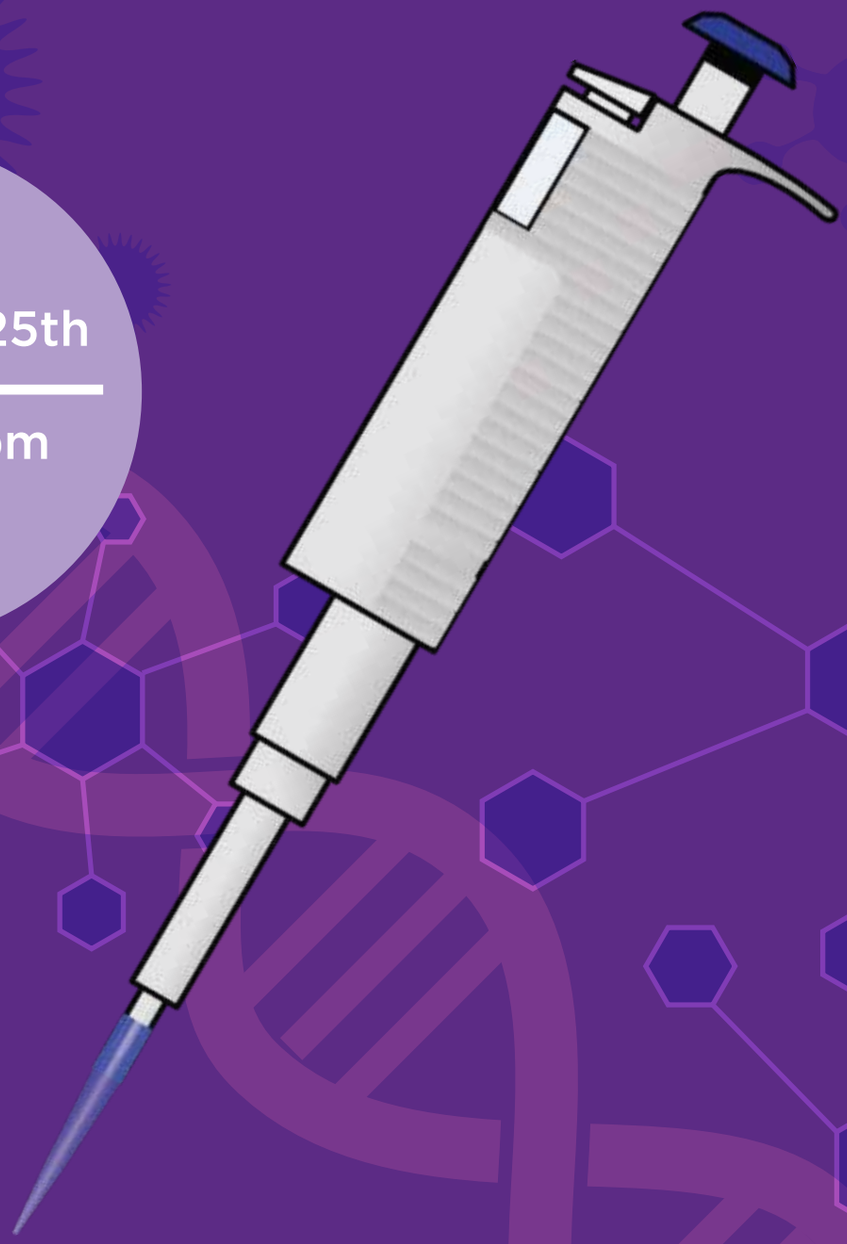
POSTDOC RESEARCH DAY 2019



September 25th

1:00-5:00pm

NYU Langone Health

OSR | OFFICE OF SCIENCE & RESEARCH

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Session 1- 1:00-2:00 pm

Abstract 1 Alice Easton, Microbiology, Changes to gut microbiome by Plasmodium vivax may be prevented by helminth infection

Alice V Easton, Mayra Raciny, Victor Liu, Erica Ruan, Ana Rodriguez, P'ng Loke*

Malaria affects the gut microbiome in mice, and both the gut microbiome and helminth infection may impact susceptibility to malaria. However, it is debated whether co-infection with helminths improves or worsen the clinical symptoms of malaria infection. We collected stool and blood samples from 130 individuals in the town of Tierralta, Colombia, split individuals into four groups: infected with Plasmodium vivax, helminths, both parasites, or neither parasite. Principal coordinates analysis (PCoA) showed that most microbiome samples were similar, but that a unique population existed among individuals infected only with malaria. We used a random forest machine learning model to identify the microbial taxa and clinical variables that were most important for differentiating between individuals in each group. Besides clinical markers of malaria infection, several microbial taxa were found to be important and investigated further. Clostridiales was significantly rarer, and Bacteroides was significantly more common, in individuals with malaria infections only (who were not co-infected with helminths). Many of the significant differences seen between the malaria-only group and the other groups were driven by the samples from the unique population of malaria-only samples identified by PCoA. This suggests that there may be a subpopulation of individuals for whom malaria infection causes large changes to the gut microbiome, but that this type of change is prevented by co-infection with helminths.

Abstract 2 Keenan Lacey, Microbiology, Investigating the host-pathogen interactions during nosocomial methicillin resistant Staphylococcus aureus pneumonia

Keenan A. Lacey, Frank Yeung, Bo Shopsin, Ken Cadwell, Victor J. Torres*

Pneumonia accounts for more deaths than any other infectious disease worldwide. Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most common causes of hospital-acquired pneumonia. To confront the growing problem of MRSA, we require a greater understanding of the host-pathogen interactions during infection. This remains poorly understood due to the lack of in vivo models relevant to infections occurring in healthcare settings. Most research to date has focused on highly virulent and cytotoxic MRSA strains, despite the fact that many nosocomial infections are caused by MRSA isolates that exhibit low cytotoxicity in ex vivo models and low virulence in mice. This study aims to functionally dissect host- and bacterial-directed mechanisms that lead to mortality in nosocomial settings. We have developed a nosocomial murine model of antibiotic conditioning, which lowers the barrier to infection, mimicking the nosocomial setting. We demonstrate that exposure to antibiotics mitigates the impact of reduced bacterial virulence in mice, allowing for a permissive environment for hospital adapted HA-MRSA isolates. These findings establish a robust model that is enabling us, for the first time, to probe the bacterial and host factors important during nosocomial infections, which are relevant to hospitalized patients that are also subjected to antibiotic regimes.

Abstract 3 Preeyam Patel, Microbiology, Eukaryotic Translation Initiation Factor 4E (eIF4E) is Required for Translation of BCL6 and Development of T Follicular Helper Cells in Infection, Autoimmunity, Allergy, and Immunization

Preeyam Patel, Beth Walters, Margo Orlen, Abhilash Gadi, Chris Park, Robert Schneider*

T follicular helper (TFH) cells require BCL6 and active mTOR for development and engagement of germinal center (GC) B cells, resulting in generation of high-affinity antibodies. When mTORC is active, eukaryotic translation initiation factor 4E (eIF4E) initiates translation. However, the requirement of eIF4E for translation of mRNAs necessary for TFH cell development has not been investigated. Disruption of eIF4E/eIF4G binding to the 5’ cap with drug 4EGI-1 inhibits TFH and GC B cell development during infection (Staphlococcus aureus), allergy (Aspergillus fumigatus), and immunization (Ovalbumin/alum), while having no effect on development of TH1, TH2, TH17, or Tregs. Silencing of eIF4E in only T cells is sufficient to inhibit formation of TFH cells, thus demonstrating the T cell intrinsic requirement for eIF4E. Mechanistically, 4EGI-1 inhibits translation of BCL6, but not other proteins required for TFH formation in both mouse and human lymph node T cells. While TFH cells have been implicated in the pathogenesis of multiple sclerosis (MS) in humans, their role in disease is unclear. 4EGI-1 administration in a mouse model of autoimmune encephalitis results in decreased infiltration of T cells into the spinal cord and demyelination. Thus, inhibiting translation of BCL6 and development of TFH cells with 4EGI-1 could be effective in treatment of autoimmunity.

Abstract 4 Karan Singh, Cell Biology, Role of Immune Cells in Marfan-Associated Thoracic Aortic Aneurysm

Karan Singh, Lior Zilberberg, Masahito Horiguchi, Colin K. L. Phoon, Daniel B. Rifkin*

Background: Marfan syndrome (MFS) is a genetic, connective tissue disorder, which can lead to thoracic aortic aneurysm (TAA). The etiology and pathogenesis of TAA is unknown, but there is an increase in elastic fiber fragmentation in the aortic media. It has been documented that increased numbers of immune cells (B cells, T cells, and macrophages) accumulate in the aortas of individuals with MFS compared to controls. However, it is unclear how or whether these cell types (and which cell types) contribute to TAA.
Methods: We investigated the recruitment of immune cells in the ascending aorta from MFS mice (Fbn1mgR/mgR) relative to wild type (Wt) mice using qRT-PCR to measure immune cell specific gene expression. To determine the role of B and T cells in TAA, we generated Fbn1mgR/mgR;Rag2-/- mice, which have no mature B and T cells. To test if the absence of these cells attenuates or ameliorates TAA, we assessed elastin fragmentation, collagen accumulation, aortic dilation, and animal survival. Results: mRNA expression of specific immune cell markers (B cells (CD19), T cells (CD3, CD4 CD8, macrophages (F4/80),) indicates higher numbers of T cells , and macrophages in the ascending aortas of Fbn1mgR/mgR relative to Wt mice. When we removed B and T cells by crossing Fbn1mgR/mgR mice with Rag2-/- mice, we observed more than 90% of Wt and Rag2-/- mice survived at 120 days, whereas more than 60% of Fbn1mgR/mgR mice died by 120 days. Interestingly, survival of Fbn1mgR/mgR;Rag2-/- mice was roughly equivalent to Wt and Rag2-/- mice. Masson's Trichrome staining demonstrated decreased collagen accumulation in the ascending aortas of Fbn1mgR/mgR;Rag2-/- mice relative to the ascending aortas of Fbn1mgR/mgR mice. However, we did not find a significant difference in the elastin fragmentation and aortic dilation between Fbn1mgR/mgR;Rag2-/- and Fbn1mgR/mgR mice.
Conclusions: Aortas from Fbn1mgR/mgR mice contain increased numbers of immune cells compared to Wt aortas as measured by gene expression. The absence of B and T cells increases survival in Fbn1mgR/mgR mice, but has no effect on elastin fragmentation. Loss of B and T cells is also associated with decreased medial collagen, implying that mediators from the immune cells are responsible for the increase in matrix. Ongoing investigations are assessing whether T cells or B cells, or both, are responsible for the aortic pathology in Fbn1mgR/mgR mice.

Abstract 5 Elizabeth Vink, Microbiology, Differential Contribution of Host Ribosomal Proteins to HSV-1 Replication and mRNA Translation

Elizabeth Vink, John Andrews, Carol Duffy, Ian Mohr*

Like all viruses, Herpes Simplex Virus 1 (HSV-1) is entirely reliant upon host cell ribosomes to produce viral polypeptides. Although the virus goes to great lengths to gain control over cellular translation initiation factors, how the ribosome itself might impact infected cell protein synthesis is unknown. Indeed, while many of the approximately 80 ribosome proteins (RPs) are dispensable for ribosome function, mounting evidence suggests that ribosome heterogeneity created by substoichiometric levels of RPs may regulate translation by influencing ribosome selectivity. Here, we present evidence that HSV-1 targets the ribosome itself to regulate infected cell protein synthesis. An siRNA screen targeting RPs revealed that while depleting individual RPs largely inhibited protein synthesis in uninfected cells, translation in HSV-1-infected cells was on average only impacted minimally. Unexpectedly, this resistance of protein synthesis in HSV-1-infected cells to RP-depletion was dependent upon expression of HSV-1 late protein VP22. In contrast to most RPs, which were dispensable for productive virus growth, the two ribosome stalk proteins, RPLP1 and RPLP2, were needed for efficient completion of the virus life cycle. Together, these results highlight the differential requirements for individual RPs during infection and hint that a viral pathogen may target ribosome function directly.

Abstract 6 Ada Weinstock, Medicine (Cardiology), Caloric restriction promotes atherosclerosis resolution and induces enrichment of phagocytic macrophages in the adipose tissue

Ada Weinstock, Emily Brown, Ozlem Tufanli Kireccibasi, Stephanie Pena, Michela L. Garabedian, Cyrus A. Nikain, Beyza Vurusaner Aktas, Bianca Scolaro, Edward A. Fisher*

Obesity is an independent risk factor for atherosclerosis, which is the main cause of death worldwide. Superficially, both obesity and atherosclerosis share many immune characteristics that drive both disease progression and its resolution, but a molecular understanding of the relationship between leukocytes in atherosclerotic lesions and adipose tissue is incomplete. In this study we investigated whether resolution of adipose tissue inflammation influences the inflammatory state in atherosclerotic lesions. To do so, we uncoupled weight loss and plasma cholesterol levels after atherosclerosis and obesity were established, using caloric restriction (achieved by feeding 30% less of the same high-fat high-cholesterol diet). After 2 weeks of treatment, aortae and visceral adipose were harvested and analyzed. Our exciting results show that caloric restriction following obesity leads to resolution of atherosclerosis, despite persistent hypercholesterolemia. Additionally, single cell analysis showed that caloric restriction promotes the enrichment of a specialized phagocytic adipose tissue macrophage population. We hypothesize that these cells may be specialized to clear the apoptotic cells that accumulate during caloric restriction, thereby shielding the systemic release of inflammatory factors that could target other tissues, including the atherosclerotic plaques.

Abstract 7 Jessica Neil, Skirball Institute, IFN-I and IL-22 mediate protective effects of intestinal viral infection

Jessica A Neil, Yu Matsuzawa-Ishimoto, Elisabeth Kernbauer-Hölzl, Simone Dallari, Timothy J Nice, and Ken Cadwell*

Intestinal bacteria evoke immune signaling pathways from their host that promote immunity and barrier function in the intestine. How enteric viruses modulate host immune responses to contribute to intestinal homeostasis is unknown. Recently, we demonstrated that infection by murine norovirus (MNV) reverses intestinal abnormalities and protects against intestinal injury in mice deficient in bacteria, indicating that an intestinal virus can provide cues to the host that are typically attributed to the microbiota. However, protection from intestinal injury by MNV requires type I interferon (IFN-I) signaling and is mechanistically distinct from the beneficial effects of bacteria. Here, we elucidate mechanisms by which MNV evokes protective responses from the host. We identify an important role for the viral protein NS1/2 in establishing local replication and an IFN-I response in the colon. We show that this IFN-I response acts on intestinal epithelial cells to increase the proportion of CCR2-dependent macrophages and enhances IL-22 expression by ILC3. These responses promote pSTAT3 signaling in intestinal epithelial cells and protection from intestinal injury. Additionally, we demonstrate that MNV provides a striking IL-22 dependent protection against early life lethal infection by Citrobacter rodentium. These findings demonstrate novel ways in which a viral member of the microbiota fortifies the intestinal barrier during intestinal injury and infectious challenges.

Abstract 8 Maud Voisin, Microbiology, Reduced Liver X Receptor a (LXRa) S196 phosphorylation in bone marrow remodels atherosclerosis plaque and protects against obesity in mouse models

Maud Voisin, Elina Shrestha, Tessa J. Barrett, Claire Rollet, Tatjana Josefs, Hye Rim Chang, Cyrus Nikain, Rachel Ruoff, Michela Garabedian, Ira J. Goldberg, Inés Pineda-Torra, Edward A. Fisher and Michael J. Garabedian*

In atherosclerosis modified lipoproteins promote inflammation by triggering an immune response. Obesity shares features with atherosclerosis, including inflammation mediated by both innate and adaptive immune cells that contribute to adipocyte dysfunction. Whereas LXRα phosphorylation at S196 modifies its target gene repertoire, the functional consequences of LXRα S196 phosphorylation in atherosclerosis and obesity has not been examined. We used a LXRα S196A knockin mice as bone marrow donors into Ldlr-/- recipient mice on western diet to interrogate LXRα phosphorylation in atherosclerosis and obesity. Plaques expressing S196A showed less inflammatory macrophage recruitment, lipid accumulation, proliferation, and apoptosis leading to a partial reduction plaque size and macrophage content relative to WT recipients. RNA seq of CD68+ cells from LXRα S196A compared to WT plaques revealed a downregulation of genes involved in inflammation and an upregulation of genes implicated in mitochondrial activity. Ldlr-/- mice reconstituted with S196A on western diet also had lower body weight and less VAT. This was driven in part by the transcriptional reprograming of adipose tissue macrophages in LXRα S196A that reduced inflammation and fat accumulation. Thus, reducing LXRα pS196 in the bone marrow improved the metabolic environment in both atherosclerosis and obesity by reprogramming the transcriptional activity of LXRα.

Session 2- 2:00-3:00pm

Abstract 9 Tasleem Samji, Microbiology, Role of Long non-coding RNAs in the differentiation of CD8+ T cells

Samji TS, Brunson JC, Nwokocha M, Vera-Licona P & Khanna KM*

Up to 90% of the human genome consists of non-coding RNAs (ncRNAs) but surprisingly little is understood about how ncRNAs function in CD8+ T cells. CD8+ T cells are a central component of cell-mediated immunity against intracellular pathogens and tumors. In addition to immediate pathogen and tumor clearance by effector CD8 T cells, they form immunological memory providing long-term protection to the host. We study the role of long ncRNAs (LncRNAs) in differentiation and function of CD8+ T cells in response to infection. We have generated an RNA-Seq dataset from antigen specific CD8+ T cells following Listeria infection. Our data indicate that LncRNAs likely control the expression of transcription factors that are critical for the differentiation of T cells into effector and memory cells. We have identified LncRNA Zeb2os that is antisense to Zeb2, which is a transcription factor that is critical to the formation of effector T cells. We have shown that a reduction in LncRNA Zeb2os expression in CD8+ T cells leads to increased memory T cells. This study will open up new avenues for understanding the role of LncRNAs in regulating CD8+ T cell function and result in new therapeutic targets for immunotherapy against infections and cancer.

Abstract 10 Ioanna Tiniakou, Pathology, Genome-wide analysis of dendritic cell differentiation

Ioanna Tiniakou, Gorkem Garipler, Esteban O Mazzoni and Boris Reizis*

Dendritic cells comprise genetically and functionally distinct subsets, including the interferon-producing plasmacytoid DCs (pDCs) and the antigen-presenting classical DCs (cDCs). In the steady state, DC development from common progenitors and subset specification in the bone marrow is driven primarily by the cytokine Flt3 ligand (Flt3L) and its receptor Flt3. It remains unclear how the same signaling pathway supports both progenitor proliferation and DC lineage commitment, necessitating a systematic search for molecular regulators of the process.
The conditionally immortalized progenitor cell line HoxB8-FL can be induced to differentiate into functional pDCs and cDCs, providing a unique tool to study Flt3L-driven DC development. We have retrofitted HoxB8-FL cells with Cas9 and used it to conduct a CRISPR-based knockout screen with a genome-wide sgRNA library. Comparison of sgRNA content between undifferentiated progenitors and their DC progeny revealed highly significant differences, identifying candidate regulators of DC differentiation. Notably, sgRNAs targeting Pten were highly enriched in differentiated cells, suggesting that Pten deletion facilitates DC differentiation. These data are consistent with our previous identification of Pten as a negative regulator of cDC development.
Our data provide a proof of principle for the bona fide genome-wide genetic analysis of DC differentiation and suggest the utility of our system for the identification of novel pathways controlling DC development.

Abstract 11 Axel Concepcion, Pathology, Functional genomics screens to define the ion channelome in T cells and adaptive immunity

Axel R. Concepcion, Jun Yang, Ulrike Kaufmann, Jingjie Zhu, Marisa Mitchell-Flack, Menghan Liu, Martin Vaeth, and Stefan Feske*

Ion channels and transporters (ICTs) are proteins that facilitate the movement of ions across cell membranes. Ionic signals are crucial to regulate many aspects of cell physiology. There are more than 600 ICTs that control ions transport across membranes but only ~10 ICTs are well established to play a functional role in T cells based on genetic evidence in mice and humans. Here we use: 1) transcriptomic analysis and 2) an unbiased in vivo functional genomics screen as independent and complementary approaches to identify novel ICTs that regulate T cell function. 1) Comparative transcriptome analysis of the ion channelome in CD4+ T cells and other cell types and tissues uncovered the specific subset of ICTs in T cells, of which only some have been shown to regulate T cell homeostasis and function. Gene knockdown of candidate ICTs in pathogenic Th17 cells demonstrates that their deletion protects mice from experimental autoimmune encephalomyelitis (EAE). 2) By using an unbiased and independent in vivo functional genomics screen with the lymphocytic choriomeningitis virus (LCMV) in CD4+ T cells, we found 10-12 novel ICTs that regulate T cell expansion and survival in antiviral immunity. Validation experiments of individual candidates in vitro and in vivo confirm the relevant role of these ICTs in clonal expansion of T cells after viral infection. Overall, our results demonstrate that functional genomics is a powerful approach to identify novel ICTs that regulate adaptive immune responses in vivo and thereby new therapeutic targets for the purpose of immunotherapy.

Abstract 12 Silvana Valtcheva, Skirball, Hypothalamic oxytocin neurons respond to infant cries via noncanonical auditory pathway

Silvana Valtcheva and Robert C. Froemke*

Motherhood is a dramatic natural experience but little is known about the neural mechanisms supporting the recognition of different infant cues. Recent studies from our lab (Marlin et al., 2015) showed that the neurohormone oxytocin promotes long-term plasticity of neural responses to infant sounds in mouse auditory cortex in vivo. Release of oxytocin from the paraventricular nucleus (PVN) of the hypothalamus might help induce recognition of different infant cues. However, it remains unknown if infant vocalizations can activate oxytocin neurons.
Here we performed unprecedented in vivo cell-attached and whole-cell recordings from optically-identified oxytocin neurons in awake maternal mice using channelrhodopsin-assisted patching. Interestingly, repeated presentation of pup calls specifically induced a gradual increase in firing of individual oxytocin neurons but not of other PVN neurons. Using anterograde and retrograde virus tracing approaches combined with channelrhodopsin-assisted circuit mapping, we identified inputs which may drive auditory responses in oxytocin neurons. We describe a novel noncanonical auditory pathway potentially relaying acoustic information about social sounds to PVN oxytocin neurons. Our data suggest that oxytocin neurons differentially integrate auditory and somatosensory information which may be critical for the recognition of different infant cues, and for mediating peripheral and central oxytocin release.

Abstract 13 Elena Spina, Cell Biology, Identification of a unipotent basal stem cell population expressing an orphan adhesion GPCR associated with poor outcome in basal breast cancer

Elena Spina, Julia Simundza, Pamela Cowin*

Treating breast cancer has long involved understanding its complex heterogeneity. Basal-like constitutes the most aggressive breast cancer subtype. Microarray analysis have suggested that this subtype may arise from a population of mammary stem cell (MaSCs) and progenitors with plastic and long-lived nature undergoing transformation. We have found that high expression of an orphan adhesion G-protein coupled receptor (Gpr) correlates with particularly poor outcome within this subtype. To understand its biology we have generated knock out and reporter mice models for this receptor. Our data show that Gpr expression coincides with mammary specification and stem cell amplification through-out mammary gland development. Combining lineage tracing and three-dimensional (3D) imaging strategies we have found that Gpr-positive cells generate a basal lineage enduring over the different stage of mammary gland development (pregnancy,lactation,involution) and in aged mice. Gpr-positive parental cells display a MaSC profile and encompass several distinct populations with regenerative capacity. Thus, Gpr is a specific universal marker of unipotent basal mammary stem cells and has potential as biomarker of poor prognosis in breast cancer.

Abstract 14 Stephanie Orstad, Medicine - Division of General Internal Medicine and Clinical Innovation, Park proximity and use for physical activity among New York City residents: The moderating role of perceived crime on associations with mental health

Stephanie L. Orstad, PhD; Kosuke Tamura, PhD; Kristin Szuhany, PhD; Melanie Jay, MD MS*

Park proximity and use are associated with better mental health among urban residents. Meta-analyses suggest that physical activity (PA) reduces depression and improves quality of life. Despite the protective effects of PA on mental health, the added benefit of park-based PA is unclear. Thus, we examined whether park use for PA mediated associations between park proximity and mental distress, and whether perceived park crime moderated these associations. We analyzed data on 3,652 New York City residents (61.4% 45+ years, 58.9% female, 56.3% non-white) who completed the Physical Activity and Transit random-digit-dial survey in 2010-2011. Measures included self-reported walking time to the nearest park from home, concern about park crime during daylight, frequency of park use for sports/exercise/PA, and number of poor mental health days in the previous month. We used multiple linear regression with bootstrap-generated 95% bias-corrected confidence intervals (BC CIs) to test for mediation and moderated mediation. Perceived park proximity was indirectly associated with fewer poor mental health days via park use for PA, but only among those not concerned about park crime (B=.04; SE=.02; 95% BC CI=.01, .10). Promoting park-based PA and improving perceptions of crime in urban neighborhoods may help to maximize the mental health benefits of nearby parks.

Abstract 15 Linda Kahn, Pediatrics, Organophosphate pesticide exposure during pregnancy associated with total gestational weight gain

Linda G Kahn*, Akhgar Ghassabian, Yelena Afanasyeva, Mrudula Naidu, Shilpi S Mehta-Lee, Sara G Brubaker, Leonardo Trasande

Background: Excessive GWG is a potentially modifiable risk factor for perinatal complications. Endocrine disrupting chemicals such as OP pesticides, which are commonly applied to food crops, are potentially obesogenic. We hypothesized that prenatal exposure to OP pesticides would increase total GWG.
Methods: Three dimethyl (DM) and three diethyl (DE) OP metabolites were measured in spot urine samples collected at <18, 18-25, and >25 gestational weeks from participants in NYU CHES, an ongoing birth cohort study. Concentrations were summed to create DM, DE, and total dialkyl phosphate metabolite groups. GWG was calculated as the difference between first measured prenatal weight and weight at delivery, extracted from the EMR. All models controlled for total gestational length; adjusted models also controlled for education and prepregnancy BMI.
Results: Although only 140 women had exposure and outcome measures, our results suggest that early pregnancy exposure to OP pesticides may increase GWG. Women with high first trimester urinary DE metabolite concentrations experienced significantly greater GWG than women with low concentrations. The effect was similar for mean exposure across pregnancy, but not when covariate-adjusted. Increasing levels of first trimester urinary DM metabolites were associated with higher odds of excessive GWG according to 2009 Institute of Medicine guidelines.

Session 3- 3:00-4:00pm

Abstract 16 Keisuke Yamamoto, Radiation Oncology, Autophagy facilitates immune evasion of pancreatic cancer by downregulating MHC class I

Keisuke Yamamoto, Anthony Venida, Douglas E. Biancur, Julian Yano, Miwako Kakiuchi, Albert S.W. Sohn, Subhadip Mukhopadhyay, Elaine Y. Lin, Seth J. Parker, Rushika M. Perera, and Alec C. Kimmelman*

Impaired antigen presentation through loss of major histocompatibility complex class I (MHC-I) is a common cause of immune evasion and resistance to immune checkpoint blockade (ICB) therapy across multiple cancer types. Unlike other types of cancer, however, no causal mutations for MHC-I loss have been identified in pancreatic ductal adenocarcinoma (PDAC), a malignancy refractory to most therapies including ICB, despite frequent loss of MHC-I expression. Here we find that MHC-I molecules are selectively targeted for lysosomal degradation in PDAC through an autophagy-dependent mechanism. In PDAC cells, MHC-I predominantly localizes in the cytosol, trapped in both autophagosomes and lysosomes, and displays reduced cell surface expression. Notably, autophagy inhibition restores surface MHC-I levels, leading to improved antigen presentation, enhanced anti-tumour T cell response and reduced tumour growth in syngeneic hosts. Moreover, depletion of CD8+ T cells or reducing surface MHC-I expression in PDAC cells mitigates the autophagy inhibition-mediated tumour suppression. Importantly, autophagy inhibition synergized with ICB and led to an enhanced anti-tumour immune response. Our findings indicate that elevated autophagy/lysosome function facilitates immune evasion of PDAC through selective targeting of MHC-I molecules and provide a rationale for the combination of autophagy inhibition and ICB as a therapeutic strategy against PDAC.

Abstract 17 Harold Elias, Pathology, Identification of Old HSCs with Preserved Self-Renewal and Long-Term Reconstitution Potential

Harold K. Elias, Mohamed A. E. Ali, Joseph Y. Shin, Sohini Chakraborty, Caryn J. Ha, Colin Konishi, Christopher Y. Park*.

Aging hematopoiesis is characterized by increased numbers of immunophenotypic HSCs, but with impaired self-renewal and long-term reconstitution potential. We previously demonstrated that young mouse HSCs (CD34-CD150+LSK) can be fractionated into subsets based on expression of c-Kit surface expression, with c-Kithi HSCs exhibiting reduced self-renewal. We therefore hypothesized that the expansion of c-Kithi HSCs in old mice could potentially explain the age-related decline in immunophenotypically defined old HSC function. Evaluation of the bone marrow of 24-month-old C57Bl/6 mice revealed that the frequency of c-Kithi HSC (out of total HSCs) is 1.5-fold higher in old mice than in 3-month old mice (P=0.04). To test the long-term reconstitution potential of aging HSCs, we competitively transplanted c-Kitlo or c-Kithi HSCs from 24-month old mice, and observed that mice receiving old c-Kitlo HSC's exhibited significantly higher donor blood chimerism levels compared to old c-Kithi HSC recipients (57.1% vs 9.4%, P=0.02) and this was concordant with a 6.4-fold higher HSC chimerism achieved by old c-Kitlo HSCs. Mechanistically, preserved properties of stemness in c-Kitlo HSCs, was attributed to lower rates of global translation, as confirmed by OP-Puro assays. Overall, our studies demonstrate a functional heterogeneity among old HSCs and identify a novel strategy to prospectively fractionate old HSCs to further investigate the molecular mechanisms of HSC aging.

Abstract 18 Christina Glytsou, Pathology, Targeting mitochondrial structure sensitizes acute myeloid leukemia to Venetoclax treatment

Christina Glytsou, Xufeng Chen, Hua Zhou, Sonali Narang, Denis E. Reyna, Andrea Lopez, Theodore Sakellaropoulos, Yixiao Gong, Andreas Kloetgen, Yoon Sing Yap, Eric Wang, Evripidis Gavathiotis, Aristotelis Tsirigos, Raoul Tibes, and Iannis Aifantis*

The BCL-2 family plays important roles in acute myeloid leukemia (AML). Venetoclax, a selective BCL-2 inhibitor, has received FDA approval for the treatment of AML. However, drug resistance ensues after prolonged treatment, highlighting the need for a greater understanding of the underlying mechanisms. Using a genome-wide CRISPR/Cas9 screen in human AML, we identified genes whose inactivation sensitizes AML blasts to Venetoclax. Genes involved in mitochondrial organization and function were significantly depleted throughout our screen, including the mitochondrial chaperonin CLPB. We demonstrated that CLPB is upregulated in human AML, it is further induced upon acquisition of Venetoclax resistance and its ablation sensitizes AML to Venetoclax. Mechanistically, CLPB maintains the mitochondrial cristae structure via its interaction with the cristae-shaping protein OPA1, whereas its loss promotes apoptosis by inducing cristae remodeling and mitochondrial stress responses. Overall, our data suggest that targeting mitochondrial architecture may provide a promising approach to circumvent Venetoclax resistance.

Abstract 19 Sarah LeBoeuf, Pathology, Activation of oxidative stress response in cancer generates a druggable dependency on exogenous non-essential amino acids

Sarah E. LeBoeuf, Warren L. Wu, Triantafyllia R. Karakousi, Burcu Karadal, Kwok-kin Wong, Volkan I. Sayin, Thales Papagiannakopoulos*

Rewiring of metabolic pathways is a hallmark of tumorigenesis as cancer cells acquire novel nutrient dependencies to support oncogenic growth. Identifying how oncogenic mutations in the context of the tumor microenvironment can drive unique nutrient requirements is crucial to uncover novel therapeutic strategies. Lung adenocarcinomas with KEAP1/NRF2-mutations, which activate the endogenous antioxidant response, can be therapeutically targeted by inhibiting glutaminolysis. Here we show that genetic, pharmacologic, and ROS-dependent activation of the NRF2 axis results in a dependency on multiple non-essential amino acids (NEAAs). This is mediated by the NRF2-dependent excretion of glutamate through the system xc- antiporter which limits availability of intracellular glutamate for NEAA synthesis. Dependency on NEAAs can be therapeutically targeted by dietary restriction or enzymatic depletion of individual amino acids. Importantly, low antioxidant tumors that lack alterations in the Keap1/Nrf2 pathway, such as Kras and p53 mutant tumors, which do not respond to known metabolic therapies, can be targeted by combination of glutaminase inhibition and dietary restriction of NEAAs. Our findings identify a novel metabolic strategy to target cancers with activation of the Nrf2 antioxidant response pathway by restricting exogenous sources of NEAAs and these vulnerabilities can be exploited in a more general fashion in low antioxidant tumors.

Abstract 20 Samik Upadhaya, Pathology, Visualization of endogenous hematopoietic stem cells in their native state

Samik Upadhaya, Oleg Krichevsky, David Fooksman, Boris Reizis*

Hematopoietic stem cells (HSC) sustain lifelong hematopoiesis owing to their unique capacity for self-renewal and multilineage differentiation. In adult mammals, HSC reside in a specialized bone marrow (BM) niche which supports their maintenance and differentiation. HSC can be mobilized from their BM residence into peripheral blood, a procedure that can be utilized in clinical BM transplantation. However, the dynamics of endogenous HSC including their morphology and interactions with the BM niche has never been characterized in live animals. We adapted a transgenic mouse strain that allows specific fluorescent labeling of endogenous HSC to visualize them in their native environment using two-photon intravital microscopy. We found that HSC exhibit dynamic morphology with a majority of them undergoing short-range processive movements in the BM. The HSC were found to be in close proximity with stem cell factor (SCF)-expressing perivascular stromal cells. Following treatment with mobilization-inducing drugs, we observed a near-complete arrest of HSC movement. Our results provide direct visual evidence of a highly dynamic nature of endogenous HSC and reveal their interaction with SCF-expressing stromal cells of the BM niche at steady state.

Abstract 21 Matthew Witkowski, Pathology, The Evolving Immune Microenvironment of B-cell Acute Lymphoblastic Leukemia

Matthew T. Witkowski, Igor Dolgalev, Nikki A. Evensen, Tiffany Chambers, Kathryn G. Roberts, Sheetal Sreeram, Yuling Dai, Anastasia N. Tikhonova, Chunxu Qu, Deqing Pei, Cheng Cheng, Gabriel A. Robbins, Joanna Pierro, Shanmugapriya Selvaraj, Valeria Mezzano, Marla Daves, Philip J. Lupo, Michael E. Scheurer, Cynthia A. Loomis, Charles G. Mullighan, Karen R. Rabin, Aristotelis Tsirigos, William L. Carroll and Iannis Aifantis*

As with most cancer types, there remains a subset of B-cell acute lymphoblastic leukemia (B-ALL) patients who will relapse and succumb to therapy-resistant disease. In many cases, tumor heterogeneity underpins therapy failure leading to a leukemia-intrinsic model of clonal evolution, however, the bone marrow microenvironment most likely also plays a role in supporting leukemia survival, progression and escape from treatment. Here, we utilize single-cell RNA sequencing (scRNA-Seq) to generate a comprehensive map of the primary human B-ALL bone marrow immune microenvironment throughout three distinct stages of the disease: leukemia diagnosis, remission and relapse. These studies show extensive re-modeling of the immune microenvironment composition throughout the course of conventional chemotherapy and uncover a role for leukemia-associated non-classical monocytes in promoting B-ALL pathogenesis in vivo. Monocyte abundance at diagnosis is predictive of B-ALL patient outcome and, when targeted in Ph+ B-ALL animal models, leads to prolonged disease remission. Our profiling of the human B-ALL bone marrow immune microenvironment provides a greater understanding of the potential extrinsic regulators of B-ALL survival and may highlight previously unknown environmental factors influencing immune-based treatment approaches to high-risk B-ALL.

Abstract 22 Shuang Zhang, Cancer Center, Genetic aberrations dictate distinct tumor immune landscape and chemosensitivity in HGSOC

Shuang Zhang, Sonia Iyer, Hao Ran, Wei wei, Robert A. Weinberg, Benjamin G. Neel*

High-grade serous ovarian cancer (HGSOC) is the most common and deadly subtype of ovarian epithelial cancer, and is known for its aggressiveness, high recurrence rate, metastasis to other sites, development of resistance to conventional chemotherapy, and general lack of response to immune checkpoint inhibitors. The absence of genomically relevant, immune competent HGSOC models represents a major barrier to developing new therapies. Taking advantage of a mouse fallopian tube organoid system that we developed, along with lentiviral gene transduction and/or CRISPR/Cas9 technology, we generated multiple new HGSOC models containing combinations of mutations seen in human HGSOC, including homologous recombination (HR)-proficient (Tp53-/-;Ccne1amp;Akt2ampand Tp53-/-;Ccne1amp;Krasamp) and -deficient (Tp53-/-;Brca1-/-;Pten-/-and Tp53-/-;Brca1-/-;Mycamp), and poorly characterized (Tp53-/-;Pten-/-;Nf1-/-) models.These models differ in proliferation, differentiation, and polarity/organoid structure in vitro, as well as tumorigenic capacity and behavior upon orthotopic injection into syngeneic mice. Organoids bearing different mutational spectra show differential sensitivity to conventional HGSOC chemotherapies, signal transduction inhibitors, and DDR inhibitors, and evoke distinctly different immune microenvironment in vivo. In particular, the immune microenvironment induced by HR-deficient tumors shows more T cell infiltration/Treg cells, whereas HR-proficient lines show lower T cell infiltration but higher levels of myeloid-derived suppressor cells and macrophages. The results of these studies suggest novel, genotype-informed combination therapies for this devastating disease.

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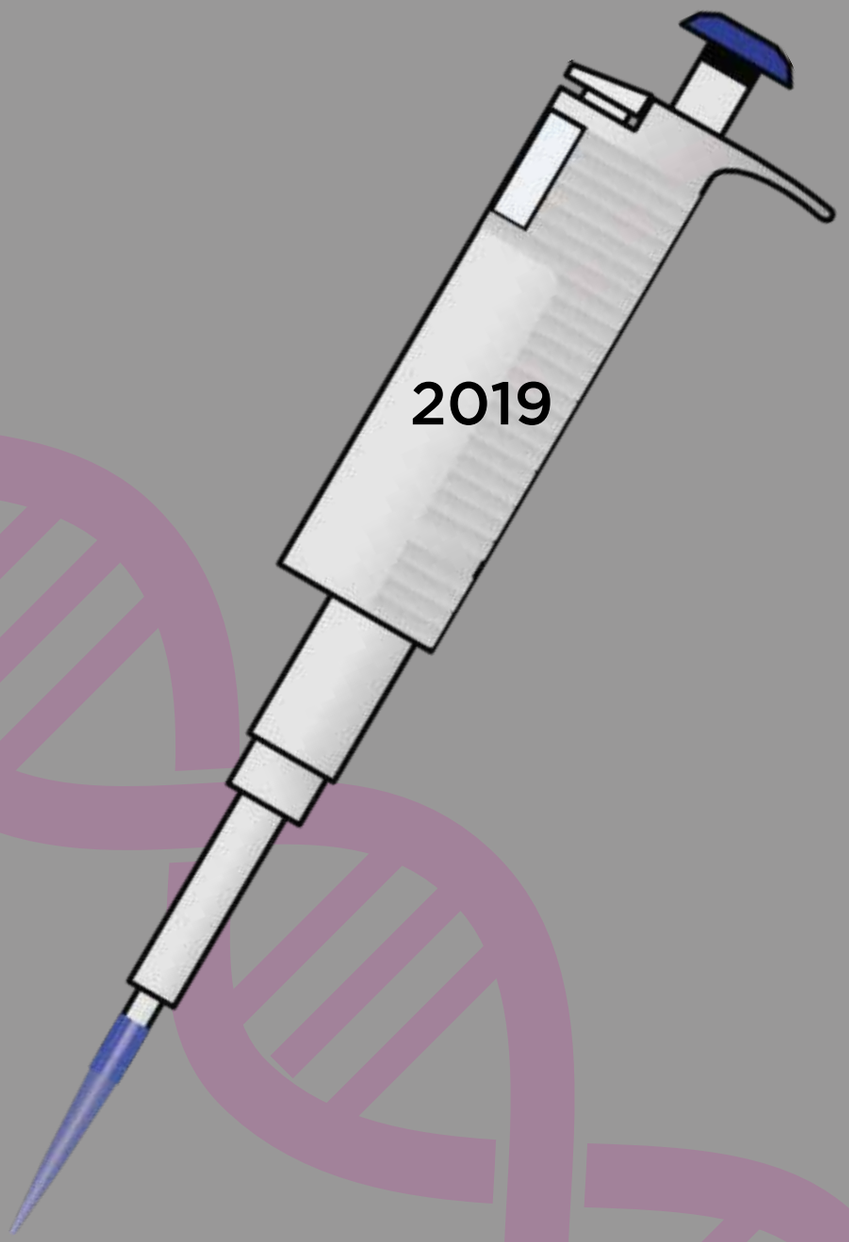

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