results: We found that live yeast are recoverable from the brain as soon as 2 days post gavage and out to at least day 58 with a trend of increasing infection over time. These colonies were polymicrobial, consisting of both yeast and bacteria, an observation that is consistent with recent analyses of AD brains that demonstrate polymicrobial brain infections involving both fungi and bacteria. *C. albicans* persists in the gut out to day 58. This data fits with our hypothesis, suggesting that gut colonization leads to increasing metastasis of polymicrobial units (fungi + bacteria) to the brain.

Conclusion: We have established a chronic model of cerebral mycosis, which presents with polymicrobial colonies which resemble AD. This model could be groundbreaking for the AD field, potentially suggesting the use antifungals as treatment and prevention for AD.

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Poster 6

STAT3 and IL-10 Inhibit Interferon Signaling in Type 1 Conventional Dendritic Cells

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Background: Conventional dendritic cells (cDCs) are the primary antigen presenting cells of the immune system, and thus control adaptive immunity. Type 1 cDCs (cDC1s) are crucial for mounting protective T cell responses against tumors and viruses. Recently, we reported that interleukin (IL)-10 inhibits polyinosinic-polycytidylic acid (pI:C)-induced maturation of cDC1s in a Signal Transducer and Activator of Transcription 3 (STAT3)-dependent manner. In addition, using a tumor vaccine approach, we demonstrated that STAT3-deficient (*Stat3*$^{\Delta/\Delta}$) cDC1s are more capable of inducing anti-tumor immunity than STAT3-sufficient (*Stat3*$^{fl/fl}$) cDC1s. Our preliminary data suggest STAT3 inhibits an autocrine interferon (IFN) signaling loop elicited during pI:C-induced maturation.

Hypothesis: We hypothesize that STAT3 and IL-10 inhibit IFN expression and signaling in pI:C-treated cDC1s.

Methods: cDC1s were generated in vitro using mouse bone marrow and cDC growth factors. Bone marrow was harvested from DC-specific *Stat3*$^{\Delta/\Delta}$, *Ifnar1*$^{-/-}$, *Ifngr1*$^{-/-}$, and control mice. cDC1s were exposed in vitro to recombinant IL-10, IFN-β, IFN-γ, IFN-λ, as well as pI:C, and IFN-λ–blocking antibody. RNA sequencing was performed by Novogene and analyzed using Gene Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis (IPA). Gene transcript abundance was also assessed using reverse transcription quantitative polymerase chain reaction. STAT phosphorylation (pSTAT) was evaluated by immunoblotting. Protein expression was measured by flow cytometry.

Results: GSEA revealed that although many inflammatory signaling pathways are enriched in cDC1s upon treatment with pI:C, only the IFN-α response pathway is inhibited by IL-10 and STAT3. Similarly, IPA identified IFNs and components of IFN receptor signaling, such as STAT1, as the primary targets of IL-10/STAT3-mediated inhibition. Exposure to specific IFNs revealed IFN-β and IFN-γ, but not IFN-λ, induce IFN-stimulated genes (ISGs) in cDC1s. Furthermore, pI:C-induced pSTAT1 accumulation was inhibited in *Ifnar1*$^{-/-}$ or IL-10–treated cDC1s but maintained in *Ifngr1*$^{-/-}$ cDC1s. In addition, pI:C-induced ISG expression was inhibited in *Ifnar1*$^{-/-}$ cDC1s but not *Ifngr1*$^{-/-}$ or IFN-λ–blocking antibody-treated cDC1s.

Conclusions: IL-10 and STAT3 inhibit IFN expression and downstream signaling in pI:C-treated cDC1s. Furthermore, cDC1s selectively require autocrine type I IFN for pI:C-induced pSTAT1 accumulation and ISG expression. Currently, studies are underway to further dissect the role of IFNs and IL-10/STAT3 in regulating cDC1 function in vitro and in vivo.

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Poster 7

Role of Intratumoral Neurotransmitter Signaling in T cell Exhaustion and Anti-tumor Responses

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Background: Immune checkpoint inhibition via CTLA-4, PD-1, and PD-L1 blocking has provided an effective and long-term durable cancer immunotherapy for many patients with diverse diseases, however, most patients do not respond to the therapy. Although pre-clinical studies of additional cell surface receptors (LAG-3, TIGIT, and TIM-3) have been promising, there are other additional inhibitory pathways that contribute to immunosuppression that have yet to be delineated.

Neurotransmitters (NTs) are small signaling molecules, generally secreted by neurons to facilitate communication between these cells and their targets. NTs are detectable in serum and present in the tissue and can be sensed by immune cells. The effects of NTs on immune cells have been immunosuppressive due to multiple distinct mechanisms. However, the role of NTs in the tumor microenvironment has not been studied, and their presence in the tumor microenvironment (TME) has not been reported.

Hypothesis: We hypothesize that intratumoral neurotransmitters provide additional immunosuppression and can be targeted pharmacologically to improve anti-tumor responses.

Methods and Results: We have detected dopamine and norepinephrine in TME of a murine melanoma model by mass cytometry and showed that dopamine beta-hydroxylase (DBH, the enzyme that converts dopamine to norepinephrine) is expressed in a subset of human T cells human melanoma biopsies by scRNAseq. High DBH expression correlated with increased expression of exhaustion markers. Additionally, these cells were confirmed in murine models.

We have showed that the inhibition of the DBH enzyme or inhibition of the adrenergic receptors that norepinephrine will act on improves the tumor growth control in animals that have received immune checkpoint blockade compared to controls.

Conclusions: We demonstrate that intratumoral neurotransmitter signaling acts as an immunosuppressive pathway and is addressable with FDA-approved compounds to modulate to improve the efficacy of immune checkpoint blockade. Future studies will focus on the molecular mechanisms that lead to Dbh expression on select immune cells and how this pathway can be expanded to other cancers.

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Poster 8

A Metabolically Engineered Bacterial Vaccine Ameliorates Collagen Induced Arthritis in Mice

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Background: Arthritis is a long-term autoimmune disease that adversely affects the quality of life of patients. Treatment options for autoimmunity include the use of chemical and biologic agents that suppress inflammation. However, these approaches can result in vulnerability to opportunistic infections. Adoptive T regulatory cell transfer can specifically block auto-inflammatory immune cells in an antigen-specific manner while keeping an intact immune response to combat pathogens. However, isolating enough Treg cells for adoptive cell transfer therapy is challenging because Treg cell populations are small among leukocytes in the blood. Therefore, improving Treg cell survival and function could have an important impact on adoptive Treg transfer therapy. The bacterial metabolite indole enhances the development of Treg populations and some live attenuated bacterial vaccines (e.g., BCG) have shown efficacy in animal models of autoimmune disease.

Goal: To explore the possibility that live attenuated bacterial vaccines that express indole can be used as an adjunctive autoimmune therapy.

Methods: We engineered bacterial tryptophanase gene into an attenuated *Brucella* vaccine strain (*Brucella melitensis ΔvjbR*), thereby enabling the bacteria (*BmΔvjbR Tna*) to produce indole. The therapeutic potential of the *BmΔvjbR Tna* was tested in a murine collagen induced arthritis (CIA) model.

Results: In CIA mice, although both Treg transfer and Treg transfer plus *BmΔvjbR Tna* injection decreased arthritis incidence and score, *BmΔvjbR Tna* injection significantly suppressed arthritis in CIA mice treated with Treg cells compared to the group treated with Tregs only. Safranin-O staining showed that CIA mice receiving *BmΔvjbR Tna* displayed less joint damage. $CD4^+FoxP3^+$ T regulatory cells and $CD8^+$ T cells in spleen and lymph nodes were increased in *BmΔvjbR Tna* treated CIA mice. *BmΔvjbR Tna* injection also promoted $CD4^+$ cell proliferation, and increased IL-2 and IFN-γ expression. The levels of some pro-inflammatory cytokines (IL-1β and IL-6) dramatically decreased in *BmΔvjbR Tna* infected bone marrow derived macrophages (BMDMs) compared to *BmΔvjbR*-infected controls.

Conclusions: These findings indicated that the indole producing *BmΔvjbR Tna* strain can ameliorate autoimmune disease in CIA mice. The vaccine may fulfill its therapeutic function by increasing Treg cell populations and proliferation while suppressing proinflammatory cytokine secretion.

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Poster 9

Promoting NK Cell Function Within the Inhibitory Tumor Microenvironment

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Background: Natural killer (NK) cells represent an attractive cellular platform for 'off-the-shelf' cancer adoptive immunotherapy. However, the efficacy of NK cellular therapies in solid tumors is limited by insufficient survival and expansion within the tumor microenvironment (TME). Solid TMEs contain cytokine mileus that induce signal transducer and activator of transcription-3 (STAT3) signaling within NK immune infiltrates, thought to negatively regulate NK survival and function. Conversely, NK activating cytokines converge on STAT5, an essential signaling node for NK survival, expansion, and anti-tumor activity. How the TME modulates the STAT3/5 signaling axes in *adoptively transferred* NK cells is under studied. Furthermore, phenotypic features differentiating NK activation from exhaustion in solid TMEs in the context of cellular immunotherapy remain poorly defined.

Hypothesis/Goals: We hypothesized that suppressive myeloid cells of the TME, such as inhibitory macrophages or myeloid-derived suppressor cells (MDSCs), induce STAT3 in NK cells that is associated with decreased survival and anti-tumor activity. In contrast, conferring NK cell-intrinsic STAT5 activation via a constitutively active IL-7 receptor (C7R) will rescue NK cell survival, expansion, and function within suppressive TMEs.

Methods: Neuroblastoma cells and PBMC-derived monocytes were co-cultured for 72 hours before addition of autologous *ex vivo* expanded C7R-modified NK cells. Flow cytometry was used to assess intracellular pSTAT3/5, cell surface activation and checkpoint markers (LAG-3, NKG2D, NKG2A, TIGIT, TIM-3), and absolute tumor and NK numbers at 6 hours, 2 days, and 5 days post-NK addition.

Results: We demonstrate that C7R-expressing NK cells exhibit increased pSTAT5 levels in the TME compared to vector control-transduced cells (mock-NK), resulting in increased survival and proliferation over 5 days. Mock-NK cells show higher levels of pSTAT3 compared to C7R-NK cells at baseline; however, pSTAT3 is downregulated in the TME. Lastly, we demonstrate that C7R-NK cells maintain an activated phenotype within the TME, while mock-NK exhibited an exhaustive phenotype.

Conclusions: Conferred STAT5 signaling via C7R represents a potential therapeutic strategy to maintain activation of adoptively transferred anti-tumor NK cells within suppressive TMEs.

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Poster 10

MicroRNA Let-7bc-Cluster Controls Treg Response and Airway Inflammation and COPD Via a SOCS1-IL6-IL10 Circuit in CD11c$^+$ Dendritic Cells and Alveolar Macrophages

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Introduction: Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the US. Emphysema is an incurable and debilitating form of COPD characterized by dyspnea, chronic airway inflammation, and destruction of the walls of the air sacs (alveoli) of the lung. Cigarette smoking (CS), atmospheric particulate matter, and fumes are the major environmental risk factors correlated with development of emphysema. We previously showed that nano-sized particles of carbon black (nCB), a constituent of smoke generated by the incomplete combustion of tobacco promotes emphysema in mice in part by aberrant stimulation of CD11c$^+$ conventional dendritic cells (cDCs) and alveolar macrophages. In this study, we investigated the importance of *let-7b and let-7c2-cluster (let-7bc2)* within CD11c$^+$ cells as a modifier of emphysema disease severity.

Methods: To determine whether *let-7b* and *let-7c* microRNAs expression might be dysregulated during emphysema, we carried out qPCR from purified CD11c$^+$ cDCs/alveolar macrophages derived from lungs of mice intranasally instilled with saline as a control vs nCB to elicit emphysema. To evaluate the *in vivo* phenotype of *let-7bc2-cluster* in emphysema within cDCs/alveolar macrophages we generated conditional knockout mice and crossed them to CD11c-Cre deleter mice. *Let-7bc2$^{f/f}$* (WT) and *let-7bc2$^{f/f}$*; CD11c-Cre (CKO) mice were evaluated in two experimental emphysema models: intranasal instillation of nCB or whole-body exposure to cigarette smoke. Mice were examined for airway inflammation and emphysema by histomorphometry, bronchoalveolar lavage fluid cell counts, flow cytometry and gene expression.

Results: MicroRNA profiling experiments from purified CD11c$^+$ cells from nCB-treated mice revealed increased expression levels of a *let-7b* and *let-7c* gene cluster. We observe that the selective deletion of this cluster in CD11c$^+$ cells exacerbates nCB-induced alveolar destruction as measured by mean linear intercept (Fig). BAL extracted from emphysematous CKO mice have enhanced infiltration of immune cells and expression of MMP9 and IL6. Mechanistically, CD11c$^+$ cells from lungs of CKO emphysematous mice show downregulation of *Socs1* gene expression along with impaired induction of IL10. Quite interestingly, CKO mice show attenuated T regulatory cell populations and decreased *Foxp3* expression.

Conclusion: Taken together, our data suggests *let-7bc2-cluster* plays a molecular braking for airway inflammation and emphysema by controlling the balance of anti-inflammatory SOCS1/IL10 and pro-inflammatory STAT3/IL6 pathways in CD11c$^+$ cells.

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Poster 11

IFNy-dependent Hematopoietic Stem Cell Activation is Mediated by Bone Marrow Stromal Antigen 2

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Background
Our lab and others have shown that chronic infections can contribute to various cytopenias and eventually bone marrow failure. The inflammatory cytokine interferon-gamma (IFNy) contributes to bone marrow failure by activating hematopoietic stem cells (HSCs) and impairing their self-renewal. The mechanisms by which IFNy drives the loss of quiescence and ultimate exhaustion of HSCs remains poorly understood, but is thought to be related to changes in the interaction between HSCs and the bone marrow (BM) microenvironment, or BM niche. HSCs are known to reside near perivascular CXCL12 abundant reticular (CAR) stromal cells which are critical for maintaining their quiescence.

Hypothesis/Goals
The goal of our work was to explore the mechanism by which IFNy alters HSC interactions within the BM niche.

Methods
We performed transcriptomic analysis of IFNy-stimulated HSCs and focused on changes in cell-surface expressed genes that may influence HSC-niche interactions. Using two reporter mouse models: CXCL12-GFP reporter mice in which CAR cells are labeled with GFP, and CXCL12-GFP Krt18-Cre LSL-tdTomato dual reporter mice in which CAR cells are labelled with GFP and HSCs are labeled with tdTomato, we performed intravital imaging in the presence and absence of IFNy. We also tested cell cycle analysis of HSCs during *Mycobacterium avium* infection.

Results
Transcriptomic analysis focused on changes in cell surface proteins in IFNy-stimulated HSCs revealed Bone Marrow Stromal Antigen 2 (BST2) as the only surface protein upregulated on HSCs upon 24-hour IFNy stimulation. After exogenously labeling and transferring HSCs into CXCL12-GFP mice or endogenously labeling HSCs in the CXCL12-GFP Krt18-Cre LSL-tdTomato mice, we observed that HSCs stimulated with IFNy were significantly distanced from CAR cells compared to pre-treated controls. We observed no change in HSC distancing from CAR cells after IFNy stimulation of IFNy-receptor deficient HSCs, suggesting that the observed HSC displacement was due to a cell autonomous mechanism. Intravital imaging using BST2-deficient HSCs revealed that BST2 KO HSCs do not re-localize from CAR cells during IFNy stimulation. Using *in vitro* plate binding assays, we found that IFNy-treatment promoted

increased HSC binding to E-selectin via BST2, as well as increased HSC homing to the bone marrow, a property that is dependent on E-selectin binding. Using cell cycle analysis, we discovered that the loss of BST2 protects against HSC activation during *Mycobacterium avium* infection. Furthermore, HSC depletion during chronic infection was mitigated in BST2 KO mice.

Conclusions

Our data identifies BST2 as a key protein that influences niche relocalization and activation in response to inflammatory stimulation. This study expands our understanding of factors that contribute to HSC activation and loss of quiescence.

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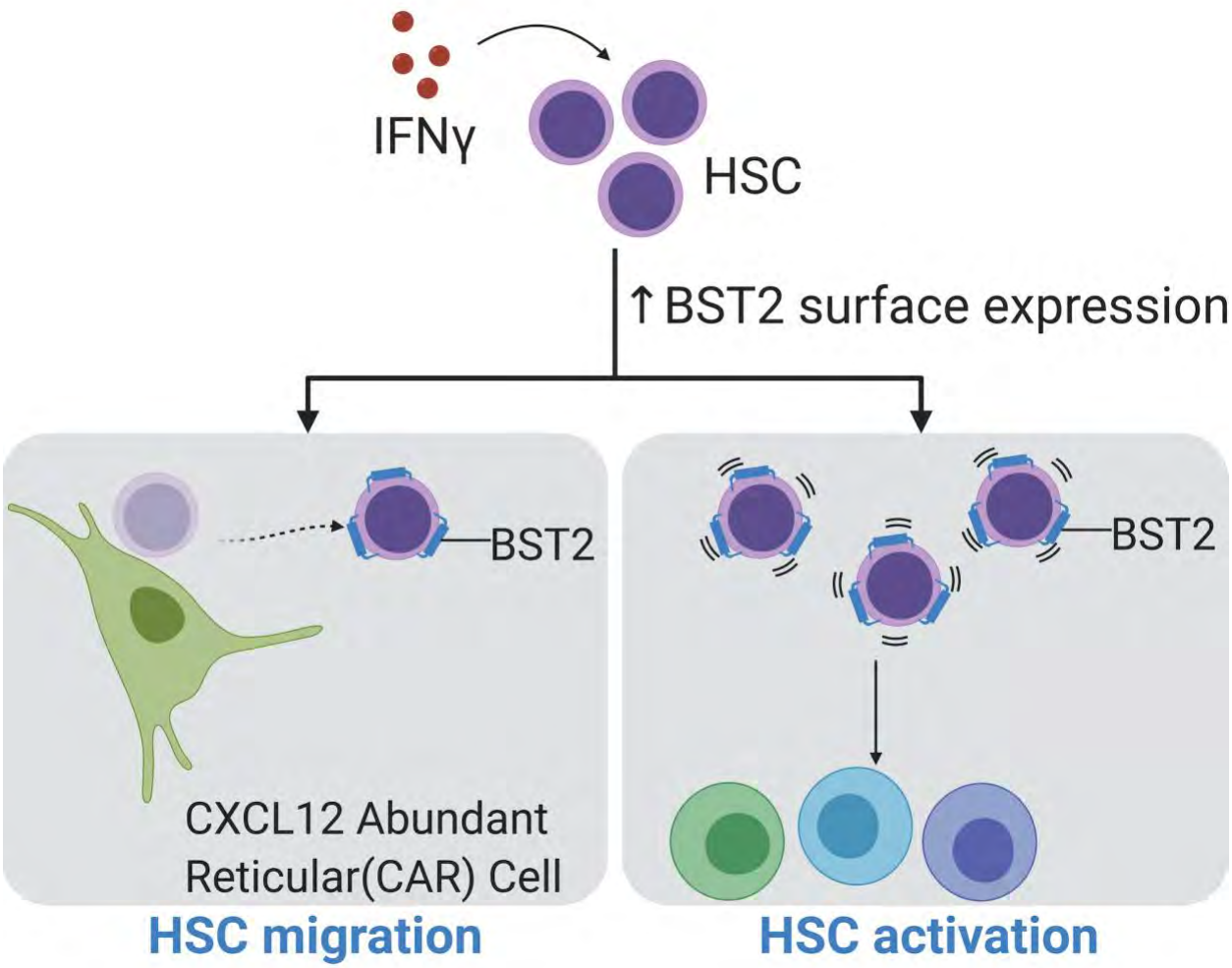

Poster 12

NF-κB Inducing Kinase Maintains T Cell Metabolic Fitness by Regulating the Redox System

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Abstract

Metabolic reprograming towards aerobic glycolysis is a pivotal mechanism shaping immune responses. Here we show deficiency in NF-*κB* B-inducing kinase (NIK) impairs glycolysis induction, rendering $CD8^+$ effector T cells hypofunctional in tumor microenvironment. Conversely, ectopic expression of NIK promotes $CD8^+$ T cell metabolism and effector function, thereby profoundly enhancing antitumor immunity and improving the efficacy of T cell adoptive therapy. NIK regulates T cell metabolism via an NF-*κB* B-independent mechanism that involves stabilization of hexokinase 2 (HK2), a rate-limiting enzyme of the glycolytic pathway. NIK prevents autophagic degradation of HK2 through controlling cellular ROS levels, which in turn involves modulation of glucose-6-phosphate dehydrogenase (G6PD), an enzyme mediating production of the antioxidant NADPH. We show that the G6PD-NADPH redox system is important for HK2 stability and metabolism in activated T cells. These findings establish NIK as a pivotal regulator of T cell metabolism and highlight a posttranslational mechanism of metabolic regulation.

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Poster 13

Does Microbiome Contribute to Immune Dysregulation in Common Variable Immunodeficiency?

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Background: Common Variable Immunodeficiency (CVID) is a B-cell defect defined by low immunoglobulins (IG), inadequate specific antibody response, and absent gut plasma cells. CVID patients present with recurrent infections. Around 60% have additional non-infectious complications (NIC) marked by autoimmunity and inflammation. Compared to infections-only, NIC-CVID patients have atypical gut microbial composition (dysbiosis), impaired gut mucosal barrier, and less diverse gut microbes.

Hypothesis/Goals: Dysbiotic gut microbiome from NIC-CVID patients promotes mucosal immune dysregulation, mucosal barrier disruption, and systemic inflammation. Our goal is to determine the impact of dysbiosis on intestinal and systemic inflammation in NIC-CVID, using fecal material transplant (FMT) from CVID patients to Germ-Free (GF) mice.

Methods: We measured IG isotypes using ELISA, immune cells from mesenteric lymph nodes (MLN) were measured using flow cytometry. 16srRNA gene sequencing was used for microbiome analysis.

Results: In this pilot study**,** GF(*C57Bl/6J)* mice (n=4/group) were orally gavaged (3 times over one week at 200µl/dose) from either NIC or infections-only CVID or healthy donors. At baseline, GF mice had deficient serum IgA levels compared to WT, while Serum IgG was not statistically significantly different between WT and GF. Thirty days following FMT, all IG isotypes increased in GF mice. Flowcytometry from MLN of NIC-FMT recipient showed a trend towards lower absolute numbers of CD19+, CD3+, CD11b+, and CD103+. Microbiome analysis and histology are pending.

Conclusions: Higher serum IgG in NIC/Infections-only FMT recipients compared to those from controls suggests that those mice developed inflammatory status post-FMT. The lower Dendritic-cell subsets and B-cells in NIC-FMT recipients suggest immune dysregulation. Together, microbiome transfer from NIC-CVID patients to GF mice might help model immune dysregulation observed in NIC-CVID.

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Poster 14

Induction of Multiple Cytotoxic Effector Responses Underlying Curative Efficacy Against Established HPV Oral and Genital Cancers by a Novel Therapeutic HPV Peptide Vaccine Formulation

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Background. High-risk type human papillomaviruses (HPV) are the underlying cause of >5% of all cancers including cervical and Head & Neck cancers in which virus-encoded E6/E7 oncoproteins serve as tumor-specific antigens. Despite the success of prophylactic HPV vaccine, therapeutic strategies targeting the E6/E7 have been ineffective in established HPV+ tumors due mainly to inadequate antigen-specific immunity. Therefore, enhancing the therapeutic potential of HPV vaccines to achieve curative efficacy is an unmet clinical need.

Hypothesis. We hypothesized that the combination of diverse acting adjuvants will stimulate potent antitumor cytotoxic immunity by therapeutic HPV peptide vaccine.

Methods. We used well-established HPV16 E6/E7 expressing mEER and TC-1 tumors in immunocompetent syngeneic murine models for oropharyngeal and cervical cancers, respectively for testing therapeutic HPV peptide vaccine formulated with novel adjuvant combinations and multi-parametric flow cytometry for the analyses of cellular immune correlates.

Results. We present here preclinical evidence for efficient clearance of HPV tumors at the vaginal (TC-1) and oral (mEER) mucosal tissues by intranasal vaccine using HPV16 E6 and E7 peptides along with the combination of clinically relevant adjuvants QS-21 and CpG-ODN. This vaccine exhibited high therapeutic efficacy with >80% mice with oral or vaginal tumors showing regression as well as significant extended survival advantage over untreated mice or mice treated with vaccine containing single adjuvants. This therapeutic effectiveness was associated with robust increases in the overall as well as antigen-specific (E7 tetramer+) cytotoxic CD8 T cells expressing Granzyme B and/or IFNg in both intra-tumoral and systemic compartments in vaccine-treated mice. The intranasal vaccination containing the combination of adjuvants induced significant frequencies of a unique subpopulation of NK cells expressing CD11c, granzyme B and IFNg, termed NKDC. Moreover, depletion experiments using anti-CD8 and anti-NK1.1 (PK136) showed both CD8 and NK cells play important roles in the mechanism of action of the vaccine.

Conclusions. Our results suggest that mucosal HPV E6/E7 peptide vaccine formulation containing QS-21+CpG-ODN adjuvants provides an effective therapeutic strategy for the treatment of patients with HPV+ oropharyngeal and cervical cancers.

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Poster 15

MicroRNA let-7bc2-Cluster controls airway inflammation and COPD in an intrinsic manner in T cells

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Background: Emphysema is a progressive and incurable endotype of chronic obstructive pulmonary disease (COPD) related to cigarette smoke (CS) and air pollutants. We recently demonstrated that nano-sized carbon black (nCB) particles generated by incomplete combustion of tobacco can trigger emphysema in mice. The mechanism for nCB-mediated emphysema development includes the differentiation of autoreactive T helper 1 (T_H1), T_H17 cells by $CD11c^+$ conventional dendritic cells (cDCs) and alveolar macrophages. Expanding on these observations we investigated the importance of *let-7b and let-7c2-cluster (let-7bc2)* within $CD4^+$ T cells in the pathogenesis of emphysema in mice.
Hypothesis: Absence of let-7bc2-cluster in CD4+ T cells enhances experimental emphysema.

Methods: To determine if *let-7b* and *let-7c* are dysregulated in an experimental emphysema model, we compared their expression in lung-derived $CD4^+$ T cells of nCB treated mice to vehicle (PBS) controls. To study the role of *let-7bc2*-cluster in $CD4^+$ T cells we use floxed *let-7bc2-cluster* mice crossed to *CD4-Cre* strain and evaluated their response in experimentally induced emphysema with nCB or cigarette smoke exposure. Mice were then analyzed for alveolar destruction, T_H1/T_H17/iTreg responses and gene expression changes in T cells.

Results: Our data revealed downregulation of *let-7bc2*-cluster expression in emphysematous $CD4^+$ T cells. Notably, compared to wild-type (WT) mice, *let-7bc2$^{f/f}$; CD4-Cre* mice showed exaggerated airway inflammation and alveolar destruction in both nCB and CS murine models of emphysema. On the other hand flow cytometric analysis of major $CD4^+$ T cell effector lineages including T_H1/T_H17/iTreg were similar to WT mice. On the other hand, *let-7bc2*-deficient $CD4^+$ T cells showed enhanced transcript levels of *Eomes* a known target of *let-7*.

Conclusion: These findings reveal an essential role of *let-7bc2-cluster* in $CD4^+$ T cells as a modulator of airway inflammation and emphysema. We are currently exploring the possibility that absence of *let-7bc2* exacerbates cytotoxic $CD4^+$ T cell response via posttrancriptional targeting of *Eomes* to promote emphysema.

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Poster 16

Roles for Leukemia Inhibitory Factor in Intestinal Immunity

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Background: Leukemia inhibitory factor (LIF) is a member of the interleukin-6 cytokine family which has the ability to repress endotoxin-induced inflammatory responses *in vitro* and induce an immunosuppressive phenotype in tumor-associated macrophages, inhibiting the antitumor response in mice. Our group has observed that LIF protein levels significantly rise in the colon and serum of mice infected with *Citrobacter rodentium*; however, roles for LIF in intestinal immunity are unknown. Single-cell RNA sequencing data generated in our lab, which focused on colonic lamina propria immune cells, suggests $CX3CR1^{+}$ macrophages and dendritic cells (cDCs) are major LIF receptor (LIFR)-expressing populations. Therefore, we generated CD11c cre^{+} $Lifr^{f/f}$ mice, which drive *Lifr* deletion in cells such as intestinal macrophages and dendritic cells. In homeostatic conditions, we observed increased $Ly6C^{lo}$ monocytes in the blood and mesenteric lymph nodes of CD11c cre^{+} $Lifr^{f/f}$ mice compared to controls. Moreover, CD11c cre^{+} $Lifr^{f/f}$ mice challenged with *C. rodentium* via oral gavage have increased bacterial dissemination to the spleen and liver and higher IL-23 protein levels in the colon compared to controls. **Hypothesis:** LIFR signaling in $CD11c^{+}$ cells promotes intestinal homeostasis and, during infection, LIFR signaling in $CD11c^{+}$ restrains excessive IL-23 production, protecting the host from increased bacterial dissemination. **Results:** We found that LIF stimulation of $CD103^{+}$ cDCs generated *in vitro*, as well as splenic cDC1s decreases tumor necrosis factor α (*Tnfa*) mRNA expression, suggesting LIFR signaling represses the expression of this pro-inflammatory cytokine. Moreover, exposure of $CD103^{+}$ cDCs to LIF results in reduced protein expression of the costimulatory molecules CD40 and CD86 compared to unstimulated controls, as measured by flow cytometry. **Conclusions:** These data suggest LIF is induced during *C. rodentium* infection to help restrain pro-inflammatory responses, and protect the host from excessive inflammation. Future experiments aim to delineate the importance of LIFR signaling in controlling *C. rodentium* infection and understanding how LIFR signaling modulates the immune landscape.

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Poster 17

A Unique Subset of Cytotoxic Effector Memory T-cells Enhances CAR T-cell Function against Pancreatic Ductal Adenocarcinoma

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In humans, the NK cell marker CD161 identifies several subsets of T-cells including a polyclonal CD8 αβ T-cell receptor expressing subset with characteristic specificity for tissue-localized viruses. This subset has also been reported to display enhanced cytotoxic and memory phenotypes. Here we characterized the biology and functional properties of this unique T-cell subset while determining its potential suitability for use in CAR T-cell therapy. In mice, gene expression profiling among the CD161 equivalent $CD8^+$ T-cell populations ($CD8^+NK1.1^+$) revealed significant upregulation of granzymes, perforin, killer lectin-like receptors and innate signaling molecules in comparison to $CD8^+NK1.1^{neg}$ T-cells. Adoptive transfer of $CD8^+NK1.1^+$ cells from previously exposed animals offered significantly enhanced protection and improved survival against melanoma tumors and influenza infection compared to $CD8^+NK1.1^{neg}$ cells. To determine if this subset might be utilized to enhance the antitumor efficacy CAR T-cell therapy in the solid tumor setting, bulk PBMC, $CD8^+CD161^{neg}$, and $CD8^+CD161^+$ T-cells were transduced with a HER2-specific CAR construct and compared. *In vitro*, $CD8^+CD161^+$ CAR-transduced T-cells killed $HER2^+$ targets faster and with greater efficiency. Similarly, *in vivo* these cells mediated substantially enhanced anti-tumor efficacy and survival in xenograft models of $HER2^+$ pancreatic ductal adenocarcinoma. The data suggest that this unique T-cell subset might present an opportunity to enhance CAR T-cell therapy for the treatment of solid tumors.

Poster 18

Batf2 Drives Depletion of Hematopoietic Stem Cells During Chronic Infection

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Patients with chronic infections, which affect over 2 billion people worldwide (CDC), often develop bone marrow (BM) suppression and pancytopenia, a depletion of platelets, red and white blood cells, resulting in increased susceptibility to infection (Achi et al., *Rev Mal Respir*, 2013). However, the molecular mechanism of infection-induced BM suppression is not fully understood. Using a mouse model, we previously demonstrated that HSCs are depleted during chronic infection due to excessive myeloid differentiation (Matatall et al., *Cell Reports*, 2016). We identified basic leucine zipper ATF-like transcription factor 2 (Batf2), an interferon-activated immune response regulator, as a key factor responsible for myeloid differentiation in chronic infection. Specifically, overexpression of BATF2 in mice promotes myeloid differentiation of HSCs. Conversely, CRISPR-based knock out of *BATF2* in human HSCs reduces interferon-gamma-dependent myeloid differentiation. However, the role of BATF2 in driving HSC exhaustion during chronic infection has never been studied.

Here, we hypothesize that BATF2 drives the depletion of HSCs through myelopoiesis in response to chronic infection. We infected WT and *Batf2* KO mice with *Mycobacterium avium* for four months. We found that compared to WT mice, *Batf2* KO mice were more resistant to thrombocytopenia, anemia, and overall loss of BM cellularity during chronic infection. Infected *Batf2* KO mice showed lesser pro-inflammatory cytokine productions, such as interferon-gamma and tumor necrosis factor-alpha, in peripheral blood than infected WT. Moreover, infected *Batf2* KO mice had smaller spleens and a reduction in granuloma size compared to infected WT. Interestingly, HSCs from *Batf2* KO mice were better conserved and maintained better transplant capacity than WT mice during chronic infection. On the other hand, the frequencies of myeloid-bias MPP3 subsets and granulocyte/monocyte progenitors in the BM were significantly lower in infected *Batf2* KO mice compared to infected WT. These results suggest that disrupting *Batf2* protects the HSC population from exhaustion during chronic infection. Interestingly, infected *Batf2* KO mice had lower bacterial loads in the spleen than WT. In future studies, we will examine Batf2-interacting proteins and transcriptional networks using co-IP and RNAseq. Understanding the mechanism underlying Batf2-mediated HSC differentiation during chronic infection will establish the foundation for a novel therapeutic approach to preserve the HSC population in the setting of chronic inflammatory stress.

Acknowledgements

- Funding: 2T32HL092332-16, R01 HL1348800, 1F31HL154991-01.

Poster 20

CD5 CAR T-cells Avoid Self-elimination By Continuously Degrading CD5 Protein

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Background

CARs specific to T-cell antigens may result in extensive fratricide of CAR T-cells, precluding their expansion. However, T-cells expressing CD5-specific CARs produce limited initial fratricide and then become resistant to self-targeting despite high CD5 expression prior to CAR transduction. Such minimal fratricide coincides with loss of detectable CD5 on the cell surface without affecting CD5 gene transcription, suggesting post-translational downmodulation of CD5 protein.

Hypothesis/Goals

We hypothesized that CD5 CAR T-cells downregulate CD5 antigen to evade fratricide. An improved understanding of these processes may guide future efforts to target additional T cell antigens that are currently excluded from consideration due to excessive fratricide.

Methods

Packing plasmids PegPAM and RD114 was used for retroviral transduction. Leica TCS SP8 confocol microscope and Fiji was used for time-lapse fluorescence microscopy. Trans-downregulation co-cultures were conducted at an effector:target ratio of 1:1 or 3:1. CRISPR knock-out was conducted using Cas9 with the Neon Transfection System. Soluble CD5 ELISA was conducted using cell supernatant from 48-hour single or co-cultures.

Results

Surface CD5 protein was rapidly aggregated and subsequently internalized in freshly transduced CD5 CAR T-cells. Western blot analysis indicated that ultimately, CD5 CAR T-cells completely remove CD5 protein ruling out epitope masking or intracellular sequestration of the antigen. Further investigation using an engineered CD5 variant containing myc-/FLAG-tags on the N-/C-termini confirmed CD5 is indeed entirely removed. Neither CAR nor CD5 signaling was required for antigen downmodulation as removal of intracellular signaling portions of each respective molecule did not ablate CD5 downregulation. CD5 CAR T-cells also induced *in trans* downmodulation of surface CD5 expression in healthy primary T-cells and malignant T-cell tumor lines suggesting this mechanism can limit availability of CD5 on target T-cells leading to resistance to cytotoxicity. Complete removal of CD5 protein *in trans* was observed in target T-cells expressing dual-tagged [N-]myc/[C-]FLAG CD5 co-cultured with CD5 CAR T-cells. Contrary to the *cis*-downmodulation, robust release of soluble CD5 protein was detected in culture supernatant during coculture of normal CD5+ T-cells with CD5 CAR T-cells, suggesting CD5 protein can also be shed or secreted from target cells upon contact with CD5 CAR T-cells.

Conclusions

This study unravels a novel mechanism of fratricide evasion in T-cells expressing a T lineage antigen-specific CAR mediated by continuous removal of target antigen. Understanding the mechanisms of fratricide resistance can inform design of other T lineage-specific CARs and improve outcomes in patients with T-cell malignancies.

Acknowledgements

Poster 20

CAGT T32, NIH NCI SPORE P50

Poster 21

LMW-E Potentiates Local Immune Responses Leading to an Immunosuppressive Microenvironment at the Early stages of Breast Tumorigenesis

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Background: Cyclin E is an independent predictor of worse outcomes and response to treatment in breast cancer. Expression of low-molecular-weight cyclin E (LMW-E) is associated with the presence of more aggressive tumor subtypes. While tumor infiltrating lymphocytes (TILs) are more abundant in LMW-E positive tumors, high-TIL/LMW-E positive tumors have lower probability of pathological complete response (pCR) to neoadjuvant chemotherapy.

Hypothesis/Goals: We hypothesized that LMW-E induces immune and inflammatory responses that alter the pre-tumor microenvironment (TME) in the mammary gland, thus promoting tumor initiation and subsequent growth. We aim to evaluate the role of mammary epithelial expression of LMW-E in producing inflammatory factors by phenotypic assessment of their cellular source and spatial localization in the mammary gland.

Methods: We generated a transgenic mouse model capable of conditionally expressing human LMW-E under the control of the MMTV promoter in a p53 heterozygous background (MPT) upon doxycycline (Dox) administration. Female MPT mice under Dox treatment for 3, 6 and 9 months and age-matched untreated controls were sacrificed at each time point. Mammary glands and peripheral immune organs were harvested to undergo immune profiling by flow cytometry and multiplex immunofluorescence microscopy (IF). Serum was also isolated from peripheral blood for cytokine/chemokine assessment. Immune profiling was performed using two different multi-color panels to assess basic immune and more specialized T-cell subsets.

Results: We report that although immune cell frequencies are changing over time, in accordance with changes in age of the mice, the total amount of CD45+ cells between Dox-on and Dox-off mice are very similar, hence indicating that immune cell accrual is independent of Dox treatment and LMW-E induction. However, the immune composition of LMW-E+ mammary gland suggests that there is a gradual enrichment in B cells, T cells (CD4+ cells, Tregs), macrophages, and pDCs over time. On the contrary, monocytes and cDC1 subsets have decreased in the pre-tumorigenic mammary gland of the 9-month LMW-E+ mice. We additionally observed an increase in PD-1+ T-cells and PD-1+/PD-L1+ macrophages mediated by LMW-E expression.

Conclusions: LMW-E potentiates local inflammatory alterations by inducing changes in the immune milieu of the mammary gland over time. Our results suggest that the immunological changes driven by LMW-E lead to an immunosuppressive microenvironment that may promote tumor formation at the early stages of breast tumorigenesis.

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Poster 22

Outcome of Concurrent Treatment with α-CTLA4 and Metronidazole in Murine Model of Colon Adenocarcinoma

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Background: Immune checkpoint blockade (ICB) therapy transformed clinical oncology by inducing durable responses and increasing survival rates in many types of cancers. However, ICB is effective in only in a subset of patients. Recent studies delineated the role of gut microbiome as both a biomarker and a therapeutic in ICB responsiveness. **Hypothesis/Goals:** We aimed to increase understanding of the microbiome-immune system axis in ICB therapy by using antibiotics to knock out certain components of gut microbiome. **Methods:** We treated MC38 colon adenocarcinoma-bearing mice with a widely-used antibiotic, metronidazole, that is effective against anaerobic and protozoal infections including *Clostridioides difficile* – a major mediator of colitis. Metronidazole was administered via oral gavage or mixed in drinking water before and after tumor injections. Mice received twice weekly treatment with α-CTLA4 ICB. **Results:** Metronidazole treatment alone slowed the growth rate of MC38 tumors, consistent with the current literature regarding colon cancer murine models. When metronidazole treatment was combined with α-CTLA4 therapy, we found ~90% complete tumor regression. In the metronidazole and α-CTLA4 combination group, we also observed an increase in the number of $CD103^+$ type 1 conventional dendritic cells (cDC1s) in colon lamina propria, which suggests enhanced antigen sampling from lumen. Also, in the mesenteric lymph nodes (mLN), we detected upregulation of CD80 and CD86 co-stimulatory molecule expression on $CX3CR1^+$ antigen presenting cells. Multiplex analysis of colon cytokines and colon pathology evaluation was comparable among groups, which implies a non-inflammatory environment in colon. Analysis of tumor-draining lymph nodes eight days after tumor injections showed higher expression of CD86 on $CD103^+$ cDC1s implying superior anti-tumor immunity. 16rRNA sequencing analysis of fecal samples revealed loss of *Lachnospiraceae* and enrichment of *Bifidobacteriaceae* and *Sutterellaceae* families in metronidazole-treated mice. **Conclusions:** Shifting microbiome composition with metronidazole treatment elicits a favorable anti-tumor immune response to α-CTLA4 treatment in murine colon adenocarcinoma.

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Poster 23

Molecular Dynamics and Ensemble Generation as a Tool to Select High Affinity T Cell Receptors Towards Cancer Immunotherapy

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Background: Cancer has a major impact on society and represents one of the biggest challenges for healthcare. Cancer immunotherapy arises as a promising intervention, which involves the "training" of the patient's own immune system to fight cancer cells, and one way to achieve better results is through the recognition of tumor-derived peptides and neoantigens by the T cell lymphocytes through T Cell Receptors (TCRs). Unfortunately, obtaining high affinity cancer-specific TCRs is a challenge. **Hypothesis/Goals:** The goal of this project is to apply a protocol involving molecular dynamics simulation and protein clustering to select an ensemble of TCR conformations, which can be used to guide the selection of higher affinity T-cells for cancer immunotherapy. **Methods:** We will select TCR sequences from T-cell pools, specifically EGFR-specific T-cells. The sequences will be modelled to obtain a three-dimensional structure. A molecular dynamics (MD) simulation will be set to produce at least three independent runs of 200 microseconds for each structure. Data form independent MD simulations will be gathered and ensembles will be built through data analysis based on dimensionality reduction with Principal Component Analysis (PCA) and K-means clustering. **Results:** We have implemented this protocol in a set of SARS-CoV-2-derived proteins as a proof-of-concept, showing that ensembles offer a better solution on docking-based approaches for drug screening. So far, we were able to sample thousands of conformations, creating highly diverse clusters of proteins. These clusters are being used as the receptor input in a server (http://dinc-covid.kavrakilab.org/) aiming to predict binding affinity with different drug targets. **Conclusions:** We show that our pipeline can be successfully applied in a system involving different proteins. The next step will be the application of the same approach in a system involving TCRs. **Acknowledgements:** This work was funded in part by the National Science Foundation (NSF Award number 2033262), by the Cancer Prevention & Research Institute of Texas (CPRIT), by the National Council for Scientific and Technological Development (CNPq, Brazil), and by Rice University funds. MMR is supported by a Computational Cancer Biology Training Program fellowship (CPRIT Grant No. RP170593).

Poster 26

Novel Antifungal Strategies for Airway Mycosis

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Background

Airway mycosis is a major underlying cause of asthma and other inflammatory diseases. Antifungal therapy is often highly effective for asthma and chronic rhinosinusitis, but a significant minority of patients are refractory to antifungal therapy, presumably due to resistance. Improved antifungal protocols are needed to more effectively treat airway mycosis-related diseases

Hypothesis/Goals

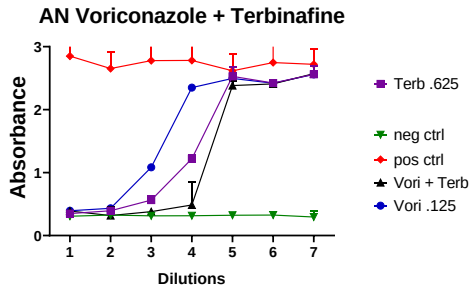
The goal of this study is to develop antifungal therapy protocols with enhanced efficacy against fungal asthma. We hypothesize that combinations of standard antifungals with synergistic activity will more effectively kill fungi in vitro.

Methods

Using an improved in vitro fungistasis assay, we determined Minimum Inhibitory Concentrations (MICs) for different drugs already used in the clinic to treat fungal-related asthma including terbinafine, voriconazole, itraconazole, fluconazole, and amphotericin B. We further included cyclosporine A and azithromycin, which reportedly have antifungal properties, but are not routinely used as antifungal agents. Fungi were grown overnight in 2-fold concentrations of each drug, after which the intravital dye WST-1 was added for 4 hours and absorbance readings were obtained as measurements of fungal growth. We compared results from *Aspergillus niger*, *Candida albicans*, and *Candida auris*. After determining the MICs for each drug and organism, we combined two or more antifungal agents to determine additive, synergistic, and antagonistic effects.

Results

We discovered that standard antifungals differ markedly in effectiveness in a fungal species-dependent manner. The combination of voriconazole and terbinafine conferred synergy (Fig. 1), whereas the combination of voriconazole and amphotericin B proved to be additive, but only for *A. niger.* All other combinations with this species proved to be antagonistic. For *Candida* species, we discovered that all combinations were antagonistic.



Conclusions

We have developed an efficient, high-throughput assay for determining MICs of antifungal drugs against common fungal pathogens. Our findings confirm previously findings and demonstrate that this assay will be useful in identifying potentially synergistic combinations of novel antifungal agents currently under development that may be useful in the future treatment of airway mycosis-related diseases.

Poster 27

Genetic Ablation of let-7bc2-cluster in Alveolar Type 2 Cells Drives COPD-like Disease in Mice

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Background: Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung pathology characterized by airway obstruction and has become the third leading cause of death in the United States. Cigarette smoke, fumes, and atmospheric pollutants are the primary causes of emphysema which is the most severe endotype of COPD characterized by irreversible destruction of the alveoli sacs. From our work and others, the Let-7 microRNA family is dysregulated in human and mouse lung tissue with COPD. In our preliminary work, absence of the *let-7bc2-cluster* in myeloid or T cells predisposes mice to emphysema. Epithelial cells are known to regulate immune responses in the airway, drive lung homeostasis, and injury repair mechanisms. In particular, alveolar type 2 (AT2) cells are critical progenitors for renewal of damaged alveolar cell communities. Here, we aim to determine whether let-7bc2-cluster also controls emphysema in AT2 cells.

Hypothesis: We hypothesize that absence of *let-7bc2-cluster* in alveolar type 2 cells will predispose mice to alveolar destruction and inflammation in context of nCB or CS-exposure but not in naïve mice.

Methods: We generated a tamoxifen-inducible AT2-specific *let-7bc2-cluster* conditional knockout mouse by crossing *Sftpc-CreER*^{T2} mice with our *let-7bc2*$^{flox/flox}$ mice (*let-7bc2*^{AT2}). Eight week old *let-7bc2*^{AT2} mice were treated by i.p. with tamoxifen every other day for five doses and then again after 4 weeks. At 16 weeks of age *let-7bc*$^{flox/flox}$ control and *let-7bc2*^{AT2} mice lung tissue was collected for histomorphometric scoring of mean linear intercept (MLI) as well as molecular gene expression.

Results: The *let-7bc2*^{AT2} mice treated with tamoxifen showed a reduction of *let-7bc2-cluster* gene expression in whole lung tissue in comparison to *let-7bc*$^{flox/flox}$ mice and re-affirms our mouse model. To our surprise, tamoxifen-treated *let-7bc2*^{AT2} provoked significant airspace enlargement in comparison to control mice upon unbiased morphometric MLI measurements. Furthermore, masson trichrome stained lung sections also revealed the presence of leukocytes/macrophages in lung parenchyma and we observed a significant induction in *Mmp12* and *IL1b* transcript levels in lungs of *let-7bc2*^{AT2} mice. Mechanistically, lungs of *let-7bc2*^{AT2} also showed dramatic downregulation of *Socs1* mRNA levels and induction of the *let-7* target *lin28a*.

Conclusions: Our data suggests that absence of *let-7bc2-cluster* in AT2 cells provokes age-dependent lung inflammation and a COPD-like phenotype. We are exploring the notion that *let-7* controls cellular senescence and regeneration via a *lin28a*/Socs1 regulatory circuit in AT2 cells.

Acknowledgments: This project was funded through the Gilson Longenbaugh Foundation, a R01 grant from NHLBI (R01HL140398-01A1), and a T32GM136554 NIGMS.

Poster 28

Removal of CD45RA PBMCs Enables the Generation of Epstein-Barr Virus Specific T-cells from Patients with EBV+ Lymphoma

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Background: In a clinical trial (NCT01555892) for EBV+ lymphoma, we have used T-cells targeting the four viral type 2 latency proteins (T2-Ags) that are expressed in these tumors. However, we were unable to manufacture T2-EBVSTs from ~25% of patients, due to lack of antigen specificity in the final cell product or failure of the cells to grow. Even EBVSTs that met release criteria had low T2-Ag specificity.

Hypothesis: We hypothesized that low antigen specificity resulted from the outgrowth of non-specific bystander cells and that enrichment of memory T-cells prior to EBVST stimulation would improve antigen specificity and hence potency.

Methods: We removed the CD45RA+ fraction of PBMC that includes naïve T-cells and NK cells, prior to two stimulations with overlapping peptide libraries representing EBV T2-Ags on days 0 and 9. From day 16, we compared the specificity and potency of CD45RA-depleted (RAD) EBVSTs with that of EBVSTs generated from whole PBMCs (W-EBVSTs).

Results: RAD-EBVSTs demonstrated a 2-10 fold higher frequency of T2-Ag specific T-cells than W-EBVSTs as measured in IFN-y ELIspot assays, correlating with enhanced proliferation, polyfunctionality and antigen specific cytotoxicity. Notably, CD45RA depletion enabled the outgrowth of T2-Ag-specific T-cells from patient PBMCs that previously failed to respond to stimulation (Fig.1). Following adoptive transfer into a murine xenograft model, RAD-EBVSTs produce more rapid tumor clearance of autologous EBV+ tumor cells, with decreased metastasis than mice receiving W-EBVSTs. TCR-β chain sequencing revealed major differences in the most abundant clonotypes of RAD-EBVSTs and W-EBVSTs, despite similar frequencies of those clonotypes in the starting PBMCs. Instead, most high frequency clonotypes in W-EBVSTs were identical to those found in "EBVSTs" derived from CD45RA+ PBMCs that lacked apparent EBV specificity.

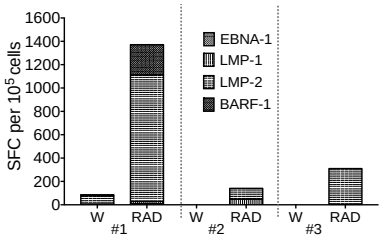

Fig.1: Bar graph demonstrating IFN-y releasing cells as a count of Spot forming cells (SFC) in response to EBV T2-Ag stimulation via ELIspot assay. Y-axis represents SFC per 10^5 cells and X-axis represent W-EBVSTs (W) and RAD-EBVSTs (RAD) generated from EBV+ lymphoma patients (Patient #1,#2,#3).

Conclusions: Removal of CD45RA+ subsets prior to EBVSTs generation enhanced the antigen specificity and potency of EBVSTs. This could be explained by the removal of proliferative bystander cells or by the presence of a component of CD45RA+ cells that inhibits the reactivation and clonal expansion of T2-Ag specific T-cells. Regardless, CD45RA depletion has increased the manufacturing success rate in our clinical trial, which has now been amended to include CD45RA depletion, and we will determine if our preclinical findings translate into increased tumor efficacy in patients.

Acknowledgment: This work was funded and supported by CPRIT RP160283- Baylor College of Medicine Comprehensive Cancer Training Program, NIH-NCI P50 CA126752, American Society of Gene and Cell Therapy (ASGCT) Career Development Award, and Tessa Therapeutics.

Poster 29

Suppressive Naïve Subsets That Inhibit EBV-Specific T-cells Are Associated with Heterogeneous Clonal Expansion

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Background: In our clinical trial using EBV-specific T-cells (EBVSTs) to treat EBV+ lymphoma, ~25% of EBVSTs failed to meet the release criterion of antigen specificity, or failed to grow at all. Removal of the CD45RA+ (RA) fraction of PBMC, that includes naïve T-cells, Tregs and NK cells, prior to EBVST generation enabled the outgrowth of T-cells with greater EBV-specificity even from patients who previously failed to produce EBVSTs.

Hypothesis & Methods: We hypothesized that RA subsets exert an inhibitory effect on the outgrowth of EBV-specific memory T-cells and that this inhibition manifests as a change in T cell receptor (TCR) repertoire. To uncover the effect of RA inhibition on EBVST generation and predict systems immunology mechanisms underlying this suppression, we used single cell ImmunoSEQ technology and clonal metrics to compare the TCR repertoire expanded from PBMCs, RA PBMCs and RA depleted PBMCs (RAD), after stimulation with EBV peptides and cytokines. Clonotypes were defined by the number of cells that share a common TCR within and between populations.

Results: Top clonotypes that expanded from RAD-PBMCs were low frequency or absent in EBVSTs derived from whole PBMCs, which surprisingly were more similar to those in EBVSTs derived from RA+PBMCs (Fig. 1A). Differences were also observed in clonality between subsets. The TCR repertoire of RAD-EBVSTs increased in clonality relative to RAD-PBMCs. This was not observed in both Whole and RA subsets, corresponding to heterogeneous clonal expansion (Fig. 1B). Fold expansion of clonotypes was greater in RAD subsets and shared clonotypes with greater than 10 fold expansion in RAD or Whole, exhibited greater expansion if they were expanded in the RAD subset.

Conclusions: CD45RA+ subsets inhibit the outgrowth of memory T-cells in W-EBVSTs, producing a heterogeneous repertoire of lower frequency clonotypes. Reasons for a difference in clonal expansion between subsets are simple outgrowth of bystander cells in the RA population or memory clonotype suppression by naiive subsets. The results suggest that PBMCs contain an RA-positive suppressive component. The role of which is unknown, but it could be important in ensuring that inappropriate, cross-reactive memory cells do not respond to new pathogens at the expense of a broad repertoire of low frequency naïve T-cells that is important for protection of the host.

Poster 29

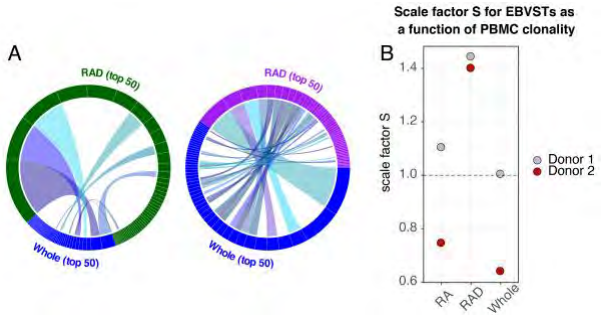

Fig. 1: A. Circos plots showing the top 50 clonotypes in EBVSTs, that were detected in PBMCs. Ribbons connecting segments indicate clonotypes shared between subsets. B. Multiplicative factor of EBVST clonality when written as a function of PBMC clonality. Points above and below S=1 correspond to an increase and decrease in clonality respectively.

Acknowledgement: This work was funded and supported by CPRIT RP160283- Baylor College of Medicine Comprehensive Cancer Training Program, NIH-NCI P50 CA126752 American Society of Gene and Cell Therapy (ASGCT) Career Development Award, and Tessa Therapeutics.

Poster 30

TRAF2 Regulates T cell Immunity by Maintaining a Tpl2-ERK Survival Signaling Axis in Effector and Memory CD8 T Cells

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Background: Generation and maintenance of antigen-specific effector and memory T cells are central events in immune responses against infections.

Hypothesis/Goals: To investigate the role of TRAF2 in antigen-specific CD8 T cell responses and immunological memory.

Methods: Naïve $CD8^+$ T cells were activated with plate bound anti-CD3 plus anti-CD28 antibodies for in vitro generation of effector and memory T cells; L. monocytogenes (LM-OVA) infection model and B16 tumor model in the wild-type and Traf2-TKO mice; Apoptosis assay and Immunoblot analysis of effector and memory $CD8^+$ T cells.

Results: We show that TNF receptor-associated factor 2 (TRAF2) maintains a survival signaling axis in effector and memory CD8 T cells required for immune responses against infections. This signaling axis involves activation of Tpl2 and its downstream kinase ERK by NF-κB-inducing kinase (NIK) and degradation of the proapoptotic factor Bim. NIK mediates Tpl2 activation by stimulating the phosphorylation and degradation of the Tpl2 inhibitor p105. Interestingly, while NIK is required for Tpl2-ERK signaling under normal conditions, uncontrolled NIK activation due to loss of its negative regulator, TRAF2, causes constitutive degradation of p105 and Tpl2, leading to severe defects in ERK activation and effector/memory CD8 T cell survival.

Conclusions: TRAF2 controls a previously unappreciated signaling axis mediating effector/memory CD8 T cell survival and protective immunity.

Acknowledgements: This study was supported by grants from the National Institutes of Health (AI64639 and GM84459), and the core facilities of MD Anderson Cancer Center are supported by the NIH/NCI Cancer Center Support Grant (CCSG) P30CA016672.

Poster 31

The Bacterial Microbiome Regulates Steady-state Hematopoiesis Via Immune Signaling Pathways

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Background: Up to 15% of patients treated with antibiotics (ABX) for ≥10 days develop neutropenia and other cytopenias, increasing medical costs and a patient's risk for deadly infections. Though a pressing medical problem, the mechanisms by which ABX induce these hematological complications remain poorly understood. Studies of germ-free mice and mouse models of ABX treatment revealed that depleting the bacterial microbiome suppressed bone marrow (BM) populations such as hematopoietic stem and progenitor cells and granulocytes. Further studies demonstrated that NOD1 and STAT1 signaling are required for the BM to receive signals from the microbiome. Absence of either microbial signals or these signaling pathways disrupts normal hematopoiesis.

Hypothesis/Goals: Both NOD1 and STAT1 signaling are associated with interferon (IFN) pathways. Thus, we hypothesized that IFNs are required for microbiome-mediated hematopoiesis.

Methods: We treated various IFN receptor knockout (KO) mice with our established ABX cocktail to assess through which IFN pathway STAT1 signals in microbiome-mediated hematopoiesis. We next tested whether small molecules NOD1 ligand (NOD1L), a motif of the bacterial cell wall component peptidoglycan, or desaminotyrosine (DAT), a metabolite that improves outcomes during infection in an IFN-α-dependent manner, were sufficient to rescue hematopoiesis in our ABX-treated mice by orally administering these small molecules to mice receiving ABX treatment.

Conclusions: Only the hematopoietic populations of IFN-alpha (IFN-α) receptor KO mice phenocopied the suppressed populations of ABX-treated mice, indicating that IFN-α signaling is required for normal hematopoiesis. Administration of DAT or NOD1L restored the granulocytic defects caused by loss of the bacterial microbiome in ABX-treated mice. Our findings demonstrate the microbiome requires IFN-α signaling to regulate hematopoiesis and identifies potential therapeutic interventions to treat neutropenia in patients on prolonged ABX regimens.

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Poster 33

TBKBP1 and TBK1 Form a Growth Factor Signaling Axis Mediating Immunosuppression and Tumorigenesis

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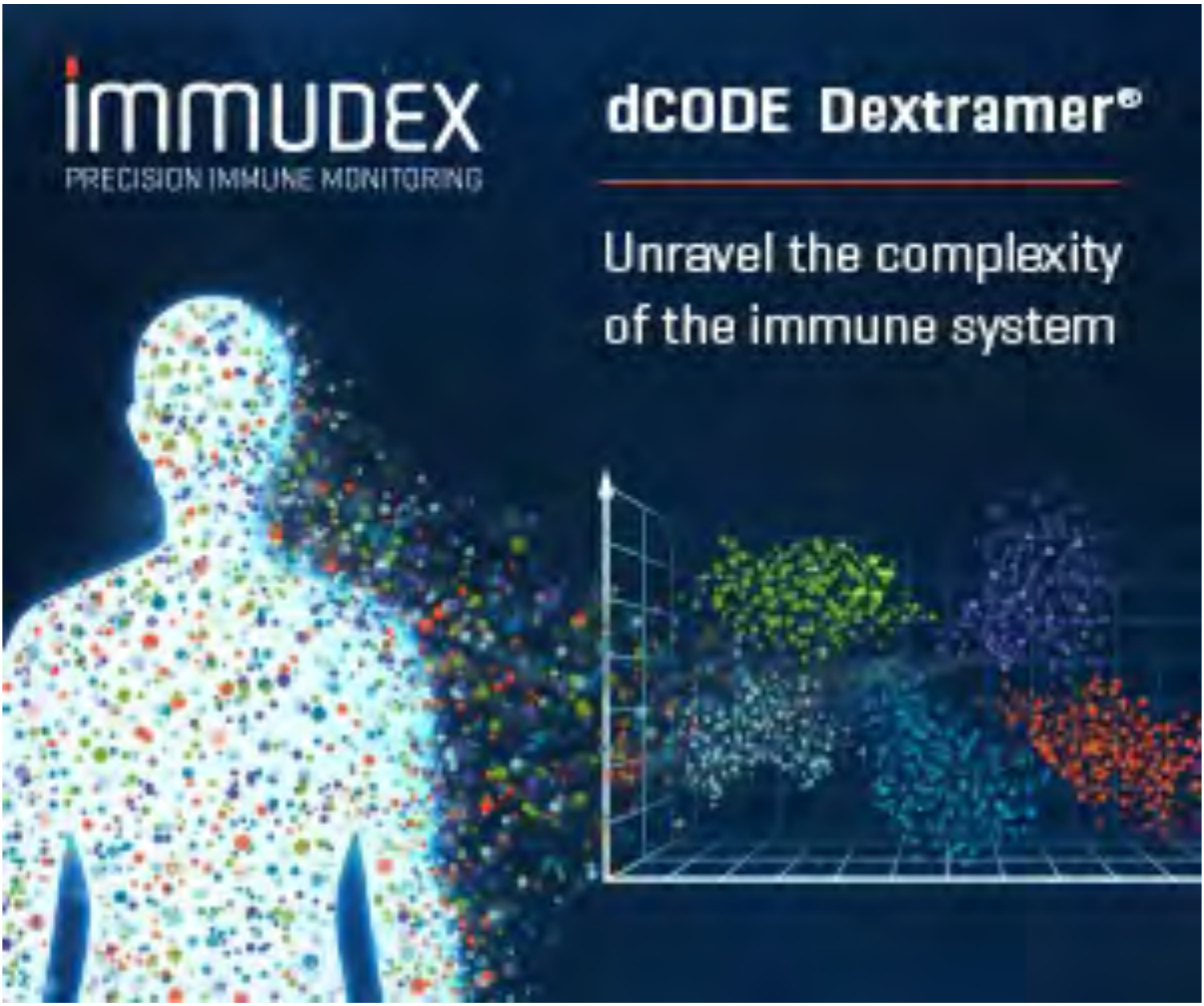
TANK-binding kinase 1 (TBK1) responds to microbial stimuli and mediates the induction of type I interferon (IFN). Here, we show that TBK1 is also a central mediator of growth factor signaling; this function of TBK1 relies on a specific adaptor—TBK-binding protein 1 (TBKBP1). TBKBP1 recruits TBK1 to protein kinase C-theta (PKCθ) through a scaffold protein, CARD10. This enables PKCθ to phosphorylate TBK1 at Ser 716, a crucial step for TBK1 activation by growth factors but not by innate immune stimuli. Although the TBK1–TBKBP1 signaling axis is not required for the induction of type I IFN, it mediates mTORC1 activation and oncogenesis. Conditional deletion of either TBK1 or TBKBP1 in lung epithelial cells inhibits tumorigenesis in a mouse model of lung cancer. In addition to promoting tumor growth, the TBK1–TBKBP1 axis facilitates tumor-mediated immunosuppression through a mechanism that involves induction of the checkpoint molecule PD-L1 and stimulation of glycolysis. Furthermore, TBK1-TBKBP1 axis mediates PD-L1 induction and glycolytic metabolism in cancer cells, which involves the mTORC1 pathway. These findings suggest a PKCθ–TBKBP1–TBK1 growth factor signaling axis that mediates both tumor growth and immunosuppression.



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