FARE 2026 Awardees

National Cancer Institute - Cancer Prevention Fellowship Program

1. Caitlin P Bailey, PhD

Group-level sleep estimates from Fitbit devices in the All of Us Research Program
Mentor: **Dr. Dana Wolff-Hughes**
Study Section: **Behavioral Science/Psychology/Psychiatry**

Study Objectives: Short sleep duration is an emerging risk factor for several cancers, including breast, colorectal, lung, and prostate. However, most existing studies examining sleep and cancer risk have relied on self-reported sleep measures, which are limited by recall and reporting biases. To better identify at-risk populations and inform targeted public health interventions, objective assessment of sleep at the population level is needed. Wearable devices offer a promising solution, but no standardized protocol currently exists for generating reliable and representative sleep estimates using this technology. Thus, this study aimed to determine the number of Fitbit monitoring days needed to produce stable population-level sleep estimates. Methods: Data from the All of Us Research Program Fitbit cohort (version 8) were used. The sample consisted of 4,984 adults (≥18y) with 7 days of Fitbit data between days 15-21 post-primary consent date. Fitbit sleep measures were total sleep duration and duration of sleep stages (light, deep, REM) in minutes. Bootstrapped simulations with 250 samples drawn per protocol were performed for 25 alternative protocols: 1-6 randoms days, Saturday plus 1-5 random weekdays, Sunday plus 1-5 random weekdays, 1 random weekend day plus 1-5 random weekdays, and both weekend days plus 1-4 random weekdays. Relative difference was calculated between the 7-day sample mean and the alternative protocol means for each sleep measure. Sensitivity analyses were conducted by age group, BMI status, race/ethnicity, and device series. Results: The 7-day sample mean for sleep duration was 411.1 ± 80.0 minutes. Alternative protocols of 1-6 random days had 0% relative difference from the 7-day mean. Protocols with nonrandom inclusion of ≥1 weekend day generally evidenced small but statistically significant deviations, with relative differences ranging from 0% to 2.3%. Results were similar across sleep stage measures and sensitivity analyses. Conclusions: One random day of monitoring is sufficient to produce stable population-level estimates of sleep duration and sleep stages, regardless of age, BMI, race/ethnicity, or device series. Nonrandom inclusion of weekend days may introduce bias, and sampling procedures should align with the specific study research questions.

2. Lydia Post, PhD

Outdoor PM2.5 components and risk of lung cancer in a large prospective U.S. cohort
Mentor: **Dr. Rena Jones**
Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Objective: Regional heterogeneity in the association between outdoor fine particulate matter (PM2.5, <2.5μm) and lung cancer suggests that PM2.5 chemical components may differentially influence risk. However, few studies have investigated these relationships. Methods: We used the U.S. Environmental Protection Agency's Community Multiscale Air Quality model (12 x 12 km) to estimate historical concentrations of outdoor PM2.5 components at residential enrollment (1995-1996) addresses of 486,101 NIH-AARP Diet and Health Study participants in eight states. Components included ammonium, nitrate, sulfate, elemental carbon (EC), organic carbon (OC), soil, and other. We used Cox models to estimate hazard ratios and 95% confidence intervals (HR [CI]) of associations between PM2.5 components (per 1 ug/m3) and lung cancer risk overall and by major histologic subtype (adenocarcinoma, squamous cell carcinoma, small cell carcinoma). We treated residential outdoor nitrogen dioxide (NO2) as an independent exposure (per 10 ppb) given its known relationship with lung cancer. We fit single pollutant models and specified the multi-pollutant mixture using elastic net regularization to account for multicollinearity of components, adjusting for age, smoking, and other key confounders. Results: 22,426 lung cancers were diagnosed over x̄=18 years of prospective follow-up. In single-pollutant models, all PM2.5 components were associated with increased risk of lung cancer overall, adenocarcinoma, and squamous cell carcinoma, with the strongest relationships observed for ammonium (HR=1.05 [1.01-1.09], HR=1.08 [1.01-1.15], and HR=1.11 [1.02-1.22], respectively) and nitrate (HR=1.03 [1.01-1.05], HR=1.05

[1.01-1.09], and HR=1.05 [1.01-1.09], respectively). Elastic net retained only OC in models of lung cancer overall (HR=1.02 [1.01-1.03]) and NO2 for adenocarcinoma (HR=1.05 [1.02-1.08]). There were no clear associations with small cell or squamous cell carcinoma. Conclusion: Our novel findings in this large cohort suggest that PM2.5 chemical constituency may be an important consideration in studies of air pollution and lung cancer.

National Cancer Institute - Center for Cancer Research

3. Etan Aber, MD, PhD

Exploration of tumor-free tissues as a means to predict metastatic risk
Mentor: **Dr. Rosie Kaplan**
Study Section: **Oncology - Development and Metastasis**

Removed at request of author

4. Vinutha Balachandra, PhD

Protein phosphatase 1 regulatory subunit PNUTS prevents CENP-A mislocalization and chromosomal instability
Mentor: **Dr. Munira Basrai**
Study Section: **Cell Biology - Cell Cycle**

Removed at request of author

5. Kylynda Bauer, PhD

Cholinergic Signaling Shapes Liver Cancer Immunity via Gut Microbes
Mentor: **Dr. Tim Greten**
Study Section: **Microbiota/Microbiome**

Immunosuppression remains a formidable challenge in liver cancer. Liver immune tolerance contributes to pro-tumor immunity, deficient immunotherapy responses, and poor overall survival. Continuously exposed to gut-derived antigens, the liver senses gut microbiota dysbiosis and shapes microbiota composition. Beyond the microbiome, neuroimmune signals also shape liver immunity. The cholinergic anti-inflammatory arc dampens immune activation via acetylcholine signaling. We assessed whether gut microbes and cholinergic signals alter tumor activation in hepatocellular carcinoma (HCC), which accounts for ~90% of primary liver cancer cases. We performed fecal microbiota transplantation (FMT) from HCC or healthy controls into healthy mice after sham vagotomy (SV) or vagal denervation (hepatic vagotomy, HV). CD8+ T cell activation was assessed in recipient mice after four FMT rounds via flow cytometry. Both control and HCC FMT recipients exhibited comparable CD8+ T cell features in non-vagotomized mice (CON-SV, HCC-SV). In contrast, HCC-HV livers exhibited increased cytokine (IFNg, TNFa) and cytotoxic (granzyme B) markers. As vagotomy alone did not alter CD8+ T cell activation (CON-HV), these results suggested that cholinergic disruption facilitates immune activation from gut dysbiosis. We next assessed whether cholinergic disruption reduced HCC tumor burden. HV mice exhibited significantly reduced tumor growth in three models of primary (RIL-175) and metastatic (B16-F10, A20) liver cancer. Moreover, tumor-bearing HV mice exhibited increased CD8+ T cell frequency. Flow cytometry and scRNA-sequencing from RIL-175 SV and HV livers reported increased CD8+ T cell granzyme subsets matching FMT studies. Moreover, HV CD8+ T cells expressed altered ACh receptor expression, namely Chrm3. Genetic depletion of Chrm3 in CD8+ T cells (CD8creChrm3flox) resulted in smaller tumors compared to littermate controls. Ongoing studies will assess whether microbiota modulation or pharmaceutical cholinergic depletion improves immune checkpoint blockade response. Collectively, these findings further understanding of systems immunity, highlighting targetable microbiota and neuroimmune pathways to improve liver cancer outcomes.

6. Amit K Behera, PhD

RNA-coupled CRISPR Screens Reveal Regulatory Factors and Mechanisms Governing Disease-Associated Alternative Splicing Events
Mentor: **Dr. Thomas Gonatopoulos-Pournatzis**
Study Section: **Omics - Genomics/Transcriptomics**

Alternative splicing is a pervasive cellular mechanism, which plays an important role in diversifying gene function enabling organisms to adapt and respond to various stimuli. Dysregulation of alternative splicing has been shown to cause several diseases such as cancer, progeria syndrome, and neuromuscular disorders. However, the lack of scalable, high throughput assays has hindered the systematic identification of splicing regulators, limiting therapeutic advancements. To address this challenge, we have developed CRASP-Seq, which is an easy to implement and cost-effective innovative method that leverages high-throughput CRISPR screens and coupled splicing reporters to systematically investigate splicing regulation. CRASP-Seq enables to simultaneously investigate the regulatory role of ~20,000 protein-coding and non-coding genes by generating pooled lentiviral CRISPR libraries and assessing their impact on splicing from a single RNA extraction sample. In parallel, we have also developed and optimized a powerful computational pipeline to efficiently analyze data and identify key splicing regulators. As a proof-of-concept, we applied CRASP-Seq to uncover regulators of an aberrant splicing event in LMNA, caused by a de novo synonymous mutation that activates a cryptic 5´ splice site, leading to the production of progerin, a truncated lamin protein responsible for Hutchinson-Gilford Progeria Syndrome. Our screen identified dozens of LMNA splicing regulators, including previously established factors, with a strong enrichment for RNA-binding proteins and splicing-related genes. Notably, we discovered ZNF207, a known mitotic spindle assembly factor, as a novel regulator of LMNA aberrant splicing. ZNF207 depletion enhances canonical LMNA splicing and reduces progerin levels in fibroblasts from progeria patients. Transcriptome-wide analyses, including RNA sequencing and enhanced crosslinking and immunoprecipitation (eCLIP), revealed that ZNF207 directly interacts with RNA targets to influence alternative splicing. Functional dissection using high-throughput mutagenesis of ZNF207 and deletion constructs demonstrated its Zinc-finger (ZnF) domain to be essential for associating with spliceosomal proteins and regulating splicing. Overall, we have developed and successfully implemented CRASP-Seq to systematically uncover splicing regulators and study disease-associated splicing mechanisms, offering a valuable tool for therapeutic discovery.

7. Megan Behrmann, Ph.D.

Elucidating an rDNA Proximity-Based Mechanism for Adaptive Gene Duplication-Amplification Antimicrobial Resistance in Staphylococcus aureus
Mentor: **Dr. Anupama Khare**
Study Section: **Bacteriology - General**

The increasing emergence of antimicrobial resistance (AMR) in clinical settings is a serious threat that demands further research into mechanisms of resistance evolution, especially in pathogens. Methicillin-resistant S. aureus (MRSA) is a drug-resistant strain and frequent source of nosocomial infections, particularly dangerous for those with chronic conditions or frequent hospital visits. Incomplete or ineffective treatment of MRSA increases the risk of systemic infection and development of new antibiotic-resistant strains. Despite this, the diverse mechanisms driving AMR are not fully understood. Delafloxacin (DLX), a dual-targeting fluoroquinolone, inhibits both DNA gyrase and topoisomerase, reducing the likelihood of resistance via enzyme modification. However, recent findings from our lab suggest that MRSA exposed to DLX evolves resistance through gene duplication-amplification (GDA), an understudied adaptation mechanism. Genomic amplification and the resulting overproduction of the drug efflux pump SdrM led to dynamic DLX heteroresistant populations, independent of target enzyme mutations. Notably, the sdrM gene resides upstream and proximal to the repetitive, highly transcribed rRNA and tRNA loci, which are targets for DNA repair and modification. Sequencing of evolved populations consistently revealed amplifications where the downstream junction was within the rDNA locus, increasing copy number and upregulating expression of the sdrM target. Relocating sdrM to a novel location adjacent to an rDNA locus yielded similar outcomes, with strains evolving high levels of DLX resistance through GDA, and junctions that mimic the wild-type strain. This suggests a loci-dependent mechanism, where proximity to highly transcribed rDNA favors amplification for adaptation and resistance. To further test this hypothesis, we are monitoring DLX evolutionary pathways in strains where the GDA target is inverted at its original location, moved distal to rDNA operons, or replaced with an alternate gene. Utilizing a novel Cas9-targeted long read sequencing

technique, we aim to quantify population heterogeneity, GDA junctions, and gene copy number in a high-resolution, high-throughput manner. Direct comparison of amplification size, acquisition rate, and stability will provide valuable insights into how gene location influences adaptive GDA. This work directly addresses antibiotic resistance evolution in MRSA, highlighting critical aspects of GDA adaptation in AMR.

8. Akhil Chameettachal, PhD

HIV-1 Gag Primarily Selects its RNA Packaging Substrate at the Plasma Membrane
Mentor: **Dr. Wei-Shau Hu**
Study Section: **HIV and AIDS Research**

HIV-1 Gag protein orchestrates both RNA packaging and virion assembly. To produce infectious virions, Gag must package HIV-1 RNA during assembly. In the absence of HIV-1 RNA, Gag packages cellular mRNAs into virions and produces non-infectious particles. Despite being a critical step in generating infectious viruses, the location where Gag selects RNA as the genome for packaging remains unknown. It has been suggested that Gag selects the packaging substrate in the cytoplasm because Gag can bind HIV-1 RNA in the cytoplasm. However, cytoplasmic Gag binds both viral and cellular mRNAs with little specificity, making the biological significance of this Gag: RNA binding unclear. We hypothesized that Gag selects RNA for packaging at the plasma membrane during assembly. To test our hypothesis, we developed a novel approach and examined whether a nonviral RNA (nvRNA) is packaged more efficiently when it is enriched at the plasma membrane. If Gag selects the packaging substrate at the plasma membrane, then enriching the nvRNA at the plasma membrane will increase its packaging. In contrast, if Gag selects RNA in the cytoplasm, then enriching the nvRNA at the plasma membrane will not facilitate its packaging. To direct nvRNA to the membrane, we used the plasma membrane-targeting abilities of HIV-1 Gag matrix (MA) domain and the N-terminal domains of Fyn and Src kinases (Fyn10 and S15). We fused these domains to an RNA-binding protein, which binds to a stem-loop on nvRNAs. We labeled these fusion proteins and nvRNAs using different fluorescent proteins, and the targeting of nvRNAs to the plasma membrane was confirmed by microscopy. Next, fluorescently labeled Gag was expressed and the frequency of nvRNA packaging into HIV-1 particles was determined by analyzing the fluorescence signals of Gag and nvRNA in individual virions. Without plasma membrane targeting, less than 1% of HIV-1 particles contained nvRNA because it had to compete with cellular mRNAs for packaging. When nvRNA was targeted to the plasma membrane, packaging of nvRNA increased approximately 20- to 35-fold. These findings show that although nvRNA is generally poorly packaged, its packaging increases significantly when targeted to the plasma membrane. Similar results were observed with other RNA substrates and membrane-targeting proteins. These results provide the first evidence that HIV-1 Gag selects its RNA packaging substrate at the plasma membrane and address a long-standing question in the field.

9. Shreya Chappidi, BA

Building clinically relevant and interpretable multi-modal machine learning algorithms to predict glioblastoma disease progression
Mentor: **Dr. Andra Krauze**
Study Section: **Bioinformatics - algorithms, packages and tools**

Removed at request of author

10. Xi Chen, PHD

MYBBP1A is part of a mechanically modulated system that alters breast cancer metastatic potential by regulating focal adhesionMYB binding protein 1a (MYBBP1A) was first identified for its ability to bind and repress the proto-oncogene c-MYB. Most res
Mentor: **Dr. Kent Hunter**
Study Section: **Oncology - Development and Metastasis**

MYB binding protein 1a (MYBBP1A) was first identified for its ability to bind and repress the proto-oncogene c-MYB. Most research focuses on its role in regulating ribosomal RNA transcription; However, its impact on metastasis remains unclear. Analysis of TCGA breast cancer patient data reveals higher MYBBP1A levels are associated with shorter survival times in breast cancer patients, while a Mybbp1a knockdown (KD) gene signature, generated by machine learning, indicates significantly improved patient survival outcomes. Using mouse spontaneous metastasis model, we confirmed that MYBBP1A loss suppresses breast cancer cell metastasis. Further analysis revealed that Mybbp1a KD cells exhibit robust abnormalities in cell morphology, which is associated with reduced cell invasion and disrupted F-actin organization. However, cell morphological abnormalities are partially rescued in type I collagen-coated high stiffness extracellular matrix (ECM) culture, indicating MYBBP1A could be part of a mechanically modulated system that alters breast cancer metastatic potential. This is supported by the observation that MYBBP1A levels exhibits a gradually increase in response to the increases of ECM stiffness, which is coupled with increased cell invasion capacity. Mechanical stimuli from the tumor microenvironment play an important role in mediating breast cancer metastasis. The pathways that cancer cells use to sense and leverage mechanical cues are largely driven by the assembly and disassembly of focal adhesions, which regulate the dynamics of F-actin stress fibers and couple F-actin with the extracellular matrix (ECM) to transduce mechano-signaling and regulate cell motility. Our data shows focal adhesion is reduced in Mybbp1a KD cells, evidenced by weakened level and number of phospho-FAK clustering at the points of F-actin. In parallel, overexpression of FAK rescues breast cancer cell aggressive phenotypes and partially restores the reduced cell metastasis capacity observed in Mybbp1a KD, suggesting MYBBP1A drives focal adhesion to promote cancer metastasis. Moreover, we noticed that MYBBP1A relocates from nucleolus to cytoplasm under stress conditions, and interacts with several well-studied breast cancer metastasis mechano-regulators identified in our lab, including nucleoporin 210 and N-acetyltransferase 10. In the future, we will keep studying the role of MYBBP1A as part of a mechanically modulated complex that is important in promoting breast cancer metastasis.

11. Achyut Dahal, PhD

Quest for Mucin-4 Glycopeptide Specific Antibodies as Potential Anti-Cancer Immunotherapies
Mentor: **Dr. Joe Barchi**
Study Section: **Clinical and Translational Research - Drug Discovery**

Removed at request of author

12. Arun Prasath Damodaran, Ph.D

A Scalable Exon Deletion Platform Integrated with Single-Cell Transcriptomics Reveals Alternative Splicing Events Shaping Gene Expression Dynamics
Mentor: **Dr. Thomas Gonatopoulos-Pournatzis**
Study Section: **Gene Expression - Transcriptional Regulation (Eukaryotic)**

CRISPR-Cas technology has transformed functional genomics, yet our understanding of how individual exons shape cellular phenotypes remains limited. To address this, we previously optimized and conducted massively parallel exon deletion screens in human cell lines using CHyMErA, a combinatorial Cas9 and Cas12a platform, identifying over 2,000 exons that regulate cellular fitness. Notably, these fitness-altering exons are disproportionately enriched in genes involved in gene expression regulation, underscoring the need for tools to systematically investigate exons that influence transcriptional phenotypes. Here, we present single-cell CHyMErA-sequencing (scCHyMErA-Seq), a novel platform that combines ultra-efficient exon deletion using CHyMErA with the simultaneous capture of Cas9 and Cas12a guide RNAs at single-cell resolution. scCHyMErA-Seq enables direct linkage of individual exon deletions—identified via gRNA sequencing—to transcriptional outcomes, measured by single-cell mRNA profiling, in a massively parallel manner. As proof-of-concept, we applied scCHyMErA-Seq to delete 224 exons across 161 genes, prioritizing exons previously implicated in cellular fitness. We profiled over 200,000 cells, achieving a median coverage of 582 cells per targeted exon, with 7,680 cells assigned to intergenic controls. Our analysis revealed that deletions of exons within the same protein complexes or pathways frequently result in clustered transcriptional profiles, offering insights into exon-specific functions. Strikingly, 50% of the profiled exons (112 out of 224) influence the expression of at least 100 genes. Among these, we identified NRF1 alternative exon-7. Focused analyses through engineering cell lines

expressing either full-length or Δexon-7 NRF1 isoforms revealed that exon-7 modulates NRF1 transcriptional activity by regulating its recruitment to the promoters of target genes. Additionally, an siRNA screen identified SRSF3 as the splicing regulator controlling NRF1 exon-7 inclusion. Altogether, our work establishes scCHyMErA-Seq as a powerful and scalable tool for systematically linking alternative splicing events to transcriptional phenotypes. The gene expression profiles generated by scCHyMErA-Seq recapitulate findings from traditional, labor-intensive approaches while offering enhanced scalability and efficiency. Overall, scCHyMErA-Seq provides a robust platform for uncovering the functional impact of alternative splicing on gene regulation.

13. Mohd Saleem Dar, PhD

Deciphering the epigenetic roles of NSD3 isoforms in HPV negative HNSCC
Mentor: **Dr. Vassiliki Saloura**
Study Section: **Epigenetics**

Removed at request of author

14. Stefano Di Giulio, PhD

A Tale of States: Decoding the Role of MYCN Amplification in ADRN-MES Plasticity
Mentor: **Dr. Carol Thiele**
Study Section: **Oncology - General**

Neuroblastoma (NB) is a lethal childhood cancer driven by chromosomal instability, with MYCN amplification (MNA) being the strongest prognostic marker. Despite initial therapeutic responses, high-risk NB frequently relapses, highlighting the importance of tumor plasticity in disease progression. NB plasticity results from the dynamic interplay between Adrenergic (ADRN) and Mesenchymal (MES) states. While ADRN cells predominate in untreated tumors, MES cells enrich post-chemotherapy, driving resistance and relapse. Despite increasing evidence of NB plasticity, key questions remain: 1) Do MES cells arise from ADRN-to-MES interconversion, or does therapy select a pre-existing, drug-resistant MES population? 2) What are the molecular and genetic mechanisms that drive NB state transitions? To answer these, we tracked the fate of NB patient-derived xenograft models after therapy using in vivo single-cell barcoding sequencing and profiled the genetic features associated with MES and ADRN states. We demonstrate that MES cells exist pre-treatment as a rare population in vivo and expand after chemotherapy through selection and interconversion. Barcode tracing confirms both mechanisms, revealing the persistence and accumulation of pre-existing barcoded MES cells after therapy, as well as ADRN cells undergoing MES reprogramming. Notably, relapsed tumors re-establish a high prevalence of ADRN cells, indicating that MES-to-ADRN interconversion occurs at relapse. MYCN expression inversely correlates with the MES state, being absent or reduced in MES cells. Genetic profiling using spectral karyotyping and multiplexed FISH reveals that MNA loss correlates with MES transition. The MYCN gene amplicon undergoes a dynamic reorganization during cell-state transitions, shifting from extrachromosomal DNA (ecDNA) to chromosomal integration in homogeneously staining regions, or being lost entirely in some MES cells. Whether these changes arise from genetic drift, differential segregation of ecDNA, or active mechanisms of ecDNA expulsion/reintegration is under study. Our findings reveal that NB resistance and relapse are driven by pre-existing heterogeneity and dynamic cell-state interconversions. We uncover the role of MNA dynamics in shaping tumor plasticity, highlighting its potential as a vulnerability. By revealing mechanisms of MYCN silencing and ecDNA dynamics, this work opens new avenues for targeting MES-associated plasticity and overcoming chemoresistance in NB.

15. Nisita Dutta, M.S.E.

Mesothelin-Targeted Nanobody-Drug Conjugates to Treat Pancreatic Cancer
Mentor: **Dr. Christine Alewine**
Study Section: **Chemistry - Drugs/Pharmaceuticals**

Background: Approximately 87% of patients suffering from pancreatic ductal adenocarcinoma (PDAC) will die from the disease within five years. Thus, development of targeted therapies that can overcome the dense stroma of the PDAC tumor microenvironment (TME) is essential to improve patient survival. Mesothelin (MSLN) is a membrane protein that is overexpressed in PDAC, making it an ideal target for therapeutics. We have recently discovered a nanobody (A101) that effectively binds to the MSLN protein. It has a small overall size of ~15 kDa that could facilitate penetration through the mechanical barrier of the PDAC TME, but previous attempts to chemically conjugate with a payload have impaired MSLN binding affinity. We hypothesize that small and highly specific MSLN-targeted nanobody-drug conjugates (NDCs) will have greater tumor penetration through the PDAC TME and improved pharmacokinetic distribution with limited toxic systemic effects compared to existing therapies, improving outcomes for PDAC patients. Methods: The structure of the A101 nanobody was computationally predicted and molecular dynamic simulations were run to understand its interaction with MSLN. After determining ideal positions for modification with a payload, we engineered the nanobody to incorporate a free cysteine at the desired site. Small linker-drug molecules were synthesized and bioconjugated to the A101 nanobody in a site-selective manner using maleimide chemistry. These include a microtubule inhibitor payload (MMAE) with various linkers that have unique release mechanisms, as well as a novel ortho-quinone prodrug with a beta-glucuronidase linker. These NDCs were tested for binding to MSLN via bio-layer interferometry assays. Internalization and cell viability were also assessed in tissue culture using several mouse and human PDAC cell lines. Results: Modified A101 nanobody binds human MSLN with a KD of 18nM and mouse MSLN with a KD of 5.6nM. A101 conjugates have increased internalization into MSLN-positive cell lines and can efficiently kill both mouse and human PDAC cells in viability assays with an IC50 in the 20-200 nanomolar range. Conclusions: We have successfully conjugated various payloads to the A101 nanobody without diminishing MSLN-binding activity. These NDCs have efficient and promising activity in PDAC cell lines. Future studies will explore NDC activity in humanized MSLN mouse models to assess for safety, biodistribution, and anti-tumor efficacy.

16. Maximilia Frazao De Souza Degenhardt, Ph.D.

HORNET: A Deep Learning and AFM-Based Method for Determining Heterogeneous RNA Structures
Mentor: **Dr. Yun-Xing Wang**
Study Section: **Biophysics**

RNA is a versatile molecule that serves various functions, including acting as a genetic carrier (e.g., mRNA), gene regulators (e.g., riboswitches), enzymes (e.g., ribozymes), protein synthesis machinery (e.g., rRNA), and amino acid carriers (e.g., tRNAs). Understanding RNA structure and dynamics is critical to deciphering its biological functions, which is essential for engineering RNA-based devices and identifying druggable RNA targets. However, the structural and dynamic basis for RNA's biological functions remains poorly understood, primarily due to the lack of comprehensive structural data. Despite the determination of the first three-dimensional (3D) tRNA structures nearly 50 years ago, relatively few RNA structures, especially those larger than 200 nucleotides, have been solved using traditional methods such as NMR, X-ray crystallography, and cryo-EM. Structural databases contain fewer than 50 unique structures of naked RNA molecules larger than 200 nucleotides, highlighting the challenges in RNA structure determination. These limitations are due to the conventional methods' reliance on signal averaging over conformationally homogeneous samples, which do not adequately capture the conformational space of RNA in physiologically conditions. We have pioneered the use of atomic force microscopy (AFM) to directly visualize individual RNA molecules in solution, revealing that a single RNA sequence can adopt multiple biologically relevant 3D conformations under physiological conditions. This finding challenge existing assumptions about RNA structure and dynamics. To address the gaps in current RNA structure-dynamics technologies, we developed HORNET. HORNET enables the study of highly heterogeneous and flexible RNA structures that were previously unattainable. This method, reported in Nature, makes use of direct surface 2D projection of single RNA molecule observed experimentally by AFM and utilizing neural networks to back calculate 3D topological structure of RNA and estimate their accuracy. HORNET provides critical insights into RNA dynamics, such as motion correlations among structural elements, invariant structural cores, and the relationships between RNA primary sequence, 3D structure, and dynamics. A recent demonstration of HORNET's utility in mapping the conformational landscape of RNase P RNA, published in Nature, led to novel and important discoveries that are likely to significantly influence our understanding of RNA structure and dynamics.

17. Nadezda Fursova, PhD

Live-cell imaging uncovers dynamic coupling between enhancer transcription and gene activation
Mentor: **Dr. Dan Larson**
Study Section: **Gene Expression - Transcriptional Regulation (Eukaryotic)**

Removed at request of author

18. Gourab Ghosh Roy, PhD

Artificial intelligence framework to identify spatial patterns of cancer immunotherapy outcomes from multiplexed proteomics imaging
Mentor: **Dr. Peng Jiang**
Study Section: **Bioinformatics - algorithms, packages and tools**

Removed at request of author

19. Jihye Golino, PhD

Identification of regulators of perilipin5 using in vivo Genome wide CRISPR/Cas9 screening
Mentor: **Dr. Natalie Porat-Shliom**
Study Section: **Cell Biology - Metabolism and Bioenergetics**

Hepatic lipid accumulation, known as Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), presents a significant risk factor for the development of liver cancer. Notably, hepatic lipid buildup can also occur during fasting, when fatty acids are mobilized from adipose tissue into the liver. Yet, the mechanisms that enable hepatocytes to handle increased lipid flux during nutrient deprivation, and the complications tied to MASLD, are unresolved. Perilipin 5 (PLIN5) is a lipid droplet (LD) - associated protein that regulates lipid storage and utilization in hepatocytes by modulating interactions between lipid droplets and mitochondria. It helps maintain lipid homeostasis by preventing excessive lipolysis while facilitating fatty acid oxidation when energy demand increases. Dysregulation of PLIN5 in the liver has been linked to metabolic disorders, including MASLD and hepatic insulin resistance, highlighting its significance in hepatic lipid metabolism and overall metabolic health. Recently, our group discovered that PLIN5 levels are upregulated in fasting but not western diet (WD)-fed mice. Augmentation of PLIN5 levels in WD-fed mice by overexpression increased mitochondria-LD contact sites, resulting in marked reductions in steatosis and oxidative stress. These results suggest that the regulation of mitochondria-LD contact sites by PLIN5 is not just beneficial, but may be crucial for metabolic health. To further investigate this hypothesis, we aim to dissect the signaling network that regulates PLIN5 expression. To achieve this, we generated a novel transgenic reporter mouse line by knocking in Neon Green to fluorescently tag the endogenous PLIN5. This line will be instrumental in a Genome-wide CRISPR/Cas9 screen combined with FACS, enabling us to pinpoint the broader network components that influence PLIN5 expression. Selected candidates will undergo rigorous validation, followed by comprehensive gain- and loss-of-function studies to establish their roles in PLIN5 regulation and the overall management of lipid metabolism. This study will aim to uncover potential mechanisms underlying PLIN5's role in liver metabolism and its impact on diseases such as cancer and MASLD.

20. Krishnendu Guin, PhD

Orderly mitotic progression re-establishes interphase genome organization
Mentor: **Dr. Tom Misteli**
Study Section: **Chromosomes, Chromatin, and Nuclear Architecture**

Genomes assume a complex 3D architecture in the interphase cell nucleus. Yet, the molecular mechanisms that determine global genome organization are only poorly understood. We used centromeres as surrogate markers to probe the molecular mechanisms that determine global genome organization. Centromeres are prominent structural elements of chromosomes that define the assembly locations of kinetochores complexes that are composed of multiple copies of ~100 conserved proteins. During cell division, the fibers of the mitotic spindle must correctly attach to kinetochores in a timely manner, as the

chromosomes align at metaphase plate, to ensure an error-free cell division. Therefore, spatial distribution of centromeres in the interphase nuclei is crucial as peripheral chromosomes take longer to align at metaphase plate. By studying an array of human cell lines and quantitively measuring centromere clustering we found that spatial distribution of centromeres is cell-type specific. To identify the molecular determinants of centromere distribution in the 3D space of interphase cell nuclei, we performed several high-throughput imaging based CRISPR knockout screens using a library of 1068 chromatin-associated proteins in multiple cell types and assessed changes in centromere distribution at single nuclei resolution. We identified components of the nucleolus, kinetochore, the cohesion and condensin complexes, and the nuclear pore complex as major regulators of spatial distribution of centromeres. We found that alterations in centromere distribution required progression through the cell cycle and probing further using degron-based protein depletion approaches, we show that changes in spatial centromere distribution are reversible. The effect of multiple centromere clustering factors with separate biological functions converged on cell division and we show that normal progression through mitosis is required to preserve spatial patterns of centromere distribution in interphase. Taken together, these results identify several molecular determinants of spatial centromere organization, and they reveal that orderly progression through cell division is required to re-establish of global interphase genome architecture.

21. Amit Gupta, PhD

Functional domains of the chromatin remodeler LSH interacting with the co-factors CDCA7 or macroH2A
Mentor: **Dr. Kathrin Muegge**
Study Section: **Biochemistry - General**

Lymphoid specific helicase (LSH) is an epigenetic regulator and plays a critical role in heterochromatin formation via deposition of macroH2A and regulation of DNA methylation. Mutations in the LSH gene lead to the Immunodeficiency Centromeric instability Facial anomalies 4 (ICF4) syndrome, a severe disease with high lethality. The precise role of LSH and its co-factors CDCA7 and macroH2A in modulating the chromatin remains unknown. To determine functional domains of LSH, several deletional LSH expression plasmids and patient mutations were generated and expressed in human cell lines and examined for interaction with chromatin, macroH2A, or CDCA7 using chromatin fractionation and co-immunoprecipitation. We found that the N-terminal region (1aa-234aa) is sufficient for interaction with the co-factor CDCA7. Within this region 30 to135 are essential for CDCA7 interaction and for association of LSH with chromatin. Since LSH and CDCA7 interact directly with each other in vitro (and it is known that CDCA7 improves association with a nucleosome) it suggests that LSH may be in part recruited by CDCA7 to chromatin. The region was further narrowed down to three amino acids of LSH (K74, Y78, S79) which are required for interaction with CDCA7. Importantly, the same region and residues contribute to the interaction of LSH with macroH2A. These results suggest a potential competitive role between the two interactive partners resulting in distinct molecular pathways. Despite sharing some similarity in interaction, we also identified regions that differ, namely region (162-174aa) which contributes to interaction with CDCA7, but not macroH2A. On the other hand, L801del and Q699R, point mutants found in patients, showed diminished interaction with macroH2A, but not CDCA7, suggesting that LSH mediated chromatin remodeling involving macroH2A is impaired in patients. To test the role of posttranslational modifications, we utilized phosphatases and found that both interactions, CDCA7 and macroH2A, were diminished suggesting that phosphorylation of critical residues promote association with co-factors. Our findings define the N-terminal region of LSH as critical for association with co-factors which modulate LSH association with chromatin and are known to modulate LSH activity. Revealing the molecular mechanisms of interaction can assist in developing targeted therapies, such as modulating LSH phosphorylation to mitigate ICF4 disease symptoms and improve patient outcomes.

22. Preeti Gupta, PhD

MOLECULAR DEFECT OF LSH REGULATED DNA METHYLATION IN THE HUMAN IMMUNODEFICIENCY-CENTROMERIC INSTABILITY-FACIAL ANOMALIES 4 (ICF4) SYNDROME
Mentor: **Dr. Kathrin Muegge**
Study Section: **Epigenetics**

Mutation of the LSH (Lymphoid Specific Helicase) gene causes the human ICF4 syndrome, a severe developmental disease with high lethality in childhood. ICF patient cells, as well as murine cells with a deletion of LSH, show a remarkable reduction of global DNA methylation. It remains unknown how LSH, which is not a DNA methyltransferase, helps to establish DNA methylation in vivo. Using whole genome bisulfite sequencing and micrococcal nuclease sequencing, we found that CpG methylation of nucleosomal DNA, but not linker DNA, is severely reduced in LSH knockout cells. This suggests that LSH is required for promoting access of DNA methyltransferases to nucleosomes in vivo. To test this hypothesis, we followed a biochemical approach wherein the nucleosome sliding/repositioning activity of recombinant LSH was investigated. We observed that LSH can reposition nucleosomes in an ATP dependent manner. The sliding activity only unfolds in the presence of CDCA7, a protein that causes a related ICF3 syndrome upon mutation. The remodeling assay suggests unidirectional sliding activity of LSH, instead of unwrapping of DNA. To understand how sliding can promote accessibility to DNA methyltransferase, we employed the DNA methylation assay using CpG methyltransferase M.SssI. First, we used an un-remodeled nucleosome and found that only linker DNA but not the DNA wrapped around the histone core underwent CpG methylation. In contrast, after LSH induced remodeling, we detected methylation of nucleosomal DNA, indicating that LSH improved access to nucleosomal DNA in vitro. Finally, we examined two LSH mutants that were derived from ICF4 patients, namely Q699R and L801del. While Q699R failed to stimulate ATPase activity of LSH and was unable to induce nucleosome sliding; the L801del mutant showed reduced capacity to hydrolyze ATP and a diminished ability to reposition nucleosomes in vitro suggesting that the chromatin remodeling activity of LSH is impaired in ICF4 patients. Moreover, a mouse model with L801del mutant protein showed intermediate CpG methylation of nucleosomal DNA in vivo confirming the findings in vitro. Our results indicate that LSH, through its nucleosome repositioning activity, provides access of DNA methyltransferase to nucleosomal DNA, and that in ICF4 patients LSH sliding activity is impaired resulting in loss of DNA methylation contributing to the pathophysiology of the disease.

23. John C Hancock, B.S.

Neuronal modulation of myeloid cells in glioblastoma
Mentor: **Dr. Masaki Terabe**
Study Section: **Immunology - Tumor Immunology**

Despite the current therapeutic standard of surgery, radiation, and chemotherapy, patients diagnosed with the primary brain tumor glioblastoma (GBM) currently have a poor prognosis, with a median overall survival of 14-17 months. Furthermore, despite proven efficacy in a variety of cancers, there are no immunotherapy clinical trials in GBM that have shown efficacy. Previous studies have identified that this is partly due to the large populations of immunosuppressive myeloid cells in the tumor microenvironment: brain resident microglia and bone marrow-derived macrophages. Additionally, recent studies demonstrate that tumor cells integrate into neural networks, forming synapses with neurons, and neuronal firing facilitates tumor growth. These neurons become overactive and co-release ADP and neurotransmitters. We hypothesized that, in addition to promoting tumor growth, neurons influence myeloid populations in GBM. If this myeloid cell-neuronal interaction occurs, it offers an opportunity to modulate this immunosuppressive cell population to potentially augment immunotherapy efficacy. Using immunofluorescent staining of human GBM tissue to explore the relationship between neurons and myeloid cells, we determined that approximately 50% of the Iba1+ myeloid cells colocalized with the presynaptic neuronal marker synapsin 1. These findings support our hypothesis that a significant interaction occurs between neurons and myeloid cells in these tumors. To identify the mechanism of this interaction, we used freshly acquired human GBM tissue to sort for the CD11b+ myeloid cells. Bulk RNA-seq of these samples revealed high expression of neurotransmitter receptors, predominantly the Gi protein-coupled P2Y12 receptor for ADP. Using calcium fluorescence imaging and calcium flow cytometry, we screened these isolated myeloid cells for responsiveness to neurotransmitters such as glutamate, GABA, acetylcholine, dopamine, and serotonin and found that the cells were only responsive to ADP. This same set of neurotransmitters was tested in a phagocytic bead assay, and only ADP statistically enhanced the phagocytic ability of these cells. We hypothesize that ADP binding to the P2Y12 receptor promotes a pro-inflammatory, anti-tumor response in these myeloid populations. We are in the process of validating these findings in vivo in two immunocompetent mouse models of GBM and are currently synthesizing a selective P2Y12 agonist, with the overarching goal of improving GBM outcomes.

24. Yuta Hikichi, Ph.D

Mutations outside integrase lead to high-level resistance to HIV-1 integrase inhibitors in cell culture and in vivo
Mentor: **Dr. Eric Freed**
Study Section: **HIV and AIDS Research**

HIV-1 integrase (IN) strand transfer inhibitors (INSTIs) are a key class of antiretrovirals with minimal side effects, high potency, and a substantial genetic barrier to resistance. INSTIs block integration by interacting with a complex of IN and viral DNA (the intasome). Since 2018, the WHO has recommended the INSTI dolutegravir (DTG) as a component of the preferred first- and second-line treatment for people living with HIV (PLWH). Resistance mutations to INSTIs are typically found in the IN-coding region, often altering drug-target interactions. However, recent clinical studies have documented numerous cases of virologic failure (VF) to INSTIs in the absence of mutations in IN, suggesting that HIV-1 takes non-canonical resistance pathways to escape inhibition by INSTIs. To investigate INSTI resistance pathways, we subjected multiple HIV-1 isolates to long-term (~1 year) propagation in primary T cells in the presence of increasing DTG concentrations. Independent of viral strain, HIV-1 acquired resistance to DTG through the sequential acquisition of mutations in the envelope glycoprotein (Env) and nucleocapsid (NC), with occasional IN mutations. We demonstrated that Env mutations confer modest to high-level resistance to INSTIs by increasing infection capacity. Also, by enhancing the efficiency of virus replication, these Env mutations increased the opportunity for HIV-1 to acquire high-level resistance mutations. NC mutations selected in the presence of DTG conferred modest but specific INSTI resistance. Virological analysis revealed that the NC mutations accelerate integration kinetics, reducing the time between the completion of reverse transcription and integration. This shortens the time window during which INSTIs can act, thereby reducing INSTI efficacy. To assess the clinical relevance of results from our cell-culture selections, we analyzed samples from PLWH experiencing VF on DTG-containing regimens. Notably, plasma HIV RNA sequences from some individuals at VF showed NC mutations similar to those observed in cell culture, and a few cases showed both NC and IN resistance mutations. Phenotypic analysis demonstrated that mutations in NC and IN act in concert to increase resistance to DTG. These findings provide novel and clinically relevant insights into mechanisms and pathways of INSTI resistance and highlight the importance of genotypic analysis outside IN in individuals experiencing VF on INSTI-containing regimens.

25. Yi Chun Huang, PhD

BreakID: A Single-Locus Proximity Labeling Approach for Mapping Proteins at DNA Double-Strand Break
Mentor: **Dr. Andre Nussenzweig**
Study Section: **DNA Replication, Damage and Repair**

Background: DNA double-strand breaks (DSBs) represent a critical threat to genome integrity, initiating complex DNA damage response (DDR). While various repair factors and chromatin modifications coordinate DSB resolution, their precise recruitment dynamics and spatial organization remain incompletely understood. Current approaches, such as ChIP-seq and CUT&Tag require prior knowledge of target protein, limiting their ability to identify novel or transient interactions. Although mass spectrometry-based proteomics is unbiased, it lacks the spatial and temporal resolution required to resolve individual break sites. To address these limitations, we developed BreakID, a CRISPR-Cas9-based proximity labeling system enabling unbiased, single-locus, time-resolved protein mapping at DNA breaks. Methods: BreakID achieves precise temporal control using a doxycycline (Dox)-inducible Cas9 fused to miniTurboID, a rapid biotin ligase that labels proteins within ~10 nm upon biotin addition. Inducible mammalian cell lines were engineered to express BreakID alongside guide RNAs (gRNAs) targeting specific genomic loci. After Dox-induced BreakID expression and DSB induction, biotin was added to activate miniTurboID. Proteins biotinylated at the repair site were isolated through biotin pulldown and subsequently analyzed by Western blot, ChIP-qPCR, and mass spectrometry. Results: BreakID efficiently generated targeted DSBs, demonstrated by localized biotinylation of known DDR proteins. Western blot and ChIP-qPCR assays confirmed robust enrichment of hallmark DDR markers such as γH2AX and 53BP1 specifically at the induced break sites. Temporal profiling revealed the rapid induction of early-stage markers like γH2AX. Extended biotin labeling enabled the detection of later-recruited proteins, such as RAD51, marking the progression of repair pathways. Ongoing mass spectrometry analyses are comparing DDR factor recruitment in heterochromatin and euchromatin using distinct gRNAs to uncover chromatin context-specific repair dynamics. Conclusion: BreakID is a robust, antibody-free method for mapping proteins at single DNA break sites, offering high spatial and temporal resolution along with

unbiased discovery capabilities. Its application can elucidate chromatin-dependent DNA repair mechanisms, uncover novel DDR regulators, and provide deeper insights into genome stability dynamics.

26. Patrick Huang, DVM

A time kinetic study of innate lymphoid cells and innate-like T cells in hepatocellular carcinoma
Mentor: **Dr. Tim Greten**
Study Section: **Immunology - Tumor Immunology**

The major focus of cancer immunotherapy has been on conventional T cells due to their unique recognition of specific peptide antigens. Despite major advances in the cancer immunotherapy field, the overall mortality rate of HCC continues to rise. Innate lymphoid cells (ILC) and innate-like T cells (ILTC) are broadly categorized within the innate lymphoid population as playing important roles in inflammation, tissue repair and immune tolerance. Their ability to react rapidly to stimulants in the local tissue environment as well as to recruit and activate a variety of other immune cells makes these cells attractive targets for anti-tumor therapy. However, previous studies looking at ILCs and ILTCs in cancer have revealed both pro- and anti-tumor functions. Furthermore, the complex interplay between these cell populations in the tumor microenvironment is not well understood, particularly over the natural course of tumor development. In this study, we aim to provide the first systematic time course analysis of ILC and ILTC populations over HCC progression by examining their kinetics and cytokine production over time as well as identifying key populations that may influence tumor progression. We performed hydrodynamic tail vein injections in mice with plasmid combinations to alter expression of MYC and TP53 in hepatocytes. Histologic analysis of livers at several time points reveal evidence of neoplastic transformation as early as day 4, progressing to macroscopic tumors over 3-4 weeks. We then designed comprehensive 29-plex spectral flow cytometry panels to assess broad kinetic and cytokine changes in all lymphoid populations. We show that in early time points (4-7 days post-injection), mucosal-associated invariant T (MAIT) cells, group 1 ILCs, ILC2s, and ILC3s expand in frequency. Cytokine analysis shows a generalized decrease in cytolytic granule production in MAITs and group 1 ILCs over time, suggesting gradual dysfunction. Finally, Zfp683-/- (Hobit) mice lacking liver ILC1s develop significantly increased tumor burden at day 4 compared with wildtype mice. These preliminary findings demonstrate that ILC and ILTC populations expand early during tumor progression but gradually decrease in functionality over time. Additionally, liver ILC1s are poised to exert strong anti-tumor function in the early stages of tumorigenesis. Further studies, including transcriptomic analysis, will focus on identifying mechanisms driving ILC1 function early in tumor progression.

27. Yue Huang, Ph.D

Overcoming Immunosuppression in Small Cell Lung Cancer: TGF-β Inhibition and Macrophage Reprogramming Synergize to Enhance Anti-Tumor Immunity and Improve Outcomes
Mentor: **Dr. Anish Thomas**
Study Section: **Oncology - Therapeutics and Translational Research**

Small cell lung cancer (SCLC) is characterized by a highly immunosuppressive tumor microenvironment (TME), contributing to poor patient outcomes. Transcriptomic analysis of an SCLC patient cohort revealed significant overexpression of TGF-β in tumor tissues compared to normal tissues, with TGF-β target genes showing a negative correlation with T lymphocyte infiltration. This suggests that activated TGF-β signaling promotes an immunosuppressive TME and is associated with worse prognosis, highlighting its potential as a therapeutic target. Based on these findings, we explored the therapeutic implications of targeting TGF-β signaling in SCLC. Recent studies demonstrate that FSL-1, a TLR2/6 agonist, reprograms tumor-associated macrophages (TAMs) from the pro-tumor M2 phenotype to the anti-tumor M1 phenotype. Combining FSL-1 with LY2157299 (LY), a TGF-β inhibitor, synergistically enhances macrophage reprogramming and suppresses TGF-β1-induced cancer-associated fibroblast (CAF) activation and tumor fibrosis by inhibiting the TGF-β/Smad signaling pathway. This dual approach remodels the extracellular matrix (ECM) and reverses immunosuppression. In vivo studies further demonstrate that the combination of FSL-1, LY, and PD-L1 blockade significantly increases intratumoral lymphocyte infiltration and inhibits tumor growth, underscoring its translational potential. These findings integrate genomics, transcriptomics, and therapeutic insights, positioning TGF-β signaling as a pivotal target for SCLC immunotherapy. By leveraging the synergistic effects of FSL-1 and LY,

this study provides a novel combinatorial strategy to reprogram the TME, enhance immune activation, and improve therapeutic outcomes in SCLC.

28. Youngkyu Jeon, PhD

Expanding the Capabilities of Combinatorial CRISPR Screening with Engineered Cas12a Nuclease and Novel Direct Repeat Variants
Mentor: **Dr. Michael Aregger**
Study Section: **Genetics - Diseases**

The development of CRISPR-Cas technologies has revolutionized functional genomics, enabling high-throughput screens to uncover genotype-phenotype relationships. While traditional CRISPR screens rely on Cas9 to target single genes, combinatorial systems are needed to address the biological complexity inherent in gene interactions. The CHyMErA platform, which integrates Cas9 and Cas12a nucleases with hybrid guide (hg)RNAs from a single promoter, enables dual targeting for efficient gene perturbation and interaction mapping. To improve CHyMErA, we optimized the efficiency of Lachnospiraceae bacterium (Lb)Cas12a by testing two engineered nucleases with a hgRNA library designed for cell fitness-based functional readouts. Both nuclease variants outperformed wild-type LbCas12a, achieving perturbation efficiency closer to Cas9 and ensuring a more balanced dual-targeting system. Next, we focused on enabling higher-order multiplexing, where more than two genetic elements are targeted simultaneously, critical for studying paralog families, complex genetic interactions beyond simple pairs, and the development of multi-drug resistance strategies. The intrinsic RNase activity of Cas12a offers a promising approach to achieve higher-order multiplexing through expression of Cas12a guide arrays. However, the use of identical direct repeat (DR) guide scaffold sequences presents several challenges: difficulties in synthesizing oligonucleotides with repeated sequences, increased recombination during lentiviral packaging and PCR amplification, and transcriptional termination triggered by T-rich regions within repeated DR sequences. To address these challenges, we designed a library of 20,000 synthetic LbCas12a DR variants by introducing nucleotide changes to disrupt the poly-T region and diversify the DR sequence. We subsequently performed high-throughput CRISPR screens using the CHyMErA platform by coupling DR variants with Cas12a spacers expected to trigger cell fitness phenotypes. We validated hits from the CRISPR screens through focused experiments involving multiple gRNA structures, ranging from one to three multiplexed spacers. Our work revealed six functional LbCas12a DR variants that enable reliable higher-order multiplexing, paving the way for complex combinatorial CRISPR screens. These optimized DRs enhance the CHyMErA platform's versatility, opening the door for interrogating intricate genetic networks and better understanding of biological complexity.

29. Dongya Jia, PhD

Quantifying the fluctuation of cell numbers in hematopoiesis
Mentor: **Dr. Gregoire Altan-Bonnet**
Study Section: **Computational Biology/Systems Biology**

Removed at request of author

30. Samanthreddy Kedika, Ph.D

Synthetic histone H3 with site specific CoA modification to unravel the complexity of p300 mediated histone acetylation.
Mentor: **Dr. Terrence Burke**
Study Section: **Chemistry - General**

A key regulator of gene expression, histone acetyltransferase p300 catalyzes histone acetylation by transferring the acetyl group from Coenzyme A (CoA) onto the lysine. Histone acetylation is pivotal in cellular processes during development and homeostasis. Dysregulation of p300 is linked to numerous diseases, including cancer and cardiovascular diseases. Despite playing a critical role in acetylation, current structures do not detail how full-length p300 protein engages on histones. Because

p300 acetylates multiple histone lysine residues, a heterogenous mixture of structures exist in equilibrium, with p300 located on different lysine residues, making structural analysis challenging. To address the structural complexity of p300 mediated acetylation, we appended CoA modification onto histone H3 lysine at 27th position (H3K27) where p300 has a preference for acetylation. This modification facilitates p300 species "capture" at single lysine residue by i) Increasing the histone H3 binding affinity towards p300, since the adduct should be able to interact with both the lysine and CoA substrate-binding pockets and ii) Inhibiting the p300 acetylation on other lysine residues. However, currently, there are no reports for site-specific modification of CoA on histone lysine residues using recombinant approaches. The chemical approaches are limited to short peptide fragments and are not suitable for histone H3, which consists of 135 amino acids. We overcome this problem by implementing a native chemical ligation strategy. Here, we divided the H3 sequence into a shorter five fragments each having N-terminal cysteine residues and C-terminal thioesters, including CoA containing peptide fragment. These fragments were subsequently ligated to produce full-length CoA appended H3 protein (H3K27CoA) and finally subjected to desulfurization of cysteine to alanine. Our one pot methodology is robust, avoids intermediate purifications, and enabled the preparation of synthetic histone H3 in multi-milligram scale. The synthetically modified histones were further characterized using mass spectrometry and chromatography techniques. Successful reconstitution of these CoA modified H3 into histone octamers further validates synthetic approach and provides the basis for ongoing biochemical and structural investigations. The ligation methodology that we developed can be translated to making other synthetic histones with site-specific CoA modifications on histone subtypes H2A and H4.

31. Bora Kim, PhD

Microbial riboflavin inhibits ceramide synthase 3-produced ceramide (d18:1/26:0) and delays colorectal cancer progression

Mentor: **Dr. Frank Gonzalez**

Study Section: **Cell Biology - Metabolism and Bioenergetics**

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide, with tumor progression influenced by genetic, environmental, and metabolic factors. Ceramide, a key regulator of mitochondrial function and sphingolipid metabolism, plays a crucial role in cell survival and inflammation. In mammals, ceramide synthesis is facilitated by six ceramide synthases (CERS1-6), which produce ceramides of varying chain lengths with distinct signaling roles. An imbalance in ceramide chain lengths is linked to cancer progression. Clinical studies in CRC show increased long-chain ceramides and decreased short-chain ceramides. However, the role of ceramides with different chain lengths in CRC remains unclear. We performed lipidomic analyses in CRC patients and mouse colon cancer models and discovered elevated ceramide d18:1/26:0 (C26) levels in CRC tumor samples. Notably, C26 was significantly increased in patients with advanced oncological characteristics and was also detected in the plasma and stool samples of CRC patients, suggesting C26 as a CRC biomarker. Functional studies demonstrated the tumor-promoting effects of C26 in MC38 colon cancer cells, patient-derived CRC organoids, and mouse xenograft models. Mechanistically, we found that C26 directly binds to the extracellular region of EGFR, activating its phosphorylation and subsequently promoting PI3K/AKT, MAPK, and RAS signaling to enhance tumor cell proliferation. The production of C26 was mediated by ceramide synthase 3 (CERS3) activity, which was significantly increased in both human and mouse CRC samples. Using patient- and mouse-derived CRC organoids, along with intestine-specific Cers3-null mice, we established a strong correlation between CERS3 activation, C26 production, and CRC progression. Interestingly, while high CERS3 expression accelerated tumor progression, some patients showed obvious heterogeneity indicating the presence of endogenous metabolites that suppress CERS3 activity. We identified riboflavin, produced by Bacteroides cellulosilyticus, as a natural inhibitor of CERS3, thereby delaying CRC progression. Furthermore, the FDA-approved drug aclidinium bromide, showed strong inhibition of CERS3, thus revealing its therapeutic potential for CRC treatment. This study unveiled a novel metabolic mechanism linking ceramide signaling and CRC progression, emphasizing CERS3 inhibition as a potent therapeutic strategy.

32. Jee Min Kim, PhD

Direct observation of transcription factor binding at target genes in living cells
Mentor: **Dr. Dan Larson**
Study Section: **Gene Expression - Transcriptional Regulation (Eukaryotic)**

Gene expression is orchestrated by transcription factors (TFs), which bind to specific DNA sequences to regulate transcription. With approximately 1,600 genes encoding for TFs, the nucleus is crowded with non-specific TFs, posing a fundamental challenge: how do genes reliably distinguish specific TFs from the vast pool of non-specific TFs? While TF dynamics in live cells have been extensively studied, their binding kinetics in the context of transcriptional state at endogenous gene loci remain largely unknown. To address this, we used single-molecule imaging to observe endogenous TF binding at native genes in living cells, coupled with a model of kinetic proofreading. We focused on the glucocorticoid receptor (GR), a ligand-inducible TF belonging to the conserved nuclear receptor superfamily. GR mediates gene transcription in response to the steroid glucocorticoid, making it an ideal model for studying TF specificity. Using single-molecule tracking (SMT), we first examined the global dynamics of endogenously Halo-tagged GR. GR residence times on chromatin significantly increases in the presence of steroid, with minimal stable binding in the inactive state, suggesting that long-lived GR binding lasting tens to hundreds of seconds is relevant for transcriptional activity. In contrast, the target search dynamics for unbound GR molecules are largely unaffected by steroid activation, suggesting that increased GR occupancy at chromatin targets is due to slower off-rates (dissociation rates) rather than enhanced on-rates (binding frequency). To spatially resolve specific from non-specific TF binding, we next tagged the GR-responsive ERRFI1 gene locus with MS2 stem loops to fluorescently mark the gene in the transcriptionally active state. Using dual-color single-molecule imaging, we found that GR exhibits substantially longer residence times near ERRFI1 compared to its non-target gene, MYH9. In combination with the conceptual framework of kinetic proofreading, these data suggest genes are 'dwell time detectors' rather than simply 'occupancy detectors.' Overall, our results support the hypothesis that dissociation rates serve as a kinetic signature to distinguish specific from non-specific TF binding events, offering new insights into the molecular mechanisms underlying TF specificity.

33. Jeongjin Kim, Ph.D.

RNA-Coupled CRISPR Screens Uncover ZNF207 as a Novel Regulator of LMNA Aberrant Splicing Linked to Progeria
Mentor: **Dr. Thomas Gonatopoulos-Pournatzis**
Study Section: **RNA Biology**

Splicing is a fundamental RNA processing mechanism essential for gene expression and cellular function. Its precise regulation is critical, and dysregulation is linked to genetic disorders and complex diseases. Despite advances in understanding pre-mRNA splicing mechanics, the precise regulatory factors and pathways governing most alternative splicing events remain poorly defined. To address this, we developed CRASP-Seq (CRISPR-based identification of Regulators of Alternative Splicing with Phenotypic Sequencing), a novel method that integrates pooled CRISPR-based genetic perturbations with deep sequencing of splicing reporters. This approach enables quantitative assessment of the impact of every human gene on alternative splicing from a single RNA extraction. As proof-of-concept, we applied CRASP-Seq to investigate a cryptic splicing event in LMNA associated with Hutchinson-Gilford Progeria Syndrome (HGPS), a rare genetic disorder characterized by premature aging. HGPS arises from a single, de novo synonymous mutation that induces aberrant LMNA splicing, leading to the production of a truncated lamin protein known as progerin. Presence of progerin increases reactive oxygen species levels, leading to cellular damage and driving symptoms of accelerated aging. Our CRASP-Seq screen identified ZNF207, a zinc-finger protein previously implicated in kinetochore formation, as a novel factor that promotes progerin isoform expression. ZNF207 depletion enhances canonical splicing of endogenous LMNA and reduces progerin levels in patient-derived cells. Transcriptomic and proteomic analyses reveal that ZNF207 broadly influences alternative splicing by interacting with spliceosome complexes and binding RNA targets, underscoring its direct role in splicing regulation. To explore ZNF207's mechanism, we performed high throughput mutagenesis, identifying and validating critical amino acids essential for ZNF207's splicing activity. Strikingly, these residues cluster within the zinc finger (ZnF) domain, which we found to be indispensable for ZNF207's role in splicing. Mechanistic studies demonstrated that the ZnF domain mediates critical interactions with U1 snRNP proteins, establishing ZNF207 as a novel U1 snRNP auxiliary factor. Collectively, this study establishes CRASP-Seq as a powerful platform for systematically identifying alternative pre-mRNA splicing regulators, with broad implications for understanding and treating splicing-related diseases, such as progeria.

34. Jessica Kindrick, DPhil

High-Throughput Screening Reveals Novel Synergistic Drug Combinations for Metastatic Castration-Resistant Prostate Cancer
Mentor: **Dr. William Figg**
Study Section: **Oncology - Therapeutics and Translational Research**

Metastatic castration-resistant prostate cancer (mCRPC) remains a significant clinical challenge, with patients often developing resistance to androgen receptor (AR) targeted therapies such as abiraterone and enzalutamide. A key resistance mechanism involves the AR splice variant AR-V7, which drives tumor progression and poor prognosis. To address the need for novel, effective treatment strategies, we conducted unbiased high-throughput screening (HTS) to discover highly synergistic drug combinations that improve therapeutic efficacy, overcome resistance mechanisms, and minimize toxicity. Leveraging a robotic automation platform, we screened 2,480 mechanistically annotated compounds from an oncology-focused library, targeting over 800 biological pathways, across 8 mCRPC cell lines representing various stages of disease progression. Single-agent cytotoxicity was assessed using an area under the 11-point dose-response curve (AUC) metric, identifying key targets relevant to mCRPC, including tubulin, MCL-1, CDK9, BRD4, and MTOR. This led to the selection of 42 compounds for pairwise study of all possible 861 dual-drug combinations in two AR-V7-expressing mCRPC cell lines, LNCaP95 and VCaPCR. Utilizing the ExcessHSA model, this large-scale analysis revealed highly synergistic combinations, particularly between MCL-1 and BcL-xL inhibitors. These results guided the development of a novel triple combination therapy to simultaneously target CDK9-regulated productive RNApol2 phosphorylation (Enitociclib), BRD4-mediated epigenetic remodeling (Pelabresib), and pro-survival BcL-xL (Navitoclax). Notably, the triple combination demonstrated robust synergy, inducing apoptosis within 4 hours post-treatment through rapid Caspase3/7 cleavage. Further mechanistic investigation through RNA-sequencing revealed downregulation of key oncogenes, including MYC, and AR-V7, alongside disruption of downstream transcriptional pathways. Importantly, the triple combination was validated in patient-derived mCRPC organoid models LuCaP167 and LuCaP136, confirming its translational potential. These findings provide a strong rationale for clinical development of multi-drug targeted therapies to overcome AR-V7-driven resistance and deliver durable responses with reduced toxicity for mCRPC patients with limited treatment options. Our approach also highlights the power of HTS to unbiasedly innovate synergistic therapies while uncovering critical biological mechanisms underlying resistance.

35. Sarah Krantz, PhD

Adora2b receptor as a novel target for skin cancer treatment
Mentor: **Dr. Ramiro Iglesias-Bartolome**
Study Section: **Oncology - Therapeutics and Translational Research**

Skin basal cell carcinoma (BCC) is the most common cancer in the United States and is caused by mutations in the Hedgehog (HH) signaling pathway. A leading treatment of advanced BCC are inhibitors of Smoothened (SMO), a HH pathway receptor. However, clinical response rates to these drugs are low and resistance is common, urging new therapeutic interventions. While BCC is mainly caused by dysregulated HH signaling, we have previously found a complementary pathway vital to BCC: the G-alpha-s (Gs) protein regulated pathway. In mouse skin, inactivating Gs or its downstream target protein kinase A (PKA) triggers BCC formation through activation of the HH pathway. Understanding how Gs and PKA regulate HH signaling and BCC formation can reveal new targets for the pharmacological intervention of cancer growth. While Gs or PKA disruption is sufficient to develop BCC, questions remained on the similarities of Gs/PKA-induced BCC with tumors arising from SMO overactivation. We utilized mouse models to compare BCC tumors by bulk and single-cell RNA sequencing. Canonical BCC formation was initiated by expressing a constitutively active form of SMO in the skin. Non-canonical BCC was triggered by skin specific knockout of Gs (GsKO) or overexpression of the PKA inhibitor protein (PKI). Single-cell RNA sequencing of tail skin revealed that tumors from all mice were almost indistinguishable, illustrating their remarkably similar gene expression signatures. Our results established that BCC tumors arising from Gs pathway inactivation are highly similar to those resulting from canonical Hedgehog signaling, indicating that this pathway could be a viable target for BCC treatment. To confirm the efficacy of this pathway in treatment, we tested activation of Gs as a therapeutic option for BCC. We targeted two Gs receptors that were

overexpressed in BCC mice from our sequencing experiments: Adrb2 and Adora2b. We initiated BCC tumors in SMO overactivation mice and then treated the mouse ears topically with agonists of each receptor. While activation of Adrb2 did not reduce tumors, we found that Adora2b activation significantly reduced tumor burden in BCC mice. Treatment with the Adora2b agonist also reduced proliferation in the epidermis as shown through PCNA staining in ear sections. Our data illustrates the feasibility of using the Gs pathway to treat BCC as well as identifying a novel druggable target for therapy.

36. Sushila Kumari, PhD

HIV-1 CAPSID-NUP98 INTERACTIONS THAT FACILITATE VIRAL CORE NUCLEAR IMPORT
Mentor: **Dr. Vinay Pathak**
Study Section: **HIV and AIDS Research**

HIV-1 infects non-dividing cells and must traverse an intact nuclear envelope to integrate into the host genome. In HIV-1 virions, conical-shaped capsids composed of capsid protein (CA) enclose the viral genomic RNA and replicative enzymes, referred to as the viral core. We recently reported that intact HIV-1 cores are imported through nuclear pore complexes (NPCs) and uncoat in the nucleus near sites of integration. The NPC is composed of 8-48 copies of ~30 different nuclear pore proteins (NUPs), and 10 of these NUPs extend intrinsically disordered domains containing up to 40 copies of phenylalanine-glycine (FG) repeats, forming a selective permeability barrier that restricts diffusion of proteins >40 kDa. We sought to determine how the HIV-1 capsid, which is >1000 times larger than the NPC passive diffusion limit, traverses the NPC. Knockdown of NUP98, a major contributor to the permeability barrier, reduced HIV-1 nuclear import by >60% compared to control siRNA-treated cells, indicating that NUP98 plays a crucial role in this process. We determined which FG motifs in NUP98 are critical for interaction with the HIV-1 capsid by replacing the capsid-binding SPRY domain of rhesus TRIM5α restriction factor with NUP98 fragments, stably expressing the fusion proteins in target cells of infection and measuring HIV-1 infectivity. Extensive mutational analyses showed that in NUP98, 8, 3, and 9 FG motifs in three fragments of the N-terminal 500 amino acids of NUP98 (F1.1, F1.2, and F1.3, respectively; 39 total) play crucial roles in binding to HIV-1 capsid and inhibiting HIV-1 infection. Mutations of the critical 8 capsid-binding FG motifs in the F1.1 mutant dramatically reduced binding of full-length NUP98 to capsid tubes. Interestingly, inhibition of the CA-cyclophilin A (CypA) interaction indicated that additional FG motifs in the F1.1 mutant that cannot bind capsid in the presence of CypA can bind in the absence of CypA. Knockdown of endogenous NUP98 with shRNA and adding back expression of shRNA-resistant WT NUP98 restored nuclear import of HIV-1 cores as well as 2XmCherry-NLS; in contrast, NUP98 mutants lacking critical capsid-binding FG motifs, restored nuclear import of 2XmCherry-NLS but not HIV-1 cores. Our results show that specific and multiple FG motifs of NUP98 play a crucial role in binding to and nuclear import of HIV-1 core and that CypA binding to the capsids interferes with the interactions of specific FG motifs with the capsid.

37. Fengchao Lang, Ph.D

ONCOMETABOLITE D-2-HYDROXYGLUTARATE IMPAIRS HOMOLOGOUS RECOMBINATION BY DISRUPTING CHROMATIN TOPOLOGY
Mentor: **Dr. Chunzhang Yang**
Study Section: **DNA Replication, Damage and Repair**

D-2-hydroxyglutarate (D-2-HG) is an abnormal metabolite produced in high concentrations in cancers with isocitrate dehydrogenase (IDH) mutations, such as glioma, acute myeloid leukemia, and chondrosarcoma. The IDH mutant enzyme exhibits neomorphic activity, driving the production of D-2-HG. This oncometabolite induces a unique oncogenic phenotype, characterized by genome-wide hypermethylation, reprogrammed metabolic landscape, and impaired DNA repair. Although these alterations contribute to tumor progression and increase therapeutic vulnerabilities, the exact mechanisms linking D-2-HG to impaired DNA repair remain unclear. In the present study, we explore how D-2-HG affects chromatin structure to impair the DNA repair pathway, focusing specifically on homologous recombination (HR). We demonstrate that D-2-HG inhibits TET (ten-eleven translocation) methylcytosine dioxygenase activity, leading to widespread CpG island hypermethylation. This epigenetic modification dissociates the chromatin architectural protein CTCF (CCCTC-binding factor) from CpG-rich DNA regions. The loss of CTCF binding disrupts higher-order chromatin contacts critical for maintaining the structural integrity at the DNA damage sites. As a result, key HR repair proteins, such as BRCA2 and RAD51, fail to be recruited to sites of DNA

damage sites, significantly impairing HR efficiency in IDH-mutated cells. Our findings provide mechanistic insights into how the cancer metabolic signature leads to DNA repair deficiency, mediated by D-2-HG-induced chromatin alterations. The disruption of chromatin topology and loss of CTCF-mediated loops extrusion at DNA damage sites abolish effective DNA repair signaling, indicating a therapeutic vulnerability in IDH-mutant cells. Our study suggests that IDH-mutant tumors may be particularly susceptible to DNA repair inhibitors, such as PARP inhibitors, or other agents that further compromise HR repair.

38. Weilin Li, PhD

Investigating the Roles of Cell Polarity Protein RACGAP1 in Regulating Gemcitabine Resistance in Pancreatic Cancer
Mentor: **Dr. Senthil Muthuswamy**
Study Section: **Cell Biology - General**

Removed at request of author

39. Xiaoyan Liu, PhD

Ceramide synthase 4 ameliorates obesity and food intake via ceramide-adipose-brain axis
Mentor: **Dr. Frank Gonzalez**
Study Section: **Biochemistry - Proteins, and Lipids**

Removed at request of author

40. Liang Liu, PhD,MD

EZH2 Inhibition Activates Notch1 to Enhance SCLC Immunotherapy
Mentor: **Dr. Nitin Roper**
Study Section: **Immunology - Tumor Immunology**

Removed at request of author

41. Meng Liu, Ph.D.

SPINK1 Regulates the Spatial Co-Dependency of Tumor and Microenvironment in Liver Cancer
Mentor: **Dr. Lichun Ma**
Study Section: **Computational Biology/Systems Biology**

Primary liver cancer (PLC) is among the most lethal malignancies worldwide, with a median overall survival of 6–10 months and a 5-year survival rate of approximately 20%. It is characterized by extensive intra-tumoral heterogeneity (ITH), creating a molecular conundrum where defining effective molecularly-targeted therapeutics is extremely difficult. Increasing evidence, including our own research, suggests that spatial context of diverse tumor cells may play a crucial role in shaping ITH. However, understanding how each tumor establishes its unique spatial landscape and what factors drive the landscape for tumor fitness remains significantly challenging. Here, we hypothesize that diverse tumor cells may spatially coordinate as tumor cell 'villages' to promote growth and enhance defense, akin to the communal mechanism of human villages. To test the hypothesis, we profiled ~2.4 million cells from 50 liver cancer biospecimens using spatial single-cell imaging and single-cell RNA sequencing. We developed a graph attention networks-based strategy to spatially map tumor cell states and the architecture surrounding them. Eight distinct tumor cell "villages" were identified, each characterized by specific coordination among different tumor cell states and supported by unique microenvironmental landscapes. Notably, these villages exhibited village-specific molecular co-dependencies between tumor cells and microenvironments and were associated with patient outcomes. Among the key regulators, SPINK1 emerged as a critical driver of spatial molecular co-dependencies within tumor cell villages linked to poor clinical outcomes. $SPINK1^{+}$ tumor cells, enriched in metastasis-related features, interacted

extensively with matrix cancer-associated fibroblasts. Perturbation of SPINK1's co-regulatory network impaired village identity predictions in a random forest model, suggesting its role in maintaining tumor village integrity. We further validated our findings using 10X Visium spatial transcriptome data from 39 HCC patients and bulk transcriptome data from 674 HCC patients. Our study provides new insights into the spatial organization of tumors and identify SPINK1 as a novel regulator of tumor landscapes in aggressive liver cancer, highlighting SPINK1 as a potential therapeutic target for developing improved treatment strategies in PLC.

42. Anthony Martini, PhD

An NADH:quinone Oxidoreductase of Staphylococcus aureus Mediates Resistance to Pseudomonas aeruginosa-derived Pyocyanin
Mentor: **Dr. Anupama Khare**
Study Section: **Microbiology and Antimicrobials**

Pathogenic bacteria can interact with co-infecting pathogens during infection, potentially altering fitness and virulence. Staphylococcus aureus is a human pathogen that frequently co-infects patients with another pathogen, Pseudomonas aeruginosa, and the simultaneous presence of both is associated with worse disease progression. P. aeruginosa produces the antimicrobial pyocyanin (PYO) that can be detected during infection and is toxic to both human cells and other microbes, including S. aureus. Since these pathogens can coexist for years within patients, we hypothesized that S. aureus could develop resistance to PYO. Using experimental evolution where we selected for S. aureus capable of enhanced growth in PYO, we identified a mutation in the predicted promoter of a putative NADH:quinone oxidoreductase, azoR (azoR*), that was present in each of our three independently evolved populations. Recapitulating the azoR* mutation in the parental strain or overexpressing azoR on a plasmid was sufficient to impart resistance to PYO, indicating that increased azoR expression mediates PYO resistance. Since AzoR is predicted to transfer electrons between NADH and quinones, we hypothesized that AzoR functions as an NADH dehydrogenase (NDH) in the electron transport chain (ETC). We found that PYO resistance was dependent on the presence of menaquinone, the low-oxygen cytochrome bd oxidase, and a functional TCA cycle, indicating that azoR* functions through NADH and the ETC to confer PYO resistance. Unexpectedly, we observed that the azoR* allele still conferred PYO resistance in a menaquinone- and respiration-deficient mutant when grown in fermentable glucose. Further, PYO alone could enhance growth of this respiration-deficient S. aureus when cultured in the presence of glucose, but not a non-fermentable carbon source. These results suggest that S. aureus can utilize PYO for extracellular electron transport like observed in P. aeruginosa, where PYO regenerates NAD+ from NDHs to promote fermentation, and that azoR* likely confers PYO resistance during fermentation by enhancing the rate of such NAD+ regeneration. Together, these data indicate that AzoR functions as an NDH whose overexpression mediates resistance to PYO by cycling electrons under both respiratory and fermentative conditions. Our work thus identifies a multifaceted metabolic adaptation to interbacterial antagonism and suggests potential mechanisms for increased fitness in polymicrobial environments.

43. Sameer A Mir, PhD

Non-virally produced hYP218 CAR T-Cells Targeting Mesothelin-Positive Solid Tumors
Mentor: **Dr. Raffit Hassan**
Study Section: **Immunology - Immunotherapy**

Background and Hypotheses: CAR T-cell therapy has revolutionized cancer treatment, however, its application in solid tumors is hindered by challenges, including immunosuppressive tumor microenvironment (TME), high manufacturing costs, and adverse events like cytokine release syndrome (CRS). Mesothelin is overexpressed in various solid tumors and is an ideal target for CAR T-cell therapy. Traditional viral vector-based CAR T-cell production has limitations, including insertional mutagenesis, high cytokine release, and T-cell exhaustion. We hypothesized that a non-viral approach targeting the specific loci to generate mesothelin-specific CAR T cells could improve cell persistence, reduce exhaustion, and enhance therapeutic efficacy while minimizing risks like insertional mutagenesis and CRS. Study Design and Methods: We developed non-viral CAR T cells using a CRISPR/Cas9 based system for targeted integration of the mesothelin targeting hYP218 CAR cassette into the AAVS1 and PD1 genomic loci. PBMCs from healthy donors were activated and nucleofected with linear DNA templates for CAR integration

along with the Cas9 and gene-specific gRNA. CAR expression, PD1 disruption, and functionality were assessed using flow cytometry, cytotoxicity, and ELISA assays. I evaluated the efficacy in mesothelin-positive ovarian (OVCAR8), mesothelioma (RH63) and pancreatic (KLM) tumor xenograft models established in NSG mice, with tumor regression, cytokine profiles, and CAR T-cell persistence analyzed. Results and Conclusions: Non-viral PD1-hYP218 CAR T cells demonstrated efficient CAR expression and robust PD1 knockout. These cells achieved tumor lysis rates of 80-90 percent in vitro and produced pro-inflammatory cytokines, including IFNg and TNFa. In vivo, PD1-hYP218 CAR T cells induced complete tumor regression in mesothelin-positive OVCAR8 and RH63 tumor models. AAVS1-hYP218 CAR T cells demonstrated slightly lower antitumor efficacy, due to differences in CAR density resulting from variable integration sites and expression levels of the CAR construct. PD1-hYP218 CAR T cells persisted within the TME and exhibited reduced expression of exhaustion markers. Mechanistic analyses revealed improved T-cell fitness and functionality, attributed to targeted PD1 disruption and stable CAR integration. This study establishes non-viral CAR T-cell therapy as a cost-effective, scalable, and safer alternative to traditional approaches for treating mesothelin-positive solid tumors.

44. Indranil Mondal, PhD

Saturated mutagenesis of hDICER1: towards unveiling the effects of genetic variants in DICER1 syndrome
Mentor: **Dr. Shuo Gu**
Study Section: **Genetics - Diseases**

Removed at request of author

45. Vivian Morris, B.A.

CRISPR-Cas9 Screens in Mice to Identify Tumor Suppressors of Aggressive Human Lymphoma
Mentor: **Dr. Louis Staudt**
Study Section: **Oncology - Development and Metastasis**

Diffuse Large B Cell Lymphoma (DLBCL), the most common form of aggressive Non-Hodgkin's Lymphoma, has been subdivided into genetic subtypes that respond differentially to therapy. The MCD subtype, characterized by MYD88 L265P and CD79B mutations, is a subtype of DLBCL with some of the worst clinical outcomes. Patients have the lowest response rates to the standard of care – R-CHOP immunochemotherapy. To study MCD biology, we have developed a mouse model harboring four MCD-associated genetic alterations. With age, these mice develop DLBCL that arises in the spleen, which suggests spontaneous splenic germinal center B Cells (GC Bs) are the cell of origin (COO) of this subtype. As MCD mice develop DLBCL with age, this suggests additional genetic events are necessary to accelerate tumor development. The MCD genetic subclass of DLBCL is enriched for mutations in many genes that could potentially function as tumor suppressors. The inclusion of a Cas9 allele in the MCD mouse model enables loss-of-function assays to study putative tumor suppressors in the premalignant COO. Screening these candidate tumor suppressors in this system revealed that loss of Setd1b, a histone methyltransferase, and Tbl1xr1, a subunit of a transcriptional repressor complex, caused the largest increase in premalignant splenic GC Bs, suggesting they play a key role in driving MCD pathogenesis. Furthermore, the combined loss of these genes causes an even larger increase in this premalignant subpopulation, indicating a functional relationship between these two genes. Single cell RNA sequencing data showed that loss of Setd1b and Tbl1xr1 alters the phenotype of splenic GC Bs, resulting in a neomorphic cell state with features of both GC Bs and memory B cells. The combinatorial loss of these genes also induced gene expression signatures characteristic of the oncogenic signaling pathways that human MCD tumors depend upon. These data point towards a cooperative functional relationship between these genes, a discovery that is further supported by analysis of human MCD tumors, in which SETD1B and TBL1XR1 are genetically inactivated in the same tumors significantly more often than expected by chance. As we continue to investigate the role of these now validated tumor suppressors in the development of MCD-DLBCL, we hope to shed valuable light on their contribution to lymphomagenesis, which may reveal new therapeutic vulnerabilities in this aggressive cancer.

46. William Nathan, Dphil

Nucleotide supplementation prevents cell death in neurons treated with platinum
Mentor: **Dr. Andre Nussenzweig**
Study Section: **DNA Replication, Damage and Repair**

Alkylating agents like cisplatin are among the most frequently used cytotoxic cancer treatments which work by inflicting DNA damage on proliferating cancer cells. However, this treatment is also associated with severe treatment-limiting side effects in primarily post-mitotic tissues like the peripheral nervous system (neuropathy). While the cytotoxic mechanisms of cisplatin in dividing cells are well known, how cisplatin affects post-mitotic cells like neurons is not, limiting the development of therapeutics to prevent the neuropathy phenotype. We have uncovered the mechanism of how cisplatin kills post-mitotic neurons. When treated with cisplatin, iPSC-derived human neurons and primary mouse cortical neurons accumulate DNA breaks which eventually drive apoptosis. These breaks are formed by nucleotide excision repair (NER) activity at sites of cisplatin-DNA damage. Quite surprisingly, neurons lacking NER have strong resistance to cisplatin, in stark contrast to dividing cells, where loss of NER has extensively been shown to induce strong sensitivity to cisplatin. We have identified that NER is toxic in neurons treated with cisplatin due to an overconsumption of limited nucleotide pools. Post-mitotic cells have very low levels of nucleotides compared to dividing cells. High levels of NER activity in response to cisplatin treatment fully consumes post-mitotic cell nucleotide pools, preventing the completion of DNA repair synthesis and leading to persistent DNA break formation at sites of DNA repair. Increasing nucleotide pools in human or mouse neurons genetically or by supplementing cells with nucleosides greatly reduced break formation and also provided durable resistance to cisplatin. In vivo, WT mice treated with cisplatin showed induction of DNA breaks in peripheral neurons within the dorsal root ganglia (DRG), the nervous system tissue most affected by cisplatin treatment. NER KO mice do not show induction of DNA breaks in DRG. Thus, we identify toxic DNA repair and low nucleotide levels as vulnerabilities responsible for cisplatin-mediated neuronal cell death and identify nucleotide supplementation as a novel therapeutic strategy to limit cisplatin-induced side effects in cancer patients.

47. Nichole S O'Neill, PhD

A Spectroscopic Study to Define Design Principles for Peptide-based Polyelectrolyte Complex Nanoparticles Formed by Flash Nanocomplexation
Mentor: **Dr. Joel Schneider**
Study Section: **Biophysics**

Nanomaterials are emerging as powerful tools for drug delivery and therapeutic intervention. Peptides possess great potential as starting materials for nanomaterials given their remarkable intrinsic property to self-assemble into noncovalent supramolecular structures. Peptides offer unique features over synthetic polymers such as inherent biocompatibility and facile chemical and biological modifications. Controlled morphology and tuning of physical and chemical properties for peptide-based materials can be achieved through simple perturbations of their primary sequence and infinite chemical diversity is available through the assortment of amino acids. There's growing interest in using peptides as nanocarriers of active pharmaceutical ingredients that possess low aqueous solubility and dissolution, properties that hinder delivery. Through peptide-drug conjugation and polyelectrolyte complexing, the drug's physicochemical properties can theoretically be tuned to increase delivery efficiency. Recognizing this, we sought to define design principles for peptide-based polyelectrolyte complex nanoparticles (PEC NP) using oppositely charged peptides as starting material. Peptide-based PEC NPs were fabricated using flash nanocomplexation, a rapid scalable method where the reacting species are homogenized in a confined impinging jet mixer, affording complete control over the assembly kinetics, NP size, surface charge, and size distribution making our systems ideal for clinical translation. A comprehensive examination of 54 peptide systems identified a minimum hydrophobic content requirement for particle formation. Hydrophobicity scores were calculated for peptides using solvent accessible area based on an extended conformation selected from an ensemble of states for the parent-peptide. Circular dichroism (CD) and photon correlation spectroscopy proved to be pivotal tools in probing the assembly of peptide monomers into PEC NPs. Interestingly, we observed the most stable PEC NPs had a CD dispersion pattern consistent with β-sheet conformation. An excess of a charged species was required for particle size stability which we corroborated with TEM micrographs. The most stable PEC NPs have a β-hairpin sequence which may restrict the assembly pathway to form highly ordered PEC NPs. Using these foundational findings, we are now developing a general method for preparing peptide-drug nanoparticles to enable delivery of drugs with otherwise poor pharmacokinetic profiles.

48. Alex Orioli, PhD

High-Throughput in vivo Telomerase Activity Probing (HT-iTAP): a new tool to measure telomerase activity
Mentor: **Dr. Eros Lazzerini Denchi**
Study Section: **Chromosomes, Chromatin, and Nuclear Architecture**

Telomerase is a ribonucleoprotein complex composed of a catalytic subunit, Telomerase Reverse Transcriptase (hTERT), and an RNA template, Telomerase RNA (hTR). Telomerase activity counteracts telomere shortening caused by the end-replication problem by adding telomeric repeats at chromosome ends. While telomerase is not expressed in somatic cells, the vast majority of human cancer cells reactivate its expression to bypass replicative senescence. Conversely, mutations that affect telomerase activity cause a set of diseases known as telomere biology disorders (TBDs), characterized by accelerated telomere attrition and stem cell depletion across multiple tissues. A subset of these mutations is found in the telomeric protein complex called Shelterin, which serves to protect chromosomal ends from DNA-damage activation. While the associations of these mutations and telomerase are clear, the overall regulation of telomerase by Shelterin and other pathways remains to be heavily explored. To bridge this gap, we developed a novel single-cell-based approach to measure telomerase activity that can be used in a high-throughput manner. We named this approach High-Throughput in vivo Telomerase Activity Probing (HT-iTAP). In this method, we introduce an inducible template-mutant of hTR to monitor telomerase's ability to incorporate new repeats at chromosome ends via fluorescence in situ hybridization (FISH). Using this approach, we have defined many aspects of telomerase regulation. We employed this method to evaluate how clinically relevant mutations in hTERT or hTR affect telomerase activity. Furthermore, we have applied this technique to uncover how telomerase activity is influenced by Shelterin subunits. Finally, we conducted a screen to identify novel players in telomerase regulation and to assess how pharmacological inhibitors affect telomerase activity. I will further characterize novel telomerase regulators with the ultimate goal of developing effective therapeutics to modulate telomerase activity.

49. Ming Qi, Ph.D.

Intestinal farnesoid X receptor deficiency protects against obesity-related metabolic dysfunction-associated steatohepatitis
Mentor: **Dr. Frank Gonzalez**
Study Section: **Biochemistry - Proteins, and Lipids**

Metabolic-associated fatty liver disease (MAFLD) is the most common chronic liver disorder in developed countries. MAFLD triggers an increased risk of metabolic dysfunction-associated steatohepatitis (MASH) and the end-stage liver diseases cirrhosis and hepatocellular cancer. Drug therapy targeting MAFLD/MASH is extremely limited. Farnesoid X receptor (FXR) is a nuclear receptor that regulates lipid homeostasis and inflammation. FXR controls lipid transport and bile acid metabolism in liver. In recent years, the intestine has emerged as a central organ participating in the control of lipid homeostasis. Interestingly, FXR expression in the intestine was significantly higher in obese humans compared lean humans. However, to date, the role of intestinal FXR in MAFLD/MASH is poorly understood. To address this issue, intestine-specific FXR knockout (Fxr-dIE) mice were examined to clarify the role of intestinal FXR in the development of MAFLD and MASH. Wild-type (WT) mice fed a 60% high-fat diet (HFD) had marked obesity and hepatic steatosis, while Fxr-dIE mice showed less body weight gain and lower hepatic steatosis. Using a high fat/cholesterol/fructose diet (HFCFD) that promotes human-like MASH pathologic features in mice, Fxr-dIE mice exhibited lower MASH activity score (MAS) and fibrosis score compared with WT mice. Consistent with a decreased MAS, hepatic genes related to inflammation and fibrosis were significantly down-regulated in HFCFD-fed Fxr-dIE mice. Mechanistically, intestinal FXR depletion reversed nutrient-excess diet-induced chemokine C-C motif ligand 28 (CCL28) expression in the intestine, thus decreasing the recruitment of pro-inflammatory macrophages via the C-C chemokine receptor type 3 (CCR3) axis and the subsequent secretion of pro-inflammatory cytokines such as IL-1beta, IL-6 and TNFalpha. Intestinal cholesterol absorption and transport were also reduced in Fxr-dIE mice via down-regulation of Niemann-pick C1-like 1 (Npc1l1) and organic anion-transporting polypeptide 3A1 (Oatp3a1, a novel direct FXR target gene), leading to lower cholesterol levels in both serum and liver of Fxr-dIE mice. These factors collectively contribute to the amelioration of obesity-related MAFLD/MASH in Fxr-dIE mice. In summary, this study revealed that intestinal FXR deficiency protected against obesity-

related MAFLD/MASH, and thus intestinal specific-FXR antagonism may be a druggable target for the treatment of MAFLD/MASH.

50. Naimur Rahman, PhD

Developing a model of extrachromosomal DNA (ecDNA) in prostate cancer
Mentor: **Dr. David Takeda**
Study Section: **Molecular Biology - General**

Increased androgen receptor (AR) activity is a key driver of therapeutic resistance in advanced prostate cancer, with AR gene amplification being the most common mechanism underlying castration-resistant prostate cancer (CRPC). Extrachromosomal DNA (ecDNA) is an established mechanism of oncogene amplification across various cancers, including prostate cancer, where it enables high AR copy numbers and worsens patient outcomes to AR-signaling inhibitors. However, the detailed role of ecDNA in AR amplification remains poorly understood due to the lack of suitable cell line models. To address this gap, we engineered a model of ecDNA containing both the AR enhancer and AR gene, spanning over 1.1 Mbp. Using a CRISPR-Cas9 sequential knock-in approach with loxP-flanked hygromycin and puromycin resistance cassettes and Cre recombinase-mediated recombination, we successfully induced ecDNA formation of the AR locus, confirmed by breakpoint PCR. To validate the model, we assessed AR copy number using qPCR, DNA Fluorescence In Situ Hybridization (FISH), and low-pass whole genome sequencing (LP-WGS), all of which confirmed AR amplification. Post-Cre clones that propagated AR-ecDNA upon puromycin selection also exhibited increased transcription and translation levels of AR and its target genes KLK3 and TMPRSS2 compared to parental lines. Furthermore, ecDNA-mediated amplification of this locus enhanced proliferation under low-androgen conditions and decreased sensitivity to enzalutamide. In the future, we will explore the oncogenic potential of engineered ecDNAs in a CRPC mouse model. Additionally, we will study effect of castration on ecDNA copy number, stability, transcriptional regulation. We anticipate this model will provide a valuable platform to investigate the regulatory mechanisms of ecDNA, its vulnerabilities, and its role in therapeutic resistance in CRPC.

51. Farid Rashidi Mehrabadi, PhD

HLA-DP1 Alleles Associate with Liver Cancer Survival via Altered Macrophage-T Cell Interaction
Mentor: **Dr. Xin Wang**
Study Section: **Oncology - General**

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are aggressive liver malignancies with poor prognoses and limited biomarkers for predicting patient outcomes. Human leukocyte antigen (HLA) variants have emerged as influential factors in cancer progression, yet their role in liver cancer survival remains poorly understood. We hypothesized that specific HLA alleles may influence liver cancer patient survival through distinct immune microenvironment interactions. In this study, we analyzed survival data from 900 patients diagnosed with HCC and CCA, identifying two critical HLA haplotypes: HLA-DPA1*01:03-DPB1*04:01 (risk allele) and HLA-DPA1*02:02-DPB1*05:01 (protective allele). Patients carrying the risk allele demonstrated significantly reduced survival compared to those with the protective allele across both cancer types. Importantly, survival differences exhibited an additive genetic pattern; homozygous risk allele carriers showed the shortest survival, heterozygotes intermediate, and homozygous protective allele carriers exhibited the longest survival. To explore mechanisms underlying this association, we performed single-cell RNA sequencing of tumor tissues from patients stratified by their HLA genotype. Our analyses revealed profound differences in macrophage polarization between these groups. Specifically, patients harboring the risk allele showed a marked reduction in macrophages expressing the chemokine CCL4L2. Furthermore, ligand-receptor interaction analysis identified significantly enhanced interactions between CCL4L2-positive macrophages and KLRB1-positive CD4 T cells via the HLA-DP1 and LAG3 axis in the risk allele group. These findings identify a novel mechanism whereby specific HLA-DP1 haplotypes shape the liver cancer immune microenvironment, influencing macrophage-T cell interactions critical for patient survival outcomes. This work not only elucidates the immunological basis of HLA-linked survival disparities in liver cancer but also highlights potential targets for personalized immunotherapeutic interventions.

52. Sounak Sahu, DPhil

Clinical Classification of BRCA2 genetic variants using Saturation Genome Editing
Mentor: **Dr. Shyam Sharan**
Study Section: **Genetics - Diseases**

Variants of Uncertain Significance (VUS) hinder the clinical utility of genetic testing in breast cancer. A notable example is BRCA2, a key DNA repair gene linked to hereditary breast and ovarian cancer, with a high carrier rate of germline single nucleotide variants (SNVs). The limited access to epidemiological data to classify these SNVs led to an exponential increase in VUSs. To comprehensively assess the function of these BRCA2 SNVs, we have developed a multiplexed assay for variant effect (MAVE) to assess thousands of variants experimentally using CRISPR-Cas9-based Saturation Genome Editing. We change each nucleotide position to all possible non-wild type nucleotides using a library of single-stranded DNA donors to essentially "saturate" every nucleotide position. Since BRCA2 is an essential gene for normal cell survival, we observed loss-of-function variants to deplete, whereas functional variants are enriched in the pool. Using deep sequencing, we measure the dropout frequency of SNVs, defined by function scores, which are subsequently analyzed using Bayesian statistics to determine their probability of pathogenicity. The likelihood of individual SNVs being pathogenic or benign is further classified into different tiers of functionality: Strong, Moderate, and Supporting. We have integrated our MAVE dataset with other available evidence and applied the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guidelines for the clinical classification of all possible SNVs. Using this framework, we have classified 437 SNVs as pathogenic, 565 as likely pathogenic, 732 as VUS, 4,478 as likely benign and 339 as benign. Subsequently, our ACMG/AMP classification showed strong concordance with the ClinVar-reported benign and pathogenic classes and around 85% of variants that were previously classified as "conflicting reports" or "VUS" were now classified. Owing to insufficient evidence based on ACMG/AMP criteria, 732 SNVs (11%) remain classified as VUS; these could be reconsidered in future when additional evidence becomes available. By integrating cellular fitness and response to DNA-damaging agents along with structural predictions, we have established that our MAVE dataset can be integrated within the ACMG/AMP clinical framework. This classification is one of the most comprehensive clinical resources for BRCA2 genetic variants, assisting physicians and genetic counselors in assessing VUS in patients.

53. Caleb K Sinclear, PhD

HUWE1 is a switch that controls the balance between B-catenin transcriptional and cell adhesion functions.
Mentor: **Dr. Andres Lebensohn**
Study Section: **Cell Biology - Organelles and Membranes**

B-catenin plays two major roles in the cell, one as the key mediator of transcriptional responses to WNT signaling in the cytoplasm and nucleus, and the other as a structural component of adherens junctions (AJs), cell-cell adhesion complexes at the plasma membrane. How the balance between these spatially separate and functionally distinct pools of B-catenin is controlled is not well understood. WNT/B-catenin signaling is a fundamental signaling pathway whose dysregulation can drive many types of cancer. Disassembly of AJ complexes often caused by reduced B-catenin at AJs is a hallmark of epithelial-mesenchymal transition (EMT), a process that promotes cancer metastasis. The goal of this study was to investigate the mechanisms that regulate the balance between the two spatially and functionally distinct pools of B-catenin. During WNT/B-catenin signaling, the main regulated step is B-catenin phosphorylation and degradation mediated by the destruction complex (DC), composed of the scaffold proteins APC and AXIN1/2, and the kinases CK1 and GSK3A/B. With CRISPR, we created two disease model cell lines from human HAP1 cells: 1. CK1 KO cells, in which the DC kinase CK1 was knocked out, and 2. B-catenin ST-A cells, containing mutations in B-catenin that render it non-degradable. In both cell lines, we observed by confocal microscopy that B-catenin accumulates in the cytoplasm and the nucleus, and promotes hyperactive WNT signaling. Importantly, we found that loss of the E3 ubiquitin ligase HUWE1 in CK1 KO cells induced a marked change in the localization of B-catenin from the nucleus to the plasma membrane, which was accompanied by a substantial reduction in WNT/B-catenin signaling. Through proximity ligation assays and a new AJ-dependent cell adhesion assay that we developed, we found that the localization of B-catenin to the plasma membrane caused by HUWE1 loss promotes B-catenin incorporation into AJs and drives an increase in its functional contribution to cell-cell adhesion. In B-catenin ST-A cells that contain non-degradable B-catenin, regulation of B-catenin subcellular localization by HUWE1 was also observed. Taken together, these results

demonstrate that HUWE1 regulates the balance between B-catenin transcriptional activity in WNT signaling and its cell adhesion functions. Therefore, regulation of B-catenin functions through HUWE1 may represent a new therapeutic strategy in cancers caused by hyperactive WNT signaling, and/or in metastasis driven by EMTs.

54. Ragini Singh, PhD

A novel lncRNA CAMAT1 interacts with the co-integrator complex to enhance the expression of MAPK signaling genes and cell migration in colorectal cancer
Mentor: **Dr. Ashish Lal**
Study Section: **RNA Biology**

Long non-coding RNAs (lncRNAs) are the largest group of genes transcribed from the human genome. Although some lncRNAs have been functional characterized, the cellular and molecular functions of the vast majority of lncRNAs remain to be elucidated. Here, we report the initial functional characterization of a poorly characterized human lncRNA LINC02582 that we termed CAMAT1 (Colorectal Adenocarcinoma Migration Associated Transcript 1). CAMAT1 is upregulated in colorectal cancer (CRC) and high CAMAT1 expression is associated with poor prognosis suggesting oncogenic functions. At the cellular level, CAMAT1 is very abundant in well-differentiated CRC cells and is predominantly localized to the nucleus. Transcriptome analysis from CAMAT1-depleted cells (using CRISPRi and CRISPR knockout) revealed that CAMAT1 activates the expression of multiple members of the S100 family of genes including S100A4 that is known to regulate actin cytoskeleton, MAPK signaling and cell migration. Consistent with this, CAMAT1-depleted cells exhibited defects in actin cytoskeleton organization, decreased ERK1/2 phosphorylation and reduced cell migration. Mechanistically, CAMAT1 RNA pulldowns followed by mass spectrometry uncovered specific binding of this lncRNA with multiple proteins of the activating signal co-integrator complex 1 (ASCC1), including the RNA-binding protein ASCC1. Importantly, silencing ASSC1 in the CAMAT1-depleted cells significantly rescued the expression of S100A4 and other CAMAT1-target genes indicating that CAMAT1 inhibits the activity of the co-integrator complex by binding to ASCC1. Collectively, our results suggest that CAMAT1 exerts its oncogenic function in CRC cells by enhancing the expression of specific genes involved in MAPK signaling and promotes cell migration by binding to ASCC1 to inhibit the activity of the co-integrator complex.

55. Emily E Steffke, BS

Tumor-specific resident memory T cells induced by viral vector vaccination mediate protection against orthotopic murine glioblastoma
Mentor: **Dr. Masaki Terabe**
Study Section: **Immunology - Immunotherapy**

Removed at request of author

56. Chiori Tabe, MD, PhD

Targeting KIF18A as a Novel Therapeutic Approach in Small Cell Lung Cancer
Mentor: **Dr. Anish Thomas**
Study Section: **Oncology - General**

Background: KIF18A, a mitotic kinesin, plays a critical role in maintaining microtubule dynamics and spindle bipolarity during mitosis. Inhibition of KIF18A results in defects in chromosome congression and the formation of multipolar spindles, leading to mitotic arrest and cell death. Small cell lung cancer (SCLC), a highly aggressive cancer characterized by neuroendocrine (NE) and non-NE features, exhibits significant chromosomal instability (CIN). Despite the potential of targeting KIF18A, its therapeutic efficacy in SCLC and the underlying factors influencing response remain unclear. Investigating these aspects could pave the way for novel KIF18A-targeted therapies in SCLC. Methods: We screened multiple SCLC cell lines with NE and non-NE features for KIF18A expression using SCLC CellMiner CDB and western blotting. Cells were treated with AM-9022, a selective KIF18A inhibitor, and classified as sensitive or resistant based on viability assays (Incucyte and ATPase assays).

Immunofluorescence and time-lapse imaging were used to assess mitotic outcomes. RNA-sequencing was performed on sensitive and resistant cell lines to explore mechanisms of resistance. KIF18A knockdown was employed to evaluate dependency across SCLC subtypes. Result: Sensitivity to AM-9022 correlated with KIF18A expression levels but was independent of the NE phenotype in SCLC cell lines. DAPI-stained metaphase analysis revealed that 95% of sensitive cells exhibited defective chromosome congression. Time-lapse imaging indicated that sensitive cells arrested in metaphase, indicating activated spindle assembly checkpoint (SAC) in these cells, while resistant cells continued through mitosis. RNA-sequencing analysis identified the E2F target pathway as significantly upregulated in sensitive cell lines, suggesting that KIF18A inhibition may activate the SAC, specifically in these cells. Conclusion: Our findings suggest that targeting KIF18A could be a promising therapeutic strategy for a subset of SCLC patients with high KIF18A expression and activated SAC signaling. These results lay the groundwork for the clinical application of novel KIF18A inhibitors, particularly in patients whose tumors exhibit elevated KIF18A levels and SAC activation.

57. Joey Toker, AB

Spatial proteomics and single-cell RNA sequencing reveal subtype-specific immune interactions in neuroblastoma
Mentor: **Dr. Javed Khan**
Study Section: **Immunology - Immunotherapy**

Infiltrating immune cells may influence neuroblastoma growth and treatment response; anti-GD2 antibody immunotherapy has benefitted patients with high-risk disease. However, outcomes vary and may depend on clinical and genetic traits, including tumor MYCN-amplification, which RNAseq studies have linked to decreased immune infiltration. We aim to understand how spatial immune-tumor interactions correlate with molecular and clinical factors, including MYCN-amplification, treatment, age, and outcome. We performed CO-Detection by indEXing (CODEX) on 5 clinically annotated tissue microarrays containing 511 neuroblastic tumor samples from 197 patients (164 with neuroblastoma, 13 with ganglioneuroblastoma, and 20 with ganglioneuroma; 84 with MYCN-amplification, 222 pre-chemotherapy). We stained for 40 tumor- and stroma-associated proteins, performed cell segmentation, phenotyped by marker intensity, and quantified neighboring stromal cells for each tumor cell. In an external scRNAseq cohort of 50 patients, we additionally compared the microenvironment by MYCN and treatment status. Preliminary analyses revealed that tumors downregulate MHC-I and feature dynamic microenvironments containing lymphocytic, myeloid, and endothelial components, whose proportions vary by MYCN and treatment status. Tumor cells with more immune interactions clustered together in proteomic space, while untreated MYCN-amplified malignant cells interacted with fewer immune cells. Interactions were generally reduced post-chemotherapy; however, MYCN-amplified tumors demonstrated persistent tumor-macrophage interactions. While T cell proportions were modestly lower, T cell-tumor interactions were almost non-existent post-treatment, regardless of MYCN status. scRNAseq showed cells expressing 17q gain and tumor-associated genes that clustered with the immune population. We utilized high-throughput, multiplex histology to investigate cell interactions within human neuroblastoma tumors, complemented by scRNAseq analyses that confirm previous results and additionally suggest a novel malignant, immune-mimicking population. By quantifying immune infiltration, we unveil distinct lymphocyte and myeloid cell interactions across patients. We are now integrating the scRNAseq and CODEX data to further uncover mechanisms associated with adverse outcomes and inform future individualized cellular immunotherapy strategies.

58. Hsi En Tsao, Ph.D.

Engineering chimeric antigen receptor (CAR) T cells targeting tumor associated exons on glypican-1 for cancer immunotherapy
Mentor: **Dr. Mitchell Ho**
Study Section: **Clinical and Translational Research - General**

Removed at request of author

59. Yicheng Wang, PhD

Circadian regulation of type 2 immune response during allergic airways inflammation
Mentor: **Dr. Chuan Wu**
Study Section: **Immunology - General**

Removed at request of author

60. Wenjuan Wang, Ph.D.

CD4+ T cell-dependent tumor control is impaired by interaction with B cells
Mentor: **Dr. Remy Bosselut**
Study Section: **Immunology - Tumor Immunology**

Removed at request of author

61. Binbin Wang, Ph.D.

Longitudinal liquid biopsy identifies an early predictive biomarker of immune checkpoint blockade response in head and neck squamous cell carcinoma
Mentor: **Dr. Eytan Ruppin**
Study Section: **Bioinformatics - algorithms, packages and tools**

Abstract Background & Approach: Immune checkpoint blockade (ICB) therapy has shown promise for treating head and neck squamous cell carcinoma (HNSCC), but only a subset of patients exhibit a meaningful response. Hence, identifying predictive biomarkers of the ICB response remains a critical roadblock. Here, for the first time, we systematically characterize the dynamic changes in the blood during ICB treatment across four different time points in a mouse HNSCC model treated with anti-PD-1 ICB. We profile serial pre and on-treatment liquid biopsies, both by single cell (SC) and bulk RNA with tandem SC-TCR sequencing to identify liquid predictors of the ICB response. Results: This study charts the host peripheral response to ICB therapy on an unprecedented scale. We observe significant differences in the clonal expansion and temporal dynamics of effector memory T cells (Tem) and B cells between responders and non-responder mice. These changes were most prominent in mice at an early on-treatment time point, highlighting this time point as optimal for assessing ICB efficacy. Remarkably, the resulting LiBIO score which integrates the Tem and B cell signatures, successfully predict ICB response in HNSCC patients across multiple tumor and blood cohorts, without any additional training. Its application using one fixed decision threshold yields a fairly high predictive odds ratio of response, which surpasses the predictive power of existing biomarkers, including a newly generated validation cohort of HNSCC patients treated with ICB. Conclusions: Collectively, our preclinical insights support the premise that peripheral immune events can serve as a foundation for biomarker discovery, offering a non-invasive, biologically grounded approach to monitor and predict immunotherapy outcomes with high fidelity in HNSCC patients. Finally, the approach presented lays a solid basis for developing similar biomarkers in other cancer indications.

62. Yangliu Xia, Ph.D.

Intestinal FABP1 underlies obesity-induced colitis
Mentor: **Dr. Frank Gonzalez**
Study Section: **Pharmacology and Toxicology**

High-fat diet (HFD) enhances proinflammatory cytokine and chemokine production and increases the incidence of inflammatory bowel disease (IBD), but the mechanisms remain unclear. Here, we found that ileal and colorectal fatty acid-binding protein 1 (FABP1) expression, as well as the levels of long-chain fatty acids (LCFA) were increased by obesity and predicted poor prognosis and worse clinical outcomes in human IBD patients. Consistently, intestinal FABP1 and LCFA levels were sharply elevated by HFD under conditions of dextran sulfate sodium (DSS)- or trinitrobenzene sulfonic acid (TNBS)-induced experimental IBD in mice. Intestinal FABP1 deficiency markedly decreased obesity-induced IBD and intestinal LCFA levels in mice. Mechanistically, lipidomics and untargeted RNAseq analyses, respectively, revealed that palmitic acid and

mRNAs encoding tight junction markers were markedly decreased, accompanied by improved anti-inflammation immune response. Palmitic acid, among the changed LCFA, at least partially contributed to the enhanced inflammation, compromised anti-inflammation immune response and tight junction disruption both in vivo and in vitro. High-throughput screening of a clinical compound library yielded 10 candidate FABP1 inhibitors, with voxilaprevir found to be a novel highly specific FABP1 inhibitor. Voxilaprevir reduces obesity-associated experimental IBD induced by DSS or TNBS accompanied by reduced intestinal LCFA levels, decreased inflammation and restored tight junction in mice, depending on the presence of intestinal FABP1 both in vivo and in vitro. Cumulatively, these results indicate that intestinal FABP1 inhibition or antagonism may be a new approach for the treatment of obesity-related IBD development via reducing fatty acids absorption.

63. Deep Kumari Yadav, PhD

Toll-like receptor 4 deletion promotes bacterial burden and cutaneous tumorigenesis in mice lacking one Ikkα allele in keratinocytes
Mentor: **Dr. Yinling Hu**
Study Section: **Immunology - General**

Toll-like receptor 4 (TLR4) is an extracellular pathogen recognition receptor (PRR) that identifies a variety of pathogens and damage-associated molecular patterns. Analysis of human TCGA database reveals many mutations of the Toll-Like Receptor 4 (TLR4) gene in human skin cancers. The exact function of PRRs in response to elevated ambient and commensal bacteria for carcinogenesis is still unknown. Recently, we found a low number of spontaneous skin squamous cell carcinomas (SCCs) in 1-year-old Ikka F/+ K5Cre mice with specific Ikka reduction in keratinocytes. Interestingly, ablation of Tlr4 enhanced SCC numbers and sizes but reduced the latency of SCC development in IkkαF/+; Tlr4-/- mice compared to Ikka F/+ mice. Both spontaneous skin SCCs obtained from Ikka F/+ and Ikka F/+; Tlr4-/- mice showed loss of wild-type (WT) Ikka allele. Thus, we hypothesize that Ikka reduction in keratinocytes alters skin microbes and Tlr4 deletion promote this condition, which lead to increased spontaneous skin SCCs. Indeed, the bacterial burden were increased in the skin of Ikka F/+ and Ikka F/+; Tlr4-/- mice compared to the skin of WT and Tlr4-/- mice predominately Gram (+) firmicutes. Further, we explore the interactions between bacteria and their hosts using skin organoids derived from these mice. Organoids are three dimensional structures recapitulating the organ's epithelial layer, linking the gap between in-vitro and in-vivo models. Skin organoids infected with heat killed gram positive bacteria reveals more DNA damage in the Ikka F/+; Tlr4-/- along with impaired bacteria clearance in these mice. Together, these findings suggest that increased bacteria cause genomic instability which leads to DNA damage. Additionally, we are investigating which bacterial pathways compromise the integrity of the skin's genome through Ikka reduction or Ikka reduction/Tlr4 deletion, as well as whether Tlr4 deletion affects immune cell function in combat against bacterial infections.

64. Leo Yamada, M.D., Ph.D.

Delta133p53α-Mediated Suppression of Cellular Senescence: A p53 Isoform-Based Strategy for Fatal Aging and Improving Immune Function
Mentor: **Dr. Curtis Harris**
Study Section: **Clinical and Translational Research - Animal Models**

Aging represents a multifaceted biological phenomenon characterized by progressive functional decline, significantly impacting morbidity and lifespan. Central to this process is cellular senescence, contributing critically to deterioration in organ functions, including the immune system. Hutchinson-Gilford progeria syndrome (HGPS), a fatal premature aging disorder caused by a de novo LMNA mutation (c.1824 C>T; p.G608G), exemplifies accelerated aging phenotypes. This mutation produces progerin, a defective nuclear lamin A protein causing DNA damage and chronic p53 activation, resulting in severe pathologies like cardiovascular disease and drastically shortened lifespan. The human TP53 gene functions beyond its traditional role as the "guardian of the genome," regulating diverse transcriptional responses via its isoforms. Among these, we identified Δ133p53α, a p53 isoform lacking the first 132 N-terminal amino acids, selectively inhibiting transcription at specific p53 response elements in a dominant-negative manner. This isoform exhibits anti-senescent effects while preserving key p53 functions, such as apoptosis and DNA damage repair, demonstrated in various human cell strains including senescent CD8+

T cells and CAR-T cells. Since Δ133p53α exists only in primates, we generated a transgenic mouse expressing human Δ133p53α using a loxP-flanked cassette driven by a CAG promoter at the ROSA26 locus, induced by tamoxifen-mediated Cre recombination. Prior to in vivo studies, MEFs from our Δ133p53α transgenic model showed reduced cellular senescence markers (p21 and SA-beta-gal). Long-term monitoring (>2 years) confirmed no tumorigenesis, emphasizing safety. Crossing these mice with the LmnaG609G HGPS model demonstrated significant anti-senescence effects, including decreased p21 expression (WB/PCR), prevention of skeletal deformities (X-ray), and preservation of vascular smooth muscle cells in the aorta (HE/IF), a leading cause of death in HGPS. Additionally, isolated T cells exhibited significantly reduced SA-beta-gal staining and decreased PD-1 expression (flow cytometry). Clinically, Δ133p53α expression in immune cells from esophageal cancer specimens (n=71) correlated positively with CD86-positive macrophages, suggesting beneficial immune activation. These findings indicate Δ133p53α's therapeutic potential against fatal aging phenotypes and immune senescence, highlighting promise for aging-associated disorders and possibly enhancing immune responses.

National Cancer Institute - Division of Cancer Epidemiology and Genetics

65. Prema Bhattacharjee, PhD

Early life body size and risks of developing biliary tract cancers
Mentor: **Dr. Jill Koshiol**
Study Section: **Epidemiology/Biostatistics - Integrative Epidemiology and Health Disparities**

Background. Biliary tract cancers (BTCs), comprising gallbladder cancer (GBC), intrahepatic bile duct cancer (IHBC), and extrahepatic bile duct cancer (EHBC), are a group of rare but highly aggressive malignancies. These cancers are characterized by a poor prognosis, largely due to late-stage diagnosis and limited effective treatment options. The distribution of BTCs varies considerably by geography and anatomical site, with GBC more commonly diagnosed in females and IHBC and EHBC more prevalent in males. Early-life body size, particularly childhood body mass index (BMI), may play a role in BTC risk. This study investigated the association between childhood BMI trajectories, z-scores, height, and the risk of BTC in a large Danish cohort. Methods. Our study included 172,113 boys and 168,503 girls born between 1930 and 1996 from the Copenhagen School Health Records Register (CSHRR). BMI values were recorded at ages 7 and 13 years, and five sex-specific BMI trajectories (below-average, average, above-average, overweight, and obesity) were identified using natural splines. Our study assessed BTC risk using Cox proportional hazards regression, stratified by sex and birth cohort, with hazard ratios (HRs) and confidence intervals (CIs). Results. During a median follow-up of 34.5 years, 635 individuals developed BTC (314 males and 321 females). In males, overweight (HR = 1.62, 95% CI: 1.08-2.42) and obesity (HR = 3.42, 95% CI: 1.71-6.84) trajectories were associated with an increased BTC risk. In females, only the obesity trajectory was notably elevated (HR = 3.01, 95% CI: 1.69-5.38). Likelihood ratio tests showed no significant sex interactions in the associations between childhood BMI z-scores, height z-scores, CDC categorization, and birthweight with BTC risk (all p-values > 0.05). Childhood height was associated with BTC risk only in males. Conclusions. Our study provides evidence that childhood overweight and obesity increase the risk of BTC, particularly in males. Our findings highlight metabolic and inflammatory pathways as key drivers of BTC and emphasize the need for further research to elucidate the underlying biological mechanisms. As childhood obesity rates continue to rise globally, targeted interventions aimed at maintaining a healthy weight trajectory may play a crucial role in reducing the future burden of BTC and other obesity-related malignancies.

66. Batel Blechter, PhD

Exposure to household air pollution and its constituents in relation to stomach and esophageal cancer
Mentor: **Dr. Qing Lan**
Study Section: **Epidemiology/Biostatistics - General**

Background: Household air pollution (HAP) is a global health burden affecting about half of the world's population, especially those in lower- and middle-income countries. In Xuanwei, China, individuals experience high concentrations of various carcinogenic HAP constituents, particularly polycyclic aromatic hydrocarbons (PAHs), from indoor cooking and heating with coal. With evidence for HAP exposure and link to disease is dominated by lung cancer research, we investigated the

association between HAP constituents and cause-specific mortality from stomach and esophageal cancer-two cancers with highest mortality rate after lung cancer. Methods: The study was conducted in a cohort of 42,420 subjects from Xuanwei, China and participants were followed from 1976 to 2011. Type of fuel used was collected through a questionnaire and categorized into lifetime smoky vs. other fuel use. Annual exposures were predicted for 43 HAP constituents using a determinant modeling approach. To address collinearity among the pollutants, we used hierarchical clustering as a method for dimension reduction in which we identified 5 pollutant clusters. Cox regression was used to estimate the association between lifetime smoky coal use and continuous pollutant clusters with stomach (n=143) and esophageal (n=39) cancer. All models were stratified by sex and birth cohort and adjusted for smoking status and education. Results: We found that lifetime smoky coal use was associated with an increased risk of stomach cancer mortality (hazard ratio [HR]=1.40, 95% confidence interval [CI]:1.00, 1.96) compared to other fuel use. Among specific combustion components, a large pollutant cluster, PAH37 (HR=1.59, 95% CI:1.19, 2.13 per standard deviation [SD]), was also associated with stomach cancer mortality. Similarly, lifetime smoky coal use (HR=2.84, 95% CI:1.38, 5.85) and PAH37 (HR=1.75, 95% CI:1.01, 3.04 per SD) were associated with esophageal cancer mortality. Stratified analyses found that the association between PAH37 and stomach cancer was higher among women compared to men (P-interaction=0.02). Conclusions: Our study is one of the first to observe an association between HAP and its constituents and risk of mortality from stomach and esophageal cancer. Future work is needed to investigate potential underlying biological mechanisms especially as they pertain to observed differences by biological sex.

67. Vicky Chang, PhD, MPH

Lifetime glyphosate use and mosaic loss of chromosome Y in buccal samples of male pesticide applicators in the Agricultural Health Study

Mentor: **Dr. Jonathan Hofmann**

Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Background: Glyphosate is the most widely applied herbicide in the United States and globally and has been linked to certain hematologic malignancies; however, the underlying biologic mechanisms remain unclear. We previously reported an association between high lifetime glyphosate use and mosaic loss of chromosome Y (mLOY) – the most common chromosomal alteration in men and a marker of genotoxicity and genomic instability that has been linked to cancer and immune dysregulation – in circulating blood of male farmers from a subcohort of the Agricultural Health Study (AHS). Here, we further investigated glyphosate use in relation to mLOY in buccal samples of pesticide applicators from a larger, independent substudy within the AHS. Methods: This investigation included 1,868 male pesticide applicators from Iowa and North Carolina who were cancer-free controls from a case-control study nested in the AHS. We detected mLOY using genotyping array intensity data [median log-R ratio (mLRR)] in the male-specific region of the Y chromosome. Prospectively collected self-reported data were used to estimate total lifetime days and intensity-weighted lifetime days (a metric accounting for exposure intensity) of glyphosate use prior to buccal sample collection. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between glyphosate use and any mLOY (mLRR ≤ -0.15) and mLOY affecting a high fraction of cells [≥median (27.55%)], adjusted for potential confounders (e.g., age, smoking). Results: mLOY was detected among 16% of pesticide applicators. Ever (vs. never) lifetime use of glyphosate was associated with having any mLOY (OR=1.65, 95%CI=1.10-2.47) and high cell fraction mLOY (OR=2.24, 95%CI=1.22-4.12). High lifetime days of glyphosate use was associated with high cell fraction mLOY (highest vs. lowest quartile, OR=1.76, 95%CI=1.04-2.99) but not any mLOY, whereas high intensity-weighted lifetime days of use was associated with both any mLOY (OR=1.54, 95%CI=1.01-2.37) and high cell fraction mLOY (OR=2.02, 95%CI=1.12-3.64). Conclusions: This is, to our knowledge, the largest investigation of glyphosate exposure in relation to mLOY. Our results from buccal-derived DNA support the previously observed association with mLOY detected in blood and provide novel evidence for a different sample type. Together, these findings can help inform our understanding of the carcinogenic potential of this widely used herbicide.

68. Jessica M Madrigal, PhD, MS

Carcinogenic industrial air emissions and lung cancer risk in a cohort of 440,000 Americans

Mentor: **Dr. Rena Jones**

Study Section: **Epidemiology/Biostatistics - Integrative Epidemiology and Health Disparities**

Background: Industrial facilities emit established carcinogens into air, but associations of these agents with cancer risk at environmental levels is unknown. We evaluated associations with a priori established lung carcinogens and other known or probable human carcinogens without prior explicit links to lung cancer. Methods: To estimate airborne exposure to 31 Group 1 and 2A agents [as classified by the International Agency for Research on Cancer (IARC)] emitted from industrial sources (1987-1995), we linked a U.S. regulatory database to geocoded residential addresses of 442,986 NIH-AARP Diet and Health Study participants (Figure 1). We estimated inverse distance- and wind-weighted average exposures within 1, 2, 5, and 10km of the enrollment residence. Using Cox regression models adjusted for smoking and other covariates, we evaluated lung cancer risk overall and by major histologic subtype (adenocarcinoma, squamous cell carcinoma, small cell carcinoma) for levels (tertiles, medians) of exposure to each agent compared to non-exposed. Results: Larger proportions in New Jersey (25%) and Pennsylvania (26%) lived within 1km of a source compared to overall (13% and 14%, respectively). Among a priori agents, risk was elevated for beryllium exposure at 5km (HR T3=1.20, 95% CI:0.94-1.55) and 10km (HR T3=1.15, 95% CI:1.01-1.31). We observed associations with cobalt that were strongest for squamous cell carcinoma (5km HR T3=1.36, 95% CI:1.15-1.61; 10km HR T3=1.24, 95% CI:1.10-1.39). Benzene, hydrazine, and nickel were also positively associated, associations were strongest for squamous cell carcinomas. Among agents without prior evidence of lung carcinogenicity, styrene was associated with risk at 1km (HR T3=1.22, 95% CI:1.00-1.48). Conclusions: Our findings show that environmental exposure to agents previously linked to lung cancer in worker populations are associated with increased risk for people living near industrial sources. However, IARC classification of the a priori agents as lung carcinogens is primarily based on occupational studies of high exposure levels. Our findings suggest, for the first time for many of these agents, that emissions at lower environmental levels may be a concern.

69. Fangya Mao, Ph.D

Natural History of Cervical Intraepithelial Neoplasia Grade 2: Evaluating Benefits and Risks of Conservative Management
Mentor: **Dr. Li Cheung**
Study Section: **Epidemiology/Biostatistics - General**

Removed at request of author

70. Chase W Nelson, Ph.D.

HPV16 genetic variation provides evidence of positive selection to avoid immune recognition
Mentor: **Dr. Lisa Mirabello**
Study Section: **Virology - General**

Human papillomavirus (HPV) causes more cancer than any other virus, including virtually all cervical cancers. For unknown reasons, HPV experiences differential success infecting and persisting in different individuals. Most HPV infections are cleared or immune-controlled without progression to precancer or cancer. Nevertheless, the virus is so prevalent that it causes ~661,000 cervical cancers (~348,000 deaths) each year, of which ~61% are due to the uniquely high carcinogenicity of just one type, HPV16. HPV16 genetic variation can be classified based on evolutionary relationships into 16 sublineages. The maximum genetic difference observed between these HPV16 sublineages is only ~2% (~150 nucleotides) of the ~7900 nucleotide viral genome, but precancer/cancer risk can vary up to 137-fold between them. To further investigate how human and virus genetic variation contribute to these wide differences in outcomes, we analyzed 4704 HPV16 whole genomes for evidence of positive natural selection favoring amino acid diversity. We identified 56 such codons with dN/dS>1, structural importance predicted by AlphaFold2, moderate-to-radical amino acid differences, evolutionary convergence, and absence of mutation bias. These codons disproportionately overlapped known cytotoxic T cell immune epitopes, particularly epitopes of the known cervical pathology risk allele, HLA-B*07:02 (OR=4.9; 95%CI=2.1-10.3; P=0.000153, Fisher's Exact Test). Positively selected codons also had more rare amino acid variation in precancers/cancers than controls, and disproportionately overlapped 158 sites at which the evolutionary sublineages of HPV16 have diverged (OR=19.1; 95%CI=10.5-34.7; P<2.2e-16). The viral oncoprotein codon E6:10 is of particular interest, as variation at this site encodes moderate and radical amino acid differences (Arginine, Glycine, Isoleucine, and Threonine) that are known to interact with human proteins p53 and E6AP

and have associations with cervical pathology. Our results suggest that a small subset of HPV16 mutations can improve viral fitness and influence infection outcomes, with escape of HLA/cytotoxic T cell immune recognition as a likely selective pressure. This may partly explain differential risks and outcomes conferred by similar or identical viral isolates. These results will help to guide the design of therapeutic vaccines and targeted immunotherapy, where human genetics and viral variation are both critical for effectiveness.

71. Fei Qin, PhD

Identification of immune cell type-specific susceptibility genes in multiple cancers using transcriptome-wide association studies
Mentor: **Dr. Kai Yu**
Study Section: **Oncology - General**

Background: Transcriptome-wide association studies (TWAS) integrate gene expression and genome-wide association studies (GWAS) to identify disease susceptibility genes. Given the substantial variation in gene expression across different cell types within a tissue, cell type-specific prediction models may enhance the power of TWAS analyses. Method: We conducted cell type-specific TWAS leveraging single-cell RNA sequencing data from the OneK1K cohort (14 immune cell types and 1.27 million cells) and GWAS summary statistics for seven cancers (>290,000 cases in total): breast, prostate, lung, melanoma, ovarian, endometrial, and diffuse large B cell lymphoma (DLBCL). To improve prediction accuracy, we developed a novel approach that incorporates gene expression correlations across cell types during model development. We identified cell-type specific significant susceptibility genes for each cancer type with an FDR < 0.05. Genes identified in TWAS but lacking significant GWAS signals within ±10 Mb of the gene boundary were classified as potential "novel" cancer susceptibility genes. Additionally, we replicated our findings for breast and prostate cancers using GWAS summary data from the UK Biobank. Existing models from GTEx for relevant tissues were also utilized to validate our findings for breast, prostate, lung, and melanoma cancers. Results: We identified 254 novel genes (106 loci) for breast cancer, 73 genes (51 loci) for prostate cancer, 14 genes (11 loci) for lung cancer, 46 genes (39 loci) for melanoma, 9 genes (9 loci) for ovarian cancer, and 2 genes (2 loci) for DLBCL. Most of these genes were cell type-specific. Gene-set association analyses confirmed that novel susceptibility genes for breast and prostate cancers were jointly associated with their respective outcomes in the UK Biobank replications. Notably, among all significant genes, 139 (7.3%) were shared across at least two cancer types, predominantly enriched in immune cell types such as CD4_NC, CD8_ET, NK, CD8_NC, and B_IN. Conclusion: Our study underscores the utility of cell type-specific TWAS in uncovering new genetic loci associated with various cancers. These findings highlight the critical role of cellular context in gene expression, demonstrating its significant influence on the identification of cancer susceptibility genes and providing distinct immune cell insights into cancer etiology.

72. Monjoy Saha, PhD

A multimodal deep learning approach to predict survival in never smoking lung cancer patients
Mentor: **Dr. Maria Teresa Landi**
Study Section: **Artificial Intelligence - General**

Purpose: Lung cancer in never-smokers (LCINS) is the fifth most common cause of cancer deaths worldwide. Identifying subjects who need more aggressive treatment could greatly help improve survival rates. We investigated a multimodal deep learning approach to predict overall survival in never-smoking lung adenocarcinoma patients. Our model integrates digital pathology (WSIs: whole slide images), censoring status, survival months, and clinical and genomic data, including EGFR, KRAS, TP53, and RBM10 mutations, as well as mutation patterns such as Kataegis. Method: The study cohort included 495 LCINS cases randomly split into training (60%), validation (15%), and testing (25%) sets. WSIs were tiled and processed through a customized VGG-19 convolutional neural network, generating high-dimensional feature vectors representing morphological patterns. Censoring status, survival months, and clinical and genomic data were combined with the CNN output (i.e., imaging features) into a shared feature space, modeled by a densely connected neural network to predict risk scores using Cox partial likelihood loss. We performed 10-fold cross-validation on the training and validation datasets, then externally validated the model on the test set, repeating the analysis with and without genomic data. Results: We achieved an

index of 0.86 for the concordance between predicted mortality risk and actual survival time on the test set using both approaches, with and without genomic data. The Kaplan-Meier curves for low- and high-risk groups, with the cutoff for the risk score determined by the median value, showed distinct survival patterns – patients predicted to have low risk exhibited a higher survival probability compared to those with higher scores. The log-rank test yielded a p-value of ≤0.05 for overall and gene-specific survival, adjusting for stage and age. Heatmaps generated using the activation map of the image model highlighted non-tumor regions as low-risk regions and tumor regions as high-risk regions. Conclusion: The multimodal approach strongly predicted overall survival across all stages and within stage I in LCINS. Specific driver gene mutations do not significantly enhance survival prediction, highlighting the benefits of modeling on multimodal information over using only molecular data. Such approaches for LCINS prognostication could be used in the future to guide effective treatment modalities.

73. Ibrahim Hossain Sajal, PhD

Two stage mendelian randomization identifies proteomic mediators of the effects of risk factors on renal cell carcinoma
Mentor: **Dr. Diptavo Dutta**
Study Section: **Omics - Metabolomics/Proteomics**

Background: Renal cell carcinoma (RCC) poses a significant global health challenge due to its increasing incidence and mortality rates. Studies of RCC have established several risk factors (RF), including obesity, blood pressure, and smoking, while blood cell counts and kidney biomarkers have also been suspected to be associated with RCC. However, the specific biological mechanisms underlying these associations are yet to be understood, limiting the identification of reliable therapeutic targets for effective treatment development. Methods: We investigated the role of plasma proteins (PP) in mediating the effect of different RFs on RCC. Using summary-level data from UK Biobank Pharma Proteomics Project (N = 34,557) for PPs, publicly available GWAS summary statistics for 14 RFs (N > 2,000,000 for each), and recently conducted largest-to-date RCC GWAS (N = 864,690, cases = 29,020), we performed a novel two-step Mendelian Randomization (MR) analysis to determine (1) which PPs are impacted by each RFs, and (2) the effect of RF-associated PPs on RCC. We conducted multiple sensitivity analyses to determine the robustness of the results and performed several in silico follow up analyses to establish the potential functional relevance of the identified proteins. Results: Among the 2940 PPs analyzed, 1845 PPs were significantly associated (p-value < 1e-5) with at least one of the 14 RFs while 343 PPs were associated with more than 5 RFs. Of these, we found 30 PPs to have significant (false discovery rate < 5%) effect on RCC, including 3 candidate cancer drivers. Notably, several known RCC-related proteins were identified to mediate effects of multiple distinct groups of RFs; e.g. SELE (odds ratio [OR; effect on RCC]=1.04, p-value=5.72e-4) and CDA (OR=1.07, p-value=7.33e-6) were associated with 9 RFs including obesity, blood pressure, and blood cell counts, while PXN (OR=0.96, p-value=5.77e-4) was associated with 7 RFs, including obesity and hypertension. The identified 30 PPs showed significant evidence of physical interaction (p-value=8.74e-4), were enriched in cancer-related pathways, and a majority (22, 73.3%) were differentially expressed in renal tumors, highlighting their relevance in RCC etiology. Conclusion: Our novel two-stage MR approach highlights the role of PPs as mediators of the effect of distinct RFs on RCC. Our results provide actionable insights into RCC etiology and nominate molecular targets for further laboratory investigation.

74. Alaina H Shreves, MS

Daily Step Counts, Step Intensity, and Mortality Risk Among U.S. Adults
Mentor: **Dr. Charles Matthews**
Study Section: **Epidemiology/Biostatistics - Integrative Epidemiology and Health Disparities**

BACKGROUND: Global physical activity guideline committees have called for prospective studies on stepping and health outcomes to inform future recommendations. While step counts have been inversely associated with mortality, the role of stepping intensity remains unclear. Most existing studies do not include populations representing adults across the U.S. This analysis investigates associations between step counts, stepping intensity, and mortality risk in a representative sample of U.S. adults. METHODS: We used data from the National Health and Nutrition Examination Survey (NHANES), a prospective analysis of adults (aged 40+) in the U.S., which was oversampled to represent the U.S. population. Participants wore wrist-

based accelerometers for up to nine days (2011–2014). We used a machine learning model ("stepcount") to ascertain median daily step counts and stepping cadence (steps per minute). All-cause mortality was recorded through December 2018. Cox proportional hazards regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI), adjusted for age, sex, race/ethnicity, body mass index, education, alcohol use, smoking status, frailty, self-rated health, mobility, and history of health conditions. RESULTS: Among 5,300 individuals (weighted N=192,396,305; mean age=60 years [standard deviation (SD)=12]; 2,777 [52%] women), the mean daily step count was 8,257 [4,893]. Over a mean follow-up of 6.7 years [1.6], there were 695 deaths. A 1,000-step increase in daily steps was associated with an 11% lower risk of mortality (HR=0.90, 95% CI: 0.88–0.93). Inverse associations were observed for a 1,000-step increase among men (HR=0.92, 95% CI: 0.89–0.96), women (HR=0.88, 95% CI: 0.84–0.93), non-Hispanic Whites (HR=0.90, 95% CI: 0.86–0.95), non-Hispanic Blacks (HR=0.89, 95% CI: 0.84–0.95), and Hispanics (HR=0.90, 95% CI: 0.83–0.98). There was no association between peak 30-minute cadence (steps per minute) and mortality risk after adjusting for step count or self-selected cadence and mortality. CONCLUSION: Higher daily step counts were associated with lower all-cause mortality across U.S. sex and racial/ethnic subgroups. These findings suggest that physical activity recommendations promoting overall step volume, rather than intensity or sustained walking bouts, may be an effective strategy for lowering mortality risk. Ultimately, findings from this study could inform future physical activity guidelines for stepping.

75. Isabelle Van Der Velpen, MD, PhD

Occupational low-dose medical radiation exposure and risk of Parkinson's disease in the U.S. Radiologic Technologists Study
Mentor: **Dr. Cari Kitahara**
Study Section: **Epidemiology/Biostatistics - General**

Background: Ionizing radiation is a known carcinogen and has recently been implicated in the etiology of neurodegenerative disorders. Parkinson's disease is a neurodegenerative disorder that is rapidly increasing in incidence globally. Recent findings from radiation worker cohorts suggested that exposure to low-dose ionizing radiation may be associated with Parkinson's disease risk, although findings for medical workers were inconclusive. Here, we studied the association between radiation work history and Parkinson's disease mortality in the U.S. Radiologic Technologists (USRT) Study. Methods: The USRT Study is a nationwide cohort of radiologic technologists exposed to protracted low-dose ionizing radiation. Work history data were collected through mailed surveys. Analyses included 89,948 participants (mean baseline age 49.1 years, 77.1% female) responding to the 2nd survey (1994-1998), which inquired about first and total years worked per decade, specific procedures performed, and number of times the participant held patients or exceeded the radiation exposure limit. Parkinson's disease-specific mortality was ascertained from the National Death Index through the end of 2021. We calculated hazard ratios (HR) and 95% confidence intervals (CI) with age as the timescale to assess Parkinson's disease-specific mortality associated with work history factors, adjusting for birth year, sex, education, income, smoking habits, alcohol consumption, physical activity, body mass index, and caffeine consumption. Results: During follow-up (maximum 27 years), 350 Parkinson's disease-specific deaths occurred. Ever assisting with interventional radiography was associated with a higher risk of Parkinson's disease-specific mortality (HR 1.46, 95%CI 1.06-2.01). Participants who assisted with interventional radiography daily during and after the 1980s had a higher risk of Parkinson's disease-specific mortality compared to participants who never or rarely assisted with these procedures (1980s: HR 2.25, 95%CI 1.26-4.00; post-1980s HR 3.32, 95%CI 1.86-5.94). Conclusion: Regularly assisting with interventional radiography procedures, known to yield relatively high doses to patients and medical staff, was associated with risk of Parkinson's disease-specific mortality, independent of established risk factors. These novel findings suggest a role of ionizing radiation in Parkinson's disease etiology and support efforts to keep occupational exposure as low as reasonably achievable.

76. Eleanor Watts, DPhil

A Multiprotein Score for Hepatocellular Carcinoma Prediction and Risk Stratification in Two Prospective Cohorts
Mentor: **Dr. Steve Moore**
Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Background: Advances in proteomics may facilitate the discovery of novel biomarkers for early detection and risk stratification of hepatocellular carcinoma (HCC). While early detection is crucial for curative treatment, current surveillance—recommended biannually for individuals with cirrhosis or chronic hepatitis—has poor compliance and modest sensitivity particularly for early-stage HCC. Using two independent prospective cohorts, we investigated whether a multiprotein score could predict HCC risk in the overall population and refine risk stratification among individuals with liver conditions. Methods: We developed a multiprotein score using LASSO regression in a nested case-control study within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, which included 118 HCC cases and 118 matched controls with a median follow-up of 9.7 years. The score was tested in the UK Biobank, which comprised 50,199 participants, including 28 who developed HCC over a 10.9-year follow-up. Circulating protein levels were measured using the Olink Explore platforms (4,003 proteins in PLCO and 2,418 in the UK Biobank). Score performance was assessed using the C-index in the overall population and among participants with cirrhosis or hepatitis B/C. These at-risk participants were further stratified into higher- and lower-risk groups using cut-points determined by Youden's index. We then used Cox models to estimate the mean cumulative risk for each risk group. Results: Our protein score comprised eight proteins, which demonstrated high discrimination in the overall population (C-Index=0.93, 95% confidence intervals [CI] 0.84-0.99) and among at-risk participants with cirrhosis (0.87, 0.80-0.95) and hepatitis B/C (0.93, 0.87-1.00). The score effectively stratified participants with liver conditions into higher- and lower-risk groups for HCC development. Among those with cirrhosis, 8-year HCC risk was 20% (higher-risk) vs 4.0% (lower-risk), while for hepatitis B/C, risks were 11% vs 1.2%, respectively. Conclusion: A multiprotein score could enhance early detection and risk stratification for HCC, identifying high-risk individuals years before diagnosis. This may enhance targeted surveillance for the highest risk groups, enable earlier interventions, and improve patient outcomes.

77. Xueyao Wu, B.Med.

Investigating the relationship between breast cancer risk factors and an AI-generated mammographic texture feature

Mentor: **Dr. Peter Kraft**

Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Background: The mammogram risk score (MRS), an AI-driven texture feature derived from digital mammograms, strongly predicts breast cancer risk independently of breast density, though underlying mechanisms remain unclear. We aimed to investigate relationships between established breast cancer risk factors, covering anthropometrics, reproductive factors, family history, and mammographic density metrics, and MRS. Methods: Using data from the Nurses' Health Study II (292 cases, 561 controls), we validated MRS's association with breast cancer using logistic regression and evaluated its relationships with risk factors through: linear regressions of MRS on observed risk factors and polygenic scores (PGS) associated with risk factors, and Mendelian randomization (MR) analysis via two-stage least squares regression. We conducted two-sample MR of MRS using summary statistics from genome-wide association studies of risk factors. Results: MRS was significantly associated with breast cancer risk before adjustment for BI-RADS density (OR=1.92 per SD increase in MRS; 95%CI:1.57-2.33; AUC=0.69) and after (OR=1.85; 95%CI:1.49-2.30). Early life body size and adult body mass index (BMI) were inversely associated with MRS, while history of benign breast disease and BI-RADS density showed positive associations; after adjusting for BI-RADS density, associations between MRS and the other three risk factors attenuated. Higher PGS for dense area was associated with increased MRS (β=0.16; 95%CI: 0.06-0.25), as was percent density (β=0.14; 95%CI:0.05-0.23). Two-sample MR identified associations between genetically predicted dense area (β=0.83; 95%CI:0.39-1.27) and percent density (β=1.14; 95%CI:0.55-1.74) with MRS. After adjusting for BI-RADS density and BMI, higher waist-to-hip ratio was significantly associated with increased MRS in PGS and two-sample MR analyses. Conclusion: We validated MRS's association with breast cancer risk in cases diagnosed 0.5-10.1 years after mammogram acquisition. Our findings reveal robust associations between breast density measures and MRS and suggest a potential impact of central obesity on MRS. Risk factor associations will be validated through expansion of analyses to the Komen Tissue Bank. Impact on screening strategies: The strong predictive value of MRS, independent of its relationship to established risk factors, suggests its potential as a valuable tool for improving breast cancer risk assessment and personalized screening approaches.

78. Pei Zhang, PhD

Estimating the Heritability of Longitudinal Rate-of-Change: Genetic Insights into PSA Velocity in Prostate Cancer-Free Individuals
Mentor: **Dr. Paul Albert**
Study Section: **Clinical and Translational Research - Diagnostics/Biomarkers**

Background: Serum prostate-specific antigen (PSA) is widely used for prostate cancer screening. While previous cross-sectional studies have estimated the heritability of PSA levels and identified genetic variants to enhance screening accuracy, the genetic basis of PSA velocity, the rate of change over time, remains unknown. The Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, a large-scale randomized study with longitudinal PSA measurements and genome-wide genotype data (N = 15,260 cancer-free male subjects, each with an average of five measurements), provides a unique opportunity to estimate the overall genetic contribution to PSA velocity. Methods: We developed a novel mixed model that incorporates genome-wide common variants to disentangle joint genetic effects on both baseline PSA levels and PSA velocity. We employ the average information restricted maximum likelihood (AI-REML) algorithm to estimate heritability metrics when sample size is moderate. However, the computation is prohibitive for analyzing the PLCO data with a large total number of observations (~100,000) due to the high dimensionality of the covariance matrix. To overcome this challenge, we introduce a novel partitioning and combining strategy: first evenly partitioning subjects into multiple subgroups, estimating parameters separately via AI-REML, and then performing a meta-analysis using a left-truncated log-likelihood method to derive final estimates and their standard errors. We evaluated this approach through simulations with realistic settings and applied it to the PLCO PSA dataset. Results: Extensive simulations show that our method provides unbiased heritability estimates, even for large datasets requiring partitioning. In PLCO PSA data, we estimated the heritability of baseline PSA at 0.31 (s.e. = 0.07) and PSA velocity at 0.45 (s.e. = 0.18), significantly greater than zero (P = 0.006), suggesting a substantial genetic contribution to PSA velocity. Conclusion: We developed a computationally efficient method (code available at GitHub) for unbiasedly estimating the overall genetic contribution to longitudinal traits by jointly modeling baseline levels and velocity. Applying this method to large-scale longitudinal PSA data from PLCO, we provide the first evidence of a strong genetic influence on PSA velocity, highlighting the potential for genome-wide association studies and the integration of genetic findings to enhance PSA-based screening strategies.

National Center for Advancing Translational Sciences (NCATS)

79. Valentine V Courouble, PhD

Development of an integrated high-throughput proteomics sample preparation platform for analysis of C. elegans
Mentor: **Dr. Christopher LeClair**
Study Section: **Omics - Metabolomics/Proteomics**

Caenorhabditis elegans (C. elegans) have long served as a eukaryotic model organism for human biology by virtue of genetic conservation and experimental tractability but their application in high-throughput screening (HTS) has been limited due to incompatibility of the labor-intensive handling and difficulty obtaining robust molecular level analyses. Progress in this area has enabled the use of laser-scanning cytometry in 384-well plate format for chemical library interrogation to screen for phenotype-modifying small molecules in these multicellular model organisms over the course of their development and several life cycles. However, deciphering the pharmacologic effect on phenotype remains a barrier to the efficient use of this new drug discovery paradigm. In response, I developed an integrated platform for the automated, HT proteomics sample preparation and mass spectrometry (MS) analysis of C. elegans. The thick cuticles of C. elegans make extraction of biomolecules, like proteins, a non-trivial task, and traditional protocols are inefficient, lack reproducibility, and are not amenable to HT workflows. Our platform incorporates novel Adaptive Focused Acoustics (AFA) technology to extract proteins from C. elegans samples through focused ultrasonication in a temperature-controlled environment. I showed for the first time that this technology is capable of extracting proteins from C. elegans samples. These samples were lysed in 8-well strips on the AFA instrument prior to manual downstream sample preparation followed by high-performance liquid chromatography with tandem MS analysis where we identified approximately 1,500 proteins from 40 worms. Subsequent optimizations using

a Bravo liquid handling system facilitated automated sample preparation process after lysis with the AFA-based system, leading to identification of approximately 2,250 proteins with a relative standard deviation below 10% from samples consisting of only 3 worms each. These results were comparable with or better than what is achieved by traditional methods using >1000 worms. Furthermore, this platform reduces overall sample preparation time from two days to eight hours and allows simultaneous processing of 384 differential samples – a 30-fold improvement in throughput. Ultimately, this automated and HT platform will allow effective utilization of C. elegans as an orthologous phenotypic model within pre-clinical therapeutic development for a wide range of human diseases.

80. Masato Ooka, Ph.D.

Identifying autophagy inducers using high-throughput screening and high-content imaging analysis
Mentor: **Dr. Menghang Xia**
Study Section: **Pharmacology and Toxicology**

Pharmacological modulation of autophagy presents significant therapeutic potential for a wide variety of diseases, including cancer and neurodegenerative disorders, due to its capacity to degrade intracellular protein aggregates. In this study, we utilized mouse embryonic fibroblasts expressing GFP-labeled LC3, a biomarker indicative of autophagy activation, to screen 3,733 clinically approved and investigational drugs. This high-throughput and high-content screening platform aimed to identify potential autophagy modulators. The primary screening and high-content imaging analysis identified 225 compounds as potential autophagy inducers. These compounds underwent further validation to confirm their ability to induce LC3. Following confirmation, we selected 3 known- and 6 potentially novel- autophagy inducers for further study based on their potency (i.e., EC50 values below 10 μM) and novelty. To elucidate the mechanisms of autophagy activation, we investigated their effects on autophagy-related pathways, including endoplasmic reticulum stress activation, p53 activation, mTOR inhibition, and caspase activation. We found that 4 compounds activated autophagy in an mTOR-dependent manner, a critical regulator of autophagy, while others activated autophagy through mTOR-independent manner. Additionally, 7 of the compounds induced caspase activity. We further assessed the clinical relevance of these compounds by focusing on amyloid protein removal and angiogenesis inhibition. Amyloid protein accumulation is a hallmark of neurodegenerative diseases. Three of the tested compounds effectively promoted amyloid removal within 48 hours in a cell-based assay. Next, their effect on angiogenesis, a key factor in tumor promotion, was evaluated using a co-culture of GFP-labeled aortic cells and mesenchymal stem cells. After the treatment with VEGF and tested compounds, the area of aortic cells was measured using high-content imager. Six compounds inhibited angiogenesis formation in this assay, suggesting their potential for cancer therapy. Among the 6 newly identified autophagy inducers, one compound consistently exhibited high potency (i.e., EC50 value below 1 μM) across the tested assays. This study identified several novel autophagy inducers with the potential for repurposing in the treatment of neurodegenerative diseases and cancer.

81. Shweta Sharma, Ph.D

Design and Development of Novel and Selective Brain Penetrant Histamine N-methyltransferase Inhibitors.
Mentor: **Dr. Samarjit Patnaik**
Study Section: **Chemistry - General**

Removed at request of author

82. Yen-Ting Tung, Ph.D

Single-cell transcriptomics on a bioprinted 3D neurovascular unit glioblastoma model identifies pharmacological interventions that selectively target tumor cells
Mentor: **Dr. Marc Ferrer**
Study Section: **Clinical and Translational Research - Drug Discovery**

The tumor microenvironment (TME) is the cellular environment in which cancer cell exist and is made up of a combination of stroma cells and extra cellular matrix (ECM), secreted biomolecules, including cytokines, from both stroma cells and tumor cells. The TME composition drives tumor growth and drug resistance. We previously established a 96-wells based bioprinted glioblastoma (GBM) model that includes a neurovascular unit (NVU) made up of endothelial cells, astrocytes and pericytes. We demonstrated its applicability for drug screening by using high content imaging system to measure tumor size and vascular integrity. We then performed single cell RNA sequencing on this bioprinted NVU-GBM model. We used the single cell transcriptomics data to investigate ligand-receptor interactions between stroma and GBM cells, to find molecular pathways that potentially drive tumor growth and be targets for pharmacological intervention as anticancer drugs. We identified top 50 ligand-receptors pairs and selected those with genes exclusively expressed in GBM cells but not in the stroma cells, to minimize potential toxic effects of drugs to the non-cancer cells. We performed a drug screen of 34 compounds targeting many of these receptors on the GBM cells, and identified 15 compounds that reduced GBM cells growth, with minimal effects on vascular integrity. This result demonstrates a promising pharmacogenomics approach to using bioengineered tumor growth tissue models that include the TME, integrated with single cell transcriptomics, to find actionable pharmacological targets that inhibit tumor growth without affecting the stroma cells.

National Eye Institute

83. Elena Daniele, PhD

An Ex Vivo Model of Aged Human Bruch's Membrane for Evaluating Cell-Based Therapeutics
Mentor: **Dr. Kapil Bharti**
Study Section: **Clinical and Translational Research - General**

The advanced stage of age-related macular degeneration (AMD) - geographic atrophy (GA) leads to irreversible vision loss and manifests in people above 60 years of age. AMD is thought happen because of retinal pigment epithelium (RPE) monolayer atrophy. RPE sits between photoreceptors and choroidal capillaries on a special membrane called the Bruch's membrane (BM), providing nutrient and functional support to the photoreceptors. RPE loss in GA stage leads to photoreceptor cell death and vision loss. Currently, no treatment is available to improve vision for the late stage of GA patients. Since AMD is a polygenic disease, with significant environmental contribution, autologous cell therapy is a viable option for these patients. Here, we developed an autologous cell replacement therapy for treating GA patients. We used induced pluripotent stem cells (iPSC) reprogrammed from patient blood cells. Patient-specific iPSCs were differentiated into pure RPE cells using a protocol previously developed in our lab. The RPE cells are matured on a biodegradable polylactic co-glycolic acid (PLGA) scaffold for five weeks as a tissue patch (iRPE-patch). Quality control assays confirmed the iRPE-patch's purity, maturity, and functionality. One challenge of late-stage AMD is the presence of inflammation induced changes in the BM making it non-conducive to RPE cell survival. This increases the uncertainty of our iRPE-patch survival in patient's eyes. To test if our transplant will survive in AMD eyes, I developed an ex vivo system with denuded BM obtained from cadaveric AMD and non-AMD eyes. iPSC-RPE were seeded either directly on to the BM or mounted as an iRPE-patch on to the BM. After five weeks of culture, cells grown directly on BM failed to fully populate it, but the iRPE-patch successfully survived and integrated in the BM. Histological analysis confirmed maturity of iRPE-patch on AMD and non-AMD Bruch's membranes. Furthermore, iRPE-patch transplanted in a retinal degeneration rat model rescued vision loss in these animals, further supporting their potential efficacy in patients. This ex vivo model of AMD BM supports the idea that the ability of RPE cells to repopulate BM in AMD is enhanced when seeded on a scaffold. The biodegradable PLGA scaffold offers optimal cues for iPSC-RPE cell survival, enabling the formation of a functional monolayer in a degenerative AMD environment, as also supported by the rat transplantation data.

84. Dominik Reichert, M.Sc.

Mutation-agnostic drug and gene therapy as a potential treatment for ciliopathy-mediated retinal degeneration
Mentor: **Dr. Kapil Bharti**
Study Section: **Clinical and Translational Research - Drug Discovery**

Primary cilia are antenna-like structures present on the surface of all cells. Hundreds of ciliary mutations have been identified, leading to a broad range of syndromic and non-syndromic developmental disorders - ciliopathies. One of the most common symptoms across ciliopathies is retinal degeneration (RD) and subsequent vision loss. Primary cilia are vital for the development and function of photoreceptors (PR), the light-sensing cell of the eye, as well as for the maturation of the adjacent Retinal Pigment Epithelium (RPE), a monolayer of polarized cells crucial for PR health. The exact mechanisms that lead to RD in ciliopathies remain unclear and no treatments are currently available. To reveal the effect of RPE defects on RD in ciliopathies and address the lack of therapies, we generated induced pluripotent stem cell (iPSC)-derived RPE (iRPE) from nine patients with various ciliopathy-associated mutations (BBS1, BBS10, BBS16, CEP290, LCA5, MYO7A, PRPF31), that affect cilia structure and function differently. All patient iPSC lines differentiated into RPE, demonstrated junctional integrity and expressed RPE maturity markers. Super-resolution imaging revealed differential effects of mutations on ciliary integrity and function. AI-based image analysis (REShAPE) revealed abnormal cell morphology in ciliopathy iRPE lines, indicating a loss of epithelial phenotype. Ultrastructural analysis (SEM/TEM) showed sparse, malformed apical microvilli and mitochondrial abnormalities, further validated by functional assays for RPE phagocytosis and mitochondrial function. To understand the molecular basis driving the shared loss of RPE phenotype, we performed RNA-Seq and identified mutation-independent dysregulated targets and pathways common across ciliopathy iRPEs. A targeted drug screen revealed two compounds that rescued the RPE phenotype in multiple ciliopathy iRPE lines. We are now using CRISPR/CAS9-mediated knockout and AAV-mediated overexpression of shared targets downstream of ciliary defects to validate these findings. This work introduces ciliopathy-associated cell-autonomous disease models and provides mechanistic insights into how ciliary mutations cause RD. We identified common molecular targets that, when addressed, rescued the disease phenotype across different patient iRPE lines. These mutation-agnostic gene and drug-based therapies address the current lack of treatments for ciliopathy-linked RD and might benefit a larger group of affected patients.

85. Sandeep K Vishwakarma, Ph.D.

Energy homeostasis mediated through mitovesicles is crucial for retinal ganglion cell survival and protection of their axons in glaucoma

Mentor: **Dr. Stanislav Tomarev**

Study Section: **Neuroscience - Therapeutics and Translational Research**

Background Retinal ganglion cells (RGCs) are highly energy-dependent and vulnerable to increased energy demands due to their high metabolic activity and limited energy reserves. This vulnerability is exacerbated under stress conditions and further research is essential to elucidate underlying mechanisms to prevent RGC degeneration in optic neuropathies like glaucoma, one of the main causes of blindness. This study elucidates the role of new type of small extracellular vesicles (sEVs), mitovesicles (mito-sEVs), in facilitating the transport of key bioenergetic components that sustain energy production, promote neurite outgrowth, and mitigate damage to RGCs. Methods Mito-sEVs were isolated from the conditioned medium (CM) of an induced pluripotent stem cell line using high-resolution buoyant density ultracentrifugation. The neuroprotective effects of mito-sEVs, unfractionated sEVs, and several fractions of sEVs were tested in mouse primary mixed retinal cell cultures (MRC). Transcriptomic analysis of mito-sEVs and unfractionated sEVs was performed for the identification of the molecular mechanisms of neuroprotection. Results Among 30 fractions of sEVs, the highest number of mito-sEVs was observed in fractions 16 to 20 (1.83x1012 per 100mL CM). Addition of mito-sEVs to MRC, improved RGC survival by ~200% compared to untreated RGCs (p<0.0001), surpassing the effects observed with growth factors (BDNF+CNTF+Forskolin), and other fractions. Mito-sEVs significantly improved RGC morphometry such as the highest number of surviving RGCs with axons and neurites (~72%), RGCs without axons (~49%), and neurites excluding axons (~59%). Mito-sEVs also significantly promoted the growth of primary (~68%), secondary (~53%), and tertiary neurites (~61%), as well as axonal tree branches (~75%). As demonstrated by gene network analysis, the mito-sEVs treatment of MRC led to upregulation of transcripts associated with pathways involved in energy production, such as oxidative phosphorylation and ATP biosynthesis. Conclusions This is the first proof-of-principle demonstration of the profound neuroprotective role of mito-sEVs in RGCs by the modulation of energy homeostasis through the activation of ATP-driven pathways. This approach provides a crucial platform for probing the molecular mechanisms driving RGC survival and neurite regeneration, offering a potentially transformative neural repair strategy to restore visual function in glaucomatous neurodegeneration.

86. Andrew E Wegerski, M.S.

Day geckos: Using novel reptile models to shed light on human fovea development
Mentor: **Dr. Brian Brooks**
Study Section: **Developmental Biology - Early Development/Embryology**

Disorders affecting the fovea, a specialized pit in the human retina necessary for high acuity vision, represent a significant healthcare burden across the globe. Degenerative diseases (e.g., macular degeneration) and developmental conditions (e.g., albinism and aniridia) affecting the fovea can lead to irreversible, central vision loss. Moreover, fovea disorders are challenging to study because there are no high-throughput, laboratory animal models that have a fovea. This has led to a surprising lack of knowledge on how the fovea develops during gestation. Although lizards are evolutionarily distant from humans, many diurnal species possess a fovea and may shed new insights on its development and relevant disease pathways in humans. Promisingly, the gold-dust day gecko, Phelsuma laticauda, has proven useful in early attempts at fovea modeling. Wild geckos were collected from invasive populations in Hawaii to establish a breeding colony to use for embryological studies. Initially, eggs were retrieved during various timepoints throughout gestation to establish a staging series to chronicle both embryo and fovea development to highlight similarities to human fovea-genesis. Eyes were fixed, sectioned, and stained to illustrate morphological changes that happen during fovea development. We found that P. laticauda, like humans and other foveate species, undergoes asymmetric changes in ocular shape that occur shortly after the onset of retina differentiation. Likewise, the region of the eye that will give rise to the fovea becomes transiently elongated and retracts as the fovea forms during the final 3rd of embryonic development, a progression consistent with humans. These initial steps to showcase fovea formation in geckos serve as a foundation to further explore the species' potential as a model for retinal disorders. While the morphological similarities of fovea development exist between geckos and humans, we are continuing to see if molecular and disease pathways are also shared. Currently, we are employing immunohistochemistry to establish molecular markers of all major retinal cell types in the developing gecko retina as well as adapting gene-editing technology to potentially replicate human fovea disorders in P. laticauda. Growing these baseline tools for geckos will undoubtedly provide new insights for understanding fovea development and disease pathways.

National Heart, Lung, and Blood Institute

87. Graham Brogden, PhD

Tertiary Lymphoid Structures form in salivary glands of enteric virus infected mice
Mentor: **Dr. Nihal Altan-Bonnet**
Study Section: **Virology - General**

Norovirus causes an enteric infection leading to diarrhea and vomiting, with the intestine and salivary glands the major sites of viral replication. The virus is transmitted via the fecal-oral route and recently, infectious virus particles have also been shown to be present in saliva, facilitating an oral-oral transmission route. However, the cell types infected and the role of saliva in virus dissemination remains to be elucidated. Mice inoculated with norovirus became positive for virus in salivary glands and intestine plus associated lymphatic tissues. Notably, enteric infection of salivary glands and associated lymph nodes resulted in little to no inflammation or cell death. Furthermore, the majority of virus shed into saliva was in the form of vesicle-cloaked viral populations, the latter a highly virulent infectious form compared to free virus particles by enabling high multiplicity of infection and shielding from antibodies. In contrast, the intestinal infections resulted in high levels of inflammation and cell death and feces was largely composed of free viruses that were released from lysed cells. The majority of virus was present and replicating in CD45+ immune cells and EpCAM+ epithelial cells. Spatio-temporal analysis of the infection revealed that the earliest and highest viral pools were associated with mandibular lymph node CD45+EpCAM+ cells, suggesting that these cells potentially play a sentinel role in disseminating norovirus. Further analysis of this unique cell population revealed similarity to Langerhans cells based on cell markers and the presence of Birbeck-like granules. Additionally, one day post infection with either norovirus or rotavirus, tertiary lymphoid structures form in the salivary glands. These structures contain T, Tregs, B, Langerhans and MHC II positive cells and persist for at least 28 days post infection. In summary, our findings suggest that viruses can have vastly differing infectious forms and thereby transmission efficiencies based on replication sites, and

tertiary lymphoid structures play an important role in either suppressing virus-induced tissue damage and/or generation of a pathogen specific antibody response.

88. Jianyi Ding, PhD

CD38 Inhibition Enhances Human Hematopoietic Stem Cell Maintenance
Mentor: **Dr. Andre Larochelle**
Study Section: **Stem Cells - General and Cancer**

Hematopoietic stem cells (HSCs) maintain a delicate balance between self-renewal and differentiation within the bone marrow, ensuring lifelong maintenance of the blood and immune systems. However, these properties are compromised under in vitro culture conditions, posing a significant barrier to investigating HSC function ex vivo and hindering clinical applications, such as ex vivo gene therapy. CD38 is commonly used as a negative selection marker to enrich human HSCs from CD34+ hematopoietic stem/progenitor cell (HSPC) population, suggesting CD38 may negatively regulate HSC function. To explore the potential impact of CD38 on HSCs, human mobilized peripheral blood CD34+ cells were cultured for 7 days with or without CD38 inhibitor 78c. Treatment with 78c resulted in a 2-fold increase of phenotypic HSCs compared to the DMSO control. To assess the engraftment potential of HSCs, we quantified human cell engraftment 4 months after transplanting NBSGW mice with 50,000 uncultured CD34+ cells or their 7-day progeny. While limited engraftment was observed in the DMSO group, 78c group exhibited engraftment levels akin to uncultured CD34+ cells. Notably, a limiting-dilution secondary transplantation assay showed that the frequency and numbers of HSCs with long-term engraftment potential were preserved in the 78c group. Proteomics analysis of CD34+ cells cultured with DMSO or 78c revealed that 78c-treated cells maintain a proteomic profile closer to uncultured cells compared to DMSO-treated cells. At 24 hours, 78c initially suppressed protein synthesis and translation, and by 72 hours, this extended to suppression of unfolded protein and stress response mechanisms. Analysis of bulk RNA sequencing from CD34+CD38low cells cultured for 24 hours with 78c highlighted downregulation of the PI3K/AKT/mTOR signaling pathway, a key regulator of cell cycle control and cell proliferation. Further analysis showed that 78c treatment reduced mTOR phosphorylation. Rapamycin, an mTOR inhibitor, replicated the effects of 78c by maintaining HSC stemness and reducing cell proliferation, confirmed by xenotransplantation assays. Collectively, this study reveals that CD38 serves not only as a negative selection marker for LT-HSCs, but also as an important target for enhancing HSC maintenance in ex vivo cultures. Inhibition of CD38 suppresses mTOR signaling and cell cycle progression, thereby safeguarding HSCs from exiting dormancy, disrupting proteostasis, and losing stemness.

89. Carolina Downie, PhD

Molecular phenogroups in heart failure: large-scale proteomics in a population-based cohort
Mentor: **Dr. Veronique Roger**
Study Section: **Omics - Metabolomics/Proteomics**

Background: Heart failure (HF) is a heterogeneous syndrome with high mortality, and the need for a new taxonomy of HF is recognized. Previous work characterizing HF subgroups primarily relied on clinical data to cluster patients, an approach constrained by the boundaries of documented clinical features. Proteomics offers potential for more precise phenotypical identification and mechanistic insights. However, few studies have used this approach to “phenomap” patients into phenogroups, and all have focused on targeted cardiovascular proteomics panels and a restricted HF ejection fraction group. Hypothesis: We hypothesized that distinct proteomics-defined phenogroups would exhibit differences in clinical characteristics and mortality. Methods: We measured over 7,000 SOMAmers (Slow off-rate modified aptamers) targeting human proteins via the SomaLogic 7K SomaScan assay in a population-based cohort of 1351 HF patients. After dimension reduction of the SOMAmers, we used unsupervised clustering analysis to identify distinct phenogroups and compared their clinical characteristics and all-cause mortality. Results: Three proteomics-defined phenogroups were identified, with substantial differences in survival (phenogroup 1 5-year survival probability: 65% [95% CI 61%, 68%], phenogroup 2: 45% [40%, 51%], phenogroup 3: 26% [22%, 30%]). In covariate-adjusted Cox regression models, membership in phenogroups 2 and 3 was significantly associated with increased rate of mortality compared to phenogroup 1. Phenogroups also exhibited differences in several measures suggesting poorer health, including N-terminal pro b-type natriuretic peptide (NT-proBNP),

kidney function, and Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) scores, but did not differ by ejection fraction or New York Heart Association class. Top proteins important for the separation of the three phenogroups have a variety of molecular functions including inflammatory signaling (CD59), cytokine-involved stress response (MIC-1/GDF-15), and protein binding (UBQL4, DYL2). Conclusions: Our study demonstrates that molecular phenomapping can stratify HF patients into distinct subgroups that go beyond pre-defined clinical classifications. These findings are important for improving phenotyping and risk stratification in HF.

90. Abigail Giles, PhD

Detection of mitochondrial-derived reactive oxygen species (ROS) in cardiac ischemia-reperfusion injury using a genetically encoded mitochondrial-targeted fluorescent peroxide sensor
Mentor: **Dr. Tish Murphy**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

Removed at request of author

91. Sara Gonzalez Hernandez, PhD

PROX1-mediated vascular permeability induces blood-brain barrier breakdown in neurovascular diseases
Mentor: **Dr. Yosuke Mukoyama**
Study Section: **Neuroscience - General**

The blood-brain barrier (BBB) is a key regulator of CNS homeostasis, controlling selective permeability and immune cell entry. However, BBB dysfunction is a hallmark of neurovascular diseases, including glioblastoma, brain arteriovenous malformations (bAVMs), and brain metastases, where vascular abnormalities exacerbate disease progression. Prox1, a key transcription factor for lymphatic differentiation, is not expressed in normal CNS endothelial cells (ECs); however, it is aberrantly induced in ECs of human glioblastoma patients. To investigate Prox1 dysregulation in neurovascular diseases, we analyzed single-cell RNA sequencing datasets from patients with glioblastoma, brain metastases, and bAVMs. Our analysis revealed that PROX1 is upregulated in CNS ECs in all these conditions, along with other lymphatic markers, as well as the vascular permeability marker PLVAP. These findings led us to hypothesize that upregulation of Prox1 contributes to vascular malformations, increased permeability, and BBB breakdown in these diseases. We next generated a mouse model to induce Prox1 in ECs (Cre-mediated Prox1 overexpression) and assess its impact on the BBB integrity. Using a pan-EC-specific Cre (Cdh5-CreERT2), we induced Prox1 in ECs postnatally, after BBB had matured, and observed severe vascular malformations, increased vascular permeability, and BBB breakdown. To ensure that these effects were specific to brain ECs, we employed a brain EC-specific Cre (Slco1c1-CreERT2), which produced the same phenotypes. Furthermore, inducing Prox1 in brain ECs during adulthood also resulted in BBB dysfunction, demonstrating that Prox1-mediated vascular defects occur independently of developmental timing. Mechanistically, we showed that Prox1 directly represses Claudin-5 and β-catenin, essential for stabilizing BBB. This repression disrupts tight junction, ultimately weakening the paracellular barrier. Additionally, the reduction in β-catenin increases transcytosis, as confirmed by electron microscopy, further increasing BBB permeability. Our findings identify Prox1 as a key factor in BBB dysfunction in neurovascular diseases, highlighting a previously unrecognized mechanism that contributes to BBB breakdown associated with these diseases. To explore a potential therapeutic approach, we plan to inactivate Prox1 in brain ECs in glioblastoma mouse models and investigate whether this intervention can restore BBB integrity and provide benefits for glioblastoma treatments.

92. Chengfei Jiang, PhD

Systemic identification of functionally conserved lncRNA metabolic regulators in human and mouse livers
Mentor: **Dr. Haiming Cao**
Study Section: **RNA Biology**

BACKGROUND & AIMS: Unlike protein-coding genes, most human long non-coding RNAs (lncRNAs) lack conservation based on their sequences, posing a challenge for investigating their role in a pathophysiological context for clinical translation. This study explores the hypothesis that non-conserved lncRNAs in human and mouse livers may share similar metabolic functions, giving rise to functionally conserved lncRNA metabolic regulators (fcLMRs). METHODS: We developed a sequence-independent strategy to select putative fcLMRs, and performed extensive analysis to determine the functional similarities of putative human and mouse LMR pairs (h/mLMRs). RESULTS: We found that several pairs of putative fcLMRs share similar functions in regulating gene expression. We further demonstrated that a pair of fcLMRs, h/mLMR1, robustly regulated triglyceride levels by modulating the expression of a similar set of lipogenic genes. Mechanistically, h/mLMR1 binds to PABPC1, a regulator of protein translation, via short motifs on either lncRNA with divergent sequences but similar structures. This interaction inhibits protein translation, activating an amino acid-mTOR-SREBP1 axis to regulate lipogenic gene expression. Intriguingly, PABPC1-binding motifs on each lncRNA fully rescued the functions of their corresponding LMRs in the opposite species. Given the elevated expression of h/mLMR1 in humans and mice with hepatic steatosis, the PABPC1-binding motif on hLMR1 emerges as a potential non-conserved human drug target whose functions can be fully validated in a physiologically relevant setting before clinical studies. CONCLUSIONS: Our study supports that fcLMRs represent a novel and prevalent biological phenomenon, and deep phenotyping of genetic mLMR mouse models constitutes a powerful approach to understand the pathophysiological role of lncRNAs in the human liver.

93. Hannah Karen Labayo, PhD

Milk IgAs of early life origin B cells have cross-neutralizing antiviral properties
Mentor: **Dr. Nihal Altan-Bonnet**
Study Section: **Virology - Pathogenesis/Therapeutics**

Immunoglobulin A (IgA) is one of the most important immune components found in mother's milk. It is vertically transmitted to infants' immature mucosal system providing protection from viral and bacterial infections including those caused by the enteric noroviruses and rotaviruses. Enteric viruses were classically known to be transmitted through the fecal-oral route but we recently demonstrated that they can also transmit via saliva. Using a lactating mouse model, we showed that saliva of a suckling norovirus or rotavirus infected infant can transmit the infection to the mother's mammary glands and induce a rapid IgA response in her milk. Now we demonstrate that these milk IgAs are protective, produced largely by early life origin (ELO) B cells located within the mammary gland and in their absence the infection in infants is enhanced in viral load and prolonged. Notably, these IgAs have cross-neutralizing antiviral properties and block a wide variety of viral infections even though the mothers' themselves have never encountered these viruses prior to their infants becoming infected. We show that these unique IgAs neutralize by binding to phosphatidylserine lipids, which are abundant in the membranes surrounding individual enveloped virus particles such as coronaviruses as well as noroviruses, rotaviruses, and picornaviruses. The latter, although "non-enveloped" are known to be transmitted en bloc in extracellular vesicles whose membranes are enriched in phosphatidylserine lipids. Our findings have broad implications for not only understanding immune responses in the context of mammalian biology but also for designing antiviral treatments based on manipulating these broadly neutralizing IgA's in milk.

94. Justin Mabin, PhD

Uncovering the isoform-resolution kinetic landscape of nonsense-mediated mRNA decay with EZbakR
Mentor: **Dr. John Hogg**
Study Section: **RNA Biology**

Transcript isoform abundance is shaped by the balance between RNA synthesis and degradation. While alternative splicing is a well-known driver of isoform expression, the role of RNA turnover remains less explored. A key contributor to isoform-specific decay is nonsense-mediated mRNA decay (NMD), which degrades transcripts with premature termination codons (PTCs) or long 3' untranslated regions (UTRs). NMD functions as both a quality control mechanism, eliminating PTC-containing mRNAs resulting from genetic mutations, transcriptional errors, and alternative splicing, as well as a regulator of endogenous gene expression under normal cellular conditions. Therefore, identifying the determinants of NMD efficiency, especially in

transcripts without PTCs, is essential for understanding its regulatory role. A major challenge in studying NMD has been quantifying isoform-specific turnover kinetics transcriptome-wide. Nucleotide-recoding RNA sequencing (NR-seq) methods such as TimeLapse-seq and SLAM-seq measure RNA synthesis and degradation without globally inhibiting transcription, but existing bioinformatic tools lack the resolution for isoform-level kinetic modeling. We introduce an enhanced NR-seq analysis framework within the EZbakR-suite, along with a transcriptome build and refinement pipeline that improves isoform-level analyses. Together, these tools provide a flexible platform for accurate kinetic modeling of gene expression. Using EZbakR-suite and acute NMD inhibition, we mapped the kinetic landscape of human NMD. Our findings validate previously identified determinants of NMD, while uncovering new regulatory features missed by standard RNA-seq analyses. We reveal unexpected heterogeneity in the decay of transcripts containing highly conserved poison exons, with half-lives varying by hours. Conversely, we identify a subset of mRNAs lacking PTCs that are nevertheless efficiently degraded, challenging the assumption that non-PTC isoforms are less susceptible to NMD. Notably, we uncover the first known cases of PTC-free alternative splicing-linked NMD (AS-NMD), where differential termination codon (TC) positioning in terminal exons extends 3'UTRs, creating TCs that are efficiently recognized by NMD. Beyond NMD, this study provides a quantitative framework for dissecting transcript-specific degradation kinetics, offering a roadmap for future studies on how decay pathways shape the human transcriptome.

95. Dennis J Michalak, PhD

Insights into clathrin lattice architecture and interaction at the plasma membrane revealed by cryo-electron tomography
Mentor: **Dr. Justin Taraska**
Study Section: **Cell Biology - Organelles and Membranes**

Clathrin is a main driver for the dominant form of endocytosis in cells, clathrin-mediated endocytosis. This process is initiated at the plasma membrane, where adaptor proteins, such as AP2, bind cytoplasmic domains of cargo receptors. Clathrin is recruited to the plasma membrane, binds to AP2, and assembles into a polyhedral lattice. Using cryo-electron tomography (cryoET), proteins can be identified within the dense cellular environment and their three-dimensional structural information averaged together to retrieve high-resolution information in a process known as subtomogram averaging. A strict requirement on sample thickness has prohibited detailed study of the plasma membrane using cryoET. We have adopted cell unroofing, a technique historically used in platinum replica electron, atomic force, and fluorescence microscopies, to isolate plasma membranes in a rapid and straight-forward manner. Our cell unroofing workflow is capable of producing basal and apical plasma membranes that are ideally thin for high-resolution cryoET. Structural studies of clathrin using cryoET have been performed in predominately in vitro systems consisting of purified or reconstituted vesicles. These systems have generally exhibited highly curved clathrin-coated membranes. The N-terminal domain of clathrin heavy chain is known to adopt alternate splaying at different curvatures. Our subtomogram average of clathrin lattice vertices results in a structure with similarities to previously reported models. The heavy chain and light chain arrangement proximal to the vertex are conserved. We find density consistent with a previously reported subtomogram average where the Beta2 appendage of the adaptor AP2 is located above the heavy chain N-terminal domain near the vertex. However, we find a displacement of the heavy chain N-terminal domain and attribute this to a lower average curvature compared to the purified or reconstituted systems. We also find significant heterogeneity which can be attributed to the various lattice configurations and endocytic stages captured within the dataset. This is particularly notable at the N-terminal domain of clathrin heavy chain, the major adaptor interaction site, and is the subject of ongoing efforts. These results demonstrate that our cryoET workflow is well-suited for further study of structures involved in clathrin-mediated endocytosis at the plasma membrane.

96. Tori Rogness, BS

Outcomes with Ibrutinib for High-Risk Chronic Lymphocytic Leukemia: 10 Year Follow-Up of Phase 2 Study"
Mentor: **Dr. Adrian Wiestner**
Study Section: **Clinical and Translational Research - Clinical Trials**

Background: Chronic lymphocytic leukemia (CLL) is a common blood cancer characterized by the accumulation of abnormal B lymphocytes. Bruton's tyrosine kinase inhibitors (BTKis), which disrupt the B-cell receptor signaling pathway essential for CLL cell survival, have significantly improved patient outcomes, particularly for patients with TP53 alterations, a genetically high-risk group. Ibrutinib, the first covalent BTKi approved, has accumulated the most extensive long-term follow-up data, providing invaluable insights into its sustained efficacy and safety. Methods: To examine these long-term outcomes, I analyzed data from a phase II clinical trial involving patients with CLL who were either aged 65 or older or had TP53 alterations. Eighty-four patients received continuous single-agent ibrutinib therapy at 420 mg daily until disease progression or unacceptable toxicity. Outcomes assessed included progression-free survival (PFS), overall survival (OS), and achievement of undetectable minimal residual disease (uMRD), defined as fewer than one detectable CLL cell per 10,000 leukocytes, determined through annual peripheral blood assessments Results: With a median follow-up exceeding 10 years, median PFS was 7.2 years, and median OS was not yet reached, indicating durable responses. Inferior PFS and OS correlated with prior treatment history, TP53 alterations, and unmutated IGHV status. Treatment discontinuations were primarily due to progressive disease (46.9%) and adverse events (36.9%), predominantly cardiac-related. Importantly, 13 patients (15.5%) achieved sustained uMRD after a median of 5 years on therapy, with some maintaining remission even after discontinuing treatment due to adverse events. Conclusion: These findings provide crucial insights into the long-term efficacy, safety, and prognostic value of continuous ibrutinib therapy in high-risk CLL populations. At 10 years, marking the longest follow-up of any study with BTKi for previously untreated patients with TP53 alterations, two-thirds of patients were estimated to be alive, and almost half remained progression-free. The prolonged and deepening clinical responses, particularly the durable uMRD status observed in a subset of patients, underscore the ongoing therapeutic potential and highlight future directions for optimizing treatment strategies in high-risk CLL.

97. Suyasha Roy, PhD

Robust anti-tumor activity of a de novo interleukin-21 mimic
Mentor: **Dr. Warren Leonard**
Study Section: **Immunology - General**

Removed at request of author

98. Ryo Sato, MD, PhD

β-III tubulin identifies anti-fibrotic state of pericytes in pulmonary fibrosis
Mentor: **Dr. Yosuke Mukoyama**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

Removed at request of author

99. Willy Sun, PhD

Elucidating the structural basis of epidermal growth factor receptor internalization by clathrin-mediated endocytosis using isolated mammalian plasma membranes.
Mentor: **Dr. Justin Taraska**
Study Section: **Cell Biology - General**

The binding of epidermal growth factor (EGF) to EGF receptor (EGFR), a receptor tyrosine kinase, initiates a signaling cascade crucial for cellular functions such as proliferation, migration, and development. Aberrant activation and misregulation of EGFR at the plasma membrane have been reported as a leading cause of various cancers, including breast, lung, and colon cancer. EGF-induced EGFR dimerization has been shown to be a key initial step. In addition, reports indicate that ligand-bound EGFR undergoes spatial reorganization in the plasma membrane, including clustering and associating with clathrin-coated pits, which enhance EGFR signaling. Fine-tuning of EGFR signaling at the plasma membrane is primarily achieved through clathrin-mediated endocytosis (CME) of EGFRs. While the EGFR signaling cascade has been studied extensively, the structural basis

of EGFR regulation via CME remains largely incomplete, mainly due to challenges associated with preparing the mammalian plasma membrane for high-resolution structural determination. Cell unroofing is an established cell thinning technique that can produce isolated plasma membranes suitable for high-resolution imaging without disrupting the molecular architectures associated with the plasma membrane. To track EGFRs in the plasma membrane, we conjugated EGF to quantum dots to take advantage of quantum dots' fluorescent properties and electron-dense cores to label and visualize EGFRs under both light and electron microscopy. Here, we combined cell unroofing, EGF-quantum dot conjugates, and electron tomography to resolve the three-dimensional structure of the clathrin-coated pit—EGFR molecular ensemble. Tomograms of platinum replicas generated from these membranes were able to capture the spatial reorganization of EGFRs in the plasma membrane. Using a pulse-chase setup, isolated plasma membranes from cells that were stimulated with EGF-quantum dot conjugates (pulse) but did not undergo the chase step showed that EGFRs are located outside clathrin-coated pits. EGFRs in cells that underwent the chase step were located within clathrin-coated structures across different stages of internalization, such as in flat, dome-shaped, and spherical structures. These observations were consistent with existing data from single-particle tracking studies. Moving forward, we will adapt this setup to cryo-electron tomography to elucidate the high-resolution structural interactions between EGFR and clathrin-coated structures.

100. Abu Syed, Ph.D.

Exploring the role of WASF3 in chemotherapy-induced cognitive dysfunction
Mentor: **Dr. Paul Hwang**
Study Section: **Clinical and Translational Research - Animal Models**

Removed at request of author

National Institute of Allergy and Infectious Diseases

101. Vanya Bhushan, PhD

Quantitative subcellular-temporal proteomics coupled with machine learning-based computational modeling to identify changes in protein signaling networks in innate immune responses
Mentor: **Dr. Aleksandra Nita-Lazar**
Study Section: **Immunology - Innate and Cell-mediated Host Defenses**

The innate immune system is the first line of defense against pathogens, shaping adaptive immunity. Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs), triggering signaling cascades that coordinate immune responses. However, subcellular localization and trafficking of signaling proteins during immune activation remain poorly understood. This study employs quantitative subcellular-temporal proteomics to track protein dynamics in macrophages stimulated with LPS (TLR4) and FLA (TLR5). By mapping protein relocalization across nine subcellular compartments over seven-time points, we uncover mechanisms driving TLR-specific immune responses. Using Dynamic Organellar Mapping (DOM) with high-resolution LC-MS/MS, we analyzed U937 macrophages fractionated into nine fractions (membrane-bound and membrane-less). Western blotting confirmed the quality of fractionation. Data was analyzed through R packages integrating Bayesian inference and machine learning to classify protein relocalization events. We identified 7,437 proteins in the temporal dataset and 4,079 in the spatial dataset. A curated set of 1,309 organelle markers was used to track localization shifts. LPS induced rapid MyD88 recruitment to endosomes, while FLA caused prolonged TRAF6 retention in the cytoplasm, demonstrating TLR-specific compartmentalization of immune signaling. Early responses (15 min–1 hr) recruited MyD88, TRIF, TRAF6, and IRAK1, whereas later time points (4 hr–12 hr) showed nuclear localization of STAT1, STAT3, and IRF3, and lysosomal enrichment of autophagy regulators (LC3B, p62, ATG5). Comparing LPS and FLA stimulation, we identified shared and unique proteome remodeling events, linking relocalized proteins to NF-κB, MAPK, and inflammasome signaling. Machine learning-based predictions highlighted key dynamic localization shifts, refining our understanding of TLR-driven immune regulation. This ongoing study still requires validation steps, including further computational refinement and experimental verification of key relocalization events. This study provides a high-resolution map of subcellular proteome remodeling during innate immune activation, integrating quantitative proteomics with computational modeling. These insights advance immune cell spatial

proteomics, offering a foundation for predicting immune responses. This research was supported by the Division of Intramural Research, NIAID, NIH.

102. Bickey Chang, MD, MHA

Safety and efficacy of repeat dosing of the anti-sporozoite monoclonal antibody L9LS in Malian children
Mentor: **Dr. Peter Crompton**
Study Section: **Clinical and Translational Research - Clinical Trials**

Anti-sporozoite monoclonal antibodies are an emerging tool for malaria prevention. A phase 2 randomized controlled trial involving Malian children aged 6-10 years demonstrated that a single subcutaneous dose of L9LS, a human IgG1 monoclonal antibody that targets highly conserved minor NVDP repeats on the Plasmodium falciparum circumsporozoite protein, was safe and provided protective efficacy against P. falciparum infection and clinical malaria over a 6-month malaria season. We conducted a 12-month extension of this trial to assess the safety and efficacy of a second subcutaneous administration of L9LS. 235 children who completed the phase 2 trial and consented to re-enrollment were randomized and received 150 mg of L9LS (n=79), 300 mg of L9LS (n=38), or placebo (n=118) before the malaria season. We found that a second administration of L9LS was well tolerated without evident safety concerns. A second dose of L9LS exhibited expected pharmacokinetics and did not elicit functionally significant anti-drug antibodies. A preliminary exploratory analysis suggests that a second dose of L9LS at 150 mg and 300 mg is efficacious compared to placebo in mediating protection against clinical malaria during a second malaria season. Moreover, preliminary analysis suggests that L9LS-mediated protection in the first year of the trial is not associated with delayed or “rebound” malaria risk among children who received placebo in the second year of the trial. Our findings indicate that repeated annual dosing of L9LS is safe and well-tolerated, supporting the continued development of L9LS as an intervention to prevent malaria among children exposed to seasonal malaria transmission.

103. Megan Dunagan, Ph.D.

Interaction of the endogenous antibody response with activating FcgRs enhance control of Mayaro virus through monocytes
Mentor: **Dr. Julie Fox**
Study Section: **Immunology - Infectious Disease**

Alphaviruses are mosquito-borne RNA viruses that have caused explosive outbreaks worldwide. Mayaro virus (MAYV), an emerging alphavirus, can cause fever, rash, myalgia, and severe polyarthritis and polyarthralgia. In a subset of infected individuals, long-term joint pain can persist for months to years following the initial infection. While there are no approved vaccines or therapeutics against MAYV disease, antibodies have been shown to be critical for protection. Therapeutic monoclonal antibody (mAb) efficacy against MAYV requires Fc-mediated activity for optimal protection, independent of time administered or neutralization potency, suggesting an importance of antibody Fc-Fc gamma receptor (FcgR) interactions for mAb-mediated protection during MAYV infection. However, the specific qualities of endogenous antibodies and the requirement of FcgRs for initial clearance and subsequent protection following natural infection has not been evaluated. To address this question, mice lacking activating FcgRs (FcRg-/-) were infected with MAYV subcutaneously and compared to wild type (WT) mice. FcRg-/- mice had prolonged foot swelling and delayed viral clearance from joint-associated tissue, despite similar levels of binding and neutralizing antibodies compared to WT mice. Similarly, FcRg-/- mice had delayed clearance follow infection with related arthritogenic alphaviruses, Ross River virus, and chikungunya virus suggesting a broad need for these receptors. Antibody Fc engagement with FcgRs was necessary for MAYV clearance, as mice lacking B cells showed a similar delay in viral clearance from the joint-associated tissue as compared to the FcRg-/- mice. Immune cell populations from the ipsilateral foot were characterized by flow cytometry and showed increased Ly6Chi monocytes and reduced Ly6Clo monocyte/macrophage in the absence of activating FcgRs. Single cell RNA sequencing showed elevated levels of inflammatory monocytes in the ipsilateral foot with increased MAYV RNA present in FcRg-/- monocytes and macrophages. While adoptive transfer of WT Ly6Chi monocytes into FcRg-/- mice did not alter viral burden, transfer of Ly6Chi monocytes lacking FcgRs into WT mice increased viral burden in the WT mice suggesting a pro-viral role for FcRg-/- monocytes during

MAYV infection. Overall, this study determined that engagement of antibody Fc with activating FcγRs promotes a protective response during MAYV infection.

104. Edmee Eyraud, PhD

Investigating a role for cachexia in Tuberculosis disease pathogenesis
Mentor: **Dr. Katrin Mayer-Barber**
Study Section: **Immunology - Infectious Disease**

Removed at request of author

105. Adam Hage, Ph.D.

The Transcriptional Regulator NONO Potentiates the Antiviral Innate Immune Response against West Nile Virus Infection
Mentor: **Dr. Sonja Best**
Study Section: **Immunology - Infectious Disease**

West Nile Virus (WNV) is a mosquito-borne orthoflavivirus capable of rapid geographical spread. WNV infection can result in a range of clinical outcomes from mild febrile illness to lethal neuroinvasive disease. The continued emergence of WNV into new areas and lack of treatments or vaccines makes WNV a priority threat to human health and highlights the need to better understand the cellular immune responses that affect the outcome of infection. To identify cellular genes that regulate antiviral innate immunity, we performed Ingenuity Pathway Analysis on an RNA-Seq dataset of orthoflavivirus-infected human dendritic cells and identified non-POU domain-containing octamer-binding protein (NONO) as a central gene in a graphical network of innate immune pathway components. NONO is a multifunctional nuclear protein that participates in numerous cellular functions including RNA metabolism and transcriptional regulation, although a role for NONO in orthoflavivirus replication is unclear. NONO CRISPR knockout (KO) A549 cells supported 10-fold higher WNV titers compared to WT cells. The type-I interferon (IFN-I) response is critical for preventing WNV replication. To determine if these differences in viral replication were from IFN-I signaling, mRNA expression levels for IFN-β, IFN-stimulated genes, and proinflammatory cytokines including IL-6 were measured and found to be significantly decreased in the WNV infected NONO KO cells. This loss in IFN-β transcripts correlated with significantly lower levels of biologically active IFN-β in cell culture supernatants. Importantly, inhibition of JAK-STAT signaling with the compound Ruxolitinib recovered WNV titers in WT cells to the same degree as the NONO KO, indicating that the effects of NONO on virus replication are dependent on IFN-I. Additionally, both NONO KO and siRNA knockdown cells stimulated with agonists of the RIG-I-like receptor (RLR) pathway (Sendai virus, Poly(I:C), or 3p-hpRNA) expressed significantly lower transcript levels for IFN-β and IL-6. Thus, NONO is a positive regulator of RLR-dependent antiviral immunity and critical for establishing an optimal antiviral state. Further defining NONO's role in immune responses may aid in development of diverse antiviral countermeasures to combat emerging threats.

106. Zohir Islam, Ph.D.

MATRIN3 deficiency leads to cGAS-STING activation
Mentor: **Dr. Stefan Muljo**
Study Section: **Immunology - Autoimmune**

MATRIN3(MATR3), is a nuclear RNA-binding protein, has been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal disorders (FTD). However, the precise mechanisms by which mutations in MATR3 contribute to ALS development remain poorly understood. In this study, we employed CRISPR/Cas9 technology to introduce mutations in MATR3 within human induced pluripotent stem cells (iPSCs) and the HAP1 cell line, facilitating the investigation of the consequences of MATR3 loss of function. To assess transcriptomic alterations, we performed RNA sequencing using both Oxford Nanopore Technologies and Illumina platforms, revealing significant upregulation of interferon-stimulated genes (ISGs) in MATR3-deficient cells. We further employed photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-

CLIP) to identify direct RNA targets of MATR3. Among the identified targets, TDRD3, a gene involved in RNA-DNA hybrid metabolism in collaboration with Topoisomerase III Beta (TOP3B), exhibited abnormal splicing and reduced expression in MATR3-deficient cells. Specifically, MATR3 loss led to the inclusion of a novel exon in the TDRD3 transcript, producing a mis-spliced isoform that triggered nonsense-mediated decay. These findings suggest that the disruption of MATR3-TDRD3-TOP3B interactions results in the accumulation of cytoplasmic R-loops, which activate the cGAS-STING pathway. This molecular cascade has profound implications, potentially elucidating the pathogenic mechanisms underlying ALS and highlighting the critical role of MATR3 mutations in the progression of neurodegenerative diseases. Our findings further provide a promising therapeutic target for MATR3-dependent interferonopathies.

107. Franziska Kaiser, DMV, PhD

Clade 1 mpox virus transmission is driven by mucosal exposure and increased shedding.
Mentor: **Dr. Vincent Munster**
Study Section: **Virology - Pathogenesis/Therapeutics**

The last decades have seen a dramatic increase in human-to-human transmitted mpox clade 1 cases in the Democratic Republic of Congo. In addition, mpox has recently spread to neighboring countries as well to the USA, Europe, Middle East and Asia. Due to this increase of mpox cases WHO declared mpox a public health emergency of international concern on Aug 14, 2024. Epidemiological data suggest that the spread of mpox virus (MPXV) Clade 1a has been largely facilitated through sexual contact. However, many aspects of the transmission kinetics of MPXV are still poorly understood. To help closing these knowledge gaps, we investigated the influence of mucosal exposure routes on disease progression and onwards transmission. We focused on virus shedding kinetics, pathogenicity, and transmission potential in a prairie dog model (Cynomys ludovicianus) following inoculation with a clade Ia (MPXV Zaire-79, 2.5x10^4 PFU). All tested mucosal inoculation routes (penile, vaginal, rectal, and intranasal; n=4) led to a productive, systemic infection. Inoculation via the urogenital route resulted in the highest virus shedding and the most severe clinical disease. In a next step, we simulated mucosal contact transmission by mechanically transferring virus from a donor to a sentinel animal. At peak disease, a swab was taken from either the nose of an intranasally inoculated or the rectum of a rectally infected donor animal and immediately transferred to the either the nose or the rectum of a sentinel. This transmission set-up enabled us to compare the transmission efficiency of the nose-to-nose transmission group with the rectum-to-rectum transmission group (n=4 transmission pairs). Both transmission routes resulted in 100% transmission efficiency. We detected high virus shedding in sentinel animals starting from the first day post transmission. Clinical signs varied by virus entry site, with predominantly respiratory signs in animals exposed to intranasal transmission and systemic disease in rectally exposed animals. These results demonstrate the increased susceptibility of the anogenital mucosa to mpox infections and early onset of virus shedding after transmission events. Pre-symptomatic shedding may be an important driver of sustained spread within communities. This knowledge can help understand the observed human to human transmission dynamics and aid the development of preemptive and prophylactic countermeasure strategies aimed at preventing transmission.

108. Prajna P Kar, PhD

A novel genetically-encoded Ca++ sensor reveals stepwise uptake into human erythrocytes infected with malaria parasites
Mentor: **Dr. Sanjay Desai**
Study Section: **Microbiology and Antimicrobials**

Malaria parasites grow in circulating erythrocytes to cause disease. The human pathogen, P. falciparum, exports many proteins into the host erythrocyte to remodel the cell and increase uptake of required nutrients and ions. While other solutes are acquired via a well-characterized parasite channel, uptake of Ca++, an ion essential for parasite growth, remains poorly characterized. Here, we designed and used a novel genetically-encoded Ca++ indicator (GECI) that capitalizes on split NanoLuc technology to yield a compartment-specific, Ca++-dependent luminescence signal. Our sensor consists of two NanoLuc fragments separated by a calmodulin-M13 domain; Ca++ binding to this domain produces conformational changes to yield a bright reporter signal by bringing the NanoLuc fragments together. An optimal Kd for Ca++ (~200 nM) was achieved

through engineered mutations of each domain. DNA transfection was then used to target the optimized sensor to parasite cytosol or to erythrocyte cytosol as a C-terminal tag on RhopH2, a protein localized exclusively in the host compartment. With both transfectants, removal of extracellular Ca++ with 2 mM EGTA, a Ca++ chelator, produced Ca++ efflux as continuously tracked with the reporter. Restoration of external Ca++ permitted kinetic tracking of Ca++ uptake and revealed stepwise uptake into the host cytosol (t1/2 < 10 min) and then the intracellular pathogen's cytosol (t1/2 = 1 h). MBX2366, a potent parasite nutrient channel inhibitor, did not alter Ca++ kinetics, indicating a distinct parasite-induced pathway for Ca++ acquisition. Vanadate, a nonspecific ATPase inhibitor, had no effect on uptake into the erythrocyte but altered Ca++ acquisition into the parasite, indicating passive uptake into infected erythrocytes and active transport at the intracellular parasite membrane. Transport kinetics into the parasite were faster in immature rings than mature trophozoites (t1/2 of 30 min vs 1 h), revealing parasite stage-dependent Ca++ acquisition. These studies report a novel luminescence-based GECI that provides compartment-specific Ca++ uptake kinetics and reveals key features of transporters at each membrane within infected erythrocytes. The reporter assay is also suitable for high-throughput inhibitor screens; such inhibitors promise to be important research tools and may be starting points for future antimalarial drugs targeting Ca++'s essential roles in parasite development.

109. Mohammad Kashif, PhD

A novel agonist of DNA transfection in human malaria implicates a conserved receptor for plasmid uptake
Mentor: **Dr. Sanjay Desai**
Study Section: **Molecular Biology - General**

DNA transfection is the cornerstone of molecular studies of drug and vaccine targets in malaria, but is plagued by low transfection efficiencies and an uncharacterized mechanism of plasmid uptake from host erythrocytes. Here, we developed a sensitive assay to track transfection in the human P. falciparum malaria parasites by expressing the NanoLuc reporter from the transfection plasmid, enabling quantification of transfection outcomes in only 48 hours. We then miniaturized this reporter assay into 384-well microplates, achieved suitable Z' statistics, and executed a high-throughput screen of 57,000 diverse small molecules to identify a single hit, NPU7, that reproducibly improves parasite transfection efficiency. NPU7 is specific for transfection because it does not increase NanoLuc reporter signals in stable transfectants carrying the episome. NPU7 also did not increase signals when the plasmid is directly electroporated into the intracellular parasite, indicating action on DNA uptake from the host cell cytosol. Improved uptake from host cells is independent of plasmid backbone and was seen with geographically divergent P. falciparum strains, implicating a conserved receptor-mediated uptake mechanism. NPU7 action also did not require circular super-coiled plasmid because linearized plasmid yielded similar increases in reporter signals at the 48 h reading. NPU7 was not effective in transfections using the phylogenetically distant P. knowlesi-rhesus monkey malaria model, implicating either a P. falciparum¬-restricted receptor or mutations in a conserved uptake mechanism. To explore how the putative receptor might interact with DNA and NPU7, we used an engineered HSP101/PTEX knockdown line defective in parasite protein export into host cell cytosol. Preserved NPU7 activity in this knockdown suggests that the putative receptor does not require HSP101/PTEX-mediated export. Our chemical screens have identified a novel reagent that provides mechanistic insights into DNA uptake by intracellular Plasmodium spp. and improves transfection of human malaria parasites for validation of drug and vaccine targets.

110. Basak Kayaoglu, PhD

Human BCMA deficiency causes common variable immunodeficiency
Mentor: **Dr. Helen Su**
Study Section: **Immunology - Infectious Disease**

Removed at request of author

111. Kathryn Laporte, PhD

The perturbation of oral tolerance during an ongoing influenza A infection
Mentor: **Dr. Andre Ballesteros-Tato**
Study Section: **Immunology - Lymphocyte Development and Activation**

At homeostasis, ingestion of food proteins triggers a tolerogenic program that maintains immune hyporesponsiveness to food antigens. In the gut, antigen-presenting cells (APCs) sample food proteins, migrate to the gut-draining mesenteric lymph nodes (mesLN), and activate CD4+ T cells, which can differentiate into peripheral regulatory T cells (pTregs), become anergic, or undergo apoptosis. Disruption of these mechanisms can lead to food allergies. While the mesLN is the primary site for food antigen sampling, studies suggest that food-derived antigens can also enter the bloodstream. Whether circulating food-allergen-derived antigens reach non-gut-draining lymph nodes (LNs) and influence food tolerance remains unclear. Here, we show that oral administration of ovalbumin (OVA) results in detectable OVA in the bloodstream within 10 minutes, indicating rapid systemic dissemination. In addition to the mesLN, OVA-specific CD4+ T cell responses are also primed in the lung-draining mediastinal lymph nodes (medLN) following antigen distribution. Under steady-state conditions, antigen-experienced CD4+ T cells in both the medLN and mesLN fail to differentiate into effector T cells, resembling tolerized cells. However, when OVA is ingested following infection with a mouse-adapted influenza A strain (PR8), the number of OVA-specific CD4+ T cells increases significantly in the medLN of PR8-infected OVA-fed mice compared to uninfected controls, while responses in the mesLN remain unchanged. Moreover, OVA-specific CD4+ T cells in the medLN adopt a T follicular helper (Tfh)-like phenotype, characterized by PD-1, CXCR5, and Bcl-6 expression. This correlates with the presence of IgE+ OVA-specific plasma cells in PR8-infected OVA-fed mice but not in controls. These findings suggest that the induction of food-specific Tfh cells and IgE production in gut distal LNs during inflammation may represent a mechanism for breaking oral tolerance. Understanding the mechanisms behind aberrant immune responses to food antigens in inflammatory environments and distal LNs will be important for developing strategies to prevent and treat food allergies and sensitivities.

112. Audrey Meloun, BS

Characterization of Ly6G/Nur77-Expressing Lung Macrophages Activated by Protease Allergens.
Mentor: **Dr. Beatriz Leon-Ruiz**
Study Section: **Immunology - Innate and Cell-mediated Host Defenses**

The immune system detects infectious pathogens, like bacteria and viruses, through pattern recognition receptor activation, triggering immune responses. Allergens, which are innocuous environmental proteins, can also activate the immune system and cause inflammation, though allergen recognition mechanisms are unclear. Despite their structurally diversity, many aeroallergens possess protease activity, which activates Protease-Activated Receptor 2 (PAR2). Previously, we identified that protease allergens activate PAR2 on an atypical Ly6G+/Nur77+ macrophage population in the lung, initiating type 2 allergic responses. Using a combination of immunofluorescence, single-cell RNA sequencing (scRNA-seq), and flow cytometry, we further characterized Ly6G+/Nur77+ macrophages. Staining for Ly6G in Nur77-GFP reporter mice, we identified that Ly6G+/Nur77+ macrophages localized within the lung alveolar instititium of naïve and allergen treated mice. Next, we sorted allergen+ cells from the lungs 24 hours after allergen exposure and performed scRNA-seq. We identified and categorized 17 distinct clusters using transcriptional signatures, including a Ly6G+/Nur77+ macrophage cluster, among other allergen+ cells, such as alveolar macrophages, CCR2+ monocytes, neutrophils, and other immune cells. In scRNA-seq data, Ly6G+/Nur77+ macrophages highly expressed signature genes like Nr4a1 (Nur77), Cx3cr1, Fcgr4 (CD16.2), Spn (CD43), and more, which we confirmed at the protein level. Gene Ontology analysis of Ly6G+/Nur77+ macrophage clusters revealed associated functions in cell adhesion, migration, and immune activation. Additionally, lineage tracing analysis indicated that Ly6G+/Nur77+ macrophages originate from Ms4a3-expressing granulocyte-monocyte progenitors and differentiate from a common CCR2+ monocyte precursor, which we validated using scRNA-seq-based developmental trajectory analysis. Gene Set Enrichment Analysis indicated that Ly6G+/Nur77+ macrophages are enriched in gene signatures of nonclassical monocytes over classical monocytes, suggesting that their differentiation may involve a transitional nonclassical monocyte state. Overall, our findings indicate that Ly6G+/Nur77+ macrophages represent a distinct monocyte-derived population residing in the alveolar instititium, where they are positioned to encounter air and blood allergen and potentially promote allergen-induced immune responses through roles in cell migration and immune activation.

113. Katie Pierce, M.S.

The Coxiella burnetii Nine Mile II deltacbu0533 strain as a suitable laboratory replacement strain
Mentor: **Dr. Carrie Long**
Study Section: **Bacteriology - General**

Coxiella burnetii is an intracellular bacterial pathogen that causes human Q fever. Livestock are the primary reservoir for C. burnetii and resulting human disease occurs following inhalation of C. burnetii-containing aerosols. Disease presentation ranges from acute, flu-like symptoms to persistent infection with severe complications. This highly infectious pathogen has several features that warrant concerns for potential bioterrorism use. Thus, the U.S. Centers for Disease Control and Prevention (CDC) have designated C. burnetii as a select agent, requiring experiments to be conducted in elevated security and containment conditions. Treatment options for chronic Q fever and vaccine development are critical research foci. C. burnetii manipulates host defense mechanisms via secretion of proteins by a Type 4B Secretion System and evades efficient immune detection by expressing lipopolysaccharide (LPS). LPS length (LPS phase) directly correlates with virulence, and bacteria expressing full length LPS are most virulent. The C. burnetii Nine Mile strain lineage is routinely studied, with Nine Mile I (NMI) expressing phase I LPS (fully virulent), Nine Mile Crazy (NMC) expressing intermediate LPS (slightly attenuated), and NMII (NMII) expressing phase II LPS (avirulent). NMII was exempt from CDC select agent regulations for many years due to a genetic deletion predicted to prevent synthesis of phase I LPS. However, recent experiments showed that NMII can undergo LPS elongation in vivo and in vitro. These findings raised concerns about the suitability of NMII as an avirulent reference strain and a “safe” biosafety level-2 (BSL-2) organism. We constructed a strain (C. burnetii NMIIdeltacbu0533) lacking the gene responsible for LPS elongation, and my project investigates suitability of NMIIdeltacbu0533 as an alternative BSL-2 strain to wild type NMII. First, growth kinetics of wild type NMII and NMIIdeltacbu0533 in axenic media and macrophage-like THP-1 cells were evaluated. After finding no significant differences in growth kinetics, fluorescence microscopy was used to visualize C. burnetii vacuole formation in THP-1 cells and primary human alveolar macrophages. Finally, Western blot analysis was used to compare manipulation of host signaling pathways by each C. burnetii strain. Results indicate NMIIdeltacbu0533 is a suitable alternative to wild type NMII for BSL-2 experiments and will be distributed to the field for use.

114. Byron Shue, Ph.D

TRIM5 alpha is a critical intrinsic barrier to Powassan virus infection of human cerebral organoids
Mentor: **Dr. Sonja Best**
Study Section: **Immunology - Innate and Cell-mediated Host Defenses**

Powassan virus (POWV) is an emerging neurotropic tick-borne orthoflavivirus endemic to North America, with no countermeasures available to treat or prevent infection. The type I interferon (IFN-I) response is critical to protect against POWV, as evidenced by the presence of auto-antibodies that neutralize IFN-I in patients with severe POWV encephalitis. Recent studies have demonstrated the IFN-stimulated gene (ISG) TRIpartite Motif protein 5 alpha (TRIM5a) directly antagonizes POWV replication and is required for the antiviral effects of IFN-I. However, how IFN-I and specific ISGs like TRIM5a coordinate cellular responses against POWV infection in the brain remains unknown. To understand both IFN-I dynamics and human TRIM5a functions in the brain in response to POWV infection, we gene edited TRIM5 knockout iPSCs and generated 40-day old cerebral organoids (COs). TRIM5-/- COs infected with POWV supported 10-fold higher virus titers by 5 days post infection (dpi) compared to WT COs. As a complementary experiment, WT COs were infected with WT POWV or a POWV variant containing a mutation that prevents recognition by TRIM5a. Both WT POWV and the mutant virus replicated rapidly in COs, although peak titers of WT POWV were delayed by 24hrs. Negative sense (replicative) viral RNA was present at 3dpi primarily in areas positive for doublecortin, a marker of immature neurons, confirming a role for TRIM5 in neuronal protection. Interestingly, POWV-infected COs did not upregulate IFN-I or ISG mRNA in response to either variant, while infection with Sendai virus induced IFN, demonstrating intact innate responses of COs to canonical stimuli. Astrocytes are an important inflammatory cell type in the CNS but are not yet present in 40-day old COs. Therefore, 6-month old COs were infected with WT POWV. These supported robust infection in cells positive for doublecortin (immature neurons) with only sporadic infection in cells positive for S100B (astrocytes) or GLAST (radial glia), but IFN-I expression remained unchanged relative to mock infected COs. Together, this work i) establishes COs as important models to study POWV biology in a human context, ii)

demonstrates the critical nature of basally expressed antiviral restriction factors for protection against neuronal infection, and iii) provides a complex 3D model to study how POWV evades the IFN-I response in the CNS.

115. Christopher Winski, PhD

Development of a human cerebral organoid model to study the effects of antivirals on Nipah virus neuropathogenesis
Mentor: **Dr. Emmie de Wit**
Study Section: **Virology - Pathogenesis/Therapeutics**

Nipah virus infections occur annually in southeast Asia. Nipah virus disease patients experience severe respiratory distress and debilitating neurological symptoms resulting in a 70% mortality rate. Currently, the models used to study Nipah virus infection include in vitro cell culture and in vivo hamster, ferret, and non-human primate models. The former is simplistic and poorly translates to human infections while the latter are skewed towards displaying respiratory disease. Given the importance of the neurological manifestations of Nipah virus, there is a strong need for a model to study Nipah virus within the human brain. We hypothesized that human cerebral organoids (hCO) can serve as a valuable model to study Nipah virus neuropathogenesis. Generated from human induced pluripotent stem cells, hCO are 3D tissue-like structures representative of a developing brain. Cellularly, our mature hCO are composed of neuronal precursors, neurons, and astrocytes, as confirmed by flow cytometry and immunofluorescence. To establish the hCO as a model of Nipah virus neuropathogenesis, we inoculated the hCO with Nipah virus and found that the virus replicated to high titers, resulting in cell death. Consistent with the limited human data, virus replication was primarily detected in neuronal populations. To explore the potential of hCO in the development of antiviral treatments against Nipah virus infection in the CNS, they were inoculated with Nipah virus and treated with either ribavirin or polyI:C for 8 days. Ribavirin, a nucleoside analog, was selected because it showed efficacy in vitro, but not in vivo. PolyI:C induces an innate immune response and showed efficacy in vitro and in vivo in a Nipah virus respiratory disease model. Interestingly, we found that both antivirals significantly reduced the replication of Nipah virus and associated cell death in the hCO. Finally, we quantified the expression of interferon stimulated genes (ISGs) in response to Nipah virus infection and antiviral treatment. Nipah virus significantly increased the expression of ISGs in the hCO and polyI:C induced the innate response in hCO. Collectively, we demonstrated that hCO are a powerful tool for studying Nipah virus neurological disease and that the lack of in vivo efficacy of ribavirin is due to its inability to cross the blood-brain barrier, rather than an inability to prevent Nipah virus replication in neurons. Thus, hCO can guide the development of novel intervention strategies.

116. Hongyu Wu, PhD

Fungal-Derived L-Asparaginase Drives Colonic Tuft Cell Expansion Independent of Type 2 Cytokine Signaling
Mentor: **Dr. Eric Dang**
Study Section: **Immunology - Mucosal Immunity**

The intestinal epithelium serves as a dynamic interface that not only absorbs nutrients but also detects microbial signals to initiate immune responses. Among its specialized cell types, tuft cells function as chemosensory sentinels, secreting IL-25 to activate type 2 innate lymphoid cells (ILC2s). In the classical model, IL-25–activated ILC2s produce IL-13, which promotes tuft cell differentiation from crypt stem cells, forming a positive feedback loop primarily observed in the small intestine. However, IL-4 administration or ILC2 activation via A20 ablation drives tuft cell expansion in the small intestine but not in the colon, suggesting distinct regulatory mechanisms in the colonic epithelium, potentially influenced by the microbiota. Here, we identify the commensal/food-derived fungus Wallemia mellicola as a driver of colonic tuft cell expansion. Oral administration of W. mellicola in mice selectively induces tuft cell expansion in the colon and drives colonic ILC2s that recirculate to the lungs. This phenotype persists in Stat6-deficient mice, indicating a tuft cell expansion circuit independent of canonical type 2 cytokine signaling. In vitro, W. mellicola supernatants promote tuft cell differentiation in intestinal organoids without IL-4 or IL-13, suggesting the secretion of a bioactive molecule that influences epithelial cell fate. Biochemical fractionation, including cut-off filtration, ammonium sulfate precipitation, and size exclusion chromatography, identified a heat- and proteinase K-sensitive protein between 30–70 kDa. Mass spectrometry analysis revealed L-asparaginase as a highly enriched component within these fractions. Surprisingly, recombinant L-asparaginase robustly induces tuft cell expansion in both wild-type and

Stat6-deficient organoids, an effect reversed by exogenous asparagine, suggesting that asparagine depletion drives tuft cell differentiation. Ongoing work aims to elucidate the effects of L-asparaginase treatment on crypt stem cell transcriptional programs and determine whether purified recombinant L-asparaginase can induce tuft cell differentiation in vivo. Given the association of W. mellicola dysbiosis with heightened asthma sensitivity, these findings reveal a novel fungal-epithelial interaction that may influence intestinal homeostasis and type 2 immune responses in the colon and distal organs such as the lungs.

117. Yufan Zheng, PhD

Coordination of type 2 immunity by oxysterols promotes latent infection in pulmonary fungal granulomas
Mentor: **Dr. Eric Dang**
Study Section: **Immunology - Mucosal Immunity**

Inhaled fungal pathogens often generate chronic, granuloma-contained subclinical infections that can reactivate to cause invasive disease in immunocompromised patients. However, the cellular and molecular basis for the inability to generate sterilizing immunity during persistent, non-lethal infection remains poorly understood. Here, using a murine Cryptococcus neoformans granuloma model, we show that an oxysterol-type 2 inflammatory circuit antagonizes fungal clearance during latent infection. Using spatial transcriptomics, we find that cryptococcal granulomas contain an inner core consisting of type 2 cytokine-responsive monocyte-derived myeloid cells surrounded by an outer layer of CD4+ T helper cells. Deletion of T helper 2 cells or removal of STAT6 in monocyte-derived cells resulted in decreased pulmonary fungal burden. Finally, we showed that granuloma macrophages expressed cholesterol-25-hydroxylase and produce oxysterols that localized CD4+ T helper cells within granulomas via the chemotactic receptor GPR183/EBI2. Deletion of GPR183 in T helper 2 cells resulted in decreased fungal burden without affecting CD4+ T cell differentiation or recruitment. We propose that an oxysterol-maintained T helper 2 cell-myeloid circuit establishes a local immunosuppressive environment driving fungal persistence and identifies new targets for immunotherapy to treat latent fungal infections.

National Institute of Allergy and Infectious Diseases - Vaccine Research Center

118. Alexandra Abu-Shmais, Ph.D.

Isolation of potently neutralizing and protective monoclonal antibodies against H5Nx influenza viruses from individuals immunized with a monovalent H5N1 vaccine
Mentor: **Dr. Sarah Andrews**
Study Section: **Virology - Pathogenesis/Therapeutics**

Removed at request of author

119. Allison N Bucsan, PhD

Visualizing the early immune response to Mtb infection in the lungs of IV BCG-immunized NHPs reveals critical functions of polyfunctional T cells in protection
Mentor: **Dr. Robert Seder**
Study Section: **Clinical and Translational Research - Animal Models**

1.25 million deaths from tuberculosis (TB) occur each year, due partly to limited protection of intradermal (ID) BCG against pulmonary TB. Previously, we showed that intravenous (IV) immunization with BCG elicits a higher frequency of antigen-specific T cell responses in the airways of rhesus macaques (RMs) and significantly higher protection against TB infection and disease compared to standard ID BCG. Importantly, there was limited detection of CFU, granulomas, or primary immunity following Mtb challenge in most IV BCG RMs, suggesting rapid clearance of Mtb post-challenge following IV BCG. Furthermore, immune correlates analysis and T cell depletion studies in RMs substantiated a major role for CD4 T cells in IV BCG-mediated protection. To understand very early immune events of Mtb control in lungs post-infection, RMs were immunized with IV or ID

BCG and challenged with Mtb ~5 months later. We used a high-dose challenge with fluorescent Mtb to facilitate visualization of Mtb and surrounding cells early post-infection. At 4d or 11d post-challenge, lungs of infected animals were interrogated using PET/CT, live-image and fixed-section confocal microscopy, flow cytometry, single-cell transcriptional analysis, and bacterial plating. PET/CT revealed early inflammation 4d post-challenge in IV BCG RM lungs, suggesting rapid induction of secondary immunity. Bacterial replication was not observed in lung or bronchoalveolar lavage (BAL) of IV BCG-vaccinated RMs between 4d and 11d post-challenge, and lung CFU was >2 log lower compared to unvaccinated RMs at 11d. Confocal microscopy revealed that IV BCG-vaccinated RMs had CD4 T cells enriched in regions containing Mtb. Flow cytometry showed that Mtb were predominantly within lung macrophages, and that lung T cells were enriched and had more TB-specific polyfunctional responses in IV BCG RMs. Using the Phenocycler Fusion, we demonstrated that IV BCG-vaccinated RMs had increased proliferative and activation signals from leukocytes near bacteria in the lungs. Overall, these data suggest that following IV BCG immunization, activated CD4 T cells are spatially enriched around infected macrophages and important for early control of Mtb infection. These data highlight the importance of enhancing the magnitude and quality of T cells in the lungs by using IV as a delivery approach for attenuated TB vaccines.

120. Francis De Souza Saraiva, PhD

Anopheles mosquitoes and the malaria parasite utilize mammalian hemopexin to mitigate heme toxicity from mosquito blood digestion
Mentor: **Dr. Joel Vega-Rodriguez**
Study Section: **Biochemistry - General**

Hematophagous insects have evolved to ingest blood, making them key vectors for blood-borne pathogens. In mosquitoes, blood digestion occurs in the midgut, releasing large amounts of labile heme, which can cause oxidative stress and cellular damage. To manage this toxicity, mammals produce hemopexin, a high-affinity heme-binding protein. Here, we show that Anopheles mosquitoes, which transmit malaria parasites, exploit mammalian hemopexin to mitigate heme toxicity during blood digestion, which consequently protects the malaria parasite. Mosquitoes fed on blood from hemopexin knockout mice exhibited midgut epithelial and peritrophic matrix damage due to increased labile heme. The peritrophic matrix, a chitinous barrier crucial for gut protection, is established by the presence of the microbiome and plays a role in microbiome homeostasis. Notably, mosquitoes ingesting blood without hemopexin displayed increased bacterial load and diversity, suggesting a disruption in microbiome balance that may impair peritrophic matrix function. Transcriptomic analysis revealed upregulation of genes associated with mitochondrial respiration, amino acid metabolism, and antioxidant responses in the midgut, fat body, and ovaries of mosquitoes fed on hemopexin-deficient blood, indicating a systemic oxidative stress response. These mosquitoes also exhibited heightened oxidative damage in the hemolymph, reduced survival, fecundity, and fertility—demonstrating hemopexin's role in enhancing mosquito vectorial capacity. Moreover, the absence of hemopexin significantly reduced Plasmodium infection in mosquitoes, an effect reversed by injecting recombinant hemopexin into knockout mice. However, infection and oxidative damage were not rescued when a heme-binding-deficient hemopexin mutant was used, confirming that heme sequestration by hemopexin protects both the mosquito and the parasite. These findings reveal how Anopheles mosquitoes adapt to manage heme toxicity and how Plasmodium parasites exploit these adaptations for successful infection. Understanding this interaction provides new insights into potential strategies for disrupting the Plasmodium lifecycle and controlling malaria transmission.

121. Dalton Hermans, PhD

Spatiotemporal dynamics of adoptively transferred stem-like T cells in the tumor microenvironment following vaccination
Mentor: **Dr. Robert Seder**
Study Section: **Immunology - Immunotherapy**

Adoptive T cell therapies (ACT) have resulted in significant clinical benefit for melanoma and hematological malignancies but have been less successful in advanced solid tumors where immunosuppressive tumor microenvironments (TME) restrict T cell function. Here, we aim to enhance the efficacy of ACT by both improving the quality of T cells used for infusion and removing

suppressive elements in the TME to support T cell infiltration and function at the tumor site. Mouse Ova-specific T cells used for ACT were expanded in the presence of an mTOR complex inhibitor, which resulted in significant expansion while maintaining a favorable PD1+TCF1+ stem-cell memory (Tscm) phenotype. When compared to less functional PD1+TCF1- effector T cells (Teff) cultured in the absence of the mTOR inhibitor, Tscm cells were highly enriched for canonical stemness programs on the RNA and protein level, indicative of future proliferative and cytotoxic function. Indeed, using a murine B16-Ova tumor model, we found that Tscm cells were capable of significantly higher in vivo expansion and tumor control than Teff cells. To improve the function of T cells within the TME following transfer, we intravenously (IV) administered an Ova peptide vaccine conjugated to a toll-like receptor 7/8 agonist. Remarkably, IV vaccination simultaneously expanded transferred Tscm cells in the blood and induced a systemic type I IFN response that altered myeloid populations in the TME. scRNAseq analysis of the TME revealed that three distinct immunosuppressive monocyte populations present in unvaccinated animals were eliminated by 24 hours after IV vaccination. Importantly, while IV vaccination remodeled the TME in mice treated with either Teff or Tscm cells, vaccination only enhanced tumor protection in mice receiving Tscm. To investigate the functional differences between Tscm and Teff in the remodeled TMEs, we performed spatial transcriptomics with the Visium HD platform. Spatial UMAPs of cellular composition and distribution within the TME varied substantially depending on the quality of transferred cells. Notably, disperse cytotoxic infiltrates deep within the tumor parenchyma containing high levels of GrnzB and IFNg were present only in TMEs that received Tscm. These data illustrate an approach to optimize ACT by improving the quality of T cells prior to transfer and subsequently using vaccination to expand transferred T cells and modulate the TME for enhanced tumor control.

National Institute of Arthritis and Musculoskeletal and Skin Diseases

122. Natalia I Acevedo Luna, Ph.D

Global transcriptional kinetic profiling during skeletal muscle stem cell lineage progression and aging at the single cell level
Mentor: **Dr. Vittorio Sartorelli**
Study Section: **Gene Expression - Transcriptional Regulation (Eukaryotic)**

Removed at request of author

123. Khalid A Garman, MD, PhD

Intersectional high-throughput screening uncovers a synergistic drug combination for a rare and lethal skin cancer
Mentor: **Dr. Isaac Brownell**
Study Section: **Oncology - Therapeutics and Translational Research**

Removed at request of author

124. Jyotirindra Maity, Ph.D

Opening a pathway to treatment for classical melorheostosis: CDK4 inhibition reduces proliferation and mineralization in MAP2K1+ melorheostosis
Mentor: **Dr. Timothy Bhattacharyya**
Study Section: **Cell Biology - Cell Cycle**

Melorheostosis is a rare disease where excessive bone overgrowth and exostoses cause deformity and pain. Somatic mutations of MAP2K1 result in hyperactivation of the ERK pathway in osteoblasts and increase in vitro mineralization. There is no effective treatment so far. Because melorheostosis osteoblasts display increased expression of cell cycle proliferation-related molecules compared to controls, we hypothesized that rapid progression through the cell cycle contributes to the bony overgrowth. We established an induced pluripotent stem cell (iPSCs) model from unaffected and affected regions of patient skin fibroblasts. We further differentiated iPSCs into induced mesenchymal stem cells (iMSCs) and then into osteoblasts. Using propidium iodide (PI) mediated cell cycle assay with flow cytometry, we recorded that affected iMSCs (and primary

patient osteoblasts) have a higher proportion of proliferative cells than unaffected. We noticed that affected osteoblasts and iMSCs have elevated expression of phospho-CDK4 and phospho-Rb, which are the crucial molecules for the G1 to S transition. Immunofluorescence confirmed a significantly higher cell proliferation marker Ki67 index in affected iMSCs and osteoblasts. EdU incorporation assays validated a higher percentage of S phase in affected cell sets. We applied the FDA-approved CDK4 inhibitor palbociclib to iMSCs and primary osteoblasts and found a significant reduction of phospho-CDK4 and phospho-Rb on western blot. Palbociclib-treated iMSCs and osteoblasts displayed elevated G0/G1 peak on flow cytometry and lowered EdU incorporation, thus confirming the blockade of cell cycle progression by limiting S phase entry. Interestingly, palbociclib treatment for an initial 5 days of a total of 21 days of osteogenic stimulation blocks mineralization in affected cells to a greater extent than the unaffected, suggesting that increased proliferation contributes to the bone growth phenotype in patients. Thus, our data suggest targeting cell cycle machinery can be a potential therapeutic approach for melorheostosis patients.

125. Jorge R Romo Tena, MD, PhD

Nerve injury induced protein-1 (Ninj1) deficiency aggravates murine lupus through modulation of macrophage polarization
Mentor: **Dr. Mariana Kaplan**
Study Section: **Immunology - Autoimmune**

Nerve injury-induced protein-1 (Ninj1) is an adhesion molecule that plays various roles in immune and stromal cells, including the modulation of inflammation and a critical role during cell death. Dysregulation of inflammatory pathways and cell death has been linked to the development of autoimmune diseases, such as systemic lupus erythematosus (SLE). Therefore, we hypothesized that Ninj1 may be involved in the pathogenesis of SLE. Ninj1-deficient (Ninj1+/-) and wild-type (WT) C57/B6 mice were induced to develop lupus-like autoimmunity using two induced models triggered by topical application of the TLR7/8 agonist imiquimod (IMQ) or by intraperitoneal injection of pristane. We assessed the mice for clinical phenotype, immune dysregulation, and organ damage. In comparison to WT mice, Ninj1+/- mice exposed to IMQ exhibited greater splenomegaly at the expense of extramedullary hematopoiesis, more severe anemia, decreased spleen lymphocyte counts, and increased spontaneous neutrophil extracellular trap (NET) formation. Although we observed no differences in autoantibody levels or proteinuria, Ninj1+/- mice developed more lupus-associated renal disease, as evidenced by increased immune complex deposition and exacerbated kidney inflammation and damage. In the pristane model, Ninj1 deficiency worsened diffuse alveolar hemorrhage and lung inflammation. Furthermore, Ninj1+/- M2 bone marrow-derived macrophages (BMDM) exposed to IMQ in vitro showed defective activation of the p38/Erk 1/2 MAPK and Akt signaling pathways, which are important in M2 macrophage polarization. Preliminary, these findings suggest that Ninj1 deficiency aggravates murine lupus, at least in part, by impairing the anti-inflammatory roles of M2 macrophages. Further experimentation is needed to identify the immune system defects associated with Ninj1 deficiency, particularly in the affected tissues, and to understand their implications for autoimmune diseases.

National Institute of Biomedical Imaging and BioEngineering

126. Aditya Josyula Venkata, Ph.D.

Damage Signals Presented by cDC1s Prime Ly49+ Killer Regulatory T cells to Prune CD62L+ T cells in Skeletal Muscle
Mentor: **Dr. Kaitlyn Sadtler**
Study Section: **Immunology - Autoimmune**

Self-antigens which are obscure to immune cells during homeostasis, become exposed following tissue damage and can trigger autoimmunity due to the quasi-random nature of thymic negative selection. Despite the escape of thymic deletion, most patients are protected from autoimmunity by multiple peripheral mechanisms of lymphocyte regulation. Using a murine model of volumetric muscle loss (VML), our lab showed that NK cell-derived XCL-1 recruits conventional type 1 dendritic cells (cDC1s) and mediates immunoregulation. Here, we identified a subset of regulatory T cells that express inhibitory Ly49 receptors and HELIOS (Ly49+ Tregs) which are primed by cDC1s after VML. Further, we observed that Ly49+ Treg recruitment

is enhanced or attenuated by implanting decellularized extracellular matrix (ECM) or polyethylene (PE) respectively at the injury site. In-vitro, cDC1s, loaded with soluble ECM proteins, stimulated and selectively induced proliferation in Ly49+ Tregs but not CD4+ T cells or CD4- Ly49- T cells. Next, using single cell RNA sequencing data from mice which underwent VML, we created gene signatures for cDC1s, CD4+ Foxp3+ Tregs and CD4- Ly49+ Tregs. Mapping these signatures onto spatial transcriptomics spots of injured muscle, we observed spatial coincidence of cDC1s and Ly49+ Tregs. Further, by extrapolating gene signatures to functional protein association networks, we identified 7 distinct pathways of communications between cDC1s and Ly49+ Tregs. In mice lacking cDC1s (Batf3 -/-), CD8+ Ly49+ Tregs were significantly depleted but CD8- Ly49+ Tregs were unchanged, suggesting a functional difference in the two subsets. Transcriptionally, Ly49+ Tregs shared gene expression signatures with CD4+ Foxp3+ Tregs as well as NK cells, leading us to hypothesize that Ly49+ Tregs regulate other lymphocytes by killing. Additionally, mice with fewer Ly49+ Tregs (PE treated or Batf3-/-) accumulated more CD62L+ naïve T cells compared to mice in which Ly49+ Tregs were abundant (ECM treated mice). This led us to evaluate selective targeting mechanisms. In-vitro, activated Ly49+ Tregs selectively targeted CD62L+ T cells for elimination in co-cultures and in-vivo, enhancing Ly49+ Treg abundance by implanting ECM resulted in significant attenuation of naïve T cell accumulation in the muscle. We propose that pruning of naïve T cells by Ly49+ Tregs is a preventive mechanism against either tertiary lymphoid structure formation or inadvertent activation of autoreactive T cells.

127. Mazen Mezher, PhD

Ezrin plays a key role in regulating the viscoelastic properties and force generation in T lymphocytes during the formation of the immunological synapse.
Mentor: **Dr. Alexander Cartagena-Rivera**
Study Section: **Biophysics**

The immunological synapse (IS), which is the specialized cell-cell junction between a T lymphocyte and an antigen-presenting cell, plays a crucial role in T cell activation. This process begins when the T-cell receptor (TCR) binds to its specific antigenic peptide. Several studies have highlighted that the mechanical properties of T cells during activation significantly impact various cellular functions, including proliferation, migration, and cytotoxic activity. The formation and maintenance of the IS are facilitated by force generation through the dynamic interaction of the actomyosin and microtubule cytoskeletal networks. However, the precise way in which force generation influences the mechanical properties of T cells remains unclear. In our study, we utilized high spatiotemporal resolution Atomic Force Microscopy to measure the viscoelastic response of T cells through common mechanical parameters (storage and loss moduli) across different timescales at the nanometer scale. Additionally, we used Traction Force Microscopy to quantify the traction stresses generated by T cells on soft silicone hydrogels during IS formation. Our findings reveal that T cells exhibit structurally diverse viscoelastic properties at the nanoscale during IS formation, triggered by CD3/CD28/LFA-1 co-stimulation. Specifically, we observed significantly higher elastic and viscous properties at the edge and center of the IS, while the peripheral transition region appeared softer and more fluid. These observations correspond to changes in the actomyosin cytoskeleton structure in these areas. Furthermore, our results demonstrated that perturbations in cytoskeletal proteins regulating filamentous actin caused substantial changes in T cell elasticity and fluidity, as well as alterations in the traction stresses generated during IS formation. Notably, we observed significant softening, fluidization, and a decrease in traction stresses when detaching the actin cortex from the plasma membrane by inhibiting Ezrin activity. In summary, understanding the relationship between key cytoskeletal structures at the IS and the mechanical property of the cell is essential for maintaining and forming the IS, offering valuable insights into potential strategies to engineer T cells with enhanced activation and cytotoxic abilities.

National Institute of Child Health and Human Development

128. Abhishek Anand, PhD

The human genome has been reduced 5 GB in size by a mechanism that truncates transposable elements
Mentor: **Dr. Henry Levin**
Study Section: **Epigenetics**

The human genome contains ~ one million copies of the non-LTR retrotransposon L1. Active copies of L1 cause disruption of germline sequences in up to 5% of newborns. Many cases of genetic diseases have been caused by L1 activity including Hemophilia, ataxia telangiectasia, β-thalassemia, and L1 expression is a hallmark of several epithelial cancers including ovarian, colon, lung, breast, and pancreatic cancers. Mechanisms of genome defense including piRNA production and DNA methylation are known to defend the genome from damage due to transposable elements. One major mechanism, thus far unexplained, is the extensive 5' truncation that occurs in >95% of L1 insertions. The L1 encoded ORF2p protein possesses endonuclease and reverse transcriptase (RT) activities that generate insertions by nicking target sites and reverse transcribing L1 RNA. During this process 95% of L1 insertions suffer extensive 5' truncation rendering them inactive due to removal of its promoter and protein coding sequences. This is likely a host defense mechanism that limits the copy number of full-length active L1s. 5' truncation has played a significant role in shaping the genome as what is a 3-gigabase genome would have otherwise expanded dramatically to be 8-gigabases. However, little is known about the mechanism of 5' truncation. To understand the mechanism, we developed a dual reporter system that expresses L1 with mCherry in the 5' UTR and GFP in the 3' UTR. Introns are included in mCherry and GFP that allow their expression only after integration occurs. Full-length L1 insertions express mCherry and GFP, but if L1 is 5' truncated, only the GFP is active. With FACS of cells expressing the L1 reporter we find 95% of insertions are 5' truncated. To identify host factors that cause 5' truncation we screened 93 candidate genes by siRNA knockdown and found RNA helicase MOV10 contributes significantly to 5' truncation. CRISPR mediated knockout of MOV10 produced similar results. MOV10 is also known to inhibit retroviruses' replication including HIV-1 by an unknown mechanism. Previous reports find MOV10 binds L1 RNA with densities that closely match where 5' truncation occurs. Our findings indicate that MOV10 moving 5' to 3' on the L1 RNA causes 5' truncation by blocking reverse transcription of L1 RNA by ORF2. Our results also suggest the possibility that MOV10 may inhibit HIV-1 by blocking its reverse transcription.

129. Amara Channell Doig, PhD

A novel measure of discretionary food intake: development and evaluation
Mentor: **Dr. Tonja Nansel**
Study Section: **Cultural/Social Sciences and Public/Environmental Health**

Background: Dietary guidelines recommend limiting foods and beverages high in added sugars, saturated fat, and sodium, and all alcoholic beverages (i.e. discretionary foods). This study developed a method to classify discretionary foods and evaluated its face validity and construct validity as compared with the Nova ultra-processed food classification method. Methods: Foods in the 2017-2018 USDA Food and Nutrient Database for Dietary Studies meeting any of the following criteria were classified as discretionary, based on existing standards: added sugar >20% of energy; sodium >460mg per serving; refined grain >50% of total grains or >10:1 carbohydrate: fiber ratio; saturated fat >20% of energy; and total fat >9% by weight (applied only to vegetables, sweets, and snacks), and all alcoholic beverages. Foods were also classified as ultraprocessed or not using Nova. Nutrient density for FNDDS foods was calculated using the Nutrient Rich Foods 9.3 score. We examined face validity by comparing the proportion of food group items classified as discretionary and ultraprocessed and evaluated construct validity by comparing the difference in nutrient density between foods classified as discretionary and ultra-processed; we also compared correlations with adherence to dietary guidelines, using the Healthy Eating Index-2020 (HEI) total scores in the U.S. population from 2017-2018 National Health and Nutrition Examination Survey data. Results: 4555 (66%) of foods were classified as discretionary; these foods contributed 77% of energy intake in U.S. children and adults. More snacks (97% vs 87%), sweets (96% vs 61%), high-fat (e.g. fried) protein and vegetables, and high-sugar fruit and dairy, were classified as discretionary than ultraprocessed. The difference in nutrient density between discretionary vs. non-discretionary foods (mean diff=74.8, 95%CI:70.6-78.9) was larger (p<0.001) than that between ultraprocessed vs. non-ultraprocessed foods (mean diff=29.7, 95%CI:25.1-34.2). The inverse correlation of HEI score with discretionary food intake (r=-0.65) was larger than that with ultraprocessed foods (r=-0.33). Conclusions: As compared with the Nova ultraprocessed classification method, the discretionary food classification method classifies non-recommended foods with lower nutrient density more effectively and is more strongly related to lower adherence to dietary guidelines in the U.S. population.

130. Megan Detels, BA

The Role of Calcium Signaling During Angiogenesis
Mentor: **Dr. Brant Weinstein**
Study Section: **Developmental Biology - Organogenesis**

Calcium signaling is a critical regulator of cellular responses in both excitable and non-excitable cells. The frequency, length, and intensity of calcium oscillations can differentially regulate a variety of different cellular responses. Endothelial cells, a non-excitable cell type, display calcium oscillations both in culture and in vivo, however the biological relevance of calcium signaling in vascular development and patterning has been largely unexplored. To address this gap, we are developing new transgenic lines to visualize and manipulate calcium signaling specifically in endothelial cells in vivo. By driving endothelial specific expression of the ratiometric calcium indicator GCaMP7s we have been able to observe calcium dynamics in the vascular endothelial cells of living, intact developing zebrafish embryos and larvae. We have also developed novel transgenic lines to drive expression of the far-red light activated channelrhodopsin ChrimsonR in endothelial cells in vivo. Preliminary results using this transgenic line have shown endothelial calcium signaling is increased upon activation, suggesting this will be a valuable tool for testing the functional role of calcium oscillations in the vasculature. In parallel studies, we are also testing the functional role of several calcium transporters expressed in endothelial cells during vascular development. Together, this research will yield important new insights into the role calcium signaling plays in regulating angiogenesis, vessel patterning, and vascular homeostasis, with potentially important implications for future therapeutic approaches to vascular pathologies.

131. Reid Doctor, BS, BA

Calcium imaging of complex behavioral control in free-swimming zebrafish
Mentor: **Dr. Harold Burgess**
Study Section: **Methods/Assay Development**

We are studying the neural dynamics that cause adaptive motor responses to variable sensory cues. While studying command neurons has yielded valuable insights into sensorimotor transforms, more complex circuits are often collaboratively controlled in ways that allow for graded output along several axes. Zebrafish have two startle responses to escape a perceived threat. The short-latency c-start (SLC) is controlled by a command neuron producing stereotyped behavior, while the long-latency c-start (LLC) is controlled by a cluster of prepontine neurons producing adaptive and energy-efficient behavior at the cost of increased processing time. The LLC circuit is a valuable model to study population encoding at the cellular level in a sensorimotor interface. However, zebrafish rarely produce LLCs when immobilized for calcium imaging using a confocal microscope.Immobilizing larvae distorts their proprioception and vestibular senses, which broadly inhibits behavior. To examine this circuit, we have built a 'swimscope': a low-cost microscope that noninvasively images neuronal activity in free-swimming zebrafish larvae. We first designate a range of starting positions from which a larva is likely to end under the imaging area after performing an escape. Once the larva is correctly positioned, we stimulate an escape using an acoustic stimulus while imaging behavior using a high-speed camera. After completing an escape, larvae pause for several hundred milliseconds. During this period, we use a high-speed z-scan to image slow-decay GCaMP fluorescence in a whole brain volume in under one second with single-cell resolution using widefield imaging. We also image unstimulated brains as references, and include GFP-expressing neurons in each larva for intensity normalization. We then compare these whole-brain snapshots to determine neural activity. This system avoids anesthesia, immobilization, and vestibular distortion and minimizes bright light exposure. We have validated this approach by confirming increased activity in the dorsal raphe of larvae when aroused. We are now using the swimscope to examine circuits which are intractable to calcium imaging in immobilized samples, notably the LLC circuit and areas of the arousal circuit outside the dorsal raphe. This will allow us to better understand complex circuits, whose activity is more commonly distorted by the invasive methods we currently use than simple circuits, and study circuits in more naturalistic settings.

132. Jian Ming Khor, PhD

Deciphering the Role of Epigenetic Reprogramming in Zebrafish Fin Regeneration
Mentor: **Dr. Brant Weinstein**
Study Section: **Epigenetics**

While is it known that epigenetic reprogramming is pivotal for kickstarting dormant developmental programs during regeneration, the regulatory mechanisms orchestrating this phenomenon remain elusive. The zebrafish, recognized for its regenerative capabilities, serves as an ideal model to explore this at the molecular level. We recently developed a novel zebrafish "EpiTag" transgenic reporter line that permits real-time visualization of tissue-specific epigenetic silencing or activation in living animals with cellular resolution. We observe early and striking activation of the EpiTag reporter in regenerating cells, including during fin regeneration after amputation, suggesting the efficacy of this line as a highly specific marker for cells undergoing epigenetic reprogramming at extremely early, otherwise inaccessible stages of regeneration. By selectively enriching early-stage regenerating cells from EpiTag fish using FACS sorting, we employ a multi-omics approach, encompassing ATAC-seq, bisulfite-seq, bulk RNA-seq, and single-cell RNA-seq analyses, to comprehensively profile chromatin accessibility, DNA methylation patterns, gene expression profiles, and single-cell transcriptomes within these regenerative cells. Our sequencing data reveal a strong enrichment of genes involved in histone modification, chromatin remodeling, and cell proliferation. To functionally characterize important epigenetic regulators, we employ Vivo-Morpholino-mediated knockdown and identify key players of chromatin remodeling complexes that are necessary for fin regeneration. To complement this, we performed small molecule inhibition of these genes to further confirm their role in regeneration. The capacity to isolate and study early-stage regenerative cells opens the door to important insights into the role of epigenetic reprogramming in regeneration and provides potential targets for therapeutic interventions.

133. June Hoan Kim, PhD

GluN1/GluN3a Excitatory Glycine Receptors as Network Modulators of Hippocampal Circuit Maturation and Memory-Related Oscillations.
Mentor: **Dr. Chris McBain**
Study Section: **Neuroscience - Cellular and Synaptic**

A subtype of NMDA receptors (NMDARs), GluN3a co-assembles with GluN1 to form an “unconventional” NMDAR, also known as an excitatory glycine receptor (eGlyR). Unlike conventional NMDARs, eGlyRs are solely gated by glycine, not glutamate, the major excitatory neurotransmitter. GluN3a expression dynamically influences hippocampal circuit development; its deletion accelerates excitatory synapse maturation, while its overexpression impairs long-term memory in adults. Despite being discovered over 30 years ago, its role in hippocampal network activity remains unclear. eGlyR function has been difficult to characterize due to desensitization caused by extracellular glycine, the agonist. Glycine binding to GluN3a activates eGlyRs, but binding to GluN1 leads to desensitization. Recently, CGP-78608, a GluN1-binding NMDAR antagonist, was shown to prevent desensitization, enabling functional investigation of eGlyRs. We hypothesized that eGlyRs regulate network excitability via specific interneuron subtypes, modulating rhythmic activities critical for synaptic maturation and memory. Analysis of scRNA-seq datasets identified two Grin3a(a gene for GluN3a)-expressing interneuron subtypes: somatostatin-expressing interneurons (SST-INs) and neurogliaform interneurons (NGF-INs). To investigate their role, we developed Grin3a conditional knockout mice (SST-Grin3a-cKO,NPY-Grin3a-cKO) and used optogenetic silencing (SST-Arch,NPY-Arch) to inhibit these interneurons. We then recorded CA3 pyramidal neurons, which receive interneuronal inputs but don’t express Grin3a. Comparisons between WT and cKO mice revealed that eGlyRs in NGF-INs and SST-INs modulate GABAergic tone, shaping synchronized rhythmic activity. In early networks, NGF-IN eGlyRs regulate GABAergic tone, modulating giant depolarizing potentials (GDPs), essential for synaptic maturation. As development progresses, SST-IN eGlyRs contribute to sharp-wave ripple (SWR) rhythmogenesis, critical for memory consolidation. Furthermore, eGlyR-mediated SWR modulation is conserved in non-human primates, suggesting an evolutionarily conserved role in hippocampal dynamics. Our findings indicate that eGlyRs do not merely affect individual neurons but act as a control parameter for hippocampal states by modulating network excitability. This study provides novel insights into the physiological role of eGlyRs, highlighting their potential as therapeutic targets for neurodevelopmental disorders linked to impaired network activity.

134. Snehal S Mahadik, Ph.D.

Characterizing the role of lysosome-related vesicles (LRV) mediated transport in motor neurons development and regeneration

Mentor: **Dr. Claire Le Pichon**
Study Section: **Neuroscience - Neurological and Neurodegenerative Disorders and Injury**

During development, neurons extend axons to establish neural connections. While most neurons lose their regenerative capacity and ability to reconnect after injury, some, such as spinal motor neurons, retain the ability to regrow axons—yet the mechanisms driving this process are still not fully understood. Recent studies in iPSC-derived cortical-like neurons (i3Neurons) highlighted the role of lysosome-related vesicles (LRVs) in mRNA transport critical for axonal homeostasis, with disruptions leading to axonal degeneration. However, the role of LRV-mediated transport in the development and regeneration remains unknown. To address this, we used CRISPR-Cas9 to generate iPSC-derived motor neurons (MN) lacking BORCS7, a subunit of the BORC complex essential for lysosome-kinesin coupling. BORCS7 KO halts the movement of LRVs and their associated mRNA into the axon, while allowing the movement of other organelles to continue. In BORCS7 knockout (KO) neurons, LAMP1-positive lysosomes were completely depleted from axons and confined to the soma, and live imaging confirmed loss of lysosome motility. This phenotype is specific, as the cytoplasmic or axonal distributions of other organelles, such as mitochondria and synaptic vesicles, are not affected, confirming BORCS7's role in LRV transport. Compared to wild-type MN, BORCS7 KO neurons also displayed reduced axonal outgrowth and smaller growth cones with fewer filopodia, implicating LRVs role in axonal development. To examine LRV function in regeneration, we performed laser axotomy. Following injury, 76% of wild-type MN axons regenerated, but only 32% of BORCS7 KO axons regenerated—similar to cortical i3Neurons (40%), which have lower regenerative capacity. This suggests that LRV transport is essential for motor neuron regeneration, potentially by delivering mRNAs for local translation. To test this, we generated Annexin A11 (ANXA11) KO neurons, which maintain lysosome motility but lack RNA-binding capacity. ANXA11 KO neurons exhibited intermediate regeneration (50%), supporting a model in which both LRV transport and LRV-mediated mRNA delivery are critical for regeneration. Our data show that LRVs are crucial for motor neuron development and regeneration, likely by coupling lysosomal and mRNA axonal transport. Ongoing research aims to uncover specific lysosomal functions in axonal repair, with potential implications for identifying therapeutic targets to enhance nerve regeneration.

135. Priyanka Mittal, Ph.D.

From weak to strong: A novel role of Mot1 in choreographing TBP dynamics to balance gene expression during stress
Mentor: **Dr. Alan Hinnebusch**
Study Section: **Molecular Biology - General**

Recruitment of TATA-binding protein (TBP) to RNA Polymerase II core promoters is essential for PIC (preinitiation complex) assembly at nearly all genes, facilitated in budding yeast by coactivator complexes SAGA and TFIID. It is thought that SAGA recruits TBP to promoters of stress-responsive genes with consensus (strong) TATA sequences, while TFIID is more involved with housekeeping genes containing non-consensus (weak) TATA-like sequences. Yeast Mot1 is a DNA-dependent ATPase that dissociates TBP from incomplete PICs in vitro but its role in regulating gene expression in vivo is incompletely understood. Previous studies indicated that Mot1 favors TFIID-dependent promoters by preferentially dissociating TBP from SAGA-regulated genes. While it was suggested that TBP bound to DNA in association with TFIID is less accessible to Mot1 compared to TBP delivered by SAGA, a structure of the TBP-TFIID-TFIIA-DNA complex revealed only indirect interaction between one TFIID subunit (Taf4) and TBP bridged by TFIIA. It is unclear therefore how Mot1 differentiates between SAGA and TFIID-dependent promoters for stable TBP-DNA binding. To better understand Mot1's role under stress, we conducted ChIP-Seq analysis to determine the effect of depleting Mot1 from nuclei on TBP binding at genes activated by transcription factor Gcn4 under amino acid starvation. Unlike previous findings in non-stress conditions, we discovered that Mot1 is necessary for strong TBP binding at highly activated Gcn4 target genes. Mot1 depletion also reduced TBP binding at other highly expressed genes transcribed constitutively while increasing TBP occupancies at weakly expressed genes, regardless of whether they rely on SAGA or TFIID in non-stressed cells. A similar pattern was found for genes highly induced by oxidative stress, which are not activated by Gcn4. Interestingly, while Mot1 depletion reduced TBP binding at highly expressed stress-induced and housekeeping genes alike, transcription (measured by RNA Pol II binding and RNA-Seq) declined only at TFIID genes. This suggests that transcription of stress-activated genes regulated by SAGA can proceed normally at reduced TBP occupancies. Our study reveals that Mot1 helps redistribute TBP from weakly- to strongly-expressed genes during stress rather than simply shifting TBP from SAGA- to

TFIID genes. We further suggest that stress-responsive genes have a unique mechanism allowing them to maintain robust transcription at reduced TBP binding.

136. Aye Chan Myo, PhD

Spatial Transcriptomics Mapping of Wnt10a-/- Molar Crown Morphogenesis
Mentor: **Dr. Sergey Leikin**
Study Section: **Developmental Biology - Early Development/Embryology**

Background: Wingless-type MMTV integration site family, Member 10A (Wnt10a) is the most commonly mutated gene linked to non-syndromic selective agenesis of permanent teeth in humans. Wnt10a-/- mice exhibited abnormal development of molars characterized by reduced size, flattened cusps, and taurodontic roots in both maxillary and mandibular regions. Despite the morphogenetic events resulting in taurodontism are extensively researched, significant gaps remain in our understanding of the molecular pathways responsible for the abnormal crown formation in Wnt10a-/- mice. Methods: Spatial transcriptome studies (Xenium in situ, 10x Genomics, Inc.) were conducted on Wnt10a-/- and wild-type (WT) mice at E14.5 and E15.5. This was followed by digital spatial profiling on dental papilla regions, utilizing Xenium and multiplex RNAscope in situ hybridization, H&E, and TUNEL assays. Student's t-test was utilized to evaluate the statistical significance. Results: In Wnt10a-/- molars, Wnt modulators (Sostdc1, Sost, Dkk1, Sfrp2, Wif1) expressions were dysregulated in comparison to WT molars; these genes typically display a negative area in the epithelial and mesenchymal cell layers immediately surrounding the enamel knot. Genes associated with extracellular matrix (ECM) (Fn1, Frem1, Col3a1, Col16a1, Fmod) exhibited considerable upregulation in Wnt10a-/- dental papilla at E14.5 compared to those in WT mice. However, by E15.5, only Postn and Col3a1 remained upregulated. Odontoblast differentiation markers (Tgfb1, Dmp1) were markedly downregulated in Wnt10a-/- molars at E14.5 and E15.5. TUNEL assay indicated reduced apoptosis in Wnt10a-/- molars at E14.5. Conclusion: Here, we discovered that molar crown dysmorphology in Wnt10a-/- mice was associated with altered gene expression patterns of Wnt modulators and ECM genes. Odontoblast differentiation markers in Wnt10a-/- molars were downregulated suggesting a delay in odontoblast differentiation. Our study elucidates the role of Wnt modulators and ECM genes in the formation of molar crowns in Wnt10a-/- mice.

137. Michael Nunneley, PhD

Uncovering the development of functional differences between intestinal smooth muscle and mesenchyme during gut development.
Mentor: **Dr. Jeffrey Farrell**
Study Section: **Developmental Biology - Organogenesis**

A fundamental question in developmental biology is how cells differentiate into specialized cell types. Our lab previously made a single cell RNA-seq atlas of zebrafish development, which led to the identification of several previously uncharacterized cell types, including multiple that surround the intestine. The goal of my project is to characterize the cells surrounding the intestine and investigate how they develop. First, we performed single-cell RNA/ATAC-seq on dissected zebrafish intestines and identified cell-type-specific enhancers. We used these to generate transgenic fluorescent reporter lines to observe the location and morphology of these undescribed cell types, including foxl1+ cells reminiscent of mammalian telocytes (an important source of signaling to the intestine), lyve1a+ cells (morphologically similar to mammalian interstitial cells that play a role regulating iSMC contractions), and podxl+ cells that may serve as the serosa layer of cells surrounding the intestine and intestinal smooth muscle. Our scRNAseq atlas also identified the first comprehensive gene expression differences between the two individual intestinal smooth muscle cell (iSMC) layers. These iSMC layers are traditionally identified by their orientation, as one layer wraps circumferentially around the gut while the other is aligned along the longitudinal axis. The differences in orientation between these layers requires external mechanical forces. We aim to test whether the transcriptional differences we identified instruct iSMC orientation, are a consequence of the mechanical forces that drive iSMC orientation, or occur independently in parallel. As a first step, we have made novel transgenic lines that mark the individual iSMC layers. I live imaged these layer specific transgenic lines along with a general smooth muscle transgenic line during the process of orientation. I found that the circular iSMC marker, kcnk18:GFP, begins to be expressed just prior to and during the orientation

of circular iSMCs. This suggests that iSMC-layer-specific transcripts are not dependent on the mechanical forces driving their orientation, and may instead be driven by the extracellular signals that specify iSMC cells. Our next steps are to use our scRNA/ATAC-seq data to infer gene regulatory networks driving the development of each of these cell types, and alter candidate signals, mechanical forces, and transcription factors to determine how each cell type differentiates.

138. Gilseung Park, PhD

Deciphering Hybrid Gene Expression States in Zebrafish Axial Mesoderm: Origins, Mechanisms and Developmental Consequences
Mentor: **Dr. Jeffrey Farrell**
Study Section: **Developmental Biology - Early Development/Embryology**

During development, cells typically activate a single cell-type-specific gene expression program. However, some cells have a "hybrid" state where they express gene expression programs of multiple cell fates. The eventual fate of these hybrid cells and the mechanisms behind their formation and possible resolution remain largely unknown. Therefore, we aim to examine the formation of hybrid cells, their developmental outcomes, and the factors that influence their behavior and fate determination during early development. To investigate these questions, we study axial mesoderm hybrid cells that co-express genes specific to late notochord specification and early prechordal plate specification (PPL). We generated notochord and PPL-specific transgenic lines to examine the formation and fate of axial mesoderm hybrid cells. The live confocal imaging and gene expression studies of the double transgenic line, which labels both late notochord and early PCP cells, will allow the tracking of hybrid cells and their non-hybrid counterparts throughout early zebrafish developmental stages and examine underlying transcriptome and chromatin accessibility changes. We find that some axial mesoderm hybrid cells differentiate into hatching gland (HG) cells, which are the final fate of PPL cells. However, these formerly hybrid HG cells demonstrated delayed expression of HG differentiation markers compared to HG cells that did not undergo a hybrid gene expression state. Since hybrid cells also showed delayed expression of the transcription factor klf17, we made klf17 mutants to test whether the delayed expression of klf17 in hybrid cells might cause the delayed expression of other HG differentiation genes. Single-cell RNA sequencing and histological studies revealed that PPL cells in klf17 mutants cannot differentiate into HG cells and have migration defects. Instead, these PPL cells showed a persistent expression of early PPL genes and late notochord genes, suggesting that they are stuck in an early PPL state and resemble axial mesoderm hybrid cells that co-express both early PPL and late notochord genes. Future work will investigate whether early notochord transcription factors, like tbxta, in PPL cells inhibit klf17 expression and potentially cause differentiation delays in hybrid axial mesoderm cells and whether restoring klf17 expression in axial mesoderm hybrid cells rescues their differentiation delay.

139. Ganesh V Shelke, PhD

Two novel ARL8 effectors, TBC1D9 and TBC1D9B, modulate exosome secretion through the RAB11A-exocyst axis
Mentor: **Dr. Juan Bonifacino**
Study Section: **Cell Biology - Intracellular Trafficking and Cell Signaling**

Removed at request of author

140. Anna Vlachos, B.S.

Identification and Interrogation of Novel Long-Range Projecting Hippocampal Somatostatin-Interneurons Across Species
Mentor: **Dr. Chris McBain**
Study Section: **Neuroscience - Cellular and Synaptic**

Inhibitory interneurons are a small, but extremely diverse cell class that critically regulate information flow and processing amongst principal, excitatory neurons of forebrain circuits. In the hippocampus, cell type diversity is imperative to understand as interneuron subsets selectively innervate postsynaptic regions and their functions are individually implicated in neural

circuit disorders including Alzheimer's Disease, epilepsy, and schizophrenia. Advances in large-scale transcriptomics allow researchers to target interneurons and uncover the roles they play in neural circuits. Importantly, evolutionary conservation of inhibitory interneuron subpopulations remains understudied, though recent research suggests that interneuron diversity is greater in higher order species. We used a novel, cell-type specific labelling AAV (Hpse cis-acting regulatory enhancer) designed to target a cortical somatostatin interneuron (SST-IN) subpopulation and injected it into the hippocampi of mice and rhesus macaques. Virally labelled cells are found in the stratum oriens layer of the hippocampus and co-stain for SST. Remarkably, these cells demonstrate unique electrophysiological properties that are novel to previously identified hippocampal SST-INs but remain consistent across species, suggesting species conservation of a discrete inhibitory interneuron subtype. In mice, we used tissue clearing to reveal virally infected SST-INs form extrahippocampal projections to the medial septum (MS). This long-range, bidirectional circuit between the hippocampus and MS is involved in several processes including learning, fear, and anxiety. Using a variant of the enhancer virus delivering channelrhodopsin, we optogenetically activated projection axons while recording post-synaptic MS cells and observed a GABAergic input, confirming a functional role in the hippocampal-septal circuit. We also identified molecular markers expressed in virally infected cells, including Cbln4, and ruled out markers expressed in known hippocampal SST-INs, further suggesting that we uncovered a novel hippocampal interneuron subpopulation. Indeed, our initial characterization of this novel subpopulation sets the foundation to probe the function of a major long-range circuit with cell-type specificity in future in-vivo experiments. Our findings provide insight to a discrete inhibitory subpopulation conserved across species with the potential to address human neural circuit disorders.

National Institute of Dental and Craniofacial Research

141. Azeez Alade, DDS, PhD

Multi-Ancestral GWAS of Malocclusion Phenotypes Identifies Multiple Risk Variants
Mentor: **Dr. Janice Lee**
Study Section: **Omics - Genomics/Transcriptomics**

Malocclusion is a common oral health problem with a strong hereditary component. Previous genetic studies were limited by small sample sizes and low genomic coverage. To address these limitations, we conducted a genome-wide association study (GWAS) using array-based genotype data in five large, multi-ancestral cohorts. The study included 7,397 participants comprising individuals from Iowa, the Northern Appalachian region, the Philippines, Puerto Rico, Colombia, and Nigeria. Class II malocclusion was defined as an Angle's Class II molar relationship with an overjet >3.5 mm, Class III malocclusion as an Angle's Class III molar relationship with a negative overjet, and Class I malocclusion (controls), as an Angle's Class I molar relationship with an overjet of 0–3.5 mm. In the vertical plane, an anterior open bite of any magnitude was classified as "open bite," while an overbite >3.5 mm was classified as "deep bite," with controls having an overbite of 0–3.5 mm. Multiplanar malocclusion included cases with both sagittal and vertical discrepancies, with controls having an Angle's Class I molar relationship, an overjet of 0–3.5 mm, and an overbite of 0–3.5 mm. GWAS was performed for each cohort, adjusting for genetic ancestry, sex, and age, followed by meta-analysis. We identified genome-wide significant ($P \leq 5E\text{-}08$) associations at 11q22.3 (rs372564249, OR = 3.12) and 3p24.3 (rs13060317, OR = 2.4) for Class II and multiplanar malocclusion, respectively. Additionally, we observed a near-significant signal at 12q15 (rs1261646, OR = 1.9, $P = 6.2 \times 10^{-8}$) for Class II malocclusion and a suggestive association at 1p21.3 (rs7541224, OR = 2.0, $P = 1.7 \times 10^{-7}$) for Class III malocclusion. The 11q22.3 and 3p24.3 loci were QTLs for the PDGFD and SGO1-AS1 genes, and transcriptomic data showed consistent expression of these genes during facial development. These findings provide valuable insights into the etiology of malocclusion and lay the groundwork for genetic risk score development.

142. Jonathan Freeman, B.S.

Fibrous Dysplasia Progression in Patients with Craniofacial Involvement of the Frontal, Zygomatic, and Sphenoid Bones

Mentor: **Dr. Alison Boyce**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

Objectives: Fibrous dysplasia (FD) is characterized by expansile fibro-osseous lesions that may occur in association with endocrinopathies as part of McCune-Albright syndrome (MAS). FD lesions in the sphenoid bone are of frequent clinical concern due to optic nerve compression. The purpose of this study was to evaluate expansion rates in craniofacial lesions associated with significant morbidity, including the frontal, zygomatic, and sphenoid bones. Methods: Patients with craniofacial FD and serial CT imaging from a natural history study at the NIH were evaluated. I performed volumetric analyses of CT scans using MIM Encore software version 7.3.3 (MIM Software Inc., Cleveland, Ohio). I performed a generalized mixed model analysis to account for intra-subject correlation, with FD lesion volume as the dependent variable. In addition to age, effects of MAS-associated endocrinopathies, sex, Skeletal Disease Burden Score (a quantitative measure of total skeletal FD involvement), and history of bisphosphonate treatment were evaluated. Results: 137 total lesions (frontal N =47, zygomatic N=44, sphenoid N=46) in 62 patients were evaluated longitudinally in serial CT scans. Frontal, zygomatic, and sphenoid lesion volume increased with age and growth hormone excess (p<0.001 & p=0.047), and expansion rates decreased over time (p<0.001). There were no associations between expansion rates and all other MAS-associated endocrinopathies, sex, Skeletal Disease Burden Score, or bisphosphonate treatment. Expansion rate from greatest to lowest was Frontal FD lesions, Sphenoid FD lesion, and finally Zygomatic FD lesions (4.01 cm3/year, 95% CI 2.9-5.12, p<0.001 vs 3.11 cm3/year, 95% CI 2.39-3.83 p<0.001 vs 1.98 cm3/year, 95% CI 1.05-2.79 p<0.001). Conclusion: Craniofacial lesion expansion rates are most rapid in younger children and decline as patients approach adulthood. FD lesions involving the frontal bone expand at a greater rate than sphenoid, zygomatic lesions, and previously reported gnathic bones. These differences in growth rates indicate that location-specific analyses are required to assess FD progression. The availability of quantitative natural history data will guide clinicians in identifying patients who are candidates for medical and surgical interventions and clinical trials for preventative therapies.

143. Robert P Ganley, PhD

The Functional Organization of Inhibitory Neurons in the Rostral Ventromedial Medulla that control pain sensitivity
Mentor: **Dr. Mark Hoon**
Study Section: **Neuroscience - Sensory**

With 20% of the population suffering from chronic pain and the fact that many painkillers are either ineffective or have adverse side effects, there is an urgent need to develop more effective pain treatments. Understanding precisely how endogenous pain control systems are organized and function may reveal new possibilities for such treatments. Descending pain control is essential for the regulation of pain sensitivity in response to external conditions, internal emotional states, and may be compromised in pain pathologies. The rostral ventromedial medulla (RVM) is an area strongly linked to descending pain control and contains descending projections that densely innervate the spinal dorsal horn, the first node in the ascending pain pathway. Although RVM neurons can both inhibit and facilitate pain, the most likely antinociceptive neurons are descending inhibitory neurons, since these can directly inhibit pain-related information from the spinal dorsal horn. However, the precise functional organization of these neurons is currently unknown. Here, we identify at least three populations of descending inhibitory neurons within the RVM. These differ in their neurotransmitter content, influence on pain behaviors, and anatomical organization. We functionally manipulated these neurons by combining an intersectional genetic strategy with chemogenetics, neuronal silencing, and CRISPR-Cas9-mediated gene deletion. This revealed that glycinergic descending neurons are required for normal control of tactile sensitivity and can reverse mechanical hypersensitivity upon activation. We used the same strategy to trace their axonal projections through the central nervous system, revealing that these descending neurons also projected into the brain. Further, we utilized the Targeted Recombination in Active Populations (TRAP) technique to demonstrate that a subset of descending neurons, located mainly in the lateral RVM, are activated by noxious stimuli and their reactivation can inhibit thermal pain. These data indicate that there is functional and anatomical heterogeneity among the descending inhibitory RVM neurons and that these play different functional roles in endogenous pain control.

144. Ioana N Ghita, DMD, PhD

M3R-DREADD (Muscarinic 3 Receptor - Designer Receptors Exclusively Activated by Designer Drugs) - a Novel Tool to Pharmacologically Address Xerostomia
Mentor: **Dr. Jay Chiorini**
Study Section: **Pharmacology and Toxicology**

Xerostomia is the perception of oral dryness that can result from salivary gland hypofunction. It is a condition affecting one out of five individuals, with approximately 40% of people over 55 years of age experiencing some kind of oral dryness symptoms. Currently, the therapeutic options for xerostomia are limited and aim to increase the salivary production using mainly muscarinic agonists pilocarpine and cevimeline with many side effects like excessive sweating, frequent urinations, airway constriction, changes in blood pressure, irregular heart rate, fluid accumulation (e.g.: pulmonary edema) etc. DREADDs are a new therapeutic tool, involving specific GPCRs mutations, which enables responses to new drugs, but not to typical agonists. The hM3Dq-DREADD, the equivalent of human M3 muscarinic receptor (M3R) is activated by DREADD activators (CNO, Compound 21 or Perlapine), but not by acetylcholine, pilocarpine or cevimeline, suggesting a new approach to avoid their side effects. In vitro experiments showed that intracellular calcium levels increased in HEK293T-cell lines transfected with hM3Dq-DREADD-AAV2, after stimulation with DREADD activators, with Perlapine inducing the highest response of 59% ± 7% compared to Ionomycin maximal response. In vivo experiments: In C57BL/6 mice, after submandibular canulation with different hM3Dq-DREADD-AAVs (AAV2, AAV5, AAV8, AAV44.9 and AAVrh10), the hM3Dq-DREADD-AAV44.9 showed significantly highest salivary response at 30 minutes after Perlapine stimulation. After canulation of parotid glands with the same hM3Dq-DREADD-AAV44.9, a similar pattern was observed. The maximal saliva production response was observed between 4-8 weeks after canulation. In AQP5-/-C57BL/6 mice after 4 weeks from hM3Dq-DREADD-AAV44.9 delivery in submandibular glands, very low or no saliva was collected followed stimulation with Pilocarpine or Perlapine, clearly indicating that as in the case of M3R, hM3Dq-DREADD requires AQP5 to induce functional response. Gene therapy with hM3Dq-DREADD-AAV44.9 delivered in submandibular glands demonstrated promising results in both AAV2-LAMP3 (Sjögren model) and radiation induced xerostomia in C57BL/6 mice at 30 minutes after Perlapine stimulation treatment. M3R-DREADD gene therapy works through the same mechanism as M3R by promoting aquaporin translocation to the apical membrane of acinar cells. This is a novel and promising treatment for xerostomia, avoiding the hardly tolerated side effects.

National Institute of Diabetes and Digestive and Kidney Diseases

145. Shahar Azar, Ph.D.

Wild-Derived Microbiota Protect Against Diet-Induced Obesity via an Immune-Mediated Effect on Brown Adipocytes
Mentor: **Dr. Barbara Rehermann**
Study Section: **Microbiota/Microbiome**

Obesity is a major risk factor for metabolic diseases and early mortality and since it already reached a pandemic scale, new approaches are needed to alleviate obesity and its associated pathologies. Obesity prevalence increased in industrialized nations in parallel to alterations of the gut microbiota. Recently, our group demonstrated in a mouse model that natural microbiota from wild mice protect against diet-induced obesity. This was shown in C57BL/6 mice that were colonized with microbiota from wild mice while maintaining the tractable genetics of C57BL/6 mice. Here, I studied the mechanisms of microbiota-induced protection against diet-induced obesity. By using single-nuclei RNA-sequencing I demonstrated a gene signature in brown adipocytes of wildling mice that was associated with increased thermogenic activity. I confirmed this functionally by measuring energy expenditure in metabolic chambers, demonstrated that wildling mice had significantly higher energy expenditure than conventional laboratory mice on the same genetic background. The increased energy expenditure and the protection from obesity lasted into old age and was independent from sex and genetics. Next, I performed co-housing experiments to examine at which age the protective phenotype can be acquired. While conventional laboratory mice acquired the protective phenotype only when cohoused with wildling mice in the first 2-weeks of life, germ-free mice did so even when cohoused with wildling mice during adult life. These results led to the hypothesis that the immune system needs to be naive when the wild-derived microbiota are first encountered. To test this hypothesis, I studied the immune landscape of brown adipose tissue by flow cytometry and found that brown adipose tissue of 12-days old wildling pups contained higher levels of

monocytes than that of lab mouse pups. Indeed, an impaired ability to recruit monocytes to adipose tissues (in CCR2-knockout wildling mice) resulted in increased body weight and fat mass along with reduced energy expenditure and no protection from diet-induced obesity. Transfer of bone marrow from wildling, but not lab mice into CCR2 KO wildling pups rescued this phenotype. Collectively, these results demonstrate that wild-derived microbiota protect against diet-induced obesity via immune-mediated induction of brown adipose tissue thermogenesis.

146. Koushik Ponnanna Cheranda Ravi, PhD

Single-cell RNA-seq analysis uncovers transcriptional signatures in pancreatic islet-like cells derived from iPSCs isogenic for the ABCC8 R1420H loss-of-function variant
Mentor: **Dr. Leslie Baier**
Study Section: **Omics - Genomics/Transcriptomics**

In a prior population-based study, we reported an R1420H substitution in SUR1 (encoded by ABCC8) causing hyperinsulinemic hypoglycemia in infancy in a homozygous carrier and doubling the risk of type 2 diabetes (T2D) in 246 heterozygous carriers. Modeling this mutation using stem-cell-derived pancreatic islets (SC-islets) showed the 1420H allele induced insulin hypersecretion in immature SC-islets (mimic fetal islets) during basal conditions, whereas mature SC-islets (mimic adult islets) with the 1420H allele have a lower insulin secretory response to glucose. The current study aims to identify transcriptomic changes in mature SC-islets responsible for the lower insulin secretory response. Single-cell sequencing was used to generate transcriptional profiles for mature SC-islets from 4 independent differentiations using iPSCs edited with CRISPR/Cas9 to create 9 isogenic cell lines that varied only by SUR1 genotype (1420RR, 1420RH, or 1420HH). Unsupervised clustering and differential expression (DE) analysis of 35,593 single cells identified GCG+ SC-alpha cells (31%), INS+ SC-beta cells (30%), TPH1+ enterochromaffin-like cells (16%), KRT19+ duct-like cells (4%), SST+ SC-delta cells (1%), DPP4+/ALDH1A1+ cells (15%), and MKI67+ proliferating cells (3%). DE analysis in SC-beta and SC-alpha cells revealed dysregulation in less than 1% of expressed genes in the 1420RH and 1420HH genotypes. Most genes exhibited consistent directional changes in the 1420RH and 1420HH genotypes, as confirmed by fold change comparisons relative to 1420RR. KEGG pathway enrichment analysis identified insulin secretion for 1420HH SC-beta and glycolysis/gluconeogenesis for 1420RH SC-beta cells. Glycolytic genes, GAPDH, GPI, TPI1, and GCK, were downregulated, while gluconeogenic genes PDK3 and G6PC2 were upregulated. These DE results were consistent between the 4 independent differentiations. The observed lower insulin secretion could be attributed to lower glycolytic flux due to downregulation of glycolytic genes or upregulation of G6PC2. Other key observations include dysregulation of ER stress response genes, immediate early response genes, and calcium signaling genes. We also observed significant upregulation of GCG expression in both 1420RH and 1420HH SC-beta cells, a hallmark of T2D and indicative of either dysfunctional or dedifferentiated beta cells. Altogether, our results provide insight into transcriptomic changes in SC-alpha and SC-beta cells due to early hyperinsulinemia.

147. Manuel Dote Montero, PhD

Metabolic Inflexibility to Macronutrient-Specific Overfeeding Challenges Predict Weight Gain and Increases Fasting Glucose in Healthy Adults
Mentor: **Dr. Jonathan Krakoff**
Study Section: **Endocrinology**

Background: Human physiology evolved under conditions of fluctuating energy and macronutrient availability, shaping the body's capacity to efficiently switch between oxidizing carbohydrates, lipids, and proteins—a process known as metabolic flexibility (MetFlex). Individuals with reduced capacity to increase lipid oxidation in response to higher fat intake (i.e., metabolically inflexible) may be more prone to weight gain, ectopic fat accumulation, and insulin resistance. Traditionally, MetFlex has been calculated as the absolute change in substrate oxidation following dietary interventions. However, this method has limitations: (i) it focuses solely on substrate oxidation without considering substrate availability, a defining feature of MetFlex; and (ii) it is heavily influenced by baseline oxidation levels, which may misclassify individuals with higher baseline lipid oxidation as metabolically inflexible when fat intake increases. To address these limitations, we investigated novel approaches for defining MetFlex and examined their associations with body composition and cardiometabolic health.

Methods: Forty-nine participants underwent 24-hour substrate oxidation assessments in a whole-room calorimeter during energy balance (EBL: 50% carbohydrate, 30% fat), fasting, and three 200% overfeeding diets—standard (STOF: 50% carbohydrate, 30% fat), high-fat (HFOF: 60% fat, 20% carbohydrate), and high-carbohydrate (HCOF: 75% carbohydrate, 5% fat). MetFlex was defined using residuals (measured – predicted) from regressions between substrate oxidation during the interventions and EBL. Body weight and fasting glucose were assessed at 12 and 24 months. Results: Metabolic inflexibility to HFOF, indicated by higher-than-predicted carbohydrate oxidation ($R^2 = 0.09$, $p = 0.03$) and lower-than-predicted lipid oxidation ($R^2 = 0.08$, $p = 0.04$), was associated with weight gain at 12 months. Metabolic inflexibility to HCOF, indicated by higher-than-predicted lipid oxidation, was linked to an increase in fasting glucose at 24 months ($R^2 = 0.13$, $p = 0.04$). These associations remained significant after adjusting for potential confounders. Conclusions: Macronutrient-specific overfeeding identified distinct MetFlex phenotypes, defined by an impaired ability to adjust lipid oxidation—increasing it during HFOF and suppressing it during HCOF. These findings underscore the critical role of lipid oxidation in regulating body weight and glucose metabolism.

148. Chi-Jui Feng, Ph.D.

Characterizing Barrier Crossing Dynamics of Protein Folding Using Single-molecule FRET in Zero-mode Waveguides
Mentor: **Dr. Hoi Sung Chung**
Study Section: **Biophysics**

Protein folding dynamics is a complex process involving conformational reorganization, competing interactions between native contacts, nonnative interactions, and desolvation. In folding dynamics, transition paths are unique single-molecule properties that provide mechanistic information of the conformational transition crossing the free energy barrier. Despite advances by theory and molecular simulations, experimental characterizations of folding transition paths are sparse due to limited time resolution to probe short-lived microsecond transition paths. Recent progress on combining nanophotonics with single-molecule Förster resonance energy transfer (smFRET) spectroscopy showed potentials of increasing the fluorescence brightness to MHz, offering opportunities to probe microsecond biomolecular kinetics. In this study, we determined folding kinetics and transition path times of two-state folding proteins using smFRET and zero-mode waveguides. Waveguides successfully reached several MHz brightness to resolve folding transition paths of a few microseconds. We surveyed several proteins with varying sizes, secondary structure archetypes, and folding rates to discuss how various physicochemical properties influence the barrier crossing dynamics. One-dimensional free energy profile model was used to obtain barrier height and diffusion coefficient from experimental kinetics and transition path times. In this model, the reaction coordinate was the fraction of native contacts, which was demonstrated to be a good coordinate in protein folding simulations. We found that the curvature modulated by the barrier height is a strong determinant of the observed transition path time. Remarkably, diffusion coefficient along the fraction of native contact coordinate is found invariant with respect to secondary structural archetypes, protein sizes, and number of native contacts. This indicates that the rate of forming native contacts indeed increases with number of native contacts and protein size, which is a manifestation of kinetic cooperativity. These experimental findings provide deeper understanding to the barrier crossing dynamics in protein folding. We expect this approach serves as an effective modality to study fast microsecond kinetics and dynamics in general protein folding and binding processes.

149. Claire Gao, PhD

Semaglutide drives weight loss via cAMP-dependent mechanisms in GLP1R-expressing hindbrain neurons
Mentor: **Dr. Michael Krashes**
Study Section: **Neuroscience - Neural Circuits**

Removed at request of author

150. Suvankar Halder, PhD

Donor-Specific Digital Twin for Living Donor Liver Transplant Recovery
Mentor: **Dr. Vipul Periwal**
Study Section: **Computational Biology/Systems Biology**

Living donor liver transplantation (LDLT) is a life-saving procedure for recipients, but its long-term success relies on the donor liver's ability to regenerate after surgery. This regeneration is a highly coordinated and complex process, essential for the complete restoration of liver mass and function. However, donor heterogeneity significantly influences recovery trajectories, making individualized monitoring crucial. With the increasing global incidence of liver diseases, ensuring safer transplant procedures and optimizing donor outcomes is more important than ever. Existing clinical markers provide limited snapshots of recovery, lacking predictive power for personalized post-surgical management. To address this gap, we developed the Personalized Progressive Mechanistic Digital Twin (PePMDT) - a deep learning-based framework that integrates mechanistic mathematical modeling with gene expression analysis to predict donor-specific recovery. We analyzed whole transcriptome RNA sequencing data from 12 healthy LDLT donors, collected over 14-time points throughout one year. Using Weighted Gene Co-expression Network Analysis, we identified liver resection-specific gene expression patterns and categorized them into distinct transcriptional clusters with unique dynamics. These gene expression patterns were then mapped to a previously developed mechanistic model of liver regeneration using deep learning techniques, enabling the development of PePMDT - a virtual representation of patient-specific liver recovery. The PePMDT successfully predicted individual recovery trajectories, demonstrating the ability to translate gene expression data into dynamic regenerative responses. This approach provides a quantitative framework for tracking and forecasting patient-specific post-surgical outcomes by linking blood-derived gene expression profiles to computational liver models. While digital twins have gained recognition in various medical applications, their role in regenerative medicine remains limited. Our study demonstrates how PePMDT functions as a disease-specific digital twin for LDLT, offering a continuous, mechanistic, and predictive approach to precision medicine. Given the complexity of donor heterogeneity, PePMDT helps clinicians anticipate variations in recovery, allowing post-surgical monitoring, ensuring safer transplants, and improving donor recovery through precise, individualized predictions of liver regeneration.

151. Dhanush H Haspula Giridhar, PhD

G12/13 signaling in AGRP neurons regulates key metabolic functions
Mentor: **Dr. Jurgen Wess**
Study Section: **Neuroscience - Molecular**

Obesity has emerged as a major threat to human health in the 21st century. Agouti-related protein (AGRP) neurons, located in the arcuate nucleus of the hypothalamus, are important regulators of feeding behavior. Under fasting conditions, AGRP neurons release AGRP and neuropeptide Y (NPY), leading to enhanced food intake and reduced energy expenditure. Besides other factors, the activity of AGRP neurons is regulated by G-protein coupled receptors (GPCRs) which are linked to four major class of heterotrimeric G proteins (Gs, Gi/o, Gq/11, and G12/13). At present, the role of GPCR-mediated activation of G12/13 signaling in AGRP neurons remains unknown. To address this question, we generated mice that selectively expressed a G12/13-coupled designer receptor (G12/13 DREADD; G12/13D) in AGRP neurons (AGRP-G12/13D mice). The G12/13D receptor can be selectively activated by deschloroclozapine (DCZ), a small synthetic drug which is otherwise pharmacologically inert. Strikingly, chronic DCZ treatment of AGRP-G12/13D mice resulted in robust weight gain, accompanied by increased adiposity and impaired insulin resistance. Additional studies showed that DCZ-treated AGRP-G12/13D mice displayed a marked increase in food intake, along with a decrease in energy expenditure. We observed similar metabolic phenotypes when we analyzed mutant mice that expressed a constitutively active version of G12 selectively in AGRP neurons. Mechanistic experiments indicated that the observed metabolic changes required G12/13-mediated activation of the ROCK-JNK signaling cascade in AGRP neurons. AGRP neurons express the ghrelin receptor, a GPCR that can couple to multiple G proteins including G12/13, at relatively highly levels. Ghrelin treatment of wild-type mice led to a robust increase in food intake, as shown in past studies. Interestingly, this response was greatly reduced in mutant mice lacking G12/13 selectively in AGRP neurons, indicating that the orexigenic activity of ghrelin is largely dependent on the activation of G12/13 signaling in AGRP neurons. Collectively, these novel findings indicate that activation of G12/13 signaling in AGRP neurons plays a central role in regulating key metabolic functions. Our data provide a rational basis for the development of novel classes of

drugs targeting G12/13-coupled receptors expressed by AGRP neurons for the treatment of obesity and related disorders in energy homeostasis.

152. Danielle S Lafferty, PhD

Amygdala projections to the pons promote motor programs of ingestion
Mentor: **Dr. Andrew Lutas**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

Overconsumption of palatable, energy-dense food is a primary driver of obesity, yet the neural circuits that allow eating past the point of satiation remain incompletely understood. Given the capacity of hypothalamic and amygdala inhibitory circuits to drive ingestive behaviors, we chose to examine projections from the central nucleus of the amygdala (CeA) to the pons region of the hindbrain. We focused on the parabrachial nucleus (PBN) due to its established role in terminating meals, and examined nearby premotor regions that control orofacial movements and for which the CeA input has not been investigated. We hypothesized that this inhibitory CeA-PBN pathway plays a critical role in overriding satiation during overconsumption. Using fiber photometry and two-photon microscopy in mice, we observed that CeA GABAergic inputs to the PBN are highly active during ingestion, with activity correlating to bout duration and persisting throughout extended feeding sessions. Notably, these inputs did not respond to food-predictive cues, suggesting real-time modulation of consummatory behaviors rather than triggering appetitive responses to external stimuli. Consistent with this hypothesis, optogenetic stimulation of CeA-PBN axons prolonged bouts of ingestion but did not induce appetitive approach behaviors when mice were physically distant from food. Importantly, photostimulation-induced overconsumption eventually ceased, indicating that mice remained sensitive to visceral satiety signals. We also observed that photostimulation caused orofacial behaviors, such as biting and licking, suggesting that orofacial motor disinhibition was likely occurring via CeA inputs to the orofacial motor control areas in the pons. To investigate this, we performed anatomical tracing using a retrograde labeling strategy, which revealed that medial and extended CeA neurons projecting to the pons collateralize widely, connecting with sensory and premotor regions throughout the pons and medulla, as well as midbrain structures like substantia nigra pars lateralis. Our findings suggest that the CeA-pons pathway promotes ingestion by permitting orofacial disinhibition while simultaneously gating visceral satiety and satiation information. This multifaceted role highlights the pathway's crucial contribution to guiding ingestive behavior, and future studies will further elucidate the full scope of this circuit’s involvement in food consumption and its implications for obesity research.

153. Zuzana Loubalova, PhD

Protecting germ cells: exploring the role of meiotic piRNAs in mammals
Mentor: **Dr. Astrid Haase**
Study Section: **Reproductive Biology**

Removed at request of author

154. Raju Mandal, PhD

Unraveling the molecular mechanisms of piggyBat-mediated DNA transposition
Mentor: **Dr. Frederick Dyda**
Study Section: **Protein Structure/Structural Biology**

DNA transposons are mobile genetic elements that can move from one location to another within the host genome. They play a significant role in genome evolution and are found in a wide range of organisms, from prokaryotes to eukaryotes. However, most DNA transposons in higher organisms, including humans are inactive. The piggyBat DNA transposon is the only known DNA transposon active in mammals, which was identified in the genome of the little brown bat (Myotis lucifugus) and is a member of the piggyBac superfamily. The piggyBat transposon system encodes a transposase that recognizes its Left End (LE) and Right End (RE) terminal inverted repeats, and catalyze the necessary DNA cleavage and joining reactions. Studies have

shown that the piggyBat transposase is active in yeast and human cells. Recently, the piggyBat transposon system has been used as a tool for genome engineering, despite the lack of mechanistic understanding of how the piggyBat transposase works. Therefore, our goal was to elucidate the molecular mechanisms of piggyBat-mediated DNA transposition using structural and cell biological experiments and to harness the knowledge obtained to optimize the transposase system for future genome engineering applications. To accomplish this, we performed DNase footprinting, transposition activity assays in HEK 293T cells and solved the structure of the fully active piggyBat strand transfer complex by single particle cryo-electron microscopy (Cryo-EM) capturing the key integration step of the transposition pathway. Our data suggest that piggyBat forms a highly asymmetric tetrameric synaptic complex that brings together the LE and RE donor DNAs, that were derived from the terminal inverted repeats. The formation of such a complex is a necessary step to transpose. Furthermore, our data indicate that the assembly we captured by cryo-EM is optimal for high transposition activity in cells. This study provides valuable insights into the mechanisms of piggyBat-mediated DNA transposition, and serves as a starting point to develop piggyBat into a clinically useful gene transfer vector system.

155. Thushani D Nilaweera, PhD

Changes in the outer membrane lipidome significantly affect the biogenesis of outer membrane proteins in vitro
Mentor: **Dr. Harris Bernstein**
Study Section: **Biochemistry - Proteins, and Lipids**

Based on a 2024 WHO report, most of the bacteria that pose a threat to humans are Gram-negative bacteria, including pathogenic strains of Escherichia coli. The presence of the unique secondary membrane, outer membrane (OM), in Gram-negative bacteria provides a barrier to many available antibiotics delivery across the OM. However, it also contains essential membrane proteins that are targets for drug discovery. Thus, the knowledge gained from OM biogenesis studies provides vital information for developing new therapeutics against these pathogens. Almost all outer membrane proteins (OMPs) have an unusual structure known as a “beta barrel” that serves as a membrane spanning domain. During the OMP biogenesis, the folding and membrane insertion of OMPs is catalyzed by a conserved heterooligomer, beta barrel assembly machinery (BAM). In vitro OMP biogenesis has been studied using unfolded OMPs and proteo-liposomes that contain purified BAM reconstituted into synthetic lipids. It is unclear, however, how resident OMPs, hierarchical OMP assemblies and native lipids (which form a unique asymmetrical lipid bilayer) affect the OMP biogenesis. To address this question, I developed a novel approach to investigate OMP biogenesis using purified native OM fractions from wild type (WT) and mutant strains of E. coli that are thought to have defects in the composition of the OM lipidome (NR698, mlaA knockout, pldA knockout and mlaA pldA double knockout strains). First, I optimized BAM activity in the native OM fractions from WT strain using a model OMP, EspP-delta-5. In the presence of the periplasmic chaperone, SurA, EspP-delta-5 is efficiently folded and inserted into the native OM fractions. As expected, EspP-delta-5 biogenesis is blocked by the BAM inhibitor, darobactin. Interestingly, compared to WT OM fractions, BAM activity is reduced in native OM fractions purified from mlaA knockout and NR698 strains and strongly impaired in native OM fractions purified from pldA knockout and mlaA pldA double knockout strains. A large number of new phospholipids (PLs) were identified in a collaborative analysis of the lipidome of each strain. By analyzing the lipidomics data, I discovered that specific PL classes (cardiolipins and lyso-PLs) show enrichment/depletion trends between WT and mutant strains. Because the mutations did not affect the OM proteome, these findings provide evidence that the OM lipidome plays a previously unidentified and surprising role in OMP biogenesis.

156. Jihoon Oh, Ph.D.

Wild-derived gut microbiota exacerbates colitis through immune modulation in a preclinical model of ulcerative colitis
Mentor: **Dr. Barbara Rehermann**
Study Section: **Clinical and Translational Research - Animal Models**

Removed at request of author

157. Suzie Stephenson-Tsoris, PhD

HDAg Co-Opts Host Polymerase-associated Factor 1 Complex and RNA Polymerase II for HDV Replication
Mentor: **Dr. T. Jake Liang**
Study Section: **Virology - General**

Hepatitis delta virus (HDV) is an unusual human pathogen of global public health concern, causing acute and chronic liver disease. This small (~1680nt) virus requires co-infection with hepatitis B virus (HBV) for productive infection. HDV-HBV co-infection is more likely to lead to liver cirrhosis, hepatocellular carcinoma (HCC) and death than HBV infection alone. Globally, there are an estimated 12 million people with anti-HDV antibodies, and about 291 million people with chronic HBV infection who are at risk of HDV superinfection. Currently, the only licensed treatment (Myrcludex B) targets HDV entry and there is no available antiviral targeting HDV replication. HDV is the only known negative-sense single-stranded RNA virus that does not code for a viral polymerase and uses host RNA polymerase(s) to replicate its RNAs that include genome, antigenome and mRNA. HDV mRNA encodes a single protein, hepatitis delta antigen (HDAg). Host RNA polymerase II (RNAPII) has been previously shown to transcribe both the HDV genome and mRNA. RNAPII is a DNA-dependent RNA polymerase and does not use single-stranded RNA as its template substrate. HDV has previously been shown to interact with RNAPII and a few transcription factors. How HDV co-opts this DNA-dependent RNA polymerase to replicate its RNA genome and whether HDV interacts with other transcription factors is not fully understood. In this study, we used the proximity biotinylation enzyme, AirID, to probe host protein interactions with HDV HDAg by proteomic mass spectroscopy. We fused AirID to the C-terminus of HDAg, allowed the fusion protein to biotinylate interacting proteins and purified the interacting proteins with streptavidin pulldown. These proteins were then identified with mass spectroscopy. Using this method, we demonstrated that HDAg interacts with multiple components of the RNAPII transcription factor complex, polymerase-associated factor 1 complex (Paf1C). We validated that knocking down components of Paf1C decreased HDV replication significantly. In addition, we performed immunofluorescence and in situ hybridization and demonstrated that Paf1C co-localized with both HDAg and HDV RNA. By interacting with the Paf1C complex, HDV co-opts host RNAPII to transcribe and replicate its genome. This interaction may provide a novel strategy to target HDV replication for antiviral development.

National Institute of Environmental Health Sciences

158. Rajesh Bhardwaj, PhD

Mitochondrial calcium channel MCU controls cellular signaling and development of psoriasis
Mentor: **Dr. Anant Parekh**
Study Section: **Cell Biology - Intracellular Trafficking and Cell Signaling**

Removed at request of author

159. Tancia Bradshaw, PhD

Endometrial Stromal PGR Signaling Affects Pregnancy and Glandular Hormone Responsiveness in the Uterus
Mentor: **Dr. Francesco DeMayo**
Study Section: **Reproductive Biology**

Progesterone receptor (Pgr) plays a major role in pregnancy by coordinating paracrine communication between the epithelium and stroma in the endometrium. Studies utilizing knockout of Pgr demonstrate that Pgr is required for embryo implantation, stromal proliferation and decidualization, and the inhibition of epithelial proliferation. Moreover, epithelial-specific deletion of Pgr phenocopies global knockout. However, investigation of genetic ablation of Pgr in the endometrial stroma have yet to be explored. Here, we conducted stroma-specific ablation of Pgr to define the role of stroma Pgr on uterine function. Ablation of stroma Pgr was accomplished by crossing Pgrf/f mice to Foxl2Cre to generate Pgrsd/sd mice. Mice were assayed for fertility, embryo implantation, and decidualization. Hormonal induction of epithelial and stromal proliferation, and the impact on the uterine transcriptome at pregnancy day (PD) 3.5 was performed. Phenotypic analysis of Pgrsd/sd mice showed female sterility

with a lack of embryo implantation and decidualization. Measurement of stromal and epithelial cell proliferation at PD 3.5 revealed that Pgrsd/sd mice had abnormal stroma proliferation. However, epithelial cell proliferation was inhibited as in the control mice. The Foxl2Cre model also ablates genes in the pituitary and ovary. Ovulation was confirmed by the presence of embryos in the uterus. However, serum progesterone levels were significantly lower but above the pregnancy threshold suggesting a potential pituitary-luteal phenotype. To determine if the impact on stroma and epithelial proliferation was not due to an ovarian defect, proliferation was measured in response to Estrogen (E2) and progesterone (P4). E2 and P4 treated Pgrsd/sd mice resulted in impaired stroma proliferation but did not impact the inhibition of epithelial proliferation. RNA sequence analysis on the PD 3.5 transcriptome demonstrated that ablation of stroma Pgr resulted in impairment of the LIF signaling required for embryo implantation. Further analysis revealed that female Pgrsd/sd mice failed artificial induction of glandular epithelial markers such as Foxa2 and Prss29, despite having normal number of uterine glands. This work highlights the importance of endometrial stroma signaling in the regulation of uterine homeostasis. Here, we show that Pgr is required in the endometrial stroma for stroma proliferation and decidualization, and for the first time, endometrial glandular hormone responsiveness.

160. Martin Estermann, PhD

Metabolic Coupling in the Fetal Testis: Glycolytic Sertoli Cells Support Oxidative Germ Cells Through Lactate Shuttling
Mentor: **Dr. Humphrey Yao**
Study Section: **Cell Biology - Metabolism and Bioenergetics**

Removed at request of author

161. Jacob Gordon, Ph.D.

Crystal structure of human SENP3-PELP1 complex visualizes a novel mechanism of SUMO protease activation
Mentor: **Dr. Robin Stanley**
Study Section: **Protein Structure/Structural Biology**

In response to different cellular cues such as stress and diseased states, cells can adapt a variety of signaling responses through SUMOylation, a transient post-translational modification of target-proteins with the Small Ubiquitin like Modifier (SUMO). Covalent SUMO attachment to a protein can alter its features leading to changes in protein-protein interaction networks and enzymatic activity. SUMO targeted proteins are in a dynamic, but controlled equilibrium of SUMOylation and deSUMOylation. Loss of control of this dynamic switching mechanism by SUMO-specific enzymes is implicated in several human diseases including cancer, neurodegeneration, and heart disease, underscoring the need to understand these enzymes. SENP3 is a SUMO protease that deSUMOylates conjugated proteins and is integrally associated with a multi-protein complex called the rixosome that functions in ribosome biogenesis and chromatin maintenance. We previously established that association of the rixosome subunit PELP1 with SENP3 enhances SUMO protease activity, leading us to hypothesize that PELP1 activates SENP3 through an allosteric mechanism. To test this hypothesis, we reconstituted the multi-subunit rixosome complex and identified an 18 amino acid SENP3 binding patch within a large intrinsically disordered region of the PELP1 subunit. We hypothesized that this PELP1 binding patch functions as a Short Linear interaction Motif (SLiM), a unique protein-protein interaction motif that force short, disordered regions of proteins into order with its binding partner. To test this, we solved a 2.93 angstrom resolution X-ray structure of the SENP3 protease domain bound to PELP1. Our structure revealed that the PELP1 SLiM peptide binds across a positively charged surface of SENP3 adjacent to its protease active site. Two small segments of the PELP1 peptide form beta-strands upon binding SENP3, supporting our SLiM hypothesis. One of these beta-strands extends an existing central beta-sheet in SENP3 that organizes key residues of the SENP3 active site. We performed differential scanning fluorimetry which showed PELP1 SLiM binding increases SENP3's thermal stability. This likely contributes to our observed increase in catalytic activity. Together, these data support PELP1 being a bona fide allosteric activator of SENP3. This discovery informed by our structure and enzyme assays opens the door for rational drug design of SENP3-specific inhibitors/regulators.

162. Ankit Gupta, PhD

Tissue-Specific RNA Decay May Hold the Key to Coronavirus Persistence in Long COVID
Mentor: **Dr. MARCOS MORGAN**
Study Section: **Virology - General**

Removed at request of author

163. Jennifer L Ish, PhD

Long-term exposure to wildfire smoke and breast cancer incidence among California women
Mentor: **Dr. Alexandra White**
Study Section: **Epidemiology/Biostatistics - General**

Background: Wildfire activity has intensified significantly over the past decade, occurring more frequently and with greater severity. As a result, long-term exposure to wildfire smoke has increased due to more intense and recurrent smoke events each year. Wildfire smoke contains carcinogenic and endocrine disrupting compounds relevant to breast cancer etiology, yet its impact on breast cancer risk remains unexplored. Therefore, our objective was to assess the relationship between long-term wildfire smoke exposure and breast cancer incidence. Methods: We included 4,412 participants from the Sister Study, a prospective cohort of women enrolled from 2003-2009, who lived in California. An ensemble machine learning approach incorporating monitoring, remote-sensing, and smoke data was used to estimate daily mean concentrations of wildfire-specific fine particulate matter (PM2.5). We linked zip code-level concentrations to participant enrollment and follow-up addresses. Breast cancer diagnoses were self-reported and verified using medical records. Using a discrete-time approach with pooled logistic regression, we evaluated the association between the odds of breast cancer and a 3-year rolling mean wildfire PM2.5 exposure; other metrics of wildfire PM2.5 characterizing peak levels and frequency of exposure were also considered. We separately evaluated associations with overall, estrogen receptor (ER)-positive and ER-negative breast cancer. Models were adjusted for calendar year and age at enrollment, race and ethnicity, income, and educational attainment. Results: During a median of 11 years of follow-up, 267 breast cancer cases, including 231 ER-positive and 32 ER-negative cases, were diagnosed. A 1-μg/m3 increase in 3-year mean wildfire PM2.5 was associated with 2.6 times increased odds of ER-negative breast cancer (95% CI: 1.1-6.6). Null associations were observed for overall (OR=0.9, 95% CI: 0.5-1.4) and ER-positive breast cancer (OR=0.8, 95% CI: 0.4-1.4). Similar patterns were observed for all outcomes across metrics of exposure frequency and peak intensity. Conclusions: In preliminary analyses of Sister Study participants in California, long-term exposure to wildfire PM2.5 was associated with increased risk of ER-negative breast cancer. Future work will extend the analysis to the entire nationwide cohort and examine potential socioeconomic disparities.

164. Jiaqi Li, Ph.D.

PHENO-Dex: Mapping Dynamic Drug Responses to Predict Tumor Heterogeneity and Resistance
Mentor: **Dr. Benedict Anchang**
Study Section: **Bioinformatics - algorithms, packages and tools**

Predicting long-term cellular responses to perturbations based on short-term exposure remains a significant challenge in oncology. Traditional models often oversimplify tumor heterogeneity by classifying drug responses as binary (responsive vs. non-responsive), overlooking the dynamic nature of cell-state transitions. To address this, we developed PHENO-Dex, an AI-driven framework that integrates time-course scRNA-seq with transcriptomics-based reference mapping. By leveraging machine learning, deep learning, and decision-tree modeling, PHENO-Dex provides a more nuanced understanding of tumor response states and their evolution over time. We applied PHENO-Dex to T47D A1-2 breast cancer (BC) cells treated with dexamethasone (Dex), performing scRNA-seq at five time points to construct a dynamic model of transcriptomic changes. PHENO-Dex identified two major response trajectories and a 72-gene panel that defines these pathways. Gene ontology and biological process analyses revealed that one trajectory exhibited dynamic hormone response, cell motility, and differentiation changes, representing an active response, while the other lacked such patterns, indicating non-responsiveness. Notably, these response states were independent of known glucocorticoid receptor (GR) signaling, uncovering an alternative regulatory

network influencing drug response. We validated PHENO-Dex by analyzing 31 BC cell lines and 59 clinical samples, revealing that cell-state heterogeneity strongly correlated with actual growth rates (r = 0.88). Principal component analysis (PCA) showed that Luminal A, Luminal B, and Her2+ BC cell lines exhibited similar Dex response patterns, while triple-negative breast cancer (TNBC) displayed distinct profiles. Notably, patient samples showed inter-patient variability but intra-patient consistency, suggesting that pre-existing non-responsive subpopulations may drive long-term resistance and relapse. In summary, PHENO-Dex integrates AI-driven modeling with single-cell analysis to predict dynamic drug responses and long-term cellular adaptations. Beyond Dex, this framework has broad applications in precision oncology, adaptive treatment strategies, and drug selection, providing deeper insights into tumor heterogeneity and resistance mechanisms.

165. Amir Lueth, PhD

Evaluating the Mediating Role of Gestational Phthalate Exposure Biomarkers in the Associations Between Race and Ethnicity and Birthweight

Mentor: **Dr. Kelly Ferguson**

Study Section: **Epidemiology/Biostatistics - Integrative Epidemiology and Health Disparities**

Background: Prenatal exposure to phthalates is higher among non-Hispanic Black (NHB) compared to non-Hispanic White (NHW) women and may contribute to the known racial/ethnic disparities in birth weight. Objective: Investigate whether prenatal exposure to phthalates mediates the association between race/ethnicity and birthweight z-scores. Methods: We analyzed data from 271 pregnancies in the Human Placenta and Phthalates Study, a prospective cohort with recruitment from 2017-2020. We measured and averaged concentrations of phthalate metabolites in urine samples from up to eight time points across pregnancy and created summary measures for metabolites of the same parent compound. Linear regression models were used to estimate the differences in phthalate exposure biomarkers by race/ethnicity, associations between race/ethnicity and birthweight z-scores, and associations between phthalate exposure biomarkers and birthweight z-scores. Mediation analysis examined whether phthalate exposure biomarkers contribute to the relationship between race/ethnicity and birthweight z-scores. Results: NHB participants exhibited higher urinary concentrations of most phthalate metabolites compared to NHW participants. For example, monoethyl-phthalate (MEP) concentrations were 250% higher (95% CI: 173, 349) in NHB compared to NHW participants. Additionally, NHB participants had 0.43 lower birthweight z-scores (95% CI: -0.67, -0.19) compared to NHW participants. Di-iso-butyl phthalate metabolites (ΣDiBP) and MEP were inversely associated with birthweight z-scores. For example, an interquartile range difference in ΣDiBP was associated with -0.16 (95% CI: 0.32, -0.002) lower birthweight z-scores. Given these associations, we performed mediation analyses for ΣDiBP, MEP and Di-n-butyl phthalate. We observed a modest and non-significant mediation of the association between race/ethnicity and birthweight z-scores by these biomarkers. Conclusions: In a diverse pregnancy cohort with well-characterized phthalate exposures, we observed differences in exposures by race/ethnicity, associations between race/ethnicity and birthweight z-scores, and associations between some exposures and birthweight z-scores. Yet, we observed only modest and non-significant mediation of the association between race/ethnicity and birthweight z-scores by phthalate exposure biomarkers.

166. Eva Marques, PhD

Heat stress exposure assessment in urban areas: a crowdsourcing approach with spatiotemporal Bayesian inference

Mentor: **Dr. Kyle Messier**

Study Section: **Cultural/Social Sciences and Public/Environmental Health**

Heat exposure constitutes an escalating concern across the United States. While urban dwellers are exposed to combined effect of global rising temperatures and urban heat islands (UHI), state-of-the-art daily gridded products fail at properly measuring temperatures in cities. To feed epidemiological studies with accurate temperature exposure data, we design a model able to provide a gridded product of hourly air temperature at 0.01 degrees × 0.01 degrees resolution which properly detects the UHI effect. A massive dataset of personal weather stations allows us to densify the observational network in populated areas. We use a spatiotemporal Bayesian Hierarchical Model designed to address the inherent uncertainty associated with the crowdsourced dataset. The model is tested on Philadelphia (PA), New York City (NY), Phoenix (AZ) and on

the Triangle area (NC), demonstrating its consistency on different climatic zones and varied urban landscapes. It is validated on several extreme events as well, including the 2023 deadly heatwave in Phoenix and a snowstorm hitting part of the United States in winter 2021. The overall Root Mean Square Error on the four urban areas is 1.06 degrees C. Thanks to its granularity, our approach accurately targets urban hotspots that were not detected with state-of-the-art gridded products. In complement to air temperature daily extrema, the hourly resolution opens the way to assess extreme exposure duration within the day. Coupled with enhanced detection of the UHI, areas exposed to long hot nights can now be targeted. The model is designed to use material available anywhere across the United States, thereby enabling nation-wide epidemiological investigations on the impact of urban heat or cold stress on human health.

167. Xiaosu Miao, PhD

Ccr4-Not-mediated mRNA deadenylation sustains the pluripotency in stem cells
Mentor: **Dr. Guang Hu**
Study Section: **Cell Biology - General**

The pluripotent state in embryonic stem cells (ESCs) is maintained by both transcriptional and post-transcriptional mechanisms. While transcriptional regulations have been extensively studied, the role of post-transcriptional processes in pluripotency maintenance remains less understood. Ccr4-Not complex is the main deadenylase in eukaryotic cells, regulating mRNA poly(A)-tail length to influence mRNA stability and/or translation. However, its role in sustaining pluripotent state in ESCs requires further investigation. To systematically explore this, we generated a series of mouse ESC lines with individual or combinatorial deletions of core subunits to assess their impact on ESCs maintenance. We found the deletions disrupting the complex or its enzymatic activity lead to ESCs differentiation, whereases deletions of non-structural or non-enzymatic subunits are dispensable. Mechanistically, loss of Ccr4-Not enzymatic subunit causes the global increases in mRNA poly(A)-tail length and stability. However, transcripts that are normally short-lived and lowly expressed are particularly susceptible to their stability changes, showing a significant elevation in their steady-state level upon Ccr4-Not complex deletion due to the increased mRNA stability. In contrast, long-lived and highly expressed transcripts, such as housekeeping or pluripotency genes, are largely controlled by transcription rather than post-transcriptional regulation. Collectively, our findings demonstrate that Ccr4-Not-mediated poly(A)-regulation selectively represses differentiation and developmental gene expression in ESCs at the post-transcriptional level, thereby stabilizing and safeguarding the pluripotent state. As Ccr4-Not complex has been implicated in many other developmental and disease processes, we propose that the same mechanism may serve as a conserved regulatory step in cell date transitions.

168. Sookjin Moon, Ph.D.

Flotillin-2 increases TCR signaling threshold to suppress sub-optimal CD8$^+$T cell activation, effector differentiation, and anti-tumor immunity in a dendritic cell-dependent manner
Mentor: **Dr. Michael Fessler**
Study Section: **Immunology - Lymphocyte Development and Activation**

Removed at request of author

169. Anastasia Robinson, Ph.D.

Heterodimerization of steroidal nuclear receptor: GR and ER
Mentor: **Dr. John Cidlowski**
Study Section: **Endocrinology**

Nuclear receptors (NRs) belong to a superfamily that act as transcription factors which regulate several processes including inflammation, reproduction and metabolism. NRs are divided into several subfamilies including group 3 which comprises of the steroid hormone receptors: glucocorticoid receptor (GR), estrogen receptor (ER), mineralocorticoid receptor (MR), androgen receptor and progesterone receptor. Classically, crosstalk affecting transcriptional regulation between these

receptors have been been postulated to be indirect through individual receptor homodimers. Recently, our group discovered that these signaling alterations are directly mediated by heterodimerization, specifically between GR and MR. Expanding on this, the GR and ER heterodimerization was examined to determine if there would be a similar outcome. GR and ER dimerization was investigated using Nanobit assay, a luciferase-based assay measuring protein-protein interaction, in HEK293 cells. The cells were transfected with ER and GR vectors, before undergoing treatment with the GR ligand Dexamethasone (Dex), the ER ligand Estradiol (E2), or both ligands. Results demonstrated that GR and ER dimerize with Dex only treatment, while both Dex and E2 displayed a potentiated effect when compared to Dex only treatment. E2 treatment shows no response indicating that no dimerization can occur with this treatment. To understand if DNA binding domain (DBD) of one or both receptors is required for heterodimerization to occur, 3 amino acids were individually mutated, 2 around the P box and one in the D-box. The P-box is a short amino acid sequence where the receptor interacts with its specific hormone response element while the D-box has classically been described as site for dimerization for nuclear receptors. GR DBD mutants with ER, all showed significantly reduced heterodimerization with C441Y demonstrating the greatest reduction. For ER DBD mutation with GR, again all showed significant inhibition of dimerization, however F208S showed a complete loss of response while the others demonstrated significantly reduced interaction between the receptors. These results demonstrate that dimerization can occur with these receptors even with single ligand activation with Dex due to ER nuclear localization and both receptors require DNA binding for dimerization. Further experiments will evaluate the impact on transcription and understanding how direct interaction mechanism functions at a DNA level.

170. Sukanya Saha, P.hD.

Bridging the cholinergic and β-amyloid hypotheses of Alzheimer's disease
Mentor: **Dr. Guohong Cui**
Study Section: **Neuroscience - Neurological and Neurodegenerative Disorders and Injury**

Removed at request of author

171. Ammad Shaukat, PhD

Proximity labeling identified novel INO80-interacting factors that are required for pluripotency maintenance
Mentor: **Dr. Guang Hu**
Study Section: **Stem Cells - General and Cancer**

Pluripotent stem cells (PSCs) can self-renew and differentiate into all cell types in the adult body. They can be cultured in different pluripotent states, corresponding to discrete stages of embryonic development. Among these, the Naïve and Primed pluripotent states are widely studied, representing epiblast cells in the pre- and post-implantation embryos, respectively. Our previous work showed that the INO80 chromatin remodeler plays an important role in PSCs. It is required for both the establishment and maintenance of the Primed state, by occupying developmental gene promoters and preventing their premature activation. Intriguingly, INO80 is dispensable in the Naïve state. Therefore, we hypothesize that additional factors may contribute to the unique roles of INO80 between the two different pluripotent states. To test this hypothesis, we endogenously tagged INO80 with the Turbo-ID biotin ligase and carried out proximity labeling followed by mass spectrometry to identify INO80-interacting factors in the Naïve vs. Primed state. This approach identified most of the known INO80 complex subunits, supporting the validity of our assay. Importantly, we identified many other chromatin factors that have not been previously studied in association with INO80, such as chromatin remodelers, transcription factors, and histone-modifying enzymes. Furthermore, we also identified several potential Naïve- and Primed-specific INO80-interacting factors. To prioritize the identified factors for further investigation, we synthesized a custom lentiviral CRISPR-KO library targeting these genes. We carried out drop-out screens in the Naïve and Primed states using this custom library to search for genes that impair PSC maintenance in either or both states. Encouragingly, we identified several novel INO80-interacting factors that are critical for maintaining the Naïve, Primed, or both pluripotent states. We hypothesize that these newly identified factors may regulate the pluripotent state via chromatin and/or transcriptional regulation of gene expression via INO80. We will test our hypothesis using various biochemical, genetic, and genomic approaches. Specifically, we plan to generate conditional knockout cell lines to further validate their roles in the Naïve and Primed states. Together, we hope this study will provide new mechanistic insights

into the function of INO80 in chromatin regulation, pluripotency maintenance, and transitions between different pluripotent states.

172. Asmita Singh, PhD

Disrupted trafficking in autophagy-deficient cells: implications for extracellular vesicle (EV) biogenesis.
Mentor: **Dr. Carlos Guardia**
Study Section: **Cell Biology - Intracellular Trafficking and Cell Signaling**

Removed at request of author

173. Zoe Wright, PhD

SARS-CoV-2 protein Nsp15 relies on spontaneous base flipping to help select its targets in viral RNA
Mentor: **Dr. Robin Stanley**
Study Section: **Molecular Biology - General**

Coronaviruses including SARS-CoV-2 hide from their host's immune system with the help of an endoribonuclease called Nsp15, which functions like a paper shredder to cut viral RNA into small, hard-to-detect pieces. Nsp15 is a promising pharmacological target, since its sequence is well conserved across coronaviruses and blocking Nsp15's activity has been shown to decrease both the severity and duration of infection in animal models. However, while Nsp15 is known to target uridine (U) in viral RNA, years of literature lack consensus on which Us Nsp15 prefers to cut and the mechanism by which Nsp15 selects its targets, with prominent investigations showing contradictory findings. To address this gap and thus clarify options for pharmacological intervention, we first used cryo-electron microscopy to determine that Nsp15 does not make sequence-specific contacts with double-stranded RNA (dsRNA) and that Nsp15's active site is only large enough to accommodate one base (its target U), which must be flipped out of the dsRNA helix in order to fit in the active site. Hypothesizing that this base flipping may be a key factor distinguishing targets from nontargets, we then designed a set of fluorinated dsRNA substrates to probe the relationship between a U's tendency to spontaneously flip (without Nsp15) and its susceptibility to cleavage by Nsp15, using parallel 1-D 19F NMR spectroscopy and nuclease assays, respectively. By screening a set of 16 oligos with Us in a variety of contexts, we determined that Nsp15 acts most efficiently on unpaired Us, particularly those that are already locked into a flipped-out conformation. We further validated these results by selecting a portion of viral RNA, called stemloop 4 (SL4), whose structure and dynamics have been described by 2-D NMR experiments, and designing SL4 oligos to characterize with our 19F NMR and assay experiments. Across all sequences tested, we found Nsp15's cleavage efficiency to be directly related to each U's tendency to spontaneously flip – with one exception. U•G wobble pairs, which flip exclusively towards the major groove of dsRNA according to prior research, are exceptionally resistant to Nsp15. This suggests that Nsp15 acts by intercepting bases that have spontaneously flipped towards the minor groove. Overall, our findings help to unify literature on Nsp15's cleavage preferences, and suggest that activity of Nsp15 during infection is partially driven by the structure of coronaviral genomic RNA itself.

174. Rebecca Yeboah, PhD

Impact of donor-specific epigenetic signatures on iPSC reprograming
Mentor: **Dr. Trevor Archer**
Study Section: **Chromosomes, Chromatin, and Nuclear Architecture**

Induced pluripotent stem cells (iPSCs) and iPSC-derived cell types have great potential for various clinical applications including regenerative medicine, disease modeling and drug discovery. The genetic and epigenetic background of donor cells are known to influence the efficiency, stability and differentiation potential of iPSCs. Such epigenetic features including histone modifications impact gene activities critical for iPSCs reprograming. While considerable research on iPSCs has been done, characterization of epigenetic signatures of donor somatic cells at the early stages of reprograming which may impact reprograming efficiency is lacking. In a recent study involving a group of 92 healthy American donors, we found reprogramming

efficiency of dermal fibroblasts to iPSCs varied considerably across different genetic populations. In this study, we sought to address the critical question of how epigenetic signatures of donor fibroblasts from different genetic backgrounds impact iPSC reprogramming. Using ChIP-seq, we profiled the extent and patterns of histone H3 lysine 27 acetylation (H3K27ac) in naïve fibroblasts and Oct4 binding in cells at day3 of reprograming in the 92 subjects. Differential peak analyses showed considerable heterogeneity in the profiles of H3K27 acetylation and Oct4 binding throughout the donor populations at several genomic loci, many of which are less studied for their roles in iPSC reprograming. We refer to these loci as differential epigenetic signature (DES) loci. Notably, some DES loci were associated with genes involved in initiation of iPSC reprograming and mesenchymal epithelial transition (MET). Interestingly, differential gene expression was consistent with the heterogeneous enrichment levels of H3K27ac and Oct4 binding at DES loci. For instance, at a DES locus that exhibit relatively low gene expression, low H3K27ac enrichment was detected. Thus, unique epigenetic signatures at DES loci impact gene expression, which may influence iPSC reprograming. To deepen our understanding of the epigenetic determinants of iPSC reprograming across various donor genetic backgrounds, we are currently examining differential DNA methylation and chromatin accessibility patterns to ascertain their roles in gene expression at DES loci. We propose that identifying and characterizing these DES loci will uncover new epigenetic and genetic factors impacting iPSC reprogramming, potentially advancing the development of personalized medicine.

National Institute of Mental Health

175. Miguel Arenivar, B.A.

Prefrontal somatostatin peptidergic transmission in regulating learning
Mentor: **Dr. Hugo Tejeda**
Study Section: **Neuroscience - General**

The prefrontal cortex (PFC) is a neuronal hub critical for decision making and goal-directed behavior. The appropriate function of PFC microcircuits relies on controlled inhibition provided by interneurons. Disruptions to inhibitory interneurons is implicated in various psychiatric disorders (post-traumatic stress syndrome, schizophrenia, and affective disorders). Somatostatin (SST)-positive interneurons, which represent a distinct subset of inhibitory cells, are identified by SST neuropeptide expression, which historically serves as solely as a cellular marker. However, SST's role as a neuropeptide neurotransmitter remains mostly unknown, due to technical challenges in monitoring and manipulating its transmission. In this study, we used a combination of genetic and viral approaches, in-vivo recordings of a novel genetically-encoded SST-receptor based sensor, and single cell imaging to determine the role that SST neuropeptide transmission plays in learning. We find that selective genetic ablation of SST in the PFC impairs cued-fear discrimination learning (CFDL). Specifically, mice with cortical SST knockout display enhanced freezing to a neutral cue. Cortical SST ablation also impacted associations between "active" and "inactive" levers in a rewarding operant task, and novel object recognition. These data suggests that ablating SST produces discrimination deficits that impair various facets of goal-directed behavior. We also utilized a novel fluorescent SST receptor-based sensor for in-vivo tracking of SST release during CFDL . SST release was noted during the acquisition phase, underscoring SST signaling may be important as animals learn outcome contingencies, but not during the expression phase. Intra-PFC SST receptor antagonism during CFDL recapitulates deficits in expression of cued fear discrimination observed in PFC SST knockout mice, consistent with the hypothesis that SST release during acquisition is critical for establishment of cued fear discrimination. Lastly, we find evidence that SST transmission is necessary for PFC circuits to generate configural representations in PFC networks associated with discrimination of neutral and salient outcomes using single cell imaging of PFC circuits. This study provides first evidence that endogenous cortical SST peptidergic transmission plays a critical role in modulating behavior, emphasizing the study of SST beyond its utility as a cellular marker.

176. Meghan E Byrne, PhD

Gamified Attention Bias Modification Training to Augment Cognitive-Behavioral Therapy for Youth Anxiety Disorders: A Double-Blind Randomized Controlled Trial

Mentor: **Dr. Daniel Pine**
Study Section: **Behavioral Science/Psychology/Psychiatry**

Exposure-based cognitive-behavioral therapy (CBT) produces meaningful reductions in anxiety symptoms among many youth. Yet, nearly 50% of patients fail to respond to treatment. Augmentation therapies could address this problem. Attention bias modification training (ABMT) was designed to engage implicit mechanistic components of threat processing that are less targeted by CBT alone. Thus, augmenting CBT with ABMT may maximize therapeutic benefit for anxiety. However, corresponding neural mechanisms are not well understood. This preregistered, double-blind, randomized controlled trial used a novel, gamified ABMT to target attentional bias towards threat, in addition to classic CBT for anxiety disorders in youth. In this study, I examined group differences between treatment arms in clinical efficacy, and associations between amygdala connectivity and treatment outcomes. Medication-free pediatric patients (N=121; 8-17 years old) with anxiety disorders were randomized to active or sham gamified ABMT alongside 12-weeks of manualized CBT. Primary outcome measures were clinician-rated Pediatric Anxiety Rating Scale (PARS) and Clinical Global Impression-Improvement (CGI-I) Scale. I also measured amygdala seed-based connectivity during a functional magnetic resonance imaging (fMRI) dot-probe attention-bias task before and after treatment in patients and across the same time interval in healthy controls. Over the course of treatment, PARS decreased significantly in both ABMT groups (F(2, 215)=82.88; p<.001, partial eta squared=0.44). Contrary to hypotheses, active ABMT did not enhance these symptom reductions (F(2, 215)=0.62; p=.54, partial eta squared <0.01). There was no difference in the proportion of CGI-I treatment responders vs. non-responders between active and sham conditions (chi-square=0.76, p=.38). Connectivity analyses revealed a positive association between pretreatment left amygdala-temporal connectivity and posttreatment PARS in the CBT+sham ABMT group (F(1, 62)=29.48; p<.001). Patterns of amygdala connectivity demonstrated poor observed test-retest reliability in healthy controls (ICC(3,1)<0.4). Hypotheses that youth receiving active gamified ABMT+CBT would show greater improvement were not supported. My work highlights the need to explore alternate attention-bias modification strategies for augmenting the clinical response to CBT for anxiety. Future research should continue to examine reliability of attention-bias tasks adapted for fMRI.

177. Franco Giarrocco, PhD

Cortico-cortical dynamics during auditory decision-making
Mentor: **Dr. Bruno Averbeck**
Study Section: **Neuroscience - Neural Circuits**

Auditory decision-making is essential for survival and effective interaction with the environment. This process relies on the brain's ability to transform acoustic stimuli into appropriate behavioral responses. Understanding how this transformation occurs in the brain is fundamental, as deficits in auditory processing have been linked to many neurological disorders. Historically, hierarchical models of brain functions assumed that decision-making mainly occurs within higher-order brain regions, particularly the prefrontal cortex (PFC). According to such models, the role of the primary auditory cortex (A1) was largely limited to early auditory processing, such as discriminating the identity and spatial location of sounds. More recent findings from human and non-human primate studies revealed that neural activity within A1 also reflects aspects of decision-making. One hypothesis is that auditory decision-making may arise from dynamic interactions between A1 and PFC, which are anatomically interconnected. Here we tested this hypothesis. We trained two male macaque monkeys in an auditory decision-making task and simultaneously recorded neuronal activity from A1 and PFC. In this task, monkeys were presented with an auditory stimulus (cue) to either their left or right side, followed by a second auditory stimulus (target) presented on the same or opposite side of the cue. To obtain a reward, monkeys were required to make a response only if the locations of the two stimuli matched. We recorded 1199 neurons over 37 sessions (monkey 1:12 sessions, monkey 2: 25 sessions). We built a computational model to predict future activity in one area based on past activity in the other, allowing us to quantify cross-area communication. We found that cue-related information initially appeared in A1 and was unidirectionally relayed to PFC (~130 ms after cue onset), supporting the role of A1 in early sensory processing. In contrast, choice signals first emerged in PFC and subsequently transferred to A1 (~340 ms after target onset). Crucially, A1 does not passively received choice-related information but actively transferred it back to PFC (~380 ms after target onset). These results provide the first evidence that A1 and PFC engage in a bidirectional exchange of information during auditory decision-making. These findings challenge classical hierarchical models, highlighting a dynamic loop between sensory and prefrontal regions during auditory decision-making.

178. Jenessa N Johnston, Ph.D.

iPSC-derived cortical organoids from treatment-resistant depression participants and healthy controls display diagnostic- and treatment-dependent differences
Mentor: **Dr. Carlos Zarate**
Study Section: **Neuroscience - Therapeutics and Translational Research**

Background: A priority in neuropsychiatric research is the development of therapeutics effective in treatment-resistant depression (TRD). Novel in vitro models such as iPSC-derived neurons may allow for improved modeling of molecular diagnostic differences and treatment-response. (2R,6R)-hydroxynorketamine (HNK) is a major ketamine metabolite under investigation as a rapid-acting antidepressant without dissociative effects. However, its exact mechanism of antidepressant action is still unclear. Methods: PBMCs from TRD participants and healthy controls (HCs) were collected for reprogramming to iPSCs. Cerebral organoids were generated following StemCell protocols and matured for 60 days. Organoids were then exposed to HNK (1um) or a vehicle control for one hour and processed after 24 hrs. Differential gene expression and gene set enrichment analyses were conducted through Dream, a modified version of limma, to account for drug-treatment effects. Ingenuity pathway analysis was also conducted on gene expression counts. Results: At baseline, TRD organoids had lower gene expression associated with neuronal CREB signaling (p=0.001; z=-0.805), glutamatergic receptor signaling (p=0.001; z=-1.483), and calcium signaling (p=0.001, z=-1.671). Stark diagnostic-dependent responses to treatment were observed. In organoids from TRD participants, HNK increased neuronal CREB signaling (p=0.001; z=3.43), TNF receptor binding (p=0.01; z=1.342), and integrin cell-surface interactions (p=0.001; z=2.53). HC organoids had increased interleukin-4 and interleukin-13 signaling (p=0.01; z=1.134) and decreased Gαq events (p=0.01; z=-0.707) after HNK treatment. Conclusions: These findings suggest that there is less baseline excitatory signaling in TRD organoids, paralleling findings in animal models. HNK partially rescues this phenotype in TRD, though an increase in inflammatory response was also found. HCs also had an upregulation of inflammation but decreased excitatory signaling. These results suggest a recapitulation of a depressive-like phenotype in in vitro cerebral organoids. The observed diagnostic disparity parallels human clinical response to ketamine, where HCs often have a depressionogenic rather than the antidepressant response observed in TRD. This study is the first to assess diagnostic disparities and treatment-response in cortical-like organoids from participants with TRD and will hopefully inform future antidepressant therapeutic targets.

179. Sharif I Kronemer, PhD

Eye metrics indicate visual neural processing and conscious perception in cerebral blindness
Mentor: **Dr. Peter Bandettini**
Study Section: **Neuroscience - Sensory**

A person with cerebral blindness experiences partial or complete loss of conscious vision following brain injury to the visual pathway (e.g., a stroke in visual cortex). A longstanding question is whether visual neural processing and visual conscious perception persists in cerebral blindness. Motivating this query are cases of cerebrally blind people who retain degraded vision or non-visual sensations and can perform visually guided tasks with and without reported conscious vision. Detecting visual conscious perception and its underlying neural activity in cerebral blindness is significant for numerous scientific and clinical applications, including guiding patient treatment and tracking recovery. However, a key challenge for achieving this goal is that subjective measures of visual conscious perception in cerebral blindness can be misleading. For example, simply asking a cortically blind person if they can “see” may overlook instances of degraded vision or non-visual sensations. Therefore, objective, physiological markers linked to conscious perception can assist in probing blind field conscious vision and non-visual sensations. Previous findings show that eye metrics are a promising marker of visual conscious perception, yet minimally studied in cerebral blindness. In this experiment, we examined visual stimulus-evoked changes in pupil size, blinking, and microsaccades in the sighted and blind field of cerebrally blind participants and healthy age-matched controls. Using standard analysis and innovative machine learning methods, our findings support that eye metrics can infer blind field visual conscious perception, even when behavioral performance on a visual perception task indicated otherwise. In a striking case from our study, a patient reported no conscious vision for images in their blind field. Yet, they showed robust blind field

image-evoked pupil, blink, and microsaccade responses. This discovery prompted subsequent testing that revealed the patient experienced degraded, blind field conscious vision for images that was linked with occipital cortical field potentials. These findings support eye metrics as a reliable marker of visual conscious perception and residual neural activity in cerebral blindness. Also, our machine learning method offers a novel approach for determining the presence of evoked eye metrics in cerebral blindness. These advances are significant because they can improve the diagnosis and treatment of cerebral blindness.

180. Samika Kumar, PhD

Slow wave-spindle coordination during sleep unwinds in depression
Mentor: **Dr. Carlos Zarate**
Study Section: **Behavioral Science/Psychology/Psychiatry**

Depression is associated with decreased sleep spindle and slow wave activity during sleep. The temporal coordination between these sleep features is thought to reflect neuroplasticity, which is also reduced in depression. Here, we hypothesized that depressive symptoms are associated with reduced slow wave-spindle coordination. We hypothesized that this association was strongest when slow wave activity is at its peak – close to sleep onset. 204 adults (41 psychiatrically healthy volunteers, 121 major depressive disorder, 42 bipolar disorder) were recruited at the NIH Clinical Center (Bethesda, MD, USA). They completed a Montgomery–Asberg Depression Rating Scale (MADRS) and sleep polysomnography. Electroencephalography (EEG) data were entered into a slow wave detection algorithm (0.4-2.5 Hz). Analysis focused on the first four hours of the sleep recording, which had fewer awakenings and was predicted to have the highest density of slow wave sleep. Phase-amplitude coupling between slow and fast EEG frequencies was calculated. We regressed MADRS score on phase-amplitude coupling measure, controlling for age and sex. The number of detected slow waves decreased with age (t=-5.8, p<0.001) but was not significantly associated with MADRS score (t=0.4, p>0.6). In hours 2-3 of the recording, higher MADRS scores predicted higher frequency (t>=2.4, p<=0.02), but not amplitude (t<=0.3, p>=0.2), of EEG slow waves. In hour 3 of the recording, higher MADRS scores predicted reduced phase-amplitude coupling between slow waves and fast spindles (13-16 Hz) (p<=0.01). In hours 2-4, higher MADRS scores predicted reduced phase-amplitude coupling between slow waves and slow spindles (8-10 Hz) (p<=0.01). We expand on the role of EEG slow waves and spindles in depression and highlight a potential mechanism linking sleep, mood disruption, and neuroplasticity. Slow waves' frequency and cross-frequency relationships may reflect sleep disturbances in depression. Future work should confirm whether the restoration of slow wave-spindle coordination during sleep reduces depressive symptoms. This causality could help identify directions for future treatments and risk stratification.

181. Haleigh Mulholland, PhD

Acetylcholine gates neuronal plasticity via targeted optogenetic stimulation in the visual cortex
Mentor: **Dr. Mark Histed**
Study Section: **Neuroscience - Sensory**

As animals and humans navigate through the world they must readily adapt to new sensory information. Sensory information in the cortex is represented as dynamic patterns of activity across networks of neurons, and these patterns can be altered and refined with learning. The classic, Hebbian theory of learning posits that coactivated neurons become functionally coupled, leading to amplified responses within the network and facilitating pattern completion. However, recent work shows that neuronal stimulation alone without behavioral engagement is not sufficient to induce plasticity, suggesting that plasticity might require both neuronal coactivation and neuromodulatory signals. Acetylcholine (ACh), involved in arousal and attention and broadly released throughout the cortex, is known to play an important role in learning and may gate plasticity in neural circuits. To test this we aimed to induce plasticity using cellular resolution holographic optogenetic photostimulation to drive activity in select patterns of randomly chosen neurons (N=30 neurons) in mouse visual cortex while evoking ACh release across the cortex via electrical stimulation of the basal forebrain. During induction, we repeatedly stimulated the full pattern of cells while simultaneously electrically stimulating the basal forebrain for approximately an hour (N=250 trials). Using in vivo two-photon imaging to measure neural activity before and after induction, we found that optogenetic stimulation of sub-

patterns (N=10 neurons) derived from the full pattern evoked significantly increased responses in the other non-targeted sub-patterns as compared to before induction. This form of pattern completion suggests a successful strengthening of neuronal coupling following repeated coactivation paired with ACh activity. Importantly, the response of neurons in the non-targeted sub-patterns was significantly greater than control cells that were never targeted for optogenetic stimulation, indicating effects of plasticity are specific to the trained neurons only. Sham experiments in which animals received optogenetic but not basal forebrain stimulation failed to produce pattern completion, demonstrating the causal role ACh plays in gating plasticity. Our results reveal the potential mechanism by which behavioral context, via ACh release, could facilitate learning in the sensory cortex, and provides a novel platform to study brain function via selectively strengthening connections between neurons.

182. Jack T Scott, PhD

The medial pulvinar supports the functional development of thalamocortical circuits subserving primate cognition
Mentor: **Dr. James Bourne**
Study Section: **Neuroscience - Developmental**

The interplay of the thalamus and prefrontal cortex (PFC) in early life is crucial for cultivating adult cognitive function in adulthood. Primates, including humans, possess a medial pulvinar (PM), a thalamic nucleus that modulates PFC activity via fast-spiking cortical interneurons expressing parvalbumin (PV). Yet, it is unknown whether this adaptation shapes cognitive development in primates. Here, we explored this by permanently inactivating PM during early or late postnatal life in the marmoset monkey (Callithrix jacchus), and examining the consequences on the adult PFC. We employed MRI-guided stereotaxic surgery to lesion the bilateral PM using excitotoxic NMDA at either infancy (postnatal day 14; lesion: n=7) or adulthood (>18 mo.; n=2), and compared longitudinal outcomes to healthy controls (n=7). First, we employed diffusion MRI across adolescence (6–18 mo.) to reveal diverging trajectories in long-range thalamocortical circuits and local PFC anatomy after early-life lesions. Next, using a custom in-cage touchscreen interface, we conducted behavioral testing of adult animals to investigate cognitive operations that implicate PFC. Compared to controls, animals with early-life lesions exhibited impaired flexible learning in a reversal learning task (p<0.01). Similarly, in a delayed response task, early-life lesioned animals exhibited worse working memory recall for stimulus features (p<0.05). Behavioral deficits were not observed after adult lesions (p>0.05), suggesting that they were developmentally mediated. To study the circuit basis of this disruption, analysis was then performed on the post-mortem PFC. Immunohistochemistry revealed a severe reduction in neurons expressing PV (NeuN+/PV+) in the thalamorecipient layer III (p<0.0001), along with reduced thalamic input to these cells (VGLUT2+/PV+; p<0.001). Reasoning that early PM input may be driving PV expression in maturing interneurons, we then targeted these cells via surgical delivery of AAV-PHP.eB-S5E2-dTomato to the PFC of adult animals with early-life lesions. Slice electrophysiology of these cells revealed reduced spiking frequency due to a deficient protracted period of repolarization (p<0.05). Collectively, these findings portray a mechanism for the thalamic regulation of cortical function during postnatal development. This primate-specific circuit presents an intriguing focus for chronic neurodevelopmental disorders that affect healthy cognition, such as schizophrenia.

183. Rachel Smith, BS

A neuro-immune axis of transcriptomic dysregulation within the subgenual anterior cingulate cortex in schizophrenia
Mentor: **Dr. Francis McMahon**
Study Section: **Omics - Genomics/Transcriptomics**

Psychiatric disorders are highly heritable, but bridging the gap between genetic variation and downstream transcriptomic dysregulation remains a challenge. Genomic complexity, environmental confounds, and alternative splicing contribute to discrepancies between genes implicated by genome-wide association studies (GWAS) and differential gene expression (DGE) analyses in post-mortem brain tissue. This disconnect impedes therapeutic development, which relies on integrating genetic and genomic insights. To address this issue, we applied a novel multivariate technique that reduces DGE bias by leveraging gene co-expression networks and controlling for confounds such as drug exposure. Deep bulk RNA sequencing was performed

in post-mortem sgACC tissue donated by individuals with bipolar disorder (BD; N=35), major depression (MDD; N=51), schizophrenia (SCZ; N=44), and non-psychiatric controls (N=55). We analyzed case-control gene expression profiles using group regularized canonical correlation analysis (GRCCA) – a multivariate regression method that integrates gene co-expression modules to account for feature interdependence – while incorporating toxicology data from 17 known compounds. We then benchmarked GRCCA results against traditional DGE using gene set enrichment analyses of neuropsychiatric risk genes, gene ontology pathways, and cell-type markers. GRCCA revealed a robust SCZ-specific transcriptional signature (Pperm=0.001; no significant BD or MDD association) enriched for SCZ risk genes (FDR<0.05), whereas traditional DGE did not (FDR>0.05). Although GRCCA gene weights correlated with DGE SCZ-control t-statistics (R=0.53; P<0.05), GRCCA exhibited stronger biological enrichment, with downregulation of immune and microglial genes and upregulation of excitatory neuronal genes (FDR<0.05). Developmental trajectory analyses revealed that genes driving this SCZ signature display dynamic expression changes, particularly in prenatal and early postnatal life. At the transcript level, GRCCA identified isoform-specific expression patterns across SCZ risk genes, emphasizing the importance of alternative splicing in neuropsychiatric transcriptomics. These findings identify a SCZ-specific sgACC gene expression pattern that uniquely highlights SCZ risk genes and implicates neuro-immune pathways, thus demonstrating the utility of multivariate approaches to integrate genetic and genomic signals.

184. Will Snyder, B.S.

Rare and common genetic variation impacts on the developmentally rooted patterning of human cortical folding
Mentor: **Dr. Armin Raznahan**
Study Section: **Neuroscience - Developmental**

Removed at request of author

185. Samantha R White, PhD

Dopaminergic signaling in the striatum differs over the lifespan of mice and macaques
Mentor: **Dr. Veronica Alvarez**
Study Section: **Neuroscience - Developmental**

Decades of research have implicated dysregulation of the dopaminergic signaling system in psychiatric disorders. The window between puberty to late adolescence is a critical peak for the onset of these disorders including depressive, obsessive-compulsive, and substance abuse disorders. Given the adolescent onset peak, studies aiming to characterize the maturation of the dopaminergic system through development emphasize peri-puberty. However, epidemiological studies have established that compulsive and substance abuse disorders are elevated through adulthood and show a second onset peak later in adulthood. In this study, we aimed to characterize the dopamine signaling system across the lifespan in two common research models: the mouse and the macaque. We examined the dorsal striatum because it is a central hub for cortical excitatory and midbrain dopaminergic inputs, and its established role in psychiatric symptomatology. Here we used fast-scan cyclic voltammetry, a well-established electrochemical technique, to measure dopamine availability under identical conditions to directly compare mouse and macaque. We also investigated the modulation of dopamine release by cholinergic interneurons and GABA receptors. We found that over the lifespan, mouse striatal dopamine levels are generally lower in puberty and old age and peak during adulthood. By contrast, macaque striatal dopamine levels peak during puberty and adolescence and decline over adulthood. In the adult mouse, we found lower inhibitory regulation of dopamine by GABA and more potentiation by cholinergic interneurons via nicotinic receptors compared to puberty or old age – this may explain the peaks in dopamine levels observed in adult mice. Further work will examine this regulation in macaques. Overall, this study contributes to our understanding of vulnerable windows across the lifespan relevant to the onset of psychiatric disorders. It also further characterizes the striatal dopamine system across species and highlights potential cross-species differences in the release and regulation of this relevant neuromodulator.

186. Huanwen Chen, MD

Endovascular versus medical management of basilar artery occlusion stroke with low NIH stroke scale: a nationwide study in the United States
Mentor: **Dr. Christopher Grunseich**
Study Section: **Clinical and Translational Research - General**

Background and objective: The safety and efficacy of endovascular treatment (EVT) for basilar artery occlusion (BAO) stroke patients with low NIH stroke scale (NIHSS<10) is unclear, and there is limited guidance on treatment decision-making. Nevertheless, studies showed that a majority of physicians may favor EVT treatment in this population. The objective of this study is to determine whether EVT is beneficial for select BAO stroke patients with low NIHSS. Methods: This was a retrospective cohort study of the 2016-22 Nationwide Readmissions Database (NRD). Adult patients with acute BAO stroke and NIHSS 1-9 were identified, and patients were grouped into EVT (thrombectomy, angioplasty, and/or stenting) and medical management (MM) arms. The primary outcomes of interest were routine discharge to home with self-care (a surrogate marker for excellent outcomes) and in-hospital mortality. Propensity score matching (PSM) for demographics, comorbidities, presenting symptoms, stroke etiology, and other covariates was performed to adjust for confounders. Subgroup analyses were performed for stroke etiology (embolic vs. non-embolic). Results: Of 9,461 included patients, 1,807 (19.1%) underwent EVT. After PSM, 2,888 MM and 1,632 EVT patients remained. EVT patients had similar rates of routine discharge (33.8% vs. 34.0%, p=0.24) and higher rates of death (16.0% vs. 7.1%, p<0.001) compared to MM patients. In subgroup analyses, NIHSS and stroke etiology significantly modulated EVT's associations with routine discharge and in-hospital death, respectively (p-interaction <0.001 and 0.013, respectively). For patients with embolic etiology, EVT was associated with higher odds of routine discharge when NIHSS>5 (OR 1.88 [95%CI 1.10-3.21], p=0.021), and it was not associated with increased mortality (OR 1.42 [95%CI 0.71-2.86], p=0.32). In contrast, for patients with non-embolic etiology, EVT was not associated with higher odds of routine discharge (OR 0.85 [95%CI 0.70-1.03], p=0.17), and it was associated with higher odds of death (OR 3.84 [95%CI 2.49-5.92], p<0.001). Conclusions: For BAO stroke patients with NIHSS<10, EVT was not associated with higher odds of excellent short-term outcomes, and it was associated with higher odds of mortality compared to MM. Subgroup analyses suggested that while BAO-EVT may be safe and effective for select low-NIHSS patients with embolic stroke etiology, it may be harmful for patients with non-embolic etiology.

187. Catherine Demarino, PhD

HIV-1 Reservoir Transcription Contributes to CNS Complications
Mentor: **Dr. Avi Nath**
Study Section: **Neuroscience - Therapeutics and Translational Research**

Human immunodeficiency virus type-1 (HIV-1) has been responsible for significant mortality and morbidity worldwide since its discovery in 1981. Despite decades of research and development of this complex drug regimen, which is highly effective in preventing new infection, cells with integrated HIV-1 provirus are capable of producing viral RNAs. I have found HIV-1 RNAs are present in extracellular vesicles (EVs) derived from cerebrospinal fluid (CSF) and plasma. EVs are small membrane bound vesicles that are released from every cell type, they are heterogeneous in size and cargo and are generally reflective of their cell of origin. During infection, EVs can be utilized to enhance the resolution of pathogen detection as they contain viral RNAs and proteins. In my recently published landmark study, I utilized seven ddPCR assays to evaluate the transcriptional status of the latent viral reservoir. My results show EV-associated viral RNA is cross-sectionally and longitudinally correlated with neurocognitive assessments including global deficient score and cognitive domain scores in 85 HIV+ individuals with neurological impairment. In the current study, I used my high-resolution viral detection assay to assess the relationship between transcription from latent viral reservoirs and brain MRI volumetrics. My analysis shows the HIV-1 viral protein, Nef, is correlated with overall brain atrophy (increased CSF volume), neuronal damage (decreased grey matter volume), and small vessel disease (increased white matter hyperintensity volume). I confirmed these results using immunohistochemistry which shows perivascular staining of Nef in postmortem HIV brain tissue. In vitro experiments suggest Nef protein can damage brain

endothelial cell tight junctions using a cell impedance assay, potentially through changes in the cytoskeleton. Additional confocal microscopy experiments confirm that Nef protein is neurotoxic, in line with our MRI findings. In this translational work, I have leveraged my findings from a large-scale observational cohort to inform in vitro work to shed light on viral mechanisms which can be exploited for new treatment strategies. My findings suggest a relationship between latent viral transcription and CNS complications of long-term disease and point to a novel mechanism that contributes to dysfunction in infected individuals that can be targeted to achieve a functional cure.

188. Fnu Eedara Prabhakararao, PhD

Altered Excitation/Inhibition Balance in Drug-Resistant Focal Epilepsy Revealed by Interictal Magnetoencephalography
Mentor: **Dr. Sara Inati**
Study Section: **Clinical and Translational Research - Diagnostics/Biomarkers**

Background: The healthy brain maintains a delicate balance between excitation (E) and inhibition (I); epilepsy is characterized by disruptions in this E/I balance. While seizures are associated with excessive excitation, E/I alterations between seizures (interictal) remain unclear. Since patients spend most of their time in the interictal state, understanding its dynamics is crucial for targeted therapies. Using 275-channel magnetoencephalography (MEG) recordings in focal epilepsy patients, we are developing biomarkers for non-invasive assessment of E/I balance, which may be present even in the absence of overt epileptiform activity (EA). We hypothesize that inhibitory processes dominate interictally, preventing excessive excitation and thereby seizures, reflected in larger aperiodic components and increased prevalence of paroxysmal slow-wave events (PSWEs) over controls. Methods: We acquired interictal MEG recordings from 55 patients with drug-resistant focal epilepsy and 55 healthy controls (N=110). We developed two potential biomarkers using MEG power spectra: 1) aperiodic 1/f components (exponent and offset), and 2) PSWEs, or epochs with a median power frequency <4 Hz for at least 5 sec. We quantified channel-level PSWEs by occurrence rate, total duration, and the number of involved channels per individual. For multi-channel PSWEs (synchronous events across channels), we computed their total duration as an indicator of event prevalence. Results: Epilepsy patients exhibited a significantly larger aperiodic exponent (p=1.9E-14, area under the curve (AUC)=0.92) and offset (p=7.1E-12, AUC=0.88) interictally compared to healthy controls. Patients also had significantly higher channel-level PSWE occurrence rate (p=1.6E-15, AUC=0.94), longer total PSWE duration (p=5.4E-16, AUC=0.95), more PSWE-involved channels (p=2.1E-13, AUC=0.91), and prolonged multi-channel PSWEs (p=2.4E-12, AUC=0.89). Conclusion: Our findings suggest a shift in interictal E/I balance toward inhibition in epilepsy patients compared to controls, with increased PSWEs reflecting intermittently elevated inhibitory tone, potentially indicating surround inhibition suppressing activity in the seizure focus. Our findings are the first to reflect these changes in MEG recordings in the absence of overt EA. Ongoing research aims to localize these findings, ideally aiding with identification of epileptogenic zones to guide surgical planning and predict surgical outcomes in drug-resistant epilepsy.

189. Elif Everest, PhD

Rare variant and pathway analyses in monozygotic twins discordant for multiple sclerosis
Mentor: **Dr. Daniel Reich**
Study Section: **Genetics - Diseases**

Multiple sclerosis (MS) is an autoimmune and neurodegenerative disease of the central nervous system and the most common nontraumatic brain disease among young adults in the U.S. Although many major histocompatibility complex (MHC) and non-MHC common and rare variants associated with MS risk have been identified, approximately half of MS heritability remains unexplained. Prioritizing variants to uncover missing heritability is one of the most challenging aspects of understanding MS genetics, as with other complex disorders. To address these issues, we examined rare variants and cellular pathways associated with MS using exome sequencing data from 104 individuals in 52 monozygotic twin pairs discordant for MS. Following genotype, sample, and variant quality control, we identified de novo variants exclusive to affected twins. Pathway analyses were conducted using Metascape v3.5 on multiple variant subsets, such as those defined by different minor allele frequency thresholds. The most significantly and consistently overrepresented cellular pathways in affected twins included

graft-versus-host disease (hsa05332), allograft rejection (hsa05330), MHC protein complex assembly (GO:0002396), and defective GALNT3 causing hyperphosphatemic tumoral calcinosis (R-HSA-5083625). We identified 16 rare, heterozygous, coding variants present in the affected twins and absent in their unaffected twin counterparts. Among these variants, some were identified as stronger candidates due to their high pathogenicity risk scores, such as 1-95072830-C-T in ALG14 (stop-gain, CADD: 36) and 18-36805406-G-A in TPGS2 (AlphaMissense: 0.87, CADD: 26). Additionally, we detected other variants in genes with promising functional implications, including EOLA1, TMEM131, and ZNF521. Notably, TMEM131 has been implicated in both the immune and nervous systems, and rare variants in this gene are associated with MS in Genebass ($p = 0.016$). Importantly, the TMEM131 protein region bearing the corresponding amino acid change (p.I1505V) is a known epitope for an MHC ligand. Analyzing discordant monozygotic twin pairs in complex diseases helps minimize the confounding effects of genetic heterogeneity. This study represents the largest monozygotic twin cohort discordant for MS, offering a substantial advantage in gene prioritization and enabling us to identify novel and compelling candidate rare risk variants and MS-relevant pathways for translational research.

190. Martha G Garcia Garcia, PhD

Neural representations of learning and switching between multiple tasks in the premotor cortex and the cerebellum
Mentor: **Dr. Mark Wagner**
Study Section: **Neuroscience - Neural Circuits**

Learning and switching between multiple tasks is fundamental to adapting in changing environments. Unlike the brain, artificial neural networks are notoriously susceptible to catastrophic forgetting: learning new tasks degrades performance on previously learned tasks. In the brain, the neocortex and the cerebellum are thought to implement computations that underlie higher learning. These two areas specialize in distinct roles, but also synergize through dense interconnections called cortico-cerebellar loops. Recent computational studies posit that cortico-cerebellar loops could help learn to acquire and switch between several tasks. Yet to date, experimental evidence is lacking, due to the technical difficulty of repeatedly recording from fixed sets of cortical and cerebellar neurons simultaneously, across the weeks required to learn several new skills. To clear this hurdle, we developed a two-photon microscopy strategy to simultaneously image activity in fixed populations of individual premotor cortex layer 5 neurons (L5N) and cerebellar granule cells (GrC), while mice learn two different behavioral tasks in parallel: (1) push a manipulandum, and (2) run in a virtual reality environment, both for water reward. While each task requires distinct actions, both share a 1 s delay period between action completion and reward, in which mice learn anticipatory licking. In a previous study, we showed that GrCs develop activity that ramps at different rates during this delay, which was essential for learning the timing of reward. Here, we find that optogenetic inhibition of GrCs in expert mice during the delay disrupted anticipatory licking in both tasks (3 mice). Imaging data in 3 expert mice revealed similar patterns of activity in L5Ns and GrCs at key points of both tasks. We hypothesize that in early learning, the neural representations of stimuli and behavioral information will overlap between tasks. As learning progresses, these neural representations will separate, allowing for task-specific behavioral responses. However, task separation mechanisms might differ between the cortex and the cerebellum, reflecting their distinct neuronal architectures and plasticity and learning rules. Additional theory and modelling will inform on how these structures jointly achieve multi-task learning. Simultaneous, dual-site neuronal recordings during multiple tasks could uncover computations supporting complex behavior and inspire improved artificial neural networks.

191. Andrii Kopach, Ph.D.

A ribosome-mTORC1 assembly associated with lysosomes regulates local translation in neurons
Mentor: **Dr. Michael Ward**
Study Section: **Cell Biology - Organelles and Membranes**

Neurons rapidly react to changes in environment by supplying proteins to distal parts of axons. Given that axons in humans can be up to around 1 meter long and molecular motors can move as fast as 1 um/s, how to facilitate timely translation at synapse? While it is known that mRNA is transported on lysosomal surface to distal parts of axons, it is unclear why lysosomes reside between RNA granules and molecular motors. We found using various techniques that ribosomes co-traffic with

lysosomes in axons of live neurons and therefore hypothesized that lysosomes might be hubs providing amino acids derived from protein degradation for synthesis of new proteins. To test this, I applied fluorescent non-canonical amino acid tagging coupled with proximity ligation assay (FUNCAT-PLA) for measuring translation on lysosomes in iPSC-derived neurons (i3Neurons). By feeding neurons with peptide containing methionine analog HPG followed by proximity ligation assay between HPG and lysosomal marker LAMP1, I showed that cells use amino acids derived from lysosomal catabolism for protein synthesis in axons and dendrites. To determine a pathway regulating translation, I used genetic perturbations and pharmacological treatments commonly used in mTOR field for activating and inhibiting regulators of mTORC1 complex and provided evidence that mTORC1 signaling regulates protein synthesis on dendritic and axonal lysosomes. We showed using SunTag system that mTORC1 component Raptor is recruited to translating lysosomes. Does the recruitment of mTORC1 activate translation? To test this, I aimed to recruit mTORC1 components to individual lysosomes using stimulation with light and subsequent measurement of translation in live neurons using fluorescence recovery after photobleaching (FRAP) of endogenously labeled proteins having a fluorescent reporter. To do this, I performed endogenous labeling of 14 mTORC1 genes using homology-directed repair (HDR) CRISPR-Cas9 with a small epitope ALFA-tag. This provides access to optogenetic tools for lysosomal recruitment and depletion using ALFA intrabodies fused to components of an opto-dimerization system allowing to perform ongoing work on uncovering novel mechanistic details of mTORC1 signaling on individual lysosomes in live neurons. Our study contributes to advancement of basic knowledge about local translation in neurons and highlights new targets for potential therapeutic strategies directed to treating lysosome-related brain disorders.

192. Yingtao Liu, Ph.D.

All-optical investigation of corticopontine adaptation for cerebellar learning
Mentor: **Dr. Mark Wagner**
Study Section: **Biochemistry - General**

Many higher cognitive functions, such as learning a new skill, require the involvement of both the neocortex and cerebellum. In all mammals, cortical information is transmitted to the cerebellum via the neocortex Layer 5 (L5) - Pontine Nuclei (PN) - cerebellar Granule Cells (GrCs) circuit. This pathway forms a bottleneck: L5 neurons substantially outnumber PN neurons, which are again drastically outnumbered by downstream GrCs. Thus, not all cortical information can transit through the PN. This raises a fundamental question about this conserved pathway: how do the PN select which cortical information to transmit to the cerebellum, and which to discard or attenuate? To date, it has not been possible to address this question due to the difficulty of causally probing neural information transmission between distant brain regions in behaving animals. Thus, we have developed an all-optical dual-site two-photon microscopy approach. With this technique, we can simultaneously and longitudinally record activity in the L5-PN-GrC pathway, as well as stimulate specific sets of individual L5 neurons, repeatably across learning. To validate this methodology, we first demonstrated simultaneous imaging of dense populations of GrCs and PN axon terminals using two-color imaging. In animals performing an operant behavior, we found ramping activity of GrCs and PN axons during motor planning (1270 GrCs, 371 PN axons, 2 mice). Next, we evaluated the effects of L5 stimulation on GrCs. We activated L5 cells via bulk optogenetic stimulation while imaging GrCs. We found a significant increase in the average calcium activity in GrCs, with some regions repeatedly showing response following each stimulation (3 mice). To more precisely assess cortico-cerebellar transmission, we implemented two-photon L5 optogenetics by incorporating a spatial light modulator stimulation laser path into our dual-arm microscope. We developed computational alignment methods to repeatably stimulate the same sets of L5 cells across days of learning. This system now allows us to stimulate many distinct sets of L5 cells (~10 at a time) with single-cell resolution, while imaging the evoked downstream activity of dense populations of GrCs and PN axons. As we scale up this technique, we will be well-positioned to test our central hypothesis: whether the cortico-PN circuit selectively transmits the most task-relevant information for downstream cerebellar learning.

193. Jia Yuan Liu, PhD

Crystal Structure of RPGR-TTLL5 targeting domains reveals molecular mechanism of disease
Mentor: **Dr. Antonina Roll-Mecak**
Study Section: **Protein Structure/Structural Biology**

Mutations in retinitis pigmentosa GTPase regulator (RPGR) cause photoreceptor degeneration, vision loss and eventually blindness. RPGR function is strictly dependent on its glutamylation by Tubulin Tyrosine Ligase-Like 5 (TTLL5) whose loss phenocopies RPGR loss in mice. Glutamylation has been intimately linked to tubulin for many decades. However, recent work points towards its use more broadly to regulate many non-tubulin substrates. TTLL5 posttranslationally adds multiple glutamates to glutamate-rich repeats in the ORF15 region of RPGR that is unique to photoreceptors. How TTLL5 is targeted to RPGR is not known, nor how mutations in either of these proteins lead to dysfunction. In general, how TTLLs recognize non-tubulin substrates is not known. My work reports the 2.3 Å X-ray crystal structure of the human TTLL5 coactivator interacting domain (CID) in complex with the RPGR basic domain (BD), showing that the C-terminal region of the RPGR BD is sufficient for TTLL5 recognition. I have identified a single, conserved helix at the C-terminus of RPGR BD sufficient for strong TTLL5 binding with sub-micromolar affinity. This helix intercalates through intimate aromatic interactions into the helical structure of the TTLL5 CID. Mutations of aromatic residues at this interface, including that of a key invariant tryptophan residue that is mutated in patients with macular degeneration abolish interaction and RPGR glutamylation by TTLL5 both in vitro as well as in mouse photoreceptors. Through structure-based analysis we were able to segregate TTLL5 mutations into tubulin versus non-tubulin pathways, and correlate these distinct phenotypes in patients cohorts. Our work illuminates mechanism of RPGR and TTLL5 function in health and disease, and also provides insights into TTLL strategies for non-tubulin substrate recognition.

194. Carolyn A Lomahan, B.S.

Hyperemia identified on MRI is associated with the final infarct in stroke post-endovascular therapy
Mentor: **Dr. Lawrence Latour**
Study Section: **Clinical and Translational Research - General**

Background Stroke is a leading cause of death and disability worldwide. This event occurs when a clot blocks blood flow to the brain. Treatment involves endovascular therapy (EVT), a procedure to remove the clot. Despite treatment, a large proportion of patients still experience poor clinical outcome due to secondary injury. MRI can elucidate injury markers that may lead to stroke infarct worsening over time. Hyperemia is an abnormal increase in cerebral blood flow after stroke. This MRI marker can be identified on two types of imaging reflecting cerebral blood flow: dynamic susceptibility contrast (DSC) and arterial spin labeling (ASL). DSC requires intravenous administration of a contrast agent; alternatively, ASL is noninvasive. Cerebral blood flow after stroke is heterogeneous and complex, and hyperemia remains underexplored in terms of whether hyperemic tissue will recover or progress to infarction. Previous work in our lab demonstrated hyperemia on DSC is associated with infarct growth. This study aims to assess hyperemia on DSC and ASL and explore the association between hyperemia at 24 hours and the final stroke infarct at 5 days after EVT. Methods This retrospective analysis included stroke patients treated with EVT. The MRI protocol included DSC and ASL at 24 hours and fluid attenuated inversion recovery (FLAIR) at 5 days post-EVT to evaluate the final infarct. Qualitative DSC and ASL assessment involved visual hyperemia identification. Quantitative assessment included hyperemia signal intensity ratios (SIR) to quantify cerebral blood flow and Dice coefficients between hyperemia and final infarct regions to determine similarity. Results Forty-one patients were included. Hyperemia was identified in 39% and 29% of patients on ASL and DSC respectively. The hyperemia SIR represented 102% increase (p=0.004) and 66% increase (p=0.004) in cerebral blood flow for ASL and DSC compared to control SIR. The Dice coefficient for hyperemia on ASL and final infarct on FLAIR was higher, 0.60 (0.54-0.69) versus 0.39 (0.34-0.49) for DSC (p=0.008). Conclusions We confirmed visually identified hyperemia is associated with increased cerebral blood flow. Furthermore, hyperemic regions on ASL may better predict final infarct volume in stroke patients after EVT compared to DSC. This study provides insight into hyperemia and cerebral blood flow changes after stroke, suggesting a potential MRI marker for future neuroprotective therapies.

195. Kathryn F McDaniel, BA, MSc

The intracellular domain of neuroligin-3 regulates transcription and upregulates ARHGAP30, a known glioma mitogen
Mentor: **Dr. Katherine Roche**
Study Section: **Neuroscience - Cellular and Synaptic**

Removed at request of author

196. Uma R Mohan, PhD

Direct electrical microstimulation of the hippocampus evokes high-frequency ripple oscillations in humans
Mentor: **Dr. Kareem Zaghloul**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

High-frequency oscillation events, known as ripples, have been shown to underly human memory encoding and retrieval. High-frequency ripples coordinated between the medial temporal lobe and association cortices have been shown to support successful memory retrieval in awake humans. However, studies have yet to utilize brain stimulation to causally explore the role of ripples in human memory. While rodent studies have shown that hippocampal stimulation holds the potential to disrupt or elongate ongoing ripples and even evoke new ripples, we do not yet understand how stimulation modulates ripples in humans. To explore the feasibility of evoking ripples with microstimulation in humans, we collected human intracranial EEG recordings from neurosurgical epilepsy patients while delivering open-loop stimulation to the hippocampus at a range of microampere-level amplitudes. We delivered stimulation at 38 distinct hippocampal or hippocampal-adjacent locations in 7 patients and at 16 distinct cortical locations in 6 patients. On each bipolar pair of electrodes, we conducted a 5-minute stimulation session consisting of five blocks of a 30-second periods without stimulation followed by a 30-second stimulation block with either 1 or 5 pulses (at 100 Hz) delivered once per second. We increased amplitudes on each successive stimulation block (1, 15, 25, 50, and 100 µAs). We found a significant increase in ripple rates during stimulation blocks compared to non-stimulated blocks and baseline, which increased with stimulation amplitude both within and across patients. Further, we observed significant increases in cortical ripple rates coinciding with stimulation of hippocampal regions, implying that ripples evoked locally to the stimulation location are able to facilitate hippocampal-neocortical dialogue involved in memory processing. We found no significant differences in the duration and amplitude of electrically evoked and physiologic ripples across all patients suggesting evoked and physiologic ripples have similar morphology. Additionally, we show that microstimulation delivered on microelectrode arrays also evokes ripple activity that co-occurs with single unit spiking activity, demonstrating a preserved relation between sequences of neural activity and ripple oscillations evoked with stimulation. This work provides direct evidence in humans that low-amplitude hippocampal stimulation locally amplifies high-frequency ripple oscillations.

197. John M Replogle, PhD

A Model of TMEM106B Fibrils in Neurodegeneration
Mentor: **Dr. Michael Ward**
Study Section: **Neuroscience - Molecular**

Removed at request of author

National Institute on Aging

198. Elikanah Olusayo Z Adegoke, Ph.D.

Deep Serum Proteomic Profiling Identifies Candidate Paracrine Mediators of Physical Dysfunction Driven by Senescent Cell Transplantation
Mentor: **Dr. Nathan Basisty**
Study Section: **Omics - Metabolomics/Proteomics**

Senescent cells accumulate in virtually all tissues during aging and contribute to diverse pathologies and physical declines. However, the specific proteins underlying these effects remain elusive. To evaluate aging-related phenotypes and circulating proteins directly associated with increasing senescent cell burden, we leveraged a senescent cell transplantation system coupled with a multi-modal assessment of phenotypes in a senescence-reporter mouse model. Twenty mice randomly divided into two groups were given either senescent cell transplant (treatment) or proliferating cells (control). Preadipocytes were

isolated from p16-3MR mice and induced to senescence by irradiation (or mock control) prior to transplant. We measured the grip strength, RotaRod, hanging and treadmill endurance, and body weight changes to evaluate the effect of transplanted senescent cells. Body composition analysis and bioluminescence imaging of senescent cell burden were assessed at baseline and two months after transplant. Serum samples were processed on the Proteograph platform (Seer) and data-independent acquisition (DIA) proteomic analysis was conducted on the Orbitrap Astral mass spectrometer. Database search, grouping, and protein quantification was performed on DIA-NN and evaluation of group changes were performed in Proteograph Analysis Suite and R. We found that grip strength, motor coordination, hanging, and treadmill endurance significantly decreased in senescent cell recipient mice after transplant. A total of 7,080 protein groups and 79,729 peptides were identified across serum samples. We identified 3,377 proteins significantly altered by cell transplantation. Compared to non-senescent cell recipient mice, aminoglycan metabolic process was distinctively identified in mice transplanted with senescent cells. Moreover, multiple proteins were uniquely increased in senescent cell recipient mice, including a classical senescent associated secretory phenotype protein (TGFβ1) and its transcriptional factor (Nfkb1). Multiple proteins that are related to musculoskeletal impairment, neural, and connective tissues dysfunction and frailty were also changed, including senescent cell surface protein marker (CD44) and CHST 3, CHST 11, CHST 14, Hs3st3b1, and Ap2a1. Collectively, we identified numerous promising protein mediators of physical decline induced by senescent cells and offer new therapeutic approaches for further research

199. Jamal Ahmad Ansari, PhD

Schizophrenia/Autism-Linked Topoisomerase 3b Inactivation induces aberrant microglial activation in mouse brain
Mentor: **Dr. Weidong Wang**
Study Section: **Neuroscience - General**

Neuroinflammation and increased microglia activation are common features of many age-associated neurological and mental health disorders. Topoisomerase 3b (TOP3B) depletion in humans has been linked to schizophrenia and autism, whereas Top3b-KO mice display behavioral and neurodevelopmental defects that overlap with mouse models of schizophrenia and/or autism. Neuroinflammation and microglia activation have been observed in both schizophrenia and autism, raising a possibility that Top3b mutations may cause these disorders by aberrantly activating microglia and neuroinflammation. Here we examined this possibility using Top3b-KO mice and obtained several lines of evidence supporting this model. First, we observed that the levels of microglia activation marker CD68 and M1 phenotype marker CD86 were higher in Top3b mutant mice as compared to wild type mice at PND22. In contrast, the levels of M2 phenotype marker Arg1 and CD206 were lower in the mutant mice, suggesting that Top3b inactivation aberrantly activates the microglia and skews them towards the M1 phenotype. Second, we detected increased expression of proinflammatory cytokines (IL6 and TNFα) and the receptor (TLR4), and decreased expression of anti-inflammatory cytokine (IL4 and IL10) in Top3b-mutant mice, which could create the local milieu hostile for neurons, consistent with our earlier findings that Top3b-KO mice display neurodevelopment defects. Third, the activated microglial phenotype, M1, is often associated with increased phagocytosis. In agreement with this, we observed decreased mRNA levels of sirp1-α and CD47, which inhibit phagocytosis; and increased levels of phagocytic receptor CD36 and TREM2 in Top3b-KO mice. Fourth, the increased phagocytosis by microglia may result in aberrant synaptic pruning, which may provide an explanation to our earlier observation that Top3b-KO mice show reduced synapse formation. In accord with this, we observed reduced levels of two synapse-associated markers, SNAP25 and PSD95 in Top3b-KO mice. In summary, our data demonstrate that Top3b-KO mice display aberrant microglial activation and synaptic pruning, which may provide one explanation for how Top3b mutations can lead to neurological and mental health disorders in both human and rodents. We are currently studying the underlying mechanisms-which genes are regulated by Top3b at transcriptional and/or post-transcriptional levels.

200. Georgiana Luisa Baca, MD

Largest Calcium Imaging Study Reveals a Novel Mechanism of Cardiac Rhythm Failure: Aging-Induced Pacemaker Network Desynchronization in the Sinoatrial Node
Mentor: **Dr. EDWARD LAKATTA**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

Introduction The sinoatrial node (SAN), the heart's natural pacemaker, undergoes structural and functional deterioration with age, contributing to arrhythmias and sick sinus syndrome, often requiring electronic pacemakers. While abnormalities in individual pacemaker cells have been identified, how these cells communicate within the intact SAN remains unclear. Aim We hypothesized that aging disrupts the synchronization of Ca^{2+} signals among pacemaker cells. In the largest vertebrate SAN aging study to date, we present evidence supporting this hypothesis. Methods High-speed ex vivo Ca^{2+} imaging was performed on intact murine SAN tissue (C57BL/6J; young: n=30, 3-4 months; old: n=30, 26-31 months). Custom computational tools quantified impulse firing rate, rhythm, spatial-temporal propagation, and network connectivity. Two-way ANOVA assessed age-related effects on pacemaker coordination and heterogeneity. Results Aged SANs exhibited reduced mean impulse frequency (young: 6.29 ± 0.81 Hz vs. old: 5.09 ± 0.72 Hz, p = 0.006) with increased variability in the old. HRV fragmentation analysis showed increased beat-to-beat variability in aged SANs (pNN5: young: 10.44%, old: 44.47%, p = 0.022). The standard deviation of inter-impulse intervals was significantly increased, indicating greater heterogeneity. Pixel analysis revealed a 35% reduction in active pacemaker cluster area and slower Ca^{2+} propagation in aged SANs. Spatial-temporal mapping identified clusters firing out of phase. Cross-correlation network analysis (r > 0.95) confirmed fragmented pacemaker coordination, leading to disorganized impulse initiation and transmission. Notably, SAN pacemaker cells exhibit small-world network properties, a feature previously attributed to the brain, facilitating efficient signal integration. Conclusion SAN aging is not merely a loss of firing rate in individual cells but results from impaired summation and synchronization of local Ca^{2+} signals among cells, with functionally different clusters, similar to the brain's small world network organization. As the largest dataset of SAN Ca^{2+} imaging in aging hearts, this study provides novel mechanistic insights and potential therapeutic targets for restoring pacemaker function.

201. Buyandelger Batsaikhan, MD, PhD

A novel derivative of pomalidomide attenuates lipopolysaccharide-induced inflammatory response through cereblon-dependent and independent mechanisms in human cells and animal models
Mentor: **Dr. Nigel Greig**
Study Section: **Chemistry - Drugs/Pharmaceuticals**

Quelling microglial-induced excessive neuroinflammation provides a potential treatment strategy across neurological disorders and can potentially be achieved by ImmunoModulatory imide Drugs (IMiDs). IMiDs, based on the backbone of thalidomide and pomalidomide (Pom), are anti-inflammatory and clinically efficacious in multiple myeloma, but are compromised by their toxicity/teratogenicity. IMiD pharmacological actions are primarily mediated via interaction with cereblon, a crucial component of the E3 ubiquitin ligase complex. IMiD-cereblon binding leads to the degradation of specific endogenous neosubstrates, such as SALL4 that is critical for embryo development. In pursuit of safer IMiDs, we synthesized 3-monothiopomalidomide (3-MP) that binds cereblon and alters its neosubstrate preference away from SALL4. We evaluated 3-MP cellular toxicity, anti-oxidative and anti-inflammatory effects vs. Pom in LPS-challenged rodent macrophages (RAW 264.7) and microglial (IMG) cells, as well as in human monocyte (THP1), macrophage (transformed THP1) and peripheral blood mononuclear cells (PBMCs). The cells were dose-dependently pretreated with 3-MP or Pom and, thereafter, challenged with LPS. At 24 h, cell viability (MTS assay), anti-oxidation (Griess nitrite assay), and cytokine levels (ELISA) were quantified in the media. Well-tolerated 3-MP doses inhibited nitrite and pro-inflammatory cytokines. This anti-inflammation was replicated in mice following systemic administration of 3-MP and 4 h LPS challenge. 3-MP was hence evaluated in an acute neurodegenerative animal model known to involve neuroinflammation: controlled cortical impact (CCI) traumatic brain injury (TBI) in mice. 3-MP dosing post-CCI TBI mitigated markers of neuroinflammation, and behavioral impairment evaluated at 14 days. Teratogenicity was not evident in the preliminary evaluation of 3-MP in chicken embryos. To investigate the underlying mechanism of the novel compound, we are performing proteomics and RNA-seq analysis on LPS-challenged or non-challenged, 3-MP treated or non-treated THP1 human macrophages. We predict that the 3MP inhibits LPS-induced inflammatory response through cereblon-dependent and independent mechanisms in human cells. These studies indicate that suppressing excessive inflammation provides a treatment strategy in TBI and potentially other neurodegenerative disorders involving excessive inflammation and identify 3-MP as a new lead IMiD drug candidate for further development.

202. Showkat A Dar, PhD

Proteogenomic identification of novel RNA isoform changes in neurodegenerative diseases: unlocking new clinical biomarkers

Mentor: **Dr. Emmanouil Maragkakis**

Study Section: **Neuroscience - Neurological and Neurodegenerative Disorders and Injury**

Alzheimer's disease and related dementias (ADRD) involve broad transcriptomic changes that are still poorly explored. To bridge this gap in knowledge, we used CRISPR/Cas9 technology to generate 15 induced pluripotent stem cells (iPSCs) harboring single-nucleotide mutations across 7 ADRD-associated genes. We subsequently differentiated the iPSC lines into neurons which we confirmed by expression of neuronal biomarkers. We then employed nanopore long-read RNA sequencing and developed a computational pipeline aiming to examine the expression of RNA isoforms in these different neuronal conditions. Long-read sequencing revealed high-resolution diversity at isoform level with novel, complex patterns of alternative splicing events. Notably, few genes such as CHGA, EPB41L1, KIF1A, and GPC2, had more than 200 isoform variants, of which 90% were not previously reported. Furthermore, we found 2,400 new open reading frames (ORFs) and 384 long non-coding RNAs (lncRNAs) with no known function. Mass spectrometry-based proteomics of the same cell lines confirmed 42 of the newly identified ORFs, indicating that many of these RNA isoforms are translationally active. To determine the clinical relevance, we analyzed short-read RNA-seq data (exon-exon junctions) from ~2,500 patients with different stages of Alzheimer's disease. We identified 45 of these junctions in clinical data that precisely matched the junctions of novel isoforms detected in our long-read data, suggesting their involvement in disease pathology. This project reveals the transcriptomic complexity in ADRD and establishes a scalable pipeline to merge long-read transcriptomics, proteomics, and clinical genomics. Next, we aim to explore the functional aspects of these novel isoforms and proteins, with the goal of identifying new biomarkers and potential therapeutic targets for the neurodegenerative diseases.

203. Jie Dong, MD, PhD

Molecularly Distinct Striatonigral Neuron Subtypes Differentially Regulate Locomotion

Mentor: **Dr. Huaibin Cai**

Study Section: **Neuroscience - Neural Circuits**

Striatonigral neurons, traditionally known for promoting locomotion, comprise diverse subtypes with distinct transcriptomic profiles. However, their specific contributions to locomotor regulation remain incompletely understood. Using the genetic markers Kremen1 and Calb1, we demonstrate in mouse models that Kremen1+ and Calb1+ striatonigral neurons exerted opposing effects on locomotion. Kremen1+ neurons displayed delayed activation at locomotion onset but exhibited increasing activity during locomotion offset. In contrast, Calb1+ neurons showed early activation at locomotion onset and decreasing activity during locomotion offset. Optogenetic activation of Kremen1+ neurons suppressed ongoing locomotion, whereas activation of Calb1+ neurons promoted locomotion. Activation of Kremen1+ neurons induced a greater reduction in dopamine release than Calb1+ neurons, followed by a post-stimulation rebound. Conversely, activation of Calb1+ neurons triggered an initial increase in dopamine release. Furthermore, genetic knockdown of GABA-B receptor Gabbr1 in Aldh1a1+ nigrostriatal dopaminergic neurons (DANs) reduced DAN inhibition and completely abolished the locomotion-suppressing effect of Kremen1+ neurons. Together, these findings reveal a cell type-specific mechanism within striatonigral neuron subtypes: Calb1+ neurons promote locomotion, while Kremen1+ neurons terminate ongoing movement by inhibiting Aldh1a1+ DAN activity via GABBR1 receptors.

204. Mostafa Elbahnasawy, Ph.D.

Dynamic antigen specific CD8+ T cell repertoire changes during differentiation and aging

Mentor: **Dr. Nan-ping Weng**

Study Section: **Immunology - Lymphocyte Development and Activation**

The interaction between the T cell receptor (TCR) and antigen-presenting major histocompatibility complex (MHC) molecules drives T cell proliferation and differentiation. Aging alters T cell activation and differentiation, contributing to the age-related decline in T cell immunity. However, the role of antigen-specific TCRs and changes in their repertoire during these processes

remains poorly understood. To address this, we analyzed the number of CD8+ T cells and the TCR repertoire recognizing a dominant epitope of chicken ovalbumin (OVA 257-264, SIINFEKL) in naïve young C57BL/6J mice and post in vivo challenge. Using tetramer staining, we identified approximately 1,000 OVA-tetramer+ CD8+ T cells in the spleens of young mice. Following infection with Listeria monocytogenes-OVA, OVA-tetramer+ CD8+ T cells expanded to nearly 90,000 at the peak of the response (day 7). By days 45–60 post-infection, these cells declined by approximately one-third. We employed single-cell TCR sequencing (scTCRseq) to profile the OVA-specific CD8+ TCR repertoire. Analyzing 65,000 OVA-tetramer+ CD8+ T cells from over 100 mice, we identified nearly 4,000 unique TCR sequences shared by at least two different cells. At the individual mouse level, the number of unique TCRs in OVA-specific CD8+ T cells peaked at approximately 90 during the effector stage, decreasing to an average of 40 in the early memory stage (days 45–60 post-infection). To examine the impact of aging on the OVA+ CD8+ TCR repertoire, we compared young (4 months) and old (27 months) mice at the effector (day 7) and memory (days 45–60) stages. At day 7, the number of OVA+ CD8+ T cells was significantly reduced in aged mice (from an average of 87,000 in young to 23,000 in old, n = 16, p < 0.0001). Similarly, at the memory stage, the number decreased from an average of 56,000 in young mice to 9,000 in old mice (n = 16, p < 0.0001). The number of unique TCRs also declined significantly, from 90 in young mice to 33 in old mice at day 7 (p = 0.02). Collectively, our findings provide a comprehensive quantification of the OVA-specific TCR repertoire in a cohort of over 100 mice and at the individual level. We demonstrate, for the first time, a significant one-third reduction in OVA+ CD8 TCR repertoire size with aging, highlighting its role in the decline of T cell immunity.

205. Apostolos Manolopoulos, MD

Effects of oral Ketone Ester on brain metabolism and cognition: a randomized controlled trial
Mentor: **Dr. Dimitrios Kapogiannis**
Study Section: **Clinical and Translational Research - Clinical Trials**

Ketone bodies serve as alternative energy substrates and signaling molecules that may enhance neuronal cellular processes, reduce excitotoxicity, and compensate for glucose hypometabolism in Alzheimer's disease (AD). Animal studies have demonstrated that exogenous Ketone Ester (KE) administration improves cognition and reduces brain amyloid and phosphorylated tau pathologies. We conducted a double-blind randomized controlled trial to provide proof-of-concept for KE's target engagement and pro-cognitive effects in individuals with metabolic syndrome, a population at increased risk for AD. Fifty cognitively intact participants over 55 years old with metabolic syndrome were randomized (1:1) to receive either 25 g of oral KE or an isocaloric dextrose placebo three times daily for four weeks. The primary outcome was brain β-hydroxybutyrate (βHB) concentration by Magnetic Resonance Spectroscopy (MRS). Secondary outcomes included additional brain metabolite levels and cognitive performance, assessed via the Preclinical Alzheimer Cognitive Composite (PACC) and NIH Toolbox. We employed linear mixed-effects models with Group (KE/Placebo), Week (0/4), Drink (Before/After), and their interactions as fixed effects, subjects as random effects, and age and education as covariates. KE increased brain βHB levels (p < 0.001), confirming brain penetrance and target engagement. Serum and urine βHB levels also increased following KE administration. Notably, KE reduced brain glutamate levels (p < 0.001), suggesting decreased excitatory neurotransmission and potential neuroprotection. Logical memory improved for both immediate verbatim and gist recall in both groups, indicating practice effects; trends for the interaction favored KE (ChisqGroup*Week = 3.77, p = 0.052 and ChisqGroup*Week = 3.18, p = 0.07, respectively). Within-group analyses revealed improvements in delayed verbatim recall and digit symbol substitution only with KE, suggesting enhanced long-term memory and executive function. KE was well tolerated, with only mild gastrointestinal adverse events reported. This study demonstrates that oral KE supplementation induces brain ketosis and reduces brain glutamate levels, thereby decreasing hyperexcitability, and improves memory and executive function, reinforcing KE's potential for AD prevention and treatment.

206. Mansi Shrivastava, PhD

Senescence-Associated Interferon Signaling: A Key Modulator of Inflammaging
Mentor: **Dr. Nathan Basisty**
Study Section: **Clinical and Translational Research - Diagnostics/Biomarkers**

Inflammaging, the age-associated decline in immune function, is driven by chronic inflammation and exaggerated cytokine responses upon pathogen exposure. We hypothesize that the age-associated accumulation of senescent monocytes contributes to this phenomenon by secreting harmful senescence-associated secretory phenotype (SASP) factors, resulting in excessive immune responses. This study investigates their role in inflammaging and explores potential therapeutic interventions by examining pathogen responses in vitro. Additionally, we analyzed circulating monocytes in humans to investigate senescence and the interferon (IFN) response contributing to inflammaging and immune system hyperactivation. Using an irradiation-induced senescence model in THP-1 monocytes, we analyzed the proteomic changes via data-independent acquisition (DIA) mass spectrometry. We assessed the impact of senescence on pathogen response by measuring LPS-induced inflammatory cytokines in senescent and non-senescent cells using a Mesoscale kit. Additionally, we tested various compounds for their ability to modulate inflammation and evaluated inflammatory signatures in monocytes. Finally, we examined proteomic profiles of circulating monocytes from 94 individuals aged 22-89 in the Genetic and Epigenetic Signatures of Translational Aging Laboratory Testing (GESTALT) study of aging to identify clinical associations between senescence and IFN signatures and aging phenotypes in vivo. Senescent THP-1 monocytes exhibited significant proteome remodeling, with increased 359 IFN-related proteins, including STAT1, OAS1, OAS2, MX1, and ISG15, while 1355 decreased proteins. When stimulated with LPS, these cells secrete elevated levels of pro-inflammatory cytokine, including interleukins and IFN-γ, consistent with our hypothesis that senescent monocytes may increase pathogen susceptibility in aging. Moreover, a therapeutic screening revealed potential interventions capable of senolytic or senomorphic effects, which may mitigate this response. Further, the proteomic analysis of circulating monocytes in the GESTALT cohort linked senescence-associated proteins with systemic inflammation and aging-related decline in vivo. Among 106 IFN-related senescence-associated proteins, 27 are quantified as potential biomarkers, correlated with CRP levels, reinforcing their role in inflammation. Overall, our results suggest targeting senescent cells or SASP to counteract inflammaging and restore immune balance.

207. Joshua A Taylor, PhD

Autoreactive antibodies selectively exacerbate plaque development in atherosclerotic mice
Mentor: **Dr. Patricia Gearhart**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

Atherosclerosis activates the immune system, including B cells which secrete antibodies into the circulation. These antibodies bind antigens released following cellular necrosis within plaques and driving a process of sterile inflammation. To identify these antibodies, we isolated activated B cells from atherosclerotic ApoE-deficient mice aortas and performed single-cell sequencing of their B cell receptors to obtain heavy and light chain pairs. We identified 224 unique pairings with select CDR3 sequences being publicly shared, supporting a common recurring response within disease. We expressed 35 pairings found to be overrepresented compared to matched splenocytes. Epitope determination was performed by proteome array and 14 protein targets were selected with significant affinity and specificity for further interrogation. Antigens were scrutinized by immunohistochemistry to confirm presence in inflamed plaques and autoreactive antibody titers were probed in diseased sera by ELISA. ApoE-deficient mice were immunized with a cocktail of the 7 most promising antigens and were successively placed on a high-fat Western Diet. Serum antibody measurement showed strong, rapid, and early onset of antigen-specific antibody responses in immunized animals. Endpoint measurements of plaque accumulation 16-weeks post-immunization showed a 1.6- and 2.1-fold increase in plaque occurring within the aortic root and descending aorta respectively compared to CGG and adjuvant-only control mice. These findings suggested that broad antibody responses to plaque antigens worsen plaque development. To identify the specific contribution of individual antigens, mice were immunized with one of two representative antigens corresponding to antibodies with high specificity. Endpoint plaque measurements showed a differential antigen-specific effect on plaque accumulation with one antigen illustrating a 1.3-fold increase in disease development and the other antigen being entirely insignificant. Collectively, these results highlight the importance of studying antibody specificity in atherosclerosis for better understanding the disease, and the potential for specific antibody responses to have relevance as new biomarkers or therapeutics.

National Institute on Alcohol Abuse and Alcoholism

208. Lenny Pommerolle, PhD

Abhydrolase domain containing 14B (ABHD14B), an orphan serine hydrolase emerges as a novel therapeutic target in pulmonary fibrosis by its newly defined and central role in activating fibrotic fibroblasts in lungs
Mentor: **Dr. Resat Cinar**
Study Section: **Clinical and Translational Research - Diagnostics/Biomarkers**

Removed at request of author

National Institute on Drug Abuse

209. Matthew Broomer, PhD

Neural representation of altered reward-seeking behavior following punishment
Mentor: **Dr. Da-Ting Lin**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

Animals may learn to inhibit reward-seeking following punishment, in which a rewarded response now earns an aversive outcome such as electric shock. Punishment is a useful model of drug abstinence, which is often motivated by avoidance of some aversive consequence of drug-seeking. Reward-seeking behavior is associated with activity in D1 and D2 receptor-expressing neurons in the nucleus accumbens (NAc). This activity tracks response initiation and reward consumption as well as the expectation of reward and how that expectation is updated following reward delivery (or omission). However, it is unclear how these neurons encode new inhibitory learning about a reward-seeking response when contingencies change and an animal must alter its behavior. We used miniscopes to record activity from D1- and D2-expressing NAc neurons and applied machine learning-assisted analyses to characterize behavioral changes during punishment. Rats were trained to lever press for food reward, then were punished for doing so. Punished responses earned a brief footshock set to 0 mA for two baseline sessions and increased 0.1 mA per session until animals stopped pressing. We recorded during four possible response types: those that earned reward, shock, both, or neither. Punishment altered population-level neural activity in the 1.5s following each response type. Each evoked similar activity patterns during baseline sessions, but distinct patterns during later punishment sessions. Activity differed due to shock delivery, but the greatest difference was not between the most and least “desirable” responses (i.e., rewarded vs. punished) but between the most and least “informative” (i.e., both outcomes vs. neither). We observed a similar trend in the 1.5s before response, suggesting neural activity tracked both expectation of outcomes, and the confirmation/violation of those expectations upon outcome delivery. We analyzed behavior videos with DeepLabCut to characterize diffuse response-adjacent behavior. We identified an “abortive” lever approach (not followed by a lever press) that increased throughout punishment, as well as increasing latency between lever approach and lever press and decreasing latency between abortive approach and departure from the lever, suggesting that punishment induced qualitative changes in how animals emit a reward-seeking response. This is a novel approach to characterizing neurobehavioral signatures of punishment learning and behavioral flexibility.

210. Ginevra Dottavio, PhD

Individual differences in preference for heroin over rewarding social interaction in male and female rats: role of duration of drug access and type of social interaction
Mentor: **Dr. Yavin Shaham**
Study Section: **Drug and Alcohol Abuse**

Background: In some individuals, opioid use leads to decreased interest in socially relevant rewards. Recent studies showed that after extended-access heroin self-administration rats strongly prefer social interaction over a single unit-dose heroin infusion. We hypothesized that this strong social preference results from access to a suboptimal heroin dose during testing,

and that individual differences in heroin versus social choice would emerge if rats were given access to their 'preferred' heroin dose. Methods: In Experiment 1, we trained male rats to lever-press for social interaction, followed by heroin self-administration under continuous-access, no-timeout schedule, which promotes burst-like heroin intake. We then tested for choice between single-unit heroin dose and 1-minute full-contact social interaction, or 5-minute heroin-access (sufficient for burst-like intake) and 5-minute social interaction. In Experiment 2, we extended the 5-minute-access procedure to female rats and tested heroin versus limited-contact (screen-based) social interaction. We also manipulated response requirements (effort) for heroin and tested the effects of buprenorphine and naltrexone. Results: When rats given a single-unit heroin dose during choice testing, they strongly preferred social interaction. In contrast, when given 5-minute heroin-access, large individual differences in heroin preference emerged. These differences were independent of sex, social-interaction conditions, and effort manipulations, and resistant to pharmacological treatments. Individual differences in heroin preference were positively associated with high heroin-taking and heroin-seeking, and burst-like heroin intake. Conclusions: Access to 'preferred' heroin-dose during the choice leads to stable individual differences in heroin preference. This procedure provides a platform to study mechanisms of resilience and vulnerability to opioid addiction.

211. Lacey K Greer, B.S.

Increased KDEL receptor expression in the rat midbrain decreases expression of dopaminergic and neuronal genes
Mentor: **Dr. Brandon Harvey**
Study Section: **Neuroscience - Molecular**

Removed at request of author

212. Anna Loften, MD, PhD

Spironolactone receipt is associated with higher remission of alcohol use disorder and lower alcohol intoxication rates in a real-world cohort
Mentor: **Dr. Lorenzo Leggio**
Study Section: **Drug and Alcohol Abuse**

Alcohol use disorder (AUD) is a prevalent chronic disease associated with major medical and societal consequences and reduced life expectancy. Despite the high prevalence and burden of AUD, the number of approved pharmacotherapies for AUD is limited, underscoring the need to develop novel pharmacotherapeutic options. Hyperactivity of the aldosterone/mineralocorticoid receptor (MR) system has been associated with increased alcohol intake and craving, suggesting that MR antagonism could be a potential treatment strategy for AUD. The non-selective MR antagonist spironolactone is approved for medical conditions such as hypertension and heart failure, and data suggest that it could be repurposed for AUD. Spironolactone has been shown to decrease alcohol intake in rats and mice, in a dose-dependent manner. Human evidence, however, is scarce. To address this gap, we conducted a nationwide retrospective multicenter cohort study, using a large database of electronic health records, the TriNetX. Individuals with comorbid AUD and hypertension who received spironolactone were compared to propensity-score-matched individuals who received other antihypertensive treatments as an active control. Alcohol-related outcomes were compared between exposure groups. Our results demonstrate that spironolactone prescription was associated with higher rates of AUD remission compared to angiotensin converting enzyme (ACE) inhibitors (hazard ratio (HR) 1.47, confidence intervals (CI): 1.32-1.63, n=3,574 for each group), angiotensin receptor blockers (HR 1.19, CI: 1.00-1.42, n=1,269 for each group), calcium channel blockers (HR 1.28, CI: 1.14-1.44, n=2,944 for each group), thiazide diuretics (HR 1.56, CI: 1.47-1.66, n=12,337 for each group), and beta blockers (HR 1.20, CI: 1.03-1.39, n=1,553 for each group). Moreover, spironolactone treatment was associated with lower rates of alcohol intoxication compared to ACE inhibitors (HR 0.83, CI: 0.70-0.98, n=3,574 for each group) and beta blockers (HR 0.75, CI: 0.58-0.98, n=1,553 for each group). This study provides real-world evidence of the beneficial effect of spironolactone, in comparison with other antihypertensive medications, on alcohol-related outcomes in individuals with AUD. This work builds the foundation for prospective clinical trials and possibly repurposing of spironolactone and/or other MR antagonists for AUD.

213. Oscar Solis Castrejon, PhD

Integration of synaptic Zn(2+) and dopamine neurotransmission by cocaine
Mentor: **Dr. Michael Michaelides**
Study Section: **Neuroscience - Cellular and Synaptic**

Zinc (Zn(2+)) is an essential trace element that regulates neurotransmission and plays a critical role in several neurophysiological processes. Dysregulation of brain Zn(2+) is associated with several mental health conditions including substance use disorder (SUD). In the brain, a subset of glutamatergic neurons corelease Zn(2+) and glutamate, particularly in the striatum, a region involved in movement, reinforcement, and reward-related behaviors. Previous studies showed that synaptic Zn(2+) modulates dopaminergic neurotransmission in the striatum and potentiates the effects of cocaine. However, changes in synaptic Zn(2+) release and its relationship with dopamine release during movement and after cocaine exposure have not been explored. In addition, the molecular identity and connectivity of Zn(2+)-releasing neurons projecting to reward-related brain regions have not been characterized. To address these questions, we injected genetically-encoded sensors for dopamine and Zn(2+) (GRABDA and GRISZ, respectively) into the striatum of mice and used fiber photometry to record changes in both neurotransmitters during locomotion, immobility, and after cocaine administration. Our results indicate that both synaptic Zn(2+) and dopamine increase during locomotion and after cocaine administration. In addition, we found that in regions such as the prefrontal cortex and amygdala, 35-45 % of glutamate-releasing neurons corelease Zn(2+). These results provide new information on molecular and brain circuit mechanisms of neurotransmission, movement, reward, and advance our understanding of the role of Zn(2+) in SUDs and related mental health conditions associated with synaptic Zn(2+) function.

214. Phil Witkowski, PhD

The role of expected reward signaling in the orbitofrontal cortex for driving reward-seeking behaviors
Mentor: **Dr. Thorsten Kahnt**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

The orbitofrontal cortex (OFC) is critical for building knowledge of our environment by forming associative links between sensory cues and outcomes. However, when dysregulated, this same function can lead to compulsive behavioral disorders such as substance use disorder (SUD). For example, the OFC is hyperresponsive to drug-related cues in substance users, with activation levels linked to their craving intensity. A significant gap in our knowledge is an understanding of the specific information within the OFC that drives cue-induced craving and reward-seeking. Addressing this issue could reveal neural targets for non-invasive therapies treating SUD and other compulsive behavioral disorders. We address this gap using a novel adaptation of the Pavlovian-to-instrumental transfer paradigm, which isolates the neural mechanisms that induce reward seeking behaviors for specific rewards (e.g., a specific drug or desirable food). We first trained hungry participants (N=15 out of 30 planned) to associate arbitrary visual cues with one of two desirable food scents via Pavlovian conditioning. Participants then learned to obtain these rewards in a “space traveler” game in which they collected rewards for their “journey through the stars”. Each of six planets was associated with one of the food scents, and each of 20 locations on the planet had a 40% chance of producing the reward. At the test session, participants played the foraging game but were unexpectedly presented with two of the conditioned cues at the start of each trial. We predicted that the cues would induce a representation of a specific reward in OFC that would motivate responses for the same reward during foraging. Choices during the task showed that participants reliably sought the food scents indicated by cues in trials were both cues indicated the same reward (t(14)=4.29, p<.001), and foraged locations on these planets more vigorously (t(14)=2.18, p<.05). Critically, the specific reward predicted by the cues could be decoded from activity in the OFC prior to foraging. When cues indicated different rewards, the strength of the decoded reward information in OFC predicted whether participants tried to obtain that reward when foraging. These results directly link specific reward expectations in the OFC to reward seeking, enhancing our understanding of the role prefrontal regions in goal-directed behavior and highlighting putative targets in the treatment of behavioral disorders such as SUD.

National Library of Medicine

215. Stanley Liang, PhD

Multi-Agent Cross-Modal Large Language Model for Integrative Analysis and Comprehensive Diagnosis on Chest X-rays

Mentor: **Dr. Sameer Antani**

Study Section: **Artificial Intelligence - Large Language Models**

A multi-agent large language model (LLM) is an artificial intelligence (AI) architecture that integrates multiple AI agents, each optimized for distinct tasks, to enhance decision-making for complex tasks. A reasoning model, such as GPT-o1, applies a chain-of-thought process to aggregate information and to improve inference reliability. We demonstrate this through a novel multi-agent LLM framework for pneumonia diagnosis using chest X-ray (CXR) images, where different independent predictions by multiple task-specific AI models are synthesized by GPT-o1 to generate the final prediction. This framework integrates GPT-o1 as a reasoning agent with specialized cross-modal AI agents, including a fine-tuned GPT-4o an image-to-text LLM to extract structured clinical insights directly from CXR images. By applying confidence-weighted reasoning, GPT-o1 dynamically assigns weights to AI agent outputs based on their performance metrics to enhance prediction accuracy. Experimental evaluation on a multi-site CXR dataset demonstrates that the multi-agent model (GPT-o1 with metrics) achieved a pneumonia detection accuracy of 0.93, surpassing all single-agent models. It also demonstrated significantly higher specificity (0.79, p < 0.05) for reducing false positives. For pneumonia severity estimation, fine-tuned GPT-4o (MAE: 3.69, R^2: 0.51) and multi-agent GPT-o1 (MAE: 4.20, R^2: 0.47) outperformed the conventional vision-based models and mitigated noise in regression tasks. Additionally, the reasoning model (GPT-o1 with metrics) achieved the highest F1-score (0.96) and Matthews Correlation Coefficient (0.79), indicating superior classification reliability. The multi-agent system also demonstrated enhanced robustness across different pneumonia severity levels, outperforming the single-agent models in detecting mild and moderate cases. The study systematically compared the multi-agent model with GPT-o1, cross-modal LLMs (fine-tuned GPT-4o & BiomedCLIP-PubmedBERT), and conventional vision-based models (Mobile ViT and DinoV2), highlighting the benefits of applying cross-modality and reasoning AI agents for medical decision-making. The findings reflect the superior diagnostic accuracy, interpretability, and adaptability of multi-agent LLMs. This new framework provides a robust, efficient, and scalable solution for automated medical imaging analysis, paving the way for AI-driven diagnostics in radiology.

216. Jaya Srivastava, Ph.D.

Regulatory risk loci link disrupted androgen response to pathophysiology of Polycystic Ovary Syndrome

Mentor: **Dr. Ivan Ovcharenko**

Study Section: **Endocrinology**

A major challenge in deciphering the complex genetic landscape of Polycystic Ovary Syndrome (PCOS) is the limited understanding of the molecular mechanisms driven by susceptibility loci identified in Genome Wide Association Studies, necessitating an investigation into the regulatory pathways perturbed across diverse cell-types which contribute to the varied reproductive and metabolic phenotypes. In this study, we hypothesized that PCOS-associated cell-types manifest disease phenotypes due to perturbations in reproductive hormone biosynthesis or their response to these hormones. We integrated molecular and epigenomic annotations across eleven proposed pathogenic cell types and employed a deep learning (DL) model to infer the cell-type-specific effects of risk variants across 50 susceptibility loci. Our analysis revealed the role of these variants in brain and endocrine cell types affecting the binding sites of key transcription factors (TFs)—FOXA1, FOXL1, WT1, SALL4, and CPEB1—which regulate ovarian development, folliculogenesis, and steroid hormone signaling, contributing to disease-associated transcriptomic profiles. Our DL model, which is strongly correlated with MPRA data, identified enhancer-disrupting activity in 20% of the risk variants, particularly affecting TFs involved in androgen-mediated signaling, shedding light on the molecular consequences of hyperandrogenemia. Using the IRX3-FTO locus as a case study, we explored the potential cell-type-specific regulatory effects of risk variants in the fetal brain, pancreas, adipocytes, and an endothelial cell-line, which suggest that disruptions in IRX3 regulation (previously linked to obesity) may contribute to PCOS pathogenesis through diverse mechanisms, including neuronal development, metabolic regulation, and folliculogenesis. Our findings underscore the value of integrating DL models with epigenomic annotations to identify disease-relevant variants and explore the pleiotropic effects of disease risk loci. Experimental characterization of these variants has the potential to pave the way for novel, symptom-targeted therapies for PCOS patients.

217. Qingqing Zhu, Ph.D.

A Multimodal Dataset and Benchmark for Evaluating AI Models in CT Lesion Analysis
Mentor: **Dr. Zhiyong Lu**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

Introduction: Advancements in artificial intelligence (AI) have transformed medical image analysis, diagnosis, and treatment planning. In particular, accurate lesion analysis in CT imaging is essential for detection and ongoing monitoring of numerous diseases. However, progress in AI-driven lesion analysis is significantly hindered by the lack of publicly accessible, well-annotated CT datasets. To address this gap, we introduce a first-of-its-kind dataset of 20,335 lesion images with lesion description, size information and bounding box annotations, along with a benchmark with 2,850 QA pairs. Methods: The dataset annotation process involved a three-stage pipeline combining GPT-4-generated pre-annotations with manual refinement by radiologists. The benchmark is designed to evaluate multi-modal question-answering models across a variety of tasks, including lesion description, image selection, bounding box localization, lesion size estimation, attribute categorization, and other essential diagnostic tasks. To further increase the challenge of these tasks, each image was paired with visually similar cases retrieved using a deep learning model, and medical experts selected three "hard negative" cases to enhance robustness. The benchmark is further divided into samples with and without bounding box annotations, enabling evaluation of models under both scenarios. The diversity of tasks and the inclusion of both annotated and non-annotated bounding box samples allow for a comprehensive evaluation of AI models in interpreting medical images and CT scans. Results: We tested multiple state-of-the-art multimodal models on our benchmark, including vision-language and medical-specific AI models. While these models demonstrated some strengths, their accuracy remained relatively low, with the highest reaching 35.17% without bounding boxes and 41.57% with bounding boxes. However, fine-tuning on our dataset significantly improved performance. For instance, a specialized vision-language model's accuracy increased from 41.57% to 61.79% after fine-tuning. Discussion and Conclusions: The introduction of this dataset and benchmark represents a significant step forward for multimodal AI in CT analysis. By providing a robust dataset and a structured evaluation framework, these resources support the development of AI models with improved diagnostic accuracy and clinical applicability.

NIH Clinical Center

218. Ricardo Bigolin Lanfredi, PhD

LEAVS: A Large Language Model Labeler for Training Abdominal Computed Tomography Artificial Intelligence Models
Mentor: **Dr. Ronald Summers**
Study Section: **Artificial Intelligence - Large Language Models**

Structured labels extracted from radiology reports describing medical scans have been used to develop artificial intelligence (AI) models to detect several types of medical conditions in radiology images simultaneously. However, most existing studies focus on the chest region, whether in chest X-rays or computed tomography (CT) scans. Few studies have explored abdominal radiology reports, likely due to the region's complex anatomy and diverse medical conditions. We propose LEAVS (Large language model Extractor for Abdominal Vision Supervision) and employ it on a public dataset containing over 1,600 CT volumes and reports. This labeler can determine whether a condition is present and how urgent it may be for nine types of abnormalities (organ absence, image quality, presence of support devices, postsurgical changes, enlargement, atrophy, anatomical findings, as well as localized or widespread findings, also known as focal or diffuse findings) for nine abdominal organs (liver, gallbladder, spleen, right kidney, left kidney, pancreas, stomach, small bowel, large bowel) on CT radiology reports. Additionally, the labeler is easily extendable to other body parts. To ensure broad coverage, we chose abnormalities that encompass most of the finding types from CT reports. Our approach employs a privacy-preserving locally-run large language model (LLM), a type of AI model trained on vast amounts of text to understand and generate language. Our method follows a structured step-by-step reasoning process, preprocesses the report with sentence extraction, and applies multiple-choice questions to categorize findings. We show that the LLM effectively extracts multiple abnormality types across abdominal organs in two test sets. It significantly outperforms competing labelers, achieving an F1 score of 0.892 compared

to 0.827 (P=.001, tested for difference). It also surpasses human performance, with an F1 score of 0.924 versus 0.894 (P=.002). Additionally, our method extracts urgency with performance comparable to human annotations. The Kendall Tau-b correlation coefficient, which measures ranking agreement, was 0.533 compared to 0.556 for humans (P=.575). Finally, we show that these abnormality labels are valuable for training a single AI model to classify multiple organs as normal or abnormal for each abnormality type. The model achieves an average AUC of 0.716, indicating some ability to distinguish normal from abnormal organs. We release code and structured annotations.

219. Negin Jarrah, MD

An MRI-based radiomics machine learning model to predict malignant renal cysts in patients with HLRCC
Mentor: **Dr. Ashkan Malayeri**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

Introduction: Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC) is an autosomal dominant syndrome caused by mutations in the FH gene, predisposing patients to aggressive renal malignancies. Due to their high metastatic rates, biannual or annual contrast-enhanced MRI is recommended for surveillance. Radiomics, a noninvasive technique extracting quantitative imaging features, has shown promise in assessing tumor characteristics and supporting personalized medicine. This study evaluates the use of radiomics-based machine learning (ML) for predicting malignancy in renal complex cysts in HLRCC patients. Methods: Seventy-one HLRCC patients with renal cysts (2002–2022) were reviewed. The exclusion criteria included lack of nephrectomy, absence of contrast-enhanced MRI, lack of pathology results, or poor image quality. The final cohort included 40 patients (mean age 47 +/- 15 years; 20 men, 20 women) with 67 histologically confirmed lesions (41 benign, 26 malignant). MRI protocol included T2-weighted and pre-/post-contrast T1-weighted sequences. Tumor segmentation was performed semi-automatically, and 8,015 radiomic features were extracted using SyngoVia Frontier Radiomics with the PyRadiomics library. LASSO regression was used for feature selection, and an Extreme Gradient Boosting (XGBoost) model was trained on randomly divided training (70%) and testing (30%) sets. Model performance was evaluated using AUC-ROC, accuracy, F1 score, precision, and recall. Results: After feature selection, eight radiomic features were used in the XGBoost model. The ML model trained on combined radiomic features from all MR sequences achieved an F1 score of 0.85 (95% CI: 0.74–0.94) and a mean AUC-ROC of 0.93. Among features, “Wavelet HLL GLRLM Run Variance” from the pre-contrast phase had the highest predictive value, as indicated by SHAP values presenting texture features of the masses. Conclusion: Radiomics effectively characterizes renal cysts in HLRCC patients and enables pre-surgical malignancy assessment. This study is the first to apply radiomics for malignancy prediction in HLRCC-associated renal cysts. ML models performed best when incorporating features from all MR sequences. These findings provide radiologists a valuable tool to improve diagnostic accuracy and optimize therapeutic decisions, potentially reducing unnecessary invasive procedures.

220. Choa Sung, PhD

Effects of Activating the Peripheral Endocannabinoid System on Sleep Architecture and the Gut Microbiome in Mice
Mentor: **Dr. Katherine Maki**
Study Section: **Microbiota/Microbiome**

Cannabis is widely used as a sleep aid, both medicinally and recreationally, due to its hypnogenic properties. However, chronic cannabis use leads to tolerance due to its sleep-promoting effects, and sleep disturbance during abstinence increases relapse risk. Most research on cannabis and sleep focuses on cannabinoid receptor 1 (CB1R) activation in the brain. However, emerging literature suggests that peripheral endogenous cannabinoids (eCBs) and CB1R activation in the gut plays a key role in gut-brain signaling, potentially influencing sleep-wake regulation. Peripheral eCBs and CB1Rs regulate various physiological processes and alter gut microbiota composition, which has been implicated in sleep-wake processes. However, little is known about how exogenous cannabinoids, such as the phytocannabinoids in cannabis, affect the gut microbiome to regulate sleep. In this study, we used a peripherally restricted CB1R agonist, CB13, which cannot penetrate the brain, to investigate how peripheral CB1R activation affects the gut microbiota and sleep. We employed a validated treatment procedure (intraperitoneal injection, 10 injections over six days, followed by six days of abstinence) previously used to induce sleep

disruption in a chronic cannabis use mouse model. We examined CB13's within- and between-subject effects on gut microbiota composition, using shotgun metagenomic sequencing and sleep states through polysomnographic recordings before, during, and after CB13 or vehicle administration. We found that CB13-treated mice exhibited lower global microbiome diversity (mean Shannon diversity) than vehicle-treated mice after the final injection and six abstinent days, indicating that microbial disruptions occurred after chronic administration and persisted into late abstinence. Regarding sleep-microbiome interactions, microbial diversity was negatively associated with NREM sleep hours in CB13 mice after the final injections, but not controls. Interestingly, in CB13 mice but not controls, the relationship between microbial diversity and NREM sleep hours shifted to a positive association in late abstinence, suggesting that peripheral CB1R activation dynamically influences sleep-microbiome interactions and contributes to this shift from chronic use to late abstinence. Overall, these results highlight the role of peripheral eCBs in sleep and support the eCB system as a physiologically relevant and promising target for probing gut-brain signaling and sleep regulation.

221. Tanjin Taher Toma, PhD

Precision 3D Segmentation for Accurate Detection of Pheochromocytoma and Paraganglioma (PPGL) Tumors in Contrast-Enhanced CT
Mentor: **Dr. Ronald Summers**
Study Section: **Methods/Assay Development**

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors that originate in the adrenal glands or extra-adrenal locations. These tumors often metastasize, with their development and progression primarily driven by genetic factors. Contrast-enhanced CT scans are commonly used for detection. Manual localization of PPGLs in 3D CT scans is highly challenging due to the considerable variability in tumor size, morphology, and their potential occurrence throughout the body, including the head, neck, chest, adrenal glands, and abdominopelvic regions. Therefore, developing an automated 3D segmentation method for PPGLs is crucial to aid radiologists in accurately identifying these tumors and guiding subsequent treatment decisions. The existing literature on automatic PPGL detection is limited, with previous methods relying on 2D or pseudo-3D annotations and coarse bounding box labels, rather than precise 3D tumor delineation. As a result, these models may localize tumors but fail to accurately quantify key characteristics, such as volume, boundary irregularity, and changes in standardized uptake values (SUV), which are crucial for assessing tumor progression. We propose a deep learning-based method for precise 3D segmentation of PPGL tumors from contrast-enhanced CT scans. We employ the state-of-the-art nnU-Net architecture as the segmentation network. A small subset of the training dataset is manually annotated by two radiologists, providing slice-by-slice annotations across the entire 3D volumes. These annotations are used to initialize network training. Subsequently, an iterative semi-supervised learning framework is applied, where pseudo-labels generated by the model for the unlabeled data are manually corrected and incorporated into the labeled dataset for subsequent training cycles, thereby refining the model progressively. Our PPGL dataset comprises portal venous phase contrast-enhanced CT scans from 289 PPGL patients screened for tumors at NIH between April 1999 and January 2022. When evaluated on 25 CT studies for adrenal tumor segmentation, our proposed method achieved a Dice index of 0.93±0.042 and a normalized surface distance of 0.94±0.051. Our findings emphasize the potential of accurate 3D segmentation for PPGL detection and highlight the opportunity to subsequently leverage segmented tumor regions to identify genetic variants of PPGLs, which could contribute to more cost-effective cancer management.

222. Xinya Wang, PhD

Leveraging Multiphase CT for Quality Enhancement of Portal Venous CT: Utility for Pancreas Segmentation
Mentor: **Dr. Ronald Summers**
Study Section: **Artificial Intelligence - General**

Multi-phase computed tomography (CT) studies are routinely obtained in clinical practice to visualize and diagnose a variety of conditions, such as cirrhosis, coronary artery disease, diabetes, and pancreatic cancer. Currently, radiologists utilize multiple complementary CT phases, such as non-contrast, arterial, portal venous (PV), and delayed sequences, to render a diagnosis. Among the individual CT phases, the PV phase is valuable for lesion detection and vessel segmentation. However,

the quality of acquired CT scans can vary across different institutions because there are many CT scanners from different manufacturers and diverse CT exam protocols in use. Patients are also scanned with low radiation doses, and the scans can often be affected by metal artifacts or motion. Abdominal CT volumes are also reconstructed with non-isotropic voxel resolutions ranging from 0.5-5 mm in the in-plane and through-plane directions. This leads to diverse appearances of organs and structures in CT. To mitigate these effects, numerous approaches have focused on CT-based denoising and CT super-resolution with promising clinical applications. However, there is no prior approach that leverages the multiple phases inherently available within a CT study for scan quality enhancement. In this proof-of-concept study, we utilized multiphase CT scans to enhance the quality of the PV phase CT at the level of abdomen. The proposed method takes three degraded (low-quality) CT phases (non-contrast, arterial, and PV) as inputs and produces a high-quality PV CT. We extended the progressive fusion and non-local (PFNL) model into 3D, and trained it with the loss function that combined an L1 loss with a 3D Sobel edge-based loss. To determine the effect of quality enhancement, a proxy pancreas segmentation task using the public TotalSegmentator tool was performed. A total of 168 studies from 168 patients were used. Our results demonstrated that leveraging multiphase CT for quality enhancement of the PV phase improved the pancreas segmentation by ~3%. Compared against low-quality images, results from 3D-PFNL on the restored PV CT were statistically significant for both Dice score ($p = .034$) and Normalized Surface Distance ($p = .025$). To the best of our knowledge, we are the first to harness multiphase CT for scan quality enhancement, and the resulting improvement in pancreas segmentation performance may lead to clinical benefits for patients with diabetes or pancreatic cancer.

223. Linlin Yao, PhD

Geometry-Aware 3D Vessel Completion for both CECT and NCCT Images
Mentor: **Dr. Ronald Summers**
Study Section: **Artificial Intelligence - General**

Background: Cardiovascular disease is a leading cause of death worldwide, necessitating early and accurate diagnosis. Contrast-enhanced CT (CECT) plays a crucial role in visualizing vascular anatomy and identifying abnormalities such as aneurysms and stenoses, while non-contrast CT (NCCT) aids in atherosclerosis diagnosis and benefits patients with contrast contraindications. Vessel segmentation is vital for accurate assessment, intervention planning, progression monitoring, and workflow optimization. However, due to the lack of contrast in NCCT and vessel tortuosity, incomplete segmentation, characterized by missing or fragmented vessels, remains challenging in both phases. Supervised deep learning-based methods have been employed to address this issue, but they require extensive labeled data, and manual annotation of NCCT is particularly difficult. To improve vessel segmentation, we leverage the geometric similarity between paired NCCT-CECT vessel masks, utilizing only CECT annotations to enhance segmentation in both phases. Methods: We applied TotalSegmentator (TS) for initial segmentation and trained a geometric deep learning model on interrupted NCCT masks, supervised by paired CECT masks, which provided better connectivity. This eliminates the need for difficult-to-obtain NCCT annotations. To enhance data diversity, we introduced synthetic interruptions in CECT annotations, mimicking patterns observed in real-world segmentations. The training data includes 64 paired NCCT-CECT scans from Renal Donors and 1,000 synthetic interruptions. Results: We evaluated our method on 17 paired CT scans from Renal Donors dataset, plus external test sets: 66 NCCT volumes from an NIH Vasculitis dataset and 23 CECT scans from an NIH Urography dataset. Compared to TS, our method improved the average Dice Similarity Coefficient by 2.0-4.6% for CECT and 9.1-10.2% for NCCT. It also reduced Betti Error, a measure of connectivity, by 0.24-1 for CECT and 1.59-2.08 for NCCT. Qualitative analysis confirmed that our method significantly reduced interruptions and improved the connectivity in both CECT and NCCT segmentations. Conclusion: Our geometric deep learning method relying solely on CECT annotations provides an efficient solution for addressing vessel interruptions in both NCCT and CECT segmentations. The method lays the necessary groundwork for more accurate assessment of intra-abdominal arteries for disorders such as aneurysms, atherosclerotic plaque, and stenoses.