FARE2025 Awardees

National Cancer Institute - Cancer Prevention Fellowship Program

1. Alexandra R Harris, Ph.D., M.P.H., M.S.

Association of early menarche with breast tumor molecular features and recurrence
Mentor: **Dr. Gretchen Gierach**
Study Section: **Epidemiology/Biostatistics – Etiology**

Background: Early menarche is an established risk factor for breast cancer but its molecular contribution to tumor biology and prognosis remains unclear.

Methods: We profiled transcriptome-wide gene expression in breast tumors (N=846) and adjacent normal tissues (N=666) from women in the Nurses' Health Studies (NHS) to investigate whether early menarche (age<12) is associated with tumor molecular and prognostic features in women with breast cancer. Multivariable linear regression and pathway analyses using competitive gene set enrichment analysis were conducted in both tumor and adjacent-normal tissue and externally validated in TCGA (N=116). PAM50 signatures were used for tumor molecular subtyping and to generate proliferation and risk of recurrence scores. We created a gene expression score using LASSO regression to capture early menarche based on 28 genes from FDR-significant pathways in breast tumor tissue in NHS and tested its association with 10-year disease-free survival in METABRIC (N=952).

Results: Early menarche was significantly associated with 369 individual genes in adjacent-normal tissues implicated in extracellular matrix, cell adhesion, and invasion (FDR≤0.1). Early menarche was associated with upregulation of cancer hallmark pathways (18 significant pathways in tumor, 23 in tumor-adjacent normal, FDR≤0.1) related to proliferation (e.g. Myc, PI3K/AKT/mTOR, cell cycle), oxidative stress (e.g. oxidative phosphorylation, unfolded protein response), and inflammation (e.g. pro-inflammatory cytokines IFN-alpha and IFN-gamma). Replication in TCGA confirmed these trends. Early menarche was associated with significantly higher PAM50 proliferation scores (beta=0.082 [0.02-0.14]), odds of aggressive molecular tumor subtypes (basal-like, OR=1.84 [1.18-2.85] and HER2-enriched, OR=2.32 [1.46-3.69]), and PAM50 risk of recurrence score beta=4.81 [1.71-7.92]). Our NHS-derived early menarche gene expression signature was significantly associated with worse 10-year disease-free survival in METABRIC (N=952, HR=1.58 [1.10-2.25]).

Conclusions: Early menarche is associated with more aggressive molecular tumor characteristics and its gene expression signature within tumors is associated with worse 10-year disease-free survival among women with breast cancer. As the age of onset of menarche continues to decline, understanding its relationship to breast tumor characteristics and prognosis may lead to novel secondary prevention strategies.

2. Rebecca L Kelly, PhD, MPH

Mosaic loss of the Y chromosome in leukocytes is associated with prostate cancer risk
Mentor: **Dr. Mitchell J Machiela**
Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Mosaic loss of the Y chromosome (mLOY), a type of clonal hematopoiesis, is the most frequent chromosomal alteration observed in leukocytes of aging men. mLOY is a putative biomarker of genomic instability with evidence of associations with risk of some solid tumors, but more studies are needed to refine associations and identify potential mechanisms for cancer risk. Prostate cancer (PCa), the most common non-cutaneous cancer in males, is also associated with aging. To investigate potential relationships between mLOY and PCa, we examined DNA derived from leukocytes of male participants in two large biobanks: the UK Biobank (UKBB; N=210,103) and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO, N=26,795). Of the 236,898 male participants without previous cancer diagnosis, 14,253 (6.0%) were diagnosed with incident PCa after DNA collection. High-density genotyping array data collected during the studies were used to create virtual karyotypes for detecting mLOY in whole blood samples utilizing a phase-based detection method in MoChA software. Analyses were restricted to men without cancer at genotyping and multivariable regression models were adjusted for age, smoking status, and genetic similarity. Men with detectable mLOY were older (mean=62 vs 56 years) and more likely to be smokers (14.8% vs 9.7%) than men without mLOY. A total of 3,848 (27.0%) men with PCa and 42,835 (19.2%) men free of PCa had detectable mLOY. mLOY clonal fraction ranged from 0.01% to 72.1% of total leukocytes. 18.7% of PCa cases and 13.6% of men free of PCa had high clonal fraction mLOY, defined as greater than 10% of leukocytes with mLOY. Fixed effect meta-analysis of multivariable models from UKBB and PLCO produced evidence for a positive association between mLOY in leukocytes and incident PCa; (Odds Ratio (OR) = 1.062, 95% Confidence Interval (CI)[1.020, 1.107], P-Value (p) = 0.004). The effect estimate was the same in men with low clonal fraction of mLOY (OR = 1.062, 95% CI[1.012, 1.113], p=0.014) and men with high clonal fraction of mLOY (OR=1.065, 95% CI [0.998, 1.137], p=0.057). Age at PCa diagnosis was not associated with mLOY or the clonal fraction of mLOY. This investigation of the relationship between mLOY and PCa in two large prospective studies provides additional evidence for an association between mLOY detected in the blood and increased PCa risk, meriting additional studies of potential biologic mechanisms underlying the relationship.

3. Kyra Mendez, PhD, MPH, BSN

The relationship between COVID severity and telomeres in the context of hematopoietic cell transplantation
Mentor: **Dr. Shahinaz Gadalla**
Study Section: **Clinical and Translational Research – General**

Background: Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment option for hematologic cancers, but it leaves some patients with long-term immune dysfunction. Almost one in four HCT recipients who contract SARS-CoV-2 develop severe COVID infection, and one in six have a COVID-related death. In the general population, short telomeres are associated with worse COVID prognosis. In this study, we examined the relationship between patient and donor leukocyte telomere length (LTL) measured before HCT and patients' COVID severity after HCT.
Methods: We included 87 HCT recipients who were hospitalized for COVID-19 infection and had pre-HCT blood samples for both the patient and matched donors available at the Center for International Blood & Marrow Transplant Research (CIBMTR). Study outcomes were: 1) COVID-19 severity defined as mild (no oxygen needed); moderate (oxygen needed, but not mechanical ventilation); or severe (mechanical ventilation needed); and 2) patient survival four months after hospitalization. Telomere Shortest Length Assay (TeSLA) was used to measure LTL. We used multinomial logistic regression and Cox regression to test the relationship between two LTL parameters— mean LTL and percent of telomeres < 3 kilobases (kb), which reflects accumulation of short telomeres– and study outcomes, controlling for patient and donor age, and patient sex.
Results: Ten patients (11%) developed severe COVID at an average of 4.7 years (SD= 3.5) after receiving HCT. Having severe COVID was associated with longer donor LTL (relative risk [RR]= 148.5, P= 0.005) and less accumulation of short telomeres in donors (RR= 0.8, P= 0.01), compared to having mild COVID. Furthermore, patients who had donors with the longest mean LTLs (≥ 5 kb) had a 21 times higher risk of mortality (95% CI= 1.5- 291; P= 0.02) than patients who had donors with the shortest mean LTLs (< 4 kb). Patient LTL parameters were not associated with COVID prognosis.
Conclusions: Our results support the hypothesis that longer donor LTL in HCT recipients with COVID may be associated with an overactive innate immune response, due to increased telomere reserves that enable neutrophil proliferation, in the presence of an HCT-related, defective adaptive immune response. This immune imbalance may result in an uncontrolled cytokine storm, which damages organs (e.g., lungs resulting in respiratory distress) and leads to death.

National Cancer Institute - Center for Cancer Research

4. Sooraj R Achar, B.S.

Engineering TCR-controlled Fuzzy Logic into CAR T Cells Enhances Therapeutic Specificity
Mentor: **Dr. Gregoire Altan-Bonnet**
Study Section: **Immunology – Immunotherapy**

Chimeric Antigen Receptor (CAR) T cell immunotherapy represents a breakthrough in the treatment of hematological malignancies, but poor specificity has limited its applicability to solid tumors. In contrast, T cells armed with T cell receptors (TCRs) display superb specificity but can also exhibit limited tumor cytotoxicity. We used a robotic-assisted high-throughput platform to systematically test whether combinations of TCR and CAR stimulation can overcome these defects. We discovered that TCR antigens continuously modulate CAR activation, with effects ranging from antagonism of CAR activation with weak TCR antigens to enhancement of CAR activation with strong TCR antigens. A mathematical model that utilized an intracellular inhibitory module to transfer information between the TCR and the CAR was able to capture the effects of this fuzzy logic in CAR T cells across a wide range of immune environments. Furthermore, this model predicted that even the low antigen densities frequently found on solid tumors would be sufficient to provoke antagonism of CAR signals. We therefore evaluated this prediction in vivo in a MHC-low mouse melanoma model, and found that that multiple aspects of CAR T cell efficacy, including tumor burden, frequencies of reactive tumor infiltrating lymphocytes, and overall survival of mice harboring this melanoma, continued to be antagonized by expression of weak TCR signals on the surface of the tumor cells. However, these findings also raised the intriguing possibility that triggering TCR/CAR antagonism in response to healthy cells could specifically reduce harmful off-target cytotoxicity against healthy tissues that express the same CAR target as tumors. To identify TCR/antigen pairs that displayed this type of behavior, we applied our mathematical model to a large dataset of TCR antigens and found that tumor neoantigens of moderate strengths were likely to have an antagonizing self-antigen counterpart. This key insight enabled us to identify a neoantigen-specific TCR that antagonized CARs when responding to its self-antigen. We then used this TCR to engineer dual TCR/CAR T cells which efficiently eradicated tumors with minimal toxicity against healthy tissue in a humanized in vivo model. These exquisitely-tuned Antagonism-Enforced Braking System (AEBS) CAR T cells, which harness pre-existing inhibitory crosstalk between receptors to enhance therapeutic specificity, pave the way for the design of more precise cancer immunotherapies.

5. Mahesh Agarwal, PhD

Nef from primary HIV-1 infection counteracts human IFITM3 and restores virion infectivity

Mentor: **Dr. Alex A Compton**
Study Section: **Immunology - Innate and Cell-mediated Host Defenses**

Interferon-induced transmembrane protein 3 (IFITM3) is a restriction factor that reduces retroviral infectivity by incorporating into virions, inhibiting Env function, and reducing virion entry into cells. We previously reported that the accessory protein of murine leukemia virus, glycosylated Gag (glycoGag), counteracted human IFITM3. glycoGag shares certain functions with HIV-1 Nef—namely, the capacity to antagonize SERINC3/5. Here, we demonstrate that Nef from primary HIV-1 also exhibits the capacity to counteract human IFITM3, while lab-adapted Nef has lost this activity.
Using Nef from diverse viral strains, we found that Nef from a primary isolate of HIV-1 (97ZA012, clade C) fully restored HIV-1 infectivity in the presence of IFITM3, while Nef from some molecular clones of HIV-1 did so to a lesser extent. By performing proximity ligation assays in intact cells and co-immunoprecipitations in lysed cells, we found that Nef 97ZA012 interacted with IFITM3, while Nef NL4.3 interacted with IFITM3 to a lesser extent. Nef 97ZA012 mutated at the myristoylation site and basic patch (both contributing to membrane association) exhibited reduced binding to IFITM3. Importantly, the degree of interaction between Nef and IFITM3 was functionally associated with the ability of Nef to restore virion infectivity. Furthermore, Nef 97ZA012 mutated at sites shown to be important for binding to AP-2 exhibited reduced counteraction of IFITM3. Therefore, how Nef counteracts IFITM3 resembles how Nef downmodulates CD4 from the cell surface. Accordingly, we found that Nef 97ZA012 reduced surface levels of IFITM3 and reduced levels of IFITM3 incorporated into virions. Mechanistically, we demonstrate that primary Nef restores HIV-1 infectivity by increasing membrane fluidity in living, virus-producing cells. Using a consensus Nef from 105 of individuals diagnosed with acute clade C HIV-1 infection, we found that antagonism of IFITM3 is a conserved feature of primary HIV-1 infection where clade C is prevalent. We also demonstrate that Nef from other, geographically distinct clades of HIV-1 also exhibit the capacity to bind and counteract the antiviral activity of IFITM3, albeit to different extents. Lastly, we found that binding to and counteraction of human IFITM3 was functionally conserved among Nef from clade B transmitted/founder HIV-1 isolates from the United States. Our results suggest that counteraction of IFITM3 may be important for the seeding of HIV-1 infection in vivo.

6. Andrea Arrieche Suarez, Predoctoral

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Mentor: **Dr. Leslie N Aldrich**
Study Section: **Chemistry**

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7. Saad Atiq, MD

Assessing the In Vivo Tolerability and Efficacy of Enfortumab Vedotin with Pembrolizumab and N-803 in Metastatic Urothelial Carcinoma
Mentor: **Dr. Andrea Apolo**
Study Section: **Clinical and Translational Research - Animal Models**

Urothelial carcinoma (UC) is associated with intrinsic and acquired resistance to chemotherapy. Enfortumab Vedotin (EV) plus pembrolizumab was recently approved based on the results of EV-302 where the combination improved overall survival (OS) compared with chemotherapy in patients with metastatic UC (mUC). N-803 is a recombinant human superagonist IL-15 complex which activates NK and CD8+ T cells. N-803 has been shown to re-invigorate immune responses in patients previously treated with immunotherapy for non-small cell lung cancer and with Bacille Calmette-Guérin (BCG)-unresponsive UC. We hypothesize N-803 will augment the immune response seen in EV plus pembrolizumab and improve outcomes. Nectin-4 expressing MB49-Luc cells were grown in culture and 2.5 million cells were injected subcutaneously into 8-week-old B6 albino mice to establish tumor growth. For the tolerability study, 4 cohorts each containing 3 mice were established and treated with EV, anti-PD-1, and N-803 monotherapy, and the fourth cohort was treated with the triplet combination. For the efficacy study, 8 cohorts of 10 mice each were established and treated with doublet combinations of the drugs as well as the triplet combination. Tumor growth was captured by direct caliper measurement and tumor growth inhibition (TGI) was calculated using Kaplan-Meier method and log-rank test to determine average tumor reduction. Mean OS was compared between cohorts using Mann-Whitney U tests. For the tolerability study, we did not observe any change in mean body weight in the mice following dosing of treatment or any unexpected toxicities. In the efficacy study, the highest TGI was seen in mice treated with the triplet regimen of EV with anti-PD-1 and N-803, with 50% experiencing complete tumor regression with no evidence of recurrence after 6 months from treatment. Furthermore, no unexpected toxicities were observed in the efficacy study. Mean OS was also highest in the group treated with the triplet regimen, with 50% of mice still alive at the 6-month timepoint. These findings suggest the safety and efficacy of the EV, anti-PD-1, and N-803 triplet therapy in murine models. Further studies will explore metastatic models to elucidate treatment differences and investigate mechanisms of resistance. Additionally, a clinical trial is planned to evaluate the role of triplet therapy in mUC patients, aiming to translate these preclinical results into clinical practice.

8. Sumirtha Balaratnam, PhD

RNA G-quadruplex structure in KRAS mRNA as a Target for Small Molecules to control the KRAS expression

Mentor: **Dr. John Schneekloth**
Study Section: **Chemistry**

KRAS is a small guanosine triphosphatase (GTPase) and stand out as one of the most prevalent gain-of-function alterations identified in many cancers, notably in pancreatic ductal adenocarcinomas, colorectal carcinomas, and lung cancers. Constitutively activated KRAS mutation activates downstream phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways and contributing to tumor maintenance. Like all RAS-family proteins, attempts to directly target KRAS with small-molecule have been largely unsuccessful due to its small size and a single binding pocket with a picomolar affinity for GTP. To date, the covalent inhibitors has shown clinical benefits against the KRASG12C mutant, but non-G12C mutants remain undruggable. An alternative approach would involve targeting the KRAS mRNA. Our current efforts have focused on targeting a structure found in its mRNA and preventing the KRAS translation. The 5' untranslated region (5'UTR) of the KRAS mRNA is reported to contain a non-canonical secondary structure G-quadruplex (GQ), that regulates the translation process of KRAS mRNA. Stabilizing the GQ structure in KRAS by small molecules provides an alternative approach to reduce KRAS expression in cancer cells. However, a major barrier in developing biologically active small molecules that bind to nucleic acids has been the identification of selective interactions. Previous approaches have generally yielded pan-GQ binding molecules, and strategies to generate selective ligands are lacking. Here we use a small molecule microarray screen to identify a small molecule that selectively bind to the GQ located 5'UTR of the KRAS mRNA. Biophysical studies, including, MST and SPR, demonstrate that compound 1 binds reversibly to the KRAS-GQ structure with lower micromolar binding affinity. A Luciferase based reporter assay indicated that compound 1 inhibits the translation of KRAS via stabilizing the KRAS-GQ. In addition, compound 1 have capacity to reduce cell viability, and decreased the cell viability in KRAS dependent cell lines. To obtain a better binder for KRAS-GQ target, we are currently performing a structure-activity relationship (SAR) study by using a series of analogs of compound 1. Here we demonstrate that the SMM approach can reveal a selective GQ binder for oncogene inhibition. Efforts toward applying SMMs to other GQ-associated oncogenes are being pursued to discover new selective binding scaffolds.

9. Alice Browne, Cancer Medicine

Investigating ECM Remodelling in Tumorigenesis: Hyaluronidase -Expressing Genetically Engineered Mesenchymal Stromal Cells Remodel the Hyaluronan-rich Tumor Extracellular Matrix
Mentor: **Dr. Rosandra Kaplan**
Study Section: **Oncology - Development and Metastasis**

The extracellular matrix (ECM) is a vital component of cancer pathophysiology. However, little is known about how to effectively modulate the ECM for therapeutic benefit. Hyaluronic acid (HA) is a polysaccharide component of the ECM with increased deposition during tissue injury or inflammation. HA in the tumor microenvironment (TME) has been linked to aggressive cancer progression. We have found enhanced ECM remodeling behavior linked to pre-metastatic niche formation and metastatic progression. To remodel this ECM-enriched microenvironment, we developed HA-targeting genetically engineered mesenchymal stromal cells (GEMesys) expressing hyaluronidase (Hyal), the enzyme responsible for HA degradation. We examined the impact of Hyal-GEMesys in osteosarcoma (F42010), rhabdomyosarcoma (M-3-9M), and pancreatic cancer (Panc02) syngeneic murine models. Hyal-GEMesys delivered to tumor-bearing mice homed to primary tumor sites and successfully degraded HA in the TME. The degradation of HA also resulted in a significant remodeling of collagen. Our findings also shed light on the immunomodulatory effects of HA. Following Hyal-GEMesy treatment, we observed significantly reduced myeloid cell populations and tumors display an activated CD4+CD44+ phenotype with a reduction of LAG3+PD1+ exhaustion markers. These alterations demonstrate we disrupt this immunosuppressive milieu, indicating a transition towards a heightened and effective anti-tumor immune response. Additionally, Hyal-GEMesy treatment results in a significant decrease in hypoxic regions within tumors and reorganization of the tumor vasculature to a more normalized phenotype, marked by diminished CD31 expression. Hyal-GEMesy treatment reduces tumor volumes in osteosarcoma, rhabdomyosarcoma, and pancreatic cancer models and enhances chemotherapeutic efficacy. These findings suggest that reorganizing the tumor ECM with Hyal-GEMesys holds the potential to dismantle the protective stroma of the tumor, which often shields malignant cells from therapeutic reach, and combining anti-cancer and anti-stroma agents can enhance anti-tumor efficacy and patient outcomes.

10. Shreya Chappidi, BA

Using natural language processing methods with varying model complexity to extract progression free survival outcomes in glioblastoma from free text electronic health record data
Mentor: **Dr. Andra V Krauze**
Study Section: **Artificial Intelligence - Machine Learning**

Introduction: Progression free survival (PFS) is a critical clinical outcome for analysis of terminal cancer subpopulations with more aggressive disease. PFS is often missing from data sets and publications due to the subjective, expert, and time-/data-intensive nature of generating these metrics. To harness PFS as an outcome endpoint, we developed and compared two natural language processing (NLP) machine learning frameworks employing free text radiology reports.

Data: 100 pathology-proven glioblastoma patients were included in the study, resulting in 1146 post-treatment brain MRI radiology reports. Ground truth PFS dates were generated by a multidisciplinary neuro-oncology team applying any available patient data to the current clinical gold standard, Response Assessment in Neuro-Oncology criteria (PFS median 269 days; range 57–4264 days).

Method: Two NLP methods with varying model complexities were employed and compared in terms of performance, usability, and decision transparency. The first method implemented a local approach using a small, tunable biomedical language model from the spaCy NLP package in Python. We pre-processed all report text, filtered for progression-related terms, and implemented contextual rules to handle negations, semantic ambiguity, sentence complexity, and historical mentions. The second large language model (LLM) method queried GPT-4 with a fine-tuned, zero-shot text summarization prompt to generate per document structured PFS labels.

Results: Both approaches revealed an accelerated progression timeline with median PFS calculated 67 [local] and 70 days [LLM] prior to ground truth PFS. The LLM pipeline had slight performance gains (accuracy 66%; precision 75%) over the local approach (accuracy 59%; precision 69%). Despite lower performance, the interactive visualization framework provided opportunities for all user types to interpret and iteratively tune the local algorithm. In contrast, the stochastic nature of LLM outputs required time-intensive verification due to a lack of decision evidence and large formatting variations.

Conclusion: This study describes a novel application and comparison of two NLP methods to generate PFS exclusively from radiology reports. Further study aimed at balancing model complexity with interpretability can generate robust PFS metrics for large scale data sets using only textual data, compared to the larger, multi-modal clinical data burden required for the current manual approach.

11. Priyank Chaturvedi, PhD

Incomplete synapsis and infertility in a mouse model of the human Immunodeficiency-Centromeric Instability-Facial Anomalies 4 (ICF4) Syndrome
Mentor: **Dr. Kathrin Muegge**
Study Section: **Genetics – Diseases**

Lymphoid specific helicase (LSH) is an epigenetic regulator and plays a critical role in heterochromatin formation. Human and mouse LSH are more than 95% homologue and are important for normal organ development. Mutations in the LSH gene lead to Immunodeficiency-centromeric instability-facial anomalies 4 syndrome, characterized by recurrent childhood infections and distinctive facial dysmorphisms. Whether LSH mutants in ICF4 patients play a role in male germ cell development remains unknown.

Utilizing a CRISPR-Cas9 engineered mouse model with a point mutation of the LSH gene (L801del), as observed in ICF4 patients, we investigated the role of LSH in germ cell maturation and its potential implications for the reproductive health of male ICF4 patients. Our fertility assessment revealed that male L801del mutant mice were infertile, with Periodic acid–Schiff (PAS) staining indicating the absence of mature spermatozoa and reduced testicular size in adult specimens. Examination of Hematoxylin and Eosin (H&E) staining on cross-sections of 15-day postpartum mice testes highlighted the absence of germ cells at the pachytene stage of meiosis. This absence was further confirmed by the lack of XY body immunostaining, an indicator of the pachytene phase. The failure to enter pachytene suggests a disruption in early meiotic events required for genetic recombination such as programmed DNA double-strand break (DSB) formation, end-resection, and the alignment of homologous chromosomes. We employed END-seq technique to profile DNA-DSBs and observed normal DSB formation at meiotic hotspots between LSH mutants and controls, indicating no defect in the generation of DSBs and their processing. However, immunostaining of chromosomal axial and transverse elements (SYCP1 and SYCP3) revealed markedly reduced synapsis in LSH mutants, implicating faulty synaptonemal complex formation. Notably, LSH mutants also displayed aberrant chromatin architecture in both autosomes and the XY body, which may contribute to the observed synapsis anomalies rather than irregularities of DSBs processing.

Our results indicate that LSH plays a pivotal role in synaptonemal complex formation and normal male germ cell development. Our study shows infertility in L801del ICF4 mutations and suggest fertility challenges in ICF4 patients, which has likely been not recognized yet, since patients are clinically treated for immunodeficiency during childhood.

12. Xuemin Chen, PhD

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Mentor: **Dr. Jordan L Meier**
Study Section: **Omics - Metabolomics/Proteomics**

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13. Zhijun Chen, PhD

Sparse autoencoder for feature extraction of metastatic prostate cancer images

Mentor: **Dr. Stephanie Harmon**
Study Section: **Bioinformatics - algorithms, packages and tools**

Advanced metastatic prostate cancer (mPCa) is characterized by substantial biological heterogeneity. Unlike the Gleason Grading System for localized prostate cancer, there is currently no established framework for the pathological assessment of mPCa. H&E images of mPCa contain rich information of cell morphologies. These morphologies are poorly differentiated, and may be associated with distinct expression profiles, such as neuroendocrine-like differentiation. We are interested in extracting features of morphologies from mPCa images and studying their correlations with patient outcomes. Deep learning-based methods are powerful tools for image analysis. However, due to heterogeneity (metastatic sites can present multiple morphologies) and lack of clinical standard, mPCa images are often unlabeled or weakly labeled. This nature makes supervised methods developed in other areas not suitable for our tasks. In this work, we proposed a novel sparsity-constrained convolutional autoencoder model for unsupervised image feature extraction. We used a dataset containing 856 cores sampled from 411 metastatic sites (bone, lymph node, lung, liver, prostate, etc) of 81 patients. In a subset of 52 patients, further molecular characterization was completed at core- and patient-level. This resulted in core-level characterization of immunohistochemical signatures for prostate cancer relevant markers, and patient-level genomic alteration labels. Each core image (size 5000x5000) was tiled at 250x250 pixels at 40x magnification. Our model was used to extract features from these tiles. Experiments showed that our model can perform feature extraction and clustering simultaneously. The feature clusters identified show association to clinically relevant molecular subtypes of mPCa and provide initial stratification of our dataset. We also fine-tuned our model for downstream tasks such as molecular tumor subtype detection and mutation prediction. Our model achieved comparable or superior performance to other benchmark models on these tasks. At core-level, our model achieved area under receiver characteristic operating curve (AUC) of 0.732 and 0.894 for androgen receptor (AR) and neuroendocrine (NE) detection respectively. At patient-level, our model achieved AUC of 0.604, 0.744 and 0.758 for PTEN, AR, RB1 alteration prediction respectively. Future work expanding to whole slide images and other downstream tasks is ongoing.

14. Desu Chen, Ph. D.

A Myosin II Lattice Directs Interstitial Neutrophil Migration in vivo by Controlling the Dynamics of the Leading Edge
Mentor: **Dr. Roberto Weigert**
Study Section: **Cell Biology – General**

Cell migration requires local membrane remodeling at the leading edge and the rear of the cell, which involve dynamic protrusions and retractions driven by cytoskeletal rearrangements. State of the art 2-photon intravital subcellular microscopy (ISMic) enables the acquisition of 4D movies of interstitial cell migration in live animals with adequate spatial and temporal resolutions to reveal submicron features. Here, I developed an automated quantification tool implemented in Python and MATLAB to investigate how local levels of non-muscle myosin IIA (NMIIA) and cell membrane remodeling dynamics drive the cell migration. By applying this tool to 4D movies, we can automatically segment, identify, and track every migrating single cell, and quantify migrating trajectory, speed, directionality, and distribution of the NMIIA-GFP for each individual cell. At the subcellular level, this tool enables automatic quantifications of the dynamics and coordination of cell membrane motion, cell membrane curvature, and cortical NMIIA localization for every local cell membrane section at around one-micron length scale and 6s time scale. The assembly, development, lifetime, and NMIIA dwelling time of each local membrane protrusion at the cell leading edge are monitored automatically, as well. With innovations in the statistical quantification dealing with both cell heterogeneity and temporal variability, all these single-cell and subcellular features are combined to define the cell migration phenotype. We found that 3D collagen gels mimic the modality of in vivo migration in terms of cell speed and membrane dynamics. In both conditions, NMIIA was recruited not only to the rear of the cell but also to leading edge. NMIIA recruitment correlated with high local membrane curvature but not local contractility. Notably, this phenotype was not observed in 2D under agarose in vitro assays where NMIIA is mostly concentrated at the rear of the cell and drives membrane retraction. Furthermore, we found that NMIIA at the leading edge ensures the persistence of cell migration direction, possibly through stabilization of the local protrusions at the leading edge. Finally, we found that the stabilization is mediated by the assembly of NMIIA into a lattice-like structure. Notably, disruption of the lattice by inhibitors of PI3 kinase activity induced reductions in the cell directional persistence and the level of NMIIA at the leading edge.

15. Achyut Dahal, PhD

Therapeutic Applications of a MUC4 Glycopeptide Targeting Monoclonal Antibody in Pancreatic Cancer
Mentor: **Dr. Joe Barchi**
Study Section: **Clinical and Translational Research - Drug Discovery**

Tumor-Associated Carbohydrate Antigens (TACAs) are aberrant glycan structures covalently linked to proteins and/or lipids on the surface of tumor cells. They are very different than the glycan repertoire presented on a normal cell phenotype, and hence can invoke an immune response that is protective against tumor growth and progression. TACAs are often presented on large glycoproteins called mucins, many that are overexpressed in different cancers. Mucin4 (MUC4) is aberrantly expressed in pancreatic ductal adenocarcinoma's (PDACs). MUC4 peptides, glycosylated with a specific TACA, the Thomsen Friedenreich antigen (Galb1-3GalNAca-O-Ser/Thr) were previously used as immunogens in vaccine constructs which led to the development of a tumor specific monoclonal antibody (mAb) referred to as F5. The present study investigated the tumor homing ability of F5 mAb in in-vivo PDAC mouse models.

F5 was fluorescently labeled with Frederick near infrared dye (F5-FNIR), and binding to the MUC4 glycopeptide was confirmed by SPR and ELISA. MUC4+ and MUC4- PDAC cell-derived xenograft models in immunocompromised mice were developed to determine the in-vivo tumor specificity of F5-FNIR. Fluorescently labelled F5 accumulated in various MUC4+ xenografts much more than those that were MUC4-. Excised organs and tumors showed significant localization of F5-FNIR in MUC4+ tumors. To assess F5 biodistribution in a more relevant model, F5-FNIR tumor homing in PDAC MUC4+ human patient derived xenografts (PDXs) was assessed. F5-FNIR specifically localized in MUC4+ PDXs compared to isotype control. These results led us to develop an F5 antibody-drug conjugate (ADC). We designed and synthesized an ADC with F5 conjugated to monomethyl auristatin E (MMAE) as a cytotoxic payload via a cathepsin B-sensitive valine-citrulline linker. Successful conjugation of MMAE to the F5 was confirmed by UV and mass spectrometry. The ADC was found to be significantly cytotoxic to MUC4+ PDAC cells compared to MUC4- cells. In addition, the ADC also exhibited cytotoxicity in 3D tumor organoids of MUC4+ PDAC cells. We are currently exploring in-vivo efficacy of F5-ADC in relevant tumor mouse model with a functioning humanized immune system with the Center for Advanced Preclinical Research (CAPR). Our data shows that F5 conjugates may have significant potential as a therapy against pancreatic cancer.

16. Whitney Do, PhD

Antigen reactivity profiles predict prognosis after immunotherapy treatment in hepatocellular carcinoma and cholangiocarcinoma
Mentor: **Dr. Xin W Wang**
Study Section: **Immunology – Immunotherapy**

While immunotherapy has become a first line treatment strategy for advanced liver cancer, efficacy remains heterogenous across both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) prompting a focus on finding biomarkers of response. Phage immunoprecipitation sequencing measures serum samples for reactivity to a comprehensive profile of viral and bacterial antigens including â‰¥115K peptides encompassing 1557 strains, providing a unique profile of immunocapacity. Here, we have examined the ability for antigen reactivity profiles (defined using the viral and bacterial strain reactivity) to stratify prognosis following immunotherapy treatment in HCC and CCA patients. The patient population derived from the NCI Clinical Center with patients undergoing immune checkpoint inhibition therapy or tyrosine kinase therapy. We divided each cohort into a training and test set and used XGBoost cox model to develop two models predicting all-cause mortality in patients with HCC (Training n=28, Testing n=7) and CCA (Training n=15, Testing n=14). The HCC and CCA models included 23 and 7 viral and bacterial strains which discriminated high vs. low survival, respectively, with two strains overlapping. The time dependent area under the curve (AUC) for the test set was 0.8 at 1 year in both models stabilizing to 0.6 (4 years) and 0.7 (1.5 years) in HCC and CCA models, respectively. The HCC model significantly predicted survival in the training set in the 4 year follow-up period (Hazard ratio [HR] Training [95% Confidence Interval {CI}]: 81.7 [9.88, 10690.96], p-value=9.9E-08; HR Testing [95% CI]: 2.36 [0.42, 12.96], p-value=0.36) and the CCA model significantly predicted survival in both the training and test set in the 2 year follow-up period (HR Training [95% CI]: 41.38 [4.67, 5475.69], p-value=9.8E-05; HR Testing [95% CI]: 3.76 [1.02, 13.88], p-value=0.04). In both models, we found the antigen score remained associated with survival after adjustment for multiple clinical and sociodemographic parameters. We tested the HCC model in an external cohort (n=30) from the Clinica Universidad de Navarra (CUN) in Pamplona, Spain. We found the HCC model could validate in this cohort, though the results were slightly above our threshold for significance (HR Validation [95% CI]: 2.38 [0.98, 5.76], p-value=0.054). Overall, these data suggest microbiome reactivity profiles could be unique non-invasive biomarkers of response to systemic therapy in liver cancer populations.

17. Ben Donovan, PhD

The Kinetic Basis of Splice Site Selection
Mentor: **Dr. Dan Larson**
Study Section: **Gene Expression - Postranscriptional Regulation**

Among the first steps of spliceosome assembly is 3' splice site (3'SS) recognition by the U2AF heterodimer. However, U2AF binds pervasively throughout the entire pre-mRNA. A long intron, for example, may contain nearly a hundred U2AF binding sites. In light of this, we sought to uncover the characteristics of U2AF binding that lead to productive spliceosome assembly at the correct location. In vitro high throughput binding assays uncovered the U2AF binding affinity at every 3'SS in the human transcriptome. Surprisingly, these data reveal that 3'SSs generally contain low-affinity U2AF binding sites. Despite this narrow distribution of RNA binding affinities, we observe a broad distribution of U2AF dwell times in live-cell single-molecule tracking assays which we show, through a combination of complementary in vitro single-molecule assays, reflects a wide range of processes, from initial binding site sampling to involvement in spliceosome assembly. Importantly, these experiments establish an approach to uncover the kinetic regulation of splice site selection (from the E- to the A-complex) on endogenous pre-mRNAs and suggest a model where specificity is refined as the spliceosome progresses towards the A-complex. To unify our data, we established a mathematical model that predicts splice site choice based on underlying U2AF binding affinity and our measurements of spliceosome assembly kinetics in live cells. This model successfully predict alternative splicing patterns observed in RNA-sequencing data and indicates that the bulk of splice site selection occurs while U2AF is in complex with the spliceosome, not during initial binding. In light of this role for U2AF during spliceosome assembly, we performed IP-MS experiments to identify proteins that coordinate splice site selection with U2AF. Surprisingly, the most enriched splicing factor is DDX42, an RNA helicase that competes with the helicase DDX46 to bind the SF3B1 component of the U2 snRNP. Interestingly in orbital tracking assays, where U2AF binding and pre-mRNA splicing are observed simultaneously, DDX42 knockdown stabilizes U2AF

binding at all sites, weakening the ability of U2AF to discriminate annotated 3’SS from ‘incorrect’ 3’SSs in coding sequences. Together, these results provide new insight into how U2AF binding is interrogated during spliceosome assembly to ensure highly-specific 3’SS recognition.

18. Vernon J Ebegboni, PhD

ETS1, a target gene of the EWSR1::FLI1 fusion oncoprotein, regulates the expression of the focal adhesion protein TENSIN3
Mentor: **Dr. Natasha Caplen**
Study Section: **Epigenetics**

The primary oncogenic event in most cases of the pediatric tumor Ewing sarcoma (EWS) involves the translocation of EWSR1 locus and one of two members of the ETS family of transcription factors, FLI1 or ERG resulting in either EWSR1::FLI1 or EWSR1::ERG (collectively referred to as EWSR1::ETS). Recent studies have highlighted the potential for heterogenous EWSR1::ETS protein levels within a tumor resulting in some cells exhibiting de-repression of genes that regulate processes associated with metastasis. Individuals with metastatic EWS have five-year survival rates of less than 30%. However, the mechanistic basis for EWS metastasis remains poorly understood as these tumors harbor few mutations beyond the chromosomal translocation that initiates the disease. In this study, we evaluated the hypothesis that EWSR1::ETS proteins inhibit the expression of transcription factors that, if de-repressed because of heterogenous fusion protein levels, or the effect of external stimuli, could contribute to EWS metastasis. An integrated analysis of the transcriptome of multiple EWS cell lines (control and EWSR1::FLI1 or EWSR1::ERG-depleted), and the assessment of EWSR1::FLI1 binding and epigenetic marks (H3K27Ac, H3K9Me3, H3K27Me3, and H3K4Me3) assayed by CUT&RUN and/or ChIP-seq, along with the expression profiles of EWS tumors, highlighted EWSR1::FLI1â€™s repression of multiple transcription factors that regulate cell differentiation. Focusing on ETS1, we detected EWSR1::FLI1 binding and a H3K27me3 repressive mark at this locus. Ectopic expression of ETS1 in TC-32 cells at levels comparable to that observed following silencing of EWSR1:FLI1 identified 5555 genes as exhibiting altered expression, including 522 that ChIP-seq analysis indicate ETS1 regulates directly. One of these ETS1-regulated genes encodes TENSIN3 (TNS3), a focal adhesion protein. EWS cell lines expressing ETS1 (CRISPRa) exhibited increased TNS3 expression and enhanced movement compared to control cells. Specifically, control cells exhibited a distributed vinculin signal and a network-like organization of F-actin. In contrast, ETS1-activated EWS cells showed an accumulation of vinculin and F-actin towards the plasma membrane. Interestingly, the phenotype of ETS1-activated EWS cell lines depleted of TNS3 resembled the phenotype of the control cells. Critically, these findings have clinical relevance as TNS3 expression in EWS tumors positively correlates with that of ETS1.

19. Arwa Fallatah, PhD

Combined MEK and RET inhibition overcomes resistance to single agent RET inhibition in Medullary Thyroid Cancer
Mentor: **Dr. Javed Khan**
Study Section: **Clinical and Translational Research - Clinical Trials**

Medullary thyroid carcinoma (MTC) is caused by activating mutations in the RET proto-oncogene, contributing to 13% of all thyroid cancer mortalities. Patients with metastatic MTC treated with RET inhibitors (RETi) develop resistance through unknown mechanisms. We investigated mechanisms of resistance to RETi to develop combination therapies to circumvent this resistance. We used the TT, MTC cell line and established two vandetanib-resistant (VanR) lines, TT_POB and TT_MW. A multi-omic approach was applied to investigate the mechanisms of resistance. This included Spectral karyotyping (SKY) and multiplex interphase Fluorescence in situ Hybridization (miFISH), to study karyotype, ploidy, and heterogeneity at a single-cell level. Whole exome and RNA-sequencing were done to identify genetic alterations, acquired mutations, copy number variations, and gene expression changes. Genome-wide CRISPR gene knockout (KO) was performed in TT cells treated with selpercatinib or vehicle to develop potential synergistic targeted combination therapy with RETi. Additionally, we performed a cytotoxicity screening using the Mechanism Interrogation Plate (MIPE6.0) library containing 2803 investigational drugs, novel agents, and FDA-approved drugs with known mechanisms of action. SKY and miFISH results showed that, for the parental TT_POB cell line, there were 4 copies of chromosome 10 (location of the RET gene), whereas there were 3 copies in TT_POB VanR. For the latter, this was associated with an increase in the RET variant allele frequency, indicating a loss of heterozygosity of the wildtype allele, which was confirmed by whole exome sequencing. For TT_MCW VanR, we identified a new solvent front RET mutation (G810S) with an increase in total RET expression. CRISPR KO demonstrated that the knockout of NF1, with increased RAS pathway activation, led to resistance to selpercatinib. The MIPE6.0 screening confirmed resistance to RETi in TT_MCW VanR and sensitivity to multiple MEK inhibitors. In vivo testing in a xenograft mouse model confirmed the synergistic/additive activity of combined selpercatinib with trametinib, a MEK inhibitor. The study concludes that resistance to RETi occurs through an adaptive increase in RET signaling through secondary mutations in RET and an increase in expression or dose of the mutant RET allele. These results are being developed into a clinical trial for patients with relapsed, refractory MTC.

20. Kristen Fousek, Ph.D.

Characterization of the anti-tumor activity of memory cytokine enriched NK cells against tumors with neuroendocrine features
Mentor: **Dr. Claudia Palena**
Study Section: **Immunology - Tumor Immunology**

Neuroendocrine neoplasms (NEN) consist of slow growing neuroendocrine tumors and highly proliferative neuroendocrine carcinomas. The incidence of NEN continues to rise, and despite the heterogeneity observed, treatments used are those approved for small cell lung cancer (SCLC), a very aggressive tumor classically known as NE. Immune checkpoint blockade (ICB) in combination with chemotherapy is approved in extensive stage SCLC, but only a subset of patients experience improved survival. Lack of response to ICB is often attributable to low expression of MHC-class I, which is needed for antigen presentation to T cells. Our group recently published that the lack of MHC-class I can instead be utilized to enable targeting by NK cells. We found that NK cells stimulated with an IL-15 cytokine superagonist (N-803) were able to effectively target SCLC of all phenotypes. This led us to hypothesize that cytokine stimulated memory-like NK cells may be effective in targeting SCLC and other types of NE tumors. In the present study, the ability of memory cytokine enriched NK cells (M-ceNK) to target human NE cell line models is evaluated. M-ceNK are derived from an apheresis product (from healthy donors or cancer patients) and exposed to a cocktail of cytokines including N-803, IL-12, and IL-18 until a highly purified CD3- CD56+ cell population results. Characterization across many donors indicates that M-ceNK are highly activated NK cells exhibiting increased natural cytotoxicity receptors (NKp30, NKp44, NKp46), minimal inhibitory markers (KLRG1, TIGIT), and elevated IFN-gamma and Granzyme B production compared to healthy donor NK cells. The functional killing capacity of M-ceNK was assessed via in vitro immune cytotoxicity assays; M-ceNK demonstrated a median of 69% lysis (range 35-89%) at an effector to target ratio of 5:1 across 6 SCLC models (DMS79, H69, H446, H1048, DMS114, H841) as well as 66% and 43% lysis respectively in NE prostate cancer (H660) and lung cancer (H720, H727) models as compared to 2% lysis (range 0-58%) with healthy donor NK cells. Furthermore, M-ceNK provided significant anti-tumor efficacy in two xenograft models of SCLC (H69, DMS79) when administered with N-803 in vivo. Patients with NE tumors have few good treatment options; these data demonstrate the potential of M-ceNK for the treatment of NE tumors, including all subtypes of SCLC. Future studies will also evaluate M-ceNK in the settings of other ICB-refractory tumors.

21. Annie K Gilbert, PhD

Identification of lncRNA binders to investigate chromosome instability in cancer
Mentor: **Dr. Yamini Dalal**
Study Section: **RNA Biology**

Centromeric protein A (CENP-A) is a histone H3 variant essential for recruiting microtubule attachment to the centromere, ensuring proper chromosome segregation during cell division. Accurate localization of CENP-A is mediated by its centromeric-specific chaperone, HJURP, and centromeric non-coding RNA. In cancer cells, CENP-A is overexpressed and deposited ectopically (outside the centromere) at fragile chromosomal sites using non-centromeric chaperones, ultimately leading to mitotic defects. Recent work showed that like centromeric non-coding RNA, oncogenic long non-coding RNA (lncRNA) from these ectopic sites facilitate CENP-A recruitment. Oncogenic lncRNA has emerged as a promising therapeutic target because of its specificity and often structure-based function that could be targeted with small molecules. Small molecule microarrays enable the screening of thousands of drug-like small molecules to identify selective lncRNA binders that could disrupt or stabilize functional interactions. Using this technology, we set out to identify small molecule lncRNA binders that target ectopic CENP-A deposition as a novel therapeutic route to treat cancer progression. We focused our work on an oncogenic lncRNA, Prostate Cancer Associated Transcript 2 (PCAT2), that has been shown to recruit CENP-A to the c-MYC locus. Using in vitro transcription, we prepared PCAT2 RNA which we subsequently fluorescently labeled allowing for small molecule screening. With the fluorescently labeled PCAT2 RNA, we screened through 21,000 drug-like small molecules using small molecule microarrays. In total, we identified 30 selective hits. We are now testing whether these hits are functional in cells. Using high-throughput imaging, we are performing DNA Fluorescence in Situ Hybridization (FISH) combined with immunofluorescence to test if CENP-A deposition at the c-MYC locus is affected in the presence of the binders. We anticipate these small molecule lncRNA binders will provide novel drugs that regulate ectopic CENP-A for the first time in cancer. Furthermore, this approach is broadly applicable and expands the chromatin toolbox for deciphering epigenetic roles of lncRNA.

22. Sarah C Hoelscher, PhD

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Mentor: **Dr. Sergio Ruiz-Macias**
Study Section: **Stem Cells - General and Cancer**

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23. Stefan Katharios-Lanwermeyer, PhD

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Mentor: **Dr. Anupama Khare**
Study Section: **Microbiology and Antimicrobials**

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24. Veer I Keizer, PhD

Getting a hold on chromatin: magnetic micromanipulation of chromatin in live cells
Mentor: **Dr. Dan Larson**
Study Section: **Chromosomes, Chromatin, and Nuclear Architecture**

To gain a better understanding of material properties of chromatin and successfully link them to chromatin organization and functions such as transcription, we need to develop tools to actively and directly interrogate these links. To address this gap, we have developed a novel method to actively manipulate a genomic locus inside the nucleus of a living human cell. By targeting iron-containing nanoparticles to a specific genomic locus and applying a controlled magnetic field, we were able to physically move chromatin through nuclear space for the first time. We observe partially reversible stretching of chromatin highlighting its' viscoelastic nature. Moreover, long-range motion of chromatin through the nucleus was possible with little hindrance.

We could accurately recapitulate the observed behavior with a Rouse model that included only a weak obstructive effect of the surrounding chromatin and nucleoplasmic material. This challenges the view that interphase chromatin is a gel-like material has important implications for conformational dynamics that may occur within the nucleus. Using the developed method and model, we describe some of the structures present in the nucleus, such as the nuclear periphery as well as the energy barrier that is potentially associated with interphases between chromatin densities. This allows us to better understand the range of motion possible within the organization of the nucleus.

Our results indicate that the forces generated by SMC complexes would be sufficient to substantially reorganize the genome in space and could dictate interactions between enhancer and promoters. To further investigate this premise and infer the causal link between chromatin organization and transcription, we extended our magnetic micromanipulation technique. In brief, by aligning the dipoles of two magnetic loci through the approach of an external magnet, two loci can be attracted to or repulsed from each other. We present a first proof-of-principle for this novel method using an in vitro reconstituted system and characterize the forces that these loci can impose upon eachother. This system allows us to control the chromatin organization that dictates enhancer-promoter interactions and visualize the direct effects of their interactions on gene expression in live cells.

25. Young-Im Kim, phD

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Mentor: **Dr. Jing Huang**
Study Section: **Immunology - Tumor Immunology**

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26. Jayeeta Kolay, Ph.D.

Unravelling Centromeric Evolution by Nucleosome Elasticity
Mentor: **Dr. Yamini Dalal**
Study Section: **Chromosomes, Chromatin, and Nuclear Architecture**

Faithful chromosome segregation, which is indispensable for the survival of all organisms, is evolutionarily conserved across most eukaryotes. Despite the function being preserved, both centromeric DNA and kinetochore are rapidly evolving, and these contrasting aspects of centromere biology are known as the "centromere paradox". This motivates us to understand the mechanistic basis of centromeric evolution. Unlike canonical histone proteins which are nearly invariant through evolution, centromeres are epigenetically defined by the fast-evolving histone H3 variant CENP-A. Recently we reported that human CENP-A nucleosomes are two-fold more elastic than canonical H3 nucleosomes and get stiffer in the presence of kinetochore protein CENP-C in vitro. In vivo studies showed that CENP-C overexpression reduced centromeric transcription. Therefore, mechanical properties of nucleosomes have its implication on the transcriptional machinery of chromatin. In this work, we intend to investigate whether CENP-A elasticity is an intrinsic property of human only or is this a conserved feature across eukaryotes and co-evolving with kinetochore proteins. As different species vary in their kinetochore composition where centromeric genes are either lost, duplicated, sometimes invented, and displaced. So, we selected CENP-A from different eukaryotic models, out of which Homo sapiens, Arabidopsis thaliana is with regional centromeres and Saccharomyces cerevisiae contains point centromere. Herein, we aimed to compare their nucleosome elasticity using single-molecule nanoindentation atomic force spectroscopy. Preliminary results indicate that human CENP-A nucleosomes are the most elastic, while budding yeast Cse4 is the least. Two distinct elasticity populations were observed for Arabidopsis CenH3 nucleosomes with the lower resembling human CENP-A, and the higher resembles human CENP-A bound to CENP-C. This could be due to the size of human centromere tandem repeat (171 bp) is in same range as that of Arabidopsis with 178 bp in length. Whereas the budding yeast point centromere is 125 bp in size with AT rich CDEII DNA elements. Therefore, CENP-A elasticity may be centromere specific, and the conserved elasticity is observed for regional centromeres, but not for point centromeres. To understand the complete journey of centromeric evolution, centromere-specific nucleosomes from other species, and different histone variants need to be explored.

27. Anson Ku, PhD

Spatial transcriptomic identified initiator of adaptive immune response in advance prostate cancer
Mentor: **Dr. Adam G Sowalsky**
Study Section: **Computational Biology/Systems Biology**

Immunotherapies have shown durable anti-tumor response in many immunogenic tumors. Tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment (TME) is a predictor of immunotherapy efficacy. Unlike melanoma, locally advanced prostate cancer (PCa) is generally poorly infiltrated by lymphocytes unless tumor DNA repair proteins are loss leading to genomic hypermutation which occurs in 3 percent of the cases. The biology underpinning this observation is poorly understood with the consequences that many immunotherapies that are efficacious in melanoma, failed in clinical trials due to the lack of clinical response. To address this knowledge gap, we compared tissues from locally advanced PCa patients with unexplained TILs and those without TILs using spatial transcriptomics. Spatial transcriptomics, like single cell RNA, can profile the gene expression of individual cells while preserving the spatial organization crucial for understanding lymphocytes function in the TME. We first identified the expected cell types in the tissue then calculated the “nearest neighbor” to the lymphocytes in both groups. To our surprise, lymphocytes preferentially interact with a little-known cell type called “club cells” in the sample with TILs. Further investigation revealed that club cells harbor proteins that is crucial for activating lympohocytes suggesting that they can activate lymphocytes independently in localized PCa. Next, we validated the close proximity interaction between club cells and lymphocytes using fluorescent imaging on tissues with TILs. Taken together, our research, utilizing spatial transcriptomics, identified an unreported interaction between lymphocytes and club cells that could potential advance the use of immunotherapies in this disease setting.

28. Ravi Kumar, Ph.D

RNA-binding protein ZMAT3 regulates mitochondrial respiration by controlling HKDC1 transcription
Mentor: **Dr. Ashish Lal**
Study Section: **Gene Expression - Transcriptional Regulation**

RNA-Binding proteins play critical roles in diverse biological processes by regulating gene expression. Recently, we and others have shown that the p53-induced RNA-binding protein ZMAT3, functions as a key splicing regulator mediating the tumor-suppressive effects of p53, the most frequently mutated protein in human cancers. Here, we unexpectedly discovered a novel function of ZMAT3 in inhibiting transcription of HKDC1 (Hexokinase Domain containing 1) to inhibit mitochondrial respiration. HKDC1 is a putative hexokinase that plays an important role in glucose metabolism and mitochondrial function. HKDC1 caught our attention because it was the most significantly upregulated protein in our quantitative proteomics and the RNA-seq from wild-type and isogenic ZMAT3-knockout HCT116 (human colorectal cancer) cells. Consistent with these data, we found that depletion of ZMAT3 in multiple cell lines resulted in significantly increased HKDC1 expression, glucose uptake, mitochondrial respiration, and proliferation. These phenotypes were significantly rescued upon concurrent knockdown of HKDC1, suggesting that HKDC1 is a critical downstream target of ZMAT3. To determine the molecular mechanism by which ZMAT3 inhibits HKDC1 expression, we utilized our recently published ZMAT3 PAR-CLIP data where we had identified transcriptome-wide RNAs directly bound by ZMAT3 in crosslinked HCT116 cells. Interestingly, we did not observe direct binding of ZMAT3 on HKDC1 RNA. However, we noticed that ZMAT3 contains zinc finger domains that are well-established DNA-binding domains. We therefore reasoned that ZMAT3 may be implicated in DNA binding as well. Indeed, interrogation of unpublished ZMAT3 ChIP-Seq data (from ENCODE) revealed that ZMAT3 binds to the promoter of hundreds of genes, including HKDC1. Importantly, we were able to confirm direct binding and transcriptional repression of HKDC1 promoter by ZMAT3 using in vitro biotinylated DNA binding assays using purified recombinant ZMAT3 protein and luciferase reporter assays. Furthermore, mapping of the ZMAT3 binding site on the HKDC1 promoter revealed a 95-nucleotide region near the transcription start site as the ZMAT3-response element. Collectively, these unpublished findings uncover a novel function of ZMAT3 in transcriptional regulation of HKDC1 thereby inhibiting mitochondrial respiration and proliferation in the p53 pathway.

29. Rajesh Kumar, Ph.D.

Chromatin accessibility identifies novel stem like subtype in relapsed small cell lung cancer
Mentor: **Dr. Anish Thomas**
Study Section: **Computational Biology/Systems Biology**

Small cell lung cancer (SCLC) is a highly heterogeneous tumor characterized by widespread metastases and chemoresistance. SCLC is commonly classified based on retained vs. loss or reduced expression of neuroendocrine genes (NE and non-NE, respectively), but causative differences in the epigenetic regulation of these subtypes are largely unknown. We hypothesized that epigenetic differences at the level of chromatin accessibility might distinguish SCLC subtypes. By leveraging a cohort of 39 metastatic and relapsed patient-derived xenografts (PDXs) from the National Cancer Institute, we employed an integrative multi-omics including chromatin accessibility profiling (ATAC-seq), RNA sequencing (RNA-seq), single-cell RNA sequencing (scRNA-seq), and immunohistochemistry. Non-negative matrix factorization (NMF) was used to cluster ATAC-seq profiles, categorizing SCLC into three distinct subgroups. The largest subgroup (n=24) showed increased accessibility at ASCL1 and NEUROD1 loci, as well as neuronal and ganglion development genesets, representing NE SCLC. A second subgroup (n=4) showed increased accessibility at KLF10 and IRF8 loci, and as well as

antigen presentation genesets, representing non-NE SCLC. Additionally, a third subgroup (n=11) was observed, characterized by a non-NE signature but with pronounced accessibility for AP1 transcription factor subunits (FOS and JUN), stem-cell pathways, hinting at a previously unidentified non-NE stem cell-like (non-NE SC) subtype. This subtype showed enrichment of multiple stem-cell gene signatures in single-sample gene set enrichment (ssGSEA) and differential gene expression analyses of scRNA-seq and RNA-seq data. scRNA further revealed that the non-NE SC subtype shows an unexpected immune landscape, with significant clusters of T-regulatory cells and heightened T-cell proportions compared to other subtypes. Cell-cell communication using CellChat revealed high tumor cell-directed TGF-beta signaling in non-NE SC subtype. Altogether, we identify a novel SCLC subtype with stemness features, potentially regulated by TGF-beta, which explain the treatment resistance and relapse shown among patients.

30. Jay Kumar, Ph.D

Insight into the crystal structure and kinetics of Wip1/PPM1D phosphatase
Mentor: **Dr. Ettore Appella**
Study Section: **Protein Structure/Structural Biology**

The Wild-type p53-induced phosphatase, also known as Wip1 or PPM1D, is a member of the serine/threonine metal-dependent protein phosphatase 2C (PP2C) family. Wip1 is induced by the tumor suppressor p53 during the DNA damage response and acts as an oncoprotein in various human cancers. Amplification or overexpression of PPM1D is associated with unfavorable outcomes in multiple types of cancer. Overexpression of Wip1 compromises tumor suppressor functions, while mice lacking Wip1 expression show resistance to tumorigenesis. Although Wip1 is a potential therapeutic target, insights into its atomic structure are challenging due to flexible regions unique to Wip1 among PP2C phosphatases. Here, we report the first crystal structure of the Wip1 catalytic domain to 1.8 Å resolution. The structure reveals the active site with two bound Mg2+ ions alongside the flap subdomain and B-loop crucial for substrate recognition and catalysis. Although Wip1 exhibits low sequence homology with PPM family members, its core structure is similar to that of other family members. Our investigation into enzyme kinetics unveiled hysteretic behavior, indicating slow conformational transitions compared to catalytic turnover. Based on our kinetic data, we propose a model that suggests the enzyme undergoes a conformational change, resulting in two distinct conformations that exhibit diverse affinities for both the substrate and Mg2+. Additionally, these conformations display different catalytic activities. Our kinetic analysis supports the idea of slow enzyme conformational transitions, which is a unique discovery within this enzyme family. Furthermore, we identify a nitrogen-oxygen-sulfur (NOS) bridge in the crystal structure, which might serve as a redox switch or protect Wip1. While the NOS bridge is present in the crystal, its occurrence under biological conditions remains unproven. We engineered a mutant version of the NOS bridge and are currently investigating its biological implications and significance. Molecular dynamics simulations and kinetic studies provide additional mechanistic insights into the regulation of Wip1 catalytic activity. In conclusion, these results advance the understanding of Wip1 function and facilitate further development of specific inhibitors and activators of Wip1 phosphatase.

31. Manjari Kundu Sil, PhD

Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) induces cytokine production in triple negative breast cancer (TNBC) cells promoting neutrophil chemotaxis and immune suppression.
Mentor: **Dr. Stanley Lipkowitz**
Study Section: **Immunology – Immunotherapy**

TRAIL is a potential cancer therapeutic that selectively induces apoptosis in cancer cells sparing the normal. However, its efficacy in clinical trials is limited, suggesting unknown modulatory mechanisms responsible for lack of TRAIL activity in patients. Here, we hypothesize that TRAIL treatment elicits transcriptional changes in TNBC cells that alter the immune milieu. To address this, we performed a time course based RNAseq study of MDA-MB-231 cells treated with TRAIL, followed by validation in various TNBC cells. TRAIL treatment of the TNBC cells significantly induced expression of cytokines, such as CXCLs 1, 2, 3, 8,11 and IL6 which are known to affect neutrophil function. Mechanistically, induction of these cytokines was predominantly mediated by death receptor 5 and caspase-8 protein, but not caspase-8 enzymatic activity. GSEA of the RNAseq data indicated that NFKB pathway was significantly enriched. Concordantly, we confirmed by silencing key proteins and protein assays that the non-canonical NFKB2 pathway is primarily responsible for induction of the TRAIL induced cytokines. Chemotaxis of healthy human donor-isolated neutrophils was enhanced by the CXCLs and IL6 produced by the TRAIL treated TNBC cells, demonstrating the functional relevance of these cytokines. TNBC xenograft tumors from mice treated with the TRAIL showed induction of the NFKB2 pathways, increased production of CXCLs and IL6, and increased neutrophil accumulation (demonstrated by intravital imaging as well as CODEX). Additionally, preincubation of human donor neutrophils with supernatants from TRAIL-treated TNBC significantly suppressed their cytotoxic effect against TNBCs and showed reduced respiratory burst. TRAIL dependent alteration of neutrophils was further, confirmed by their transcriptomic analysis. Neutrophils incubated with either TRAIL or supernatant of TRAIL treated TNBC revealed significant enrichment of expression of inflammatory cytokine, immune modulating, and immune checkpoint genes (e.g., PDL1 and SILEC5). Functional studies with these neutrophils confirmed their suppressive effect on T cell function and increase in T Reg suppression. TRAIL also delayed neutrophil apoptosis. Collectively, our study suggests the novel role of TRAIL-induced NFKB2-dependent cytokine production promoting neutrophil chemotaxis and immune suppression. This study implies that alterations in the innate immune system may modulate the effects of TRAIL on TNBC tumors.

32. Yevgen Levdansky, Ph.D.

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Mentor: **Dr. Eugene Valkov**
Study Section: **Protein Structure/Structural Biology**

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33. Haojian Li, Bachelor

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Mentor: **Dr. Urbain Weyemi**
Study Section: **Cell Biology - Cell Cycle and Metabolism**

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34. Bilal Lone, Ph.D

Functional and clinical consequences of hotspot non-coding mutations upstream of the LEPROTL1 gene in bladder cancer
Mentor: **Dr. Rouf Banday**
Study Section: **Genetics – Diseases**

Recent whole-genome sequencing studies have unveiled intriguing hotspot mutations within non-coding genomic regions, yet their implications in cancer remain unknown. Here, we characterized the role of twin hotspot non-coding mutations upstream of the LEPROTL1 gene in bladder cancer: chr8: 29,952,919 G>A and chr8: 29,952,919 C>T. Either mutation is found in approximately 20% of bladder tumors, and the mutations do not typically co-occur. Through a comprehensive analysis integrating ChiP-seq, ATAC-seq, and Hi-C data from bladder cancer cell lines, we observed that these mutations manifest within an enhancer that interacts with three nearby genes - LEPROTL1, DCTN6, and SARAF. In vitro dual luciferase reporter assays showed that both mutations are functional, affecting promoter and enhancer activity. CRISPR-Cas9-mediated knocking out of the putative enhancer region identified LEPROTL1, DCTN6, and SARAF as target genes that are co-regulated, which was further supported by strong positive correlations of their mRNA expression in TCGA data. Isogenic cell clones generated by CRISPR-mediated base-editing showed that the mutations result in simultaneous upregulation of the three target genes, which were also expressed at higher levels in TCGA tumors harboring the non-coding mutations. Little is known about the function of LEPROTL1, DCTN6, and SARAF. Using base-edited cell lines and siRNA knockdown models complemented by live-cell imaging, viability assays, and cell cycle analysis, we found that these genes play a role in cell proliferation and cell cycle progression. The mutations were also associated with shorter disease-specific survival in a TCGA cohort, and LEPROTL1 and DCTN6 mRNA expression showed a negative association with overall survival. These results underscore the clinical significance of the non-coding mutations. Currently, we are evaluating the effect of these mutations on tumor growth and cisplatin response in vivo using xenograft mouse models. In conclusion, our data suggest that non-coding enhancer mutations near LEPROTL1 contribute to bladder carcinogenesis by increasing the expression of three co-regulated novel oncogenes, all of which were previously uncharacterized in bladder cancer. Since we found the mutations to be clinically significant, our study also raises the possibility of using the non-coding mutations and their target genes as clinical biomarkers and potential therapeutic drug targets.

35. Tania L Lopez Silva, Ph.D. in Chemistry

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Mentor: **Dr. Joel P Schneider**
Study Section: **Biophysics and Biomedical Engineering**

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36. Federico Machinandiarena, Pd.D.

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Mentor: **Dr. Kumaran Ramamurthi**
Study Section: **Cell Biology – General**

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37. Pankaj S Mahajan, PhD

Design, Synthesis and Evaluation of N-Substituted Bicyclic Carbamoyl Pyridones (BiCAPs) as Potent Integrase Strand Transfer Inhibitors (INSTIs) Against Cabotegravir-resistant HIV-1 Integrase Mutants

Mentor: **Dr. Terrence R Burke Jr.**
Study Section: **HIV and AIDS Research**

HIV-1 enzyme integrase (IN) catalyzes the insertion of the double-stranded viral DNA generated by reverse transcriptase into the host genome via two strand transfer (ST) reactions. Integrase strand transfer inhibitors (INSTIs) block the ST reactions by binding in the active site and chelating the two Mg2+ catalytic cofactors. The recently FDA-approved INSTI cabotegravir (CAB, 2021) is being used in long-duration formulations; however, missed doses can result in the persistence of unacceptably low levels of CAB. Low levels of the drug can select for resistant mutant forms of IN and subsequent virological failure in people living with HIV. Mutations near the IN active site can affect the positioning of the Mg2+ ions, impacting INSTI binding and reducing inhibitory potency. Our goal is to develop compounds that retain the ability to bind tightly to the active sites of resistant mutant forms of IN. We envisioned that the metal-chelating triad of heteroatoms contained within the bicyclic carbamoyl pyridone (BiCAP) of CAB could serve as the basis for simplified analogs. We removed the third ring of CAB and replaced it with simpler N-substituted modifications. This should allow the C—N bond with a greater rotational flexibility which may allow the metal-chelating triad of compounds to bind to drug-resistant IN mutants with changes in the active site arising from mutations in the IN protein. We designed N-substituted BiCAPs and developed a four-step one-pot synthetic protocol for their synthesis that gave ≥ 99% HPLC purity with only a single purification at the last step. We found that many of these new compounds potently inhibit WT and resistant mutant forms of HIV-1 IN in single-round replication assays without causing measurable cytotoxicity in cultured cells. Among the new BiCAPs, 7 exhibits the best antiviral profile against a panel of CAB-resistant mutants. Compound 7 is more potent than CAB against the clinically important drug-resistant mutants E138K/Q148K (> 12-fold) and G140S/Q148R (> 36-fold). Some of the new BiCAP-based compounds show greater than two-fold better efficacies than CAB against the important R263K and E138K/G140C/Q148R mutants. The antiviral data supports our original design hypothesis and provides information that can be used in the continued development of BiCAPs that are broadly effective against drug-resistant variants. Such molecules could potentially be used in long-acting formulations.

38. Marta A Markiewicz Potoczny, PhD

Nonsense-Mediated mRNA Decay (NMD) and its novel role in telomere protection in pluripotent stem cells
Mentor: **Dr. Eros L Lazzerini Denchi**
Study Section: **Gene Expression - Postranscriptional Regulation**

Chromosomes in eukaryotic cells ends up with “telomeres” – structures that consist of G-rich repetitive DNA and a DNA-associated protein complex. During ageing telomeres shorten progressively, but if they become too short the telomere lengthening may take place, or telomeres may stick together - both aberrant mechanisms can lead to disease, including cancer. To ensure organismal homeostasis mammalian cells protect their telomeres primarily by TRF2 protein that binds specifically to telomeric repeats. In somatic cells, TRF2 depletion is sufficient to induce end-to-end chromosome fusions, DNA damage response (DDR) activation and cell death. Surprisingly, our recent work showed that TRF2 is not involved in telomere protection in mouse embryonic stem cells (mESCs). To identify the alternative mechanisms of telomere protection in mESCs, we performed a synthetic lethal genome-wide CRISPR/Cas9-knockout screen in TRF2 deficient and TRF2-proficient cells. Amongst the identified pathways that could participate in telomere protection the top hit was NMD pathway. The NMD is an RNA quality control and gene regulatory mechanism conserved across eukaryotes with yet not fully understood function at telomeres. To test whether NMD pathway was involved in telomere protection in mESCs we knocked out 6 of NMD factors in mESCs where TRF2 can be conditionally deleted. We confirmed that co-depletion of TRF2 and NMD results in cell death, and found high levels of telomere de-protection, with accumulation of DDR factors at telomeres and frequent end-to-end fusions. Transcriptome analysis revealed that NMD controls the expression of several telomere-specific genes, such as TRF1 that performs telomere protective function along with TRF2. We observed that NMD-depleted mESCs have high levels of TRF1 transcript where exon 8 is excluded. Translation of such transcript results in loss of the DNA-binding MYB domain and abrogates TRF1 telomere protection function. Through western-blotting experiments we confirmed the presence of full-length and truncated TRF1 protein variants in NMD-depleted mESCs. We hypothesize that the levels of TRF2 and TRF1 must be tightly regulated in mESCs to ensure telomere protection. Now we are planning to recapitulate our findings with overexpression experiments where wild type and truncated TRF1 will be expressed in TRF2-deficient mESCs. Finally, we want to understand the role of NMD in telomere protection in differentiated cells.

39. Anthony Martini, Microbiology

Staphylococcus aureus Adapts to an Antimicrobial Produced by a Co-Infecting Pathogen Through Metabolic Suppression and an Enhanced Response to Peroxide Stress
Mentor: **Dr. Anupama Khare**
Study Section: **Microbiota**

During infection, pathogenic bacteria can interact with commensal microbes and co-infecting pathogens, potentially leading to altered fitness and virulence. Staphylococcus aureus is a human pathogen prevalent in chronic, polymicrobial infections such as chronic wounds and lung infections in people with cystic fibrosis. S. aureus frequently co-infects patients with another pathogen, Pseudomonas aeruginosa, and the simultaneous presence of both pathogens 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 coexist for years within patients, we hypothesized that S. aureus could develop tolerance to PYO as

part of this coexistence. Using experimental evolution where we repeatedly exposed S. aureus to PYO and selected for surviving cells, we identified mutations in a global regulator, codY, that were present in all our evolved isolates. Evolved isolates exhibited a 10 to 100-fold increase in survival upon PYO exposure compared to the parental strain. Deletion of the wild-type (WT) codY gene in the parental strain, or substitution with one of the evolved codY mutations, increased survival similarly to the evolved isolates, indicating that loss of codY specifically mediates PYO tolerance. We subsequently determined that a codY mutation provides protection against hydrogen peroxide and that mutants deficient in their response to peroxides were more sensitive to PYO, suggesting that the survival phenotype is due to an enhanced response to peroxides generated by PYO. Transcriptional analysis in the presence of PYO indicated that the codY mutant decreased expression of metabolic genes and increased expression of stress-responsive genes compared to WT. Artificially depleting ATP to reduce metabolism or supplementing cultures with a peroxide scavenger were both able to protect WT from PYO-mediated killing. Additionally, individual overexpression of genes that protect against peroxides was sufficient to increase WT survival to levels similar to the codY mutant. Together, our results indicate that loss of codY facilitates metabolic suppression and a stronger response to peroxide stress in the presence of PYO, thereby leading to increased survival. Our work thus reveals adaptations of S. aureus to PYO and suggests potential mechanisms for increased fitness in polymicrobial environments.

40. Tamara L McErlain, MSc

Pericytes influence disseminated tumor cell fate decisions
Mentor: **Dr. Meera Murgai**
Study Section: **Oncology - Development and Metastasis**

Metastasis is responsible for 90% of cancer related mortality. The formation of a pre-metastatic niche, a site that is considered favorable to tumor cell seeding and proliferation, promotes metastasis. Perivascular cells, including pericytes, promote the early development of a pre-metastatic microenvironment upon activation by circulating primary tumor derived factors. The microenvironmental changes associated with perivascular cell activation influence disseminated tumor cell (DTC) fate decisions, promoting metastasis. By live extravital imaging I observed that DTC interact with pericytes as they extravasate into the lung parenchyma. Therefore, it was hypothesized that direct interaction between pericytes and tumor cells in the early metastatic microenvironment could confer a survival advantage to DTC. Co-culture experiments were used to investigate the consequences of this interaction and revealed that primary lung pericytes transfer lipid containing contents to metastatic breast tumour cells (4T1), but not to non-metastatic cells (67NR). Gene expression data from metastatic 4T1 cells after direct co-culture with pericytes demonstrated activation of pathways related to syncytium formation, as well as inhibition of cell death pathways. In normal physiology, pericytes regulate blood flow by responding to mechanochannel activation induced by changing blood pressure. It was hypothesized that direct contact with tumor cells activates mechanosensitive pericytes to initiate lipid transfer. Lipid transfer was dependent on direct cell contact and was not observed with transwell assays or co-culture conditioned medium. Further, the use of mechanosensitive calcium channel inhibitors demonstrated a significant reduction in the transfer of lipids to tumor cells. When tumor cells isolated from co-culture are intracardiac injected into mice, increased colonization is observed in the lung, indicated by enrichment in the number of small lesions (less than 6 cells) compared to the monoculture group, and had low proliferation indicated by reduced ki67 status compared to lesions of the same size from the monoculture group. These data suggest that tumor cells are reprogrammed by direct pericyte contact in the early metastatic lung to aid persistence and colonization. Understanding the mechanisms by which pericytes aid tumor cell survival in early metastatic colonization may uncover new therapeutic targets to reduce metastasis.

41. Payel Mondal, Ph.D.

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Mentor: **Dr. Jing Huang**
Study Section: **Chromosomes, Chromatin, and Nuclear Architecture**

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42. Ameera Mungale, B.S. Microbiology

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Mentor: **Dr. Joseph Ziegelbauer**
Study Section: **Virology – General**

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43. Bega Murray, PhD

SWI/SNF ATPase Modulation of the DNA Damage Response in Malignant Peripheral Nerve Sheath Tumors Induces a Therapeutic Vulnerability in this Disease.
Mentor: **Dr. Jack Shern**
Study Section: **Oncology - Therapeutics and Translational Research**

Malignant peripheral nerve sheath tumors (MPNST) are rare and aggressive sarcomas of the peripheral nervous system. MPNST is typically therapeutically resistant; complete surgical resection with wide negative margins is the only treatment that statistically improves overall survival of patients. Therefore, the need to identify novel treatment options for MPNST is urgent. The PRC2 repressive complex is mutated in up to 80% of MPNST cases, leading us to theorize that an altered epigenetic regulatory network offers therapeutic opportunity in MPNST. This study investigated core components of SWI/SNF epigenetic regulatory complexes as therapeutic targets in MPNST.
Short-interfering RNA (siRNA) knockdown (KD) of multiple core SWI/SNF components identified that loss of SMARCA4 ATPase decreased MPNST cell growth and viability. Western blotting of SMARCA4 validated protein expression in 9 MPNST cell lines, while immunohistochemistry staining of 3 MPNST patient-derived xenograft tumors found high SMARCA4 expression. siRNA KD of SMARCA4 reduced viability across a panel of MPNST cell lines from 20-40% (p-val<0.001) compared to non-targeting (NT) controls. Additionally, inhibition of SMARCA4 via small molecule BRM014 reduced MPNST cell proliferation in a dose-dependent manner (IC50=8.07uM).
To establish the biological mechanism underlying SMARCA4 contribution to MPNST cell viability, RNA sequencing (RNAseq) of SMARCA4 KD versus NT control was carried out in 3 MPNST lines. Cell cycle and DNA replication KEGG pathways, as well as the E2F target pathway, were found to be significantly (p-adj<0.05) downregulated upon SMARCA4 KD. EDU/DAPI staining confirmed these findings using flow cytometry, where SMARCA4 KD caused G1 phase arrest. Further, these results could be mimicked using BRM014 inhibitor, as MPNST cells containing a fluorescent cell cycle marker arrested in the G1 phase under single-agent treatment.
Due to the identified role of SMARCA4 in MPNST cell cycle and DNA replication, DNA damaging agents were screened in combination with BRM014 to highlight synergistic compounds. Etoposide, a TOP2A poison, had synergy with BRM014 at low doses, increasing the induction of G1 arrest through the ATM DNA damage response pathway.
This study identifies SWI/SNF ATPases as novel therapeutic targets in MPNST. Further, it identifies a synergistic therapeutic regimen in combination of etoposide and BRM014 in MPNST treatment, which is currently being tested in vivo.

44. Yuta Myojin, MD, Ph.D

Comprehensive analysis of patients with hepatocellular carcinoma treated with anti-PD-L1 plus anti-CTLA-4 combination therapy.
Mentor: **Dr. Tim F Greten**
Study Section: **Oncology - Therapeutics and Translational Research**

Background: Hepatocellular carcinoma (HCC) is a significant contributor to cancer-related mortality, with immunotherapy utilizing durvalumab (Dur) and tremelimumab (Trem) now established as standard treatment for advanced cases. However, comprehensive studies on immune responses are lacking. This study aimed to investigate immune responses in HCC patients using clinical samples. Methods: In a phase 2 trial, 41 HCC patients were enrolled and administered Dur and Trem in conjunction with intervention therapies. Blood and tumor samples were collected pre- and post-treatment. Peripheral blood mononuclear cells (PBMC) and serum samples from 28 patients were analyzed. Tumor biopsies from 24 patients underwent RNA sequencing, whole exon sequencing, and 37-plexed immunofluorescence (CODEX). Orthotopic liver tumor mouse models were utilized to assess responses to anti-PD-L1 and anti-CTLA4 therapy. Results: Patients exhibited median progression-free survival (PFS) and overall survival (OS) of 4.9 and 20.2 months, respectively. Patient characteristics, including liver function and tumor stage, related with treatment response. To examine immune cells in the tumor microenvironment by spatial context, we developed a 37-plexed CODEX panel. The cellular composition observed in the images matched with predictions from RNA sequencing and regulatory T cells increased after treatment. Analysis of cellular neighborhoods revealed an increase in macrophages-enriched and lymphoid-enriched neighborhoods in post-treatment. Tissue RNA sequencing demonstrated enhancement of interferon responses in both responder and non-responder groups, with a more pronounced effect in responders. The antigen presentation gene signature was enhanced in responders and TGF-beta late signature was enriched in non-responders. In the peripheral blood analysis, levels of monocytes in PBMC increased in responders, while regulatory T cells in PBMC increased in non-responders. Serum cytokine analysis indicated elevated IL18 and CCL22 levels in responders and increased IL6 and TNFa levels in non-responders. Animal studies showed reductions in tumor size, T cell infiltration, an increase of cytotoxicity in tumors and liver, and an increase in monocytes in PBMC with combination therapy. Conclusion: Combination therapy utilizing anti-PD-L1 and anti-CTLA-4 is efficacious against HCC, promoting the expansion of CD8 T cells and monocytes in peripheral blood.

45. Nupur Nigam, PhD

Ptpn1 deficiency collaborates with a NUP98::HOXD13 fusion gene to generate B cell precursor acute lymphoblastic leukemia
Mentor: **Dr. Peter Aplan**
Study Section: **Oncology - Therapeutics and Translational Research**

Background: Acute lymphoid leukemia is characterized by inherited or acquired mutations that effect critical differentiation and proliferation pathways. We have shown that Mcm2 deficient mice developed T cell acute lymphoblastic leukemia, due to copy number variations, most commonly interstitial deletions throughout the genome. When crossed with mice that expressed a NUP98::HOXD13 (NHD13) fusion gene, Mcm2-NHD13 mice developed B cell precursor ALL (BCP-ALL). A majority of these BCP-ALL had acquired homozygous deletions of Ptpn1, a protein tyrosine phosphatase, leading to the hypothesis that Ptpn1 deficiency combined with NHD13 expression leads to BCP ALL. Objective: To investigate the role of Ptpn1 deletion in BCP-ALL development using mouse models.

Methods: Mice expressing an NHD13 fusion gene were crossed with Ptpn1 knockout mice, generating 6 possible genotypes. Mice were followed for 18 months. Mice with signs of leukemia were characterized by clinical evaluation, CBC, flow cytometry, and IHC. Primary BCP-ALL and derived BCP-ALL cell lines were also evaluated with RNA-seq and molecular pathway analysis. Results: NHD13+Ptpn1-/- mice developed BCP-ALL with 65% penetrance, characterized by hyperleukocytosis, anemia, thrombocytopenia, and invasion of non-hematopoietic tissues. Similar to human BCP-ALL, NHD13+Ptpn1-/- BCP-ALL had clonal IGH as well as clonal Tcrd gene rearrangements. Flow cytometry revealed CD19 and/or B220 expression. NHD13+Ptpn1+/- mice with BCP-ALL frequently lost the wild-type (WT) Ptpn1 allele in leukemic cells, reinforcing the hypothesis that Ptpn1 can function as tumor suppressor gene in this context. Whole exome sequencing revealed acquired mutations in B-cell differentiation genes (Pax5 or Bcor) and activating hotspot mutations in tyrosine kinase genes (Jak1/3 and Flt3). Transcription signature analysis showed significant upregulation of Hoxa/b gene clusters, RNase12 and LncRNAs subsets. Conclusion: This study demonstrates that Ptpn1 loss combined with expression of NHD13 fusion gene leads to highly penetrant BCP-ALL in mice, suggesting a role for Ptpn1 in preventing malignant transformation. These findings present a collaborative model for BCP-ALL in which the NHD13 transgene leads to increased stem cell self-renewal, somatic Bcor or Pax5 mutations block normal B cell differentiation, and somatic signaling mutations (Jak1/3,Flt3) lead to hyperproliferation, which is potentiated by Ptpn1 deficiency.

46. Yuuki Ohara, M.D., Ph.D.

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Mentor: **Dr. Stefan Ambs**
Study Section: **Oncology - Therapeutics and Translational Research**

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47. Woo Yong Park, PhD

Tumor nuclear expulsion induces T cell activation and treatment resistance
Mentor: **Dr. Yang Li**
Study Section: **Immunology - Tumor Immunology**

One of the most challenging issues in cancer biology and therapy is treatment relapse and resistance, which often occur following various treatments such as chemotherapy, radiation, or immunotherapy. While these treatments cause a massive apoptotic cell death, our understanding of the dying signals and their impact on surviving cancer cells remains incomplete. In our previous study, we discovered that apoptotic cancer cells expressing high Padi4 undergo nuclear expulsion and release a DNA/protein structure (Nuclear expulsion products; NEPs) into the extracellular space that is critical for metastasis. However, much remains to be elucidated regarding the role of nuclear expulsion during therapy. Building upon previous work, here we confirm that nuclear expulsion occurs in tumor cells after the treatment of chemo reagents and Padi4 plays a role as a nuclear expulsion switch in chemo-treatment mouse models. The presence of chemo-mediated nuclear expulsion was also verified in the human PDX model. By using nuclear expulsion switch model, we showed that NEPs drive chemoresistance in tumor-bearing mice that were treated with cisplatin and gemcitabine in Padi4 dependent manner. In addition, by comparing immune competent (balb/C) and athymic immune deficient (nu/nu) mouse models and utilizing an anti-CD8 neutralization antibody, we discovered that the Padi4 mediated chemoresistance is CD8 T cell dependent. Of interest, the infiltrated CD8 T cells exhibited higher expression of activation markers such as GrnzB, IFNg, PD1 and Tim3 as well Ki67, in chemo treated Padi4 wildtype tumor compared to knockout tumor. Our proteomic analysis further revealed a specific abundance of Vimentin as NEP component, and we found that loss of Vimentin in NEPs diminished CD8 T cell activation and chemo-resistant phenotype, implying the importance of Vimentin as a crucial mediator. Lastly, in exploring the potential application of NEPs as a chemoresistance indicator, we developed NEP detection method using NEP components such as Vimentin and we found it specifically detects NEPs in chemo treated tumor in mouse and human models. In summary, our studies provide the insight for the molecular mechanisms by which tumor cell death can impact neighboring living tumor cells during chemotherapy. We anticipate that predicting the occurrence of nuclear expulsion or targeting NEP will contribute to enhancing therapeutic efficacy.

48. Shadab Parvez, PhD

Intact HIV-1 Proviruses are Disproportionally Found in KRAB-ZNF Genes in People Living with HIV on Long-Term ART
Mentor: **Dr. Mary F Kearney**
Study Section: **HIV and AIDS Research**

The viral reservoir in people with HIV (PWH) is principally comprised of CD4+ T cells harboring intact, replication competent proviruses – viral DNAs integrated at myriad, indeterminate sites in the host cell genome. During suppressive antiretroviral therapy (ART), which halts ongoing viral replication, reservoir cells become increasingly rare but are never completely eradicated. In working to characterize the HIV reservoir, several groups have anecdotally reported that, after years on ART, intact proviruses are disproportionately found integrated into Krüppel-associated box Zinc-finger (KRAB-ZNF) genes. If the universality of this phenomenon can be demonstrated by rigorous statistical analysis, it will allow us to characterize and better understand the determinants enabling long-term HIV persistence, the principal barrier to a cure. Toward this end, we have compared (i) theoretical expected HIV-1 integration site frequencies assuming

random integration into genes, (ii) measured frequencies in primary CD4+ T cells infected ex vivo, and (iii) measured frequencies in peripheral blood mononuclear cells (PBMCs) from PWH on long-term ART (>10 years). In the latter samples, we also measured integration site frequencies considering only proviruses capable of contributing to the HIV reservoir, i.e., those confirmed to be intact (1-5% of all proviruses). We found that when proviral intactness was not considered, the frequency of integration into KRAB-ZNF genes in samples from PWH on ART (2.4%) was 1.8-fold higher than that observed for cells infected ex vivo (1.3%; p<10^-5) and 4-fold higher than expected if integration events were random (0.6%; p<10^-5). We attribute these differences to the incremental effects of integration site favorability, lineage fitness, and immune avoidance. More significantly, among only those T cells harboring intact proviruses, 60% were found integrated into KRAB-ZNF genes – 25-fold more than observed for samples from the same donors when not considering proviral intactness and 100-fold more than would be expected if integration were random. Our analysis demonstrates the marked survival advantage conferred by integration into KRAB-ZNF genes, and that it is much more evident when the proviruses harbored in CD4+ T cells are intact. These results show that the HIV reservoir during ART is primarily comprised of intact proviruses integrated into KRAB-ZNF genes and reveal a new target for the design of strategies towards a cure for HIV.

49. Vijaya Kumar Pidugu, Ph.D

Development and Characterization of a CRISPR-Edited AT2 Organoid Models for Investigating the Genomic Landscape and Therapeutic Responses in Lung Adenocarcinoma
Mentor: **Dr. ji luo**
Study Section: **Stem Cells - General and Cancer**

Lung adenocarcinoma (LUAD) is characterized by frequent co-mutations in key driver genes, including KRAS, TP53, KEAP1, and LKB1. Understanding the impact of co-occurring mutations on genomic landscapes and therapeutic responses is crucial for improving patient prognosis. In this study, we hypothesized that Kras mutant alveolar type 2 (AT2) cells with co-mutations in Trp53, Keap1, and Lkb1 exhibit distinct alterations in Kras effector pathway activity, cell metabolism, cell survival, and drug sensitivity. To this end, we optimized AT2 organoid models derived from KP (KrasLSL-G12D/WT: Trp53fl/fl) mice and characterized their morphological and proliferative properties. RNAseq analysis of gene expression in organoids treated with the KRASG12D inhibitor MRTX1113 revealed significant alterations in Kras signaling, interferon alpha, and inflammatory response genes. Nanostring analysis further demonstrated an upregulation of canonical AT2 lineage markers and a concurrent downregulation of Kras signature genes and developmental markers upon MRTX1113 treatment. Ras-G-LISA assay on KP-AT2 organoids indicated a decrease in RAS-GTP levels in response to MRTX1113, validating the inhibitory effect. Western blot analyses corroborated these findings, showing inhibition of the RAS-MAPK pathway and the expression of apoptosis markers following MRTX1113 treatment. To assess in vivo tumor formation capacity, intratracheal instillation of KP-AT2 organoids into mice lungs was conducted, revealing the formation of tumors in vivo. This comprehensive data establishes that KP-AT2 organoids exhibit consistent genotype and phenotype both in vitro and in vivo, emphasizing their reliability as models and studying the effects of Kras inhibition. To further dissect the impact of co-mutations, we are in the process of establishing a multiplex CRISPR editing system to target the Kras (K), Trp53 (P), Keap1 (E), and Lkb1 (L) genes to generate isogenic AT2 organoid models with different combinations of genetic mutations (K, KP, KE, KL, KPE, KPL, and KPEL). Our future studies involve the transplantation of compound mutant organoid models into syngeneic hosts to characterize their tumorigenic capability and drug sensitivity in an immunocompetent setting. This system opens new avenues to explore the basic pathophysiology of LUAD and assess innovative therapeutic approaches in preclinical settings, offering valuable insights for the development of novel strategies to reduce LUAD mortality.

50. Gauri Prasad, PhD

Cell type-specific effects of common germline variation on gene expression and pancreatic cancer risk
Mentor: **Dr. Efsun Arda**
Study Section: **Genetics – Diseases**

Pancreatic ductal adenocarcinoma (PDAC) stands out as an exceptionally aggressive and often lethal cancer. It originates from the exocrine compartment of the human pancreas, which is composed of acinar and duct cells. Pinpointing genes affecting PDAC risk in these cells holds promise for early detection, prevention, and effective therapies. While high-risk PDAC mutations are found in protein coding regions, common variants identified through Genome Wide Association Studies typically reside in non-coding regions, complicating the identification of their target genes. Previous studies on noncoding variations often used bulk tissue samples, potentially masking cell-specific genetic effects. Understanding genetic effects in distinct exocrine pancreas cells can delineate the molecular and cellular origins of pancreatic cancer. With this goal, we employed a flow cytometry-based approach to purify human acinar and duct cell populations from healthy donors. To pinpoint genes influenced by non-coding risk variants, we conducted cell type-resolved analyses, including expression quantitative trait loci (eQTL), chromatin accessibility quantitative trait loci (caQTL), and methylation quantitative trait loci (meQTL) analyses in purified pancreatic acinar and ductal cells. Using RNA-Seq data and imputed genotypes from 96 acinar and 94 duct samples, we performed eQTL analyses, testing associations between ~6M imputed SNPs and expression of 19,185 genes in acinar and 22,115 genes in duct cells. We identified 66,471 cis-eQTLs (2,288 eGenes) in acinar and 114,723 cis-eQTLs (4,010 eGenes) in duct samples (FDR<0.1), with approximately 20% specific to each cell type. Almost 48% of acinar eQTLs were shared with duct samples, and 28% of duct eQTLs were shared with acinar samples. Surprisingly, 30 eGenes in one cell type exhibited an effect in opposite direction in the other cell type. This implies a reverse-regulation of gene expression influenced by same genetic

variant in two distinct cell types, suggesting a complex interplay of genetic variants in shaping pancreatic cellular fate, a phenomenon we wouldn't have been able to unravel without a cell type-specific QTL dataset. Our findings reveal novel genes and potential regulatory mechanisms that are specific to exocrine pancreas cell types, shedding light on their role in PDAC risk. This cell type-resolved approach offers enhanced precision than conventional bulk tissue studies, yielding targeted therapeutic insights.

51. Beibei Ru, PhD

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Mentor: **Dr. Peng Jiang**
Study Section: **Computational Biology/Systems Biology**

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52. Ipsita Saha, PhD

Decoding the decisive dynamics of proteins in the Rab11-Rab8 cascade
Mentor: **Dr. Christopher J Westlake**
Study Section: **Cell Biology - Intracellular Trafficking and Cell Signaling**

Cellular membrane trafficking is fundamentally important in development and normal homeostasis, and its dysregulation causes numerous human diseases. Continuous cargo transport through various cellular membrane compartments is orchestrated by Rab GTPase cascades, whereby an upstream GTP-bound Rab recruits a guanine nucleotide exchange factor (GEF) to activate a GDP-bound Rab functioning in the next step of membrane transport. Upstream Rab-GTP hydrolysis, promoted by a GTPase activating protein, helps complete the exchange of one Rab for another on the same membrane. Remarkably, only two Rab cascades have been described for the more than 60 known Rabs in humans, and the only direct demonstration of protein dynamics was observed for the Rab5-Rab7 cascade, essential in endosome to lysosome conversion. The Rab11-Rab8 cascade, involving the GEF Rabin8, is important in primary cilium formation, epithelial cell apical lumen formation, and neurite outgrowth, and was first described over a decade ago. A major challenge in characterizing the protein dynamics of this cascade is the spatial and temporal behavior of associated membranes. Here we investigated the Rab11-Rab8 cascade in novel biophysical cellular systems involving active membrane trafficking by time-lapse and super resolution fluorescence microscopy. We unraveled a previously unknown requirement for this cascade in long tubular membrane (LTM) assembly. LTMs are associated with ciliogenesis, early stages of cell migration and we show here is conserved in zebrafish embryos. Strikingly, by monitoring actively forming LTMs we demonstrated membrane exchange dynamics of Rab11 and Rabin8 on Rab8 LTMs and revealed its dependency on Rabin8 GEF activity. Using an inducible exogenous expression system, we showed nascently translated Rab8 actively loading onto Rab11-Rabin8 membrane structures. Finally, we showed Rab8a trafficking into the primary cilium requires Rab11 and Rabin8 using fluorescence ablation and recovery studies (FRAP). We discovered Rab11 is recruited into cilia in a Rab8-dependent manner, a process we link to cascade protein membrane exchange by time-lapse super resolution microscopy (Elyra7 SIM2). Together, these studies establish that the Rab11-Rab8-Rabin8 cascade utilizes a membrane conversion mechanism. We expect the findings of this study and the experimental approaches used will stimulate future investigation into this, and other, Rab cascades important in normal and diseased cellular states.

53. Sounak Sahu, DPhil

AVENGERS: Analysis of Variant Effects using Next Generation sequencing to Enhance BRCA2 Stratification
Mentor: **Dr. Shyam K Sharan**
Study Section: **Omics - Genomics/Transcriptomics**

Accurate interpretation of genetic variation is a critical step toward realizing the potential of precision medicine. Sequencing-based genetic tests have uncovered a vast array of BRCA2 sequence variants. However, due to limited clinical, familial, and/or epidemiological data, thousands of variants are considered to be variants of uncertain significance (VUS). The clinical variant database, ClinVar, has reported a total of 13,201 BRCA2 Single Nucleotide Variants (SNVs) to date, with 59% still considered to be VUS. Assessing pathogenicity by linking genotype to phenotype is challenging as many of the VUSs are rare and may only be identified in a single person or family. Hence, multiplexed assays for variant effect (MAVE) are necessary to assess a large number of variants experimentally. The advent of CRISPR-based saturation genome editing (SGE) has facilitated the creation of programmed variants enabling high-throughput variant screening and multiplexing using Next Generation Sequencing (NGS). To determine the functional impact of VUSs, here we develop AVENGERS: Analysis of Variant Effects using NGs to Enhance BRCA2 Stratification, utilizing SGE in a humanized-mouse embryonic stem cell line. Loss of BRCA2 being cell lethal, we show a significant drop in the frequency for pathogenic variants of BRCA2, whereas functional variants (neutral) are enriched in the pool. The dropout frequency of SNVs is defined as function scores which were analyzed using a Gaussian-mixture-model to calculate their probability of pathogenicity. We have generated the function scores for 6270 SNVs, covering 95.5% of possible SNVs in exons 15-26 spanning residues 2479-3216, including 1069 unique missense VUS, with 81% functional and 14% found to be nonfunctional. Our classification aligns strongly with pathogenicity data from ClinVar, orthogonal functional assays, and computational meta-predictors. Our statistical classifier exhibits 92.2% sensitivity and 96% specificity in distinguishing clinically benign and pathogenic variants recorded in ClinVar. Furthermore, we

offer proactive evidence for 617 SNVs being non-functional and 3396 SNVs being functional demonstrated by impact on cell growth and response to DNA-damaging drugs like cisplatin and olaparib. In summary, AVENGERS represents one of the largest functional assays for BRCA2, providing a valuable resource for precision medicine and genetic counseling in breast cancer susceptibility.

54. Subhash Sethi, Ph.D.

Genome-wide screening identifies NuA4 HAT complex members EP400 and KAT5 as critical regulators of CENP-A mislocalization and chromosomal instability
Mentor: **Dr. MUNIRA A BASRAI**
Study Section: **Epigenetics**

Aneuploidy, a hallmark of many cancers, arises due to chromosomal instability (CIN). CIN is an unequal distribution of chromosomes into two daughter cells and/or structural rearrangements of the chromosomes. Centromeric localization of evolutionarily conserved histone H3 variant, CENP-A is essential for chromosomal stability. We previously showed that mislocalization of overexpressed CENP-A to non-centromeric regions contributes to CIN in human cells and xenograft mouse model. This observation is clinically relevant because overexpression of CENP-A is observed in several cancers, and this correlates with poor prognosis and therapeutic resistance. However, the molecular mechanisms that prevent CENP-A mislocalization remain poorly defined. Here, we present results from an image-based genome-wide high-throughput siRNA screen designed to identify gene depletions that show high nuclear levels of CENP-A. Among the top ten candidates were EP400 and KAT5, which are components of the multisubunit NuA4 histone acetyl transferase (HAT) complex. We hypothesized that defects in the ATP-dependent chromatin remodeling activity of EP400 and HAT activity of KAT5 contribute to mislocalization of CENP-A. We validated the results of the screen, performed in-depth studies and uncovered roles for EP400 and KAT5 in preventing CENP-A mislocalization and CIN using near stable diploid RPE1 cells overexpressing GFP-CENP-A. Our results showed mislocalization of CENP-A to non-centromeric regions on mitotic chromosomes and this correlates with enrichment of CENP-A in chromatin fraction of EP400 and KAT5 knockdown (KD) cells. Cells expressing an EP400 mutant that is defective for ATP-dependent chromatin remodeling activity showed increased stability and mislocalization of CENP-A with CIN phenotypes such as lagging chromosomes and micronuclei. Depletion of the histone H3.3 chaperone DAXX suppressed CENP-A mislocalization in EP400 KD cells. We conclude that ATPase activity of EP400 prevents mislocalization of CENP-A and CIN and defects in EP400 lead to mislocalization of CENP-A by factors such as DAXX. In summary, our studies define novel regulators of CENP-A localization and highlight the multifaceted roles of chromatin remodelers such as EP400 and KAT5 in preventing CENP-A mislocalization and CIN. We propose that expression of EP400 and KAT5 may serve as important prognostic biomarkers for CENP-A overexpressing cancers.

55. Yuto Shiode, Ph.D.

Mitochondrial-Associated DNAJA3 Variant and Its Role in MASH-Driven Hepatocellular Carcinoma
Mentor: **Dr. Xin W Wang**
Study Section: **Gene Expression - Transcriptional Regulation**

Metabolic dysfunction-associated fatty liver disease (MAFLD) is increasingly recognized as a major risk factor for hepatocellular carcinoma (HCC). Despite the well-known progression from MAFLD to metabolic dysfunction-associated steatohepatitis (MASH) and eventually HCC, the genetic underpinnings driving this transition remain underexplored, with limited treatment options available. To identify genes influencing MASH and MASH-related HCC, we conducted a comprehensive genomics assessment, performing a genome-wide study of variants linked to body fat distribution in 344,369 individuals. Upon correlation, significant variants associated with MASH and HCC emerged in a cohort of 1,009 participants from the NCI-UMD study, prompting further eQTL analysis for candidate variants. Our investigation revealed that the rs3747579-TT variant is significantly associated with MASH-related HCC, acting as an eQTL for mitochondrial DnaJ Heat Shock Protein Family (Hsp40) Member A3 (DNAJA3). HCC patients carrying this variant exhibited reduced DNAJA3 expression, correlating with an unfavorable prognosis. Subsequently, we explored the regulatory mechanism of DNAJA3 by rs3747579. As rs374759 is 30 kb away from the promoter region of DNAJA3, our focus turned to the regulatory mechanism mediated by the three-dimensional structure of DNA. Examination of the 3D genome architecture identified potential chromatin topology sites influencing DNAJA3 regulation by rs3747579. Notably, enhancer loops exhibited allele-specific interactions with rs3747579 in DNAJA3 regulation. Chip-seq analysis further revealed that the rs3747579-CC variant is part of a binding site of RBFOX2. In addition to its known RNA splicing functions, RBFOX2 may impact the chromatin landscape, influencing chromatin dynamics and gene transcription. RBFOX2 knockdown experiments demonstrated significantly reduced DNAJA3 mRNA expression, while luciferase assays highlighted increased activity for the rs3747579-CC allele compared to its TT counterpart. In summary, our findings highlight the rs3747579-TT variant as a potential oncogenic factor in DNAJA3, shedding light on its role in the genesis of MASH and progression to HCC. This understanding holds promise for novel therapeutic interventions in HCC stemming from MAFLD/MASH.

56. Pradeep Shrestha, Doctorate

A pH-Activatable Imaging Strategy Beyond 1000 nm for Fluorescence Guided Surgery and Quantification of Antibody-Drug Conjugate Linker Cleavage
Mentor: **Dr. Martin Schnermann**
Study Section: **Chemistry**

Introduction: Altered pH homeostasis is a hallmark of cancer. The ability to visualize changes in pH in complex organisms could enable new fluorescence-guided surgical strategies that will allow precise tumor margin delineation, facilitating thorough resection while sparing healthy tissue. Such approaches might also help track the fate and activation of targeted-drug delivery strategies, such as antibody drug conjugates. To realize this goal, we hypothesize that responsive, protein-targeted probes in the short-wave infrared (SWIR) region (1000 nm to 1400 nm) would be enabling technology. SWIR imaging enables improved resolution and tissue penetration, though existing probes are unsuitable for our goals. Key Experimental Results: We have designed and synthesized a series of benzofused pegylated norcyanine derivatives. These include BF-PEG24-pH, a cyanine with absorption maxima at 980 nm and an emission maxima of 1000 nm. When conjugated with Panitumumab (an anti-EGFR antibody), targeted imaging reveals a remarkable tumor-to-background ratio of approximately 25-fold. Validation experiments using the isotype control Obinutuzumab confirm the specificity of the EGFR in JIMT1 triple-negative breast cancer tumors. While useful, activatable probes such as these pose a unique challenge at early time points because the dye accumulation signal conflicts with the pH-responsive signal's overlap. To overcome this challenge, we introduced a mixture of two mAb probe dyes: FNIR-766 (785 nm excitation) representing dye accumulation and BF-PEG24-pH (890 nm excitation) as the pH-activatable probe. The 890/785 emission ratio correlated with pH-dependent activation, as observed in vivo. This strategy also demonstrates the multiplexing capabilities of SWIR imaging. In addition, we compared protease cleavable linker activity with a Val-Cit cleavable linkers to find a maximal signal ratio of 0.4, significantly higher than the ratio of 0.03 observed in non-cleavable linkers. Conjugation of BF-PEG24-pH with albumin facilitated rapid tumor accumulation and clearance, offering a fast and efficient imaging approach for tumor visualization. Conclusion: These novel pH-activatable SWIR probes are promising approaches for in vivo tumor visualization during surgery and quantifying antibody-drug conjugates linker cleavage.

57. Martin Stortz, PhD

Optogenetic manipulation of specific chromatin-chromatin contacts with a CRISPR/dCas9-based tool
Mentor: **Dr. Tom Misteli**
Study Section: **Chromosomes, Chromatin, and Nuclear Architecture**

The 3D organization of the human genome inside the cell nucleus gives rise to distinct architectural features at different scales. Architectural protein factors and DNA elements favor specific 3D genome configurations by controlling the chromatin-chromatin contacts that occur across the chromatin polymer. A key question in the genome biology field is whether the structure of the genome influences its functions, such as gene regulation. Traditional studies aimed to understand this relationship usually involve perturbing genome structure and performing bulk population measurement to correlate changes in genome organization with changes in gene expression, without being able to distinguish if structure drives function or the other way around. Therefore, we need new tools to interrogate the functional role of genome organization which allow controlled manipulation of chromatin structure without mutating key regulatory factors or sequences, ideally at the single cell level to reflect the dynamic nature and high variability of genome organization within a cell population. For this purpose, we are developing an optogenetic system to induce interactions between specific genome regions in a controlled fashion and study how these contacts influence gene activation. In this approach, we fused a nuclease-dead Cas9 (dCas9) with CRY2, a protein from plants capable of clustering upon blue light illumination. In this way, we use light to induce contacts between specific genome regions targeted by the dCas9-CRY2 fusion protein. As proof of principle, we tested the ability of this construct to induce interactions between two repetitive loci separated by âˆ¼1.2 Mb by recruiting dCas9-CRY2 with specific guide RNAs targeting 35 copies of each repeat and activating CRY2 with pulsed light. We demonstrate by DNA-FISH a significative increase in the contacts between these two loci. We are now applying this approach to study â€˜telomere position effect over long distancesâ€™ (TPE-OLD), a phenomenon described for some genes, including human telomerase (TERT) regulated by long-range contacts with telomeres. We found by DNA-FISH that upon illumination, dCas9-CRY2-induced interactions form between the TERT locus and the chromosome 5p arm subtelomere, separated by âˆ¼1.3 Mb. We will next examine whether we can regulate TERT expression by manipulating these chromatin contacts, in this way addressing the cause-effect relationship between genome organization and genome function.

58. Shiran Su, PhD

Exploring Direct and Indirect Transcriptional Outcomes of MeCP2 Depletion using the dTAG System
Mentor: **Dr. Lisa Boxer**
Study Section: **Neuroscience – Developmental**

MeCP2 regulates brain development and maintains the function of mature neurons throughout adulthood. Maintaining an appropriate balance of MeCP2 levels is crucial: loss-of-function mutations in MeCP2 result in Rett syndrome (RTT), and MeCP2 overexpression causes MeCP2 duplication syndrome. MeCP2 is expressed in all tissues but reaches near-histone levels in neurons. During postnatal neuronal maturation, MeCP2 builds up and reaches high levels in mature neurons at the young adult stage. MeCP2 central nervous system (CNS) dysfunction is the major contributor to RTT-like pathogenicity. Because MeCP2 binds broadly throughout the genome to methylated CG and CA sites, it is hard to link MeCP2 binding to the repression of specific genes. When MeCP2 function is disrupted, there are only small-magnitude up and down changes in gene expression. Thus, it is challenging to understand how MeCP2 regulates transcription and what are the primary or secondary transcriptional consequences of MeCP2 loss. To distinguish the primary and secondary consequences of MeCP2 loss, we are using the dTAG system for rapid protein degradation. In this system, dTAG13

molecules are used to induce degradation of target proteins. dTAG13 molecules bind to the FKBP12F36V-tagged target proteins, recruit CRBN and E3 ubiquitin ligase complex, and induce target protein ubiquitination and degradation. An MeCP2-dTAG mouse line was established by knocking-in FKBP12F36V to the Mecp2 gene in C57BL/6 WT mice. Using the MeCP2-dTAG mice, we induced in vivo and in vitro degradation with dTAG13 molecules and performed RNA-seq experiments to study the direct and indirect consequences of MeCP2 loss. In cultured neurons, we showed MeCP2 degradation thirty minutes post dTAG13 treatment. At day two and day fourteen post degradation, we found hundreds of up and down regulated genes. The up-regulated genes are long and enriched for CA DNA methylation (mCA), similar to the genes up-regulated in MeCP2 knock-out neurons. In vivo MeCP2 degradation was achieved by intravenous injection of dTAG13 molecules to mice. MeCP2 can be degraded in the brain within 3hrs of dTAG13 injection with differential gene expression changes. The up-regulated genes are also long and mCA enriched. We are validating our findings about MeCP2 degradation and exploring more direct and indirect effects of MeCP2 loss such as histone modifications and mRNA splicing in specific cell types, different time points and different brain regions.

59. Meijie Tian, Ph.D

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Mentor: **Dr. Javed Khan**
Study Section: **Oncology - Therapeutics and Translational Research**

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60. David Turner, PhD

Unkempt regulates cellular morphology via direct interaction with the CCR4-NOT mRNA decay complex.
Mentor: **Dr. Eugene Valkov**
Study Section: **RNA Biology**

Cellular morphology is a crucial factor in cellular function, but its specification remains a mystery. Neurons, for instance, depend on a highly polarized cellular morphology for information transmission within the nervous system. In this context, the evolutionarily conserved RNA binding protein Unkempt plays a pivotal role. It is indispensable for establishing the bipolar morphology of neuronal cells. Notably, the ectopic expression of Unkempt in nonneuronal cells is enough to induce a bipolar morphology, achieved through the translational repression of target mRNA transcripts.

Here, we show that Unkempt regulates morphology by recruiting the modular CCR4-NOT complex, which is a central hub for post-transcriptional regulation of gene expression by repressing both mRNA stability and translational efficiency. In pulldown assays with recombinant proteins, we determined that Unkempt's low-complexity domain (LCD) mediates direct interactions with the NOT9 module and the NOT2/3 module of the CCR4-NOT complex. Mutagenesis analysis revealed that Unkempt LCD forms a bipartite interaction with both the concave RNA binding groove and defined tryptophan-binding pockets on the convex surface of NOT9. Interestingly, Unkempt utilizes the same residues involved in the interaction with NOT9 to interact with the NOT2/3 module. Analysis of the axial ratio of HeLa cells demonstrated that the morphogenetic potential of Unkempt to polarize nonneuronal cells depends on just three residues that also maintain interaction with the CCR4-NOT complex.

Furthermore, using ribosome profiling to measure translational efficiency revealed that for Unkempt to repress target transcripts translationally, it must recruit the CCR4-NOT complex. Finally, using mass photometry, we determined that Unkempt is an obligate dimer and that it binds the CCR4-NOT complex at 2:1 stoichiometry in solution. Our mutational analysis demonstrates that Unkempt homodimerizes through a C-terminal coiled-coil adjacent to the LCD, and this association is critical for Unkempt’s function.

Together, our data support a model in which an Unkempt homodimer recruits the CCR4-NOT complex via both the NOT9 module and the NOT2/3 module. This interaction leads to translational repression of a program of transcripts involved in specifying cellular morphology.

61. Maria Vega Sendino, PhD

Functional characterization of transcriptional condensates during totipotency-to-pluripotency transition
Mentor: **Dr. Sergio Ruiz-Macias**
Study Section: **Developmental Biology - Early Development/Embryology**

The homeobox transcription factor DUX is a pioneer activator transiently expressed in the totipotent 2-cell (2C) mouse embryo that promotes the expression of stage-specific genes and transposable elements. Importantly, the transcriptional program induced by DUX is efficiently silenced following cleavage to the 4C embryo, an event required to exit from totipotency. Expression of DUX in mouse embryonic stem cells (ESC) triggers a reprogramming to 2C-like cells (2CLC), which display transcriptional and chromatin features of 2C embryos. This in vitro reprogramming system has been used to study the transition from totipotency to pluripotency. In this work, we revealed the formation of transcriptional DUX-induced nuclear condensates. Biomolecular nuclear condensates play a fundamental role

in diverse cellular processes including regulation of transcription during development and cell fate determination. However, the precise role of DUX-induced nuclear condensates during 2CLC conversion and exit from totipotency is unclear. We first examined the role of the histone acetyltransferases (HAT) EP300/CBP in the formation of DUX-dependent condensates as DUX directly recruits them to mediate gene activation. dTAG-mediated degradation of CBP in EP300-knockout ESC demonstrated the relevance of HAT in promoting the formation of DUX-dependent condensates. Next, to identify proteins enriched in these condensates we employed a biotin ligase-related proximity labeling method using the TurboID domain. Based on the specific translocation of the protein TRIM24 to DUX-induced condensates, we used TRIM24-TurboID to biotinylate proteins at condensates. Streptavidin pulldown followed by mass spectrometry analyses revealed an enrichment of 2C-associated proteins at DUX-induced condensates. We hypothesized that progressive accumulation of proteins at these condensates could induce phase separation promoting transcriptional silencing over time. Thus, we used fluorescence recovery after photobleaching (FRAP) to examine the biophysical properties of condensates. Our results showed progressive phase separation over the life of the condensate. Moreover, RNA-FISH analysis of ZSCAN4 genes, a DUX-induced gene cluster, revealed that despite a quick activation when condensates are formed, transcription decreases over condensate maturation. In summary, our data suggest that transcriptional activation mediated by DUX-induced condensates is followed by phase separation and efficient silencing.

62. Anushka Wickramaratne, Ph.D.

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Mentor: **Dr. Sue Wickner**
Study Section: **Biochemistry – General**

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63. Leo Yamada, M.D., Ph.D.

p53 Isoform-Based Therapeutic Strategy for Physiological and Pathological Aging.
Mentor: **Dr. Curtis C Harris**
Study Section: **Stress, Aging, and Oxidative Stress/Free Radical Research**

Human TP53 gene is more than just a 'guardian of the genome' and regulates diverse transcriptional responses through its isoforms. We identified a novel function for Δ133p53α, an isoform lacking 132 N-terminal amino acids, which inhibits transcription at specific p53 response elements in a dominant-negative manner and exhibits anti-senescence effects.Exploring Δ133p53α potential, we focused on Hutchinson-Gilford Progeria Syndrome (HGPS), a premature aging disease caused by a de novo point mutation (c.1824 C>T; p.G608G) in LMNA gene, leading to the production of progerin, a defective laminA protein which causes HGPS pathologies such as cardiovascular disease and shorten the lifespan. In our study, Δ133p53α showed multiple anti-aging roles. It modulated p53 signaling, inhibited cell cycle arrest, and promoted DNA repair in HGPS fibroblasts. Additionally, it significantly reduced IL-6, an inflammatory cytokine that accelerates aging, regardless unchanged progerin levels and extends replicative lifespan, despite unchanged progerin levels. This supports our hypothesis that ∆133p53a inhibits aging phenotypes and extends lifespan in vivo. Given that this p53 isoform exists only in humans and primates, but not in mice through evolutionary process, we established human Δ133p53α knocked in transgenic mouse model using a loxp-Δ133p53αcassette, crossbreeding with Cre-recombinase mice and inducing with tamoxifen. In parallel to pursuing the effect on natural aging, we crossbred with LmnaG609G mice, equivalent to HGPS, which has a shortened lifespan.We confirmed Δ133p53α's anti-senescent roles in the mouse embryonic fibroblasts and tissues with progeria pathologies through CDKN1A and pro-apoptotic BAX and BBC3 downregulation while keeping DNA repair genes (p53R2, RAD51) by qRT-PCR. SA-β-gal staining and the decrease of γH2AX foci in immunofluorescent support in vitro data as well. The lifespan follow-up is ongoing, yet the Δ133p53α group exhibits sustained physiological functions and a delayed onset of HGPS characteristic symptoms. Moreover, a significant decrease in serum IL-6 levels in LmnaG609G suggests Δ133p53α's potential for anti-aging effects. Contrary to HGPS-specific drugs, or senolytics, Δ133p53α may offer a wider therapeutic scope by restoring senescent cells to the proliferative and functional state. Insights from this study could offer significant promise for future clinical application towards common aging disorders and extending human health lifespan.

National Cancer Institute - Division of Cancer Biology

64. Marco Heydecker, PhD

Spatial and Temporal Coordination of Force-generating Actin based Modules Drives Membrane Remodeling In Vivo
Mentor: **Dr. Roberto Weigert**
Study Section: **Cell Biology - Intracellular Trafficking and Cell Signaling**

Mechanical forces are one of the main drivers of membrane remodeling, a fundamental process involved cellular functions. Dysregulation of membrane remodeling is associated with multiple diseases, however the mechanisms regulating these complex force

generating machineries are still unclear, especially in living multicellular organisms. The actin cytoskeleton and its regulation are key components providing these mechanical forces necessary in the remodeling of membranes.During regulated exocytosis in exocrine organs the membrane of large secretory granules is integrated after fusion with the apical plasma membrane (APM). We previously showed that during regulated exocytosis in salivary glands and pancreas of live mice, F-actin and non-muscle MyosinIIA are recruited on the membranes and are necessary for membrane integration.Here we further identified how F-actin is arranged in three distinct modules and identified their function during membrane remodeling. Using super-resolution microscopy, we found that after the fusion of the granules with the APM, the formin mDia1 nucleates a F-actin lattice (module1) of linear actin filaments. We inhibited mDia1 both pharmacologically and by genetic ablation resulting in severe delay or failure of membrane integration, resulting in vacuoles. Next, branched actin filaments nucleated by the Arp2/3 complex rapidly polymerize on the granule beneath the lattice (module2). We pharmacologically and genetically impaired selected components of the Arp2/3 complex, which inhibited the assembly of module2 and significantly delayed membrane integration, without affecting module1. Module3 is constituted by a ring of F-actin accumulated at the actin/membrane interface. We found that Ezrin, a cross-linker between membranes and actin, is recruited on secretory granules membrane. Pharmacologically decoupling Ezrin resulted in a severe delay in membrane integration and disruption of the F-actin ring, without interfering with module1 and module 2 assembly. Based on our findings, we propose a new model for membrane remodeling in vivo based on the spatio-temporal coordination of 3 modules: Module1, composed of linear F-actin lattice, provides a scaffold that counteracts the convective flow of membranes from the APM to the granules; Module2, composed of a network of branched F-actin controls early membrane integration; and Module3, an actin ring anchored to the membrane by Ezrin, which enables force-transmission to the granule membranes.

National Cancer Institute - Division of Cancer Control and Population Sciences

65. Catherine Pichardo, PhD

Association of Neighborhood Deprivation and Lung Cancer in African and European Americans in the NCI-University of Maryland Case Control Study
Mentor: **Dr. Stefan Ambs**
Study Section: **Cultural, Social and Behavioral Sciences**

Importance: African American men (AA) have a higher risk of developing lung cancer than European American (EA). Studies revealed mixed associations between neighborhood deprivation and lung cancer risk; but data is sparce for AA men.

Objective: To examine the association between neighborhood deprivation and lung cancer risk.

Setting and Participants: An age and race- matched case-control study of 1,946 AA and 3,412 EA from The NCI-University of Maryland recruited between 1998-2019.

Exposure: 2000 Census-tract standardized neighborhood deprivation index as continuous, dichotomized at the median (≤ median vs. > median), and quintiles (Q). Covariates included age, education, individual income, family history lung cancer, smoking status, body mass index at study entry, and chronic obstructive pulmonary disease diagnosis.

Outcome: Lung cancer status.

Results: In risk adjusted logistic regression models (OR, 95% Confidence Interval (CI)), residing in neighborhoods with higher neighborhood deprivation was associated with increased disease odds compared to neighborhoods with low deprivation (Q1) among African American (continuous OR 1.25, 95%CI: 1.10-1.43; median OR 1.37, 95%CI: 1.01-1.85) and European American adults (Q2 vs. Q1 OR 1.48, 95%CI: 1.17- 1.88; Q3 OR vs. Q1 1.42, 95% CI: 1.13-1.81; Q4 vs. Q1 OR 1.36, 95%CI: 1.06-1.75). In sex and race-stratified analysis, this association was only found among African American women (continuous, OR 1.34, 95% CI 1.09 – 1.66) and European American men (Q2 vs Q1 OR 1.36, 95%CI: 1.21-2.39; Q5 vs. Q1 OR 1.44, 95% CI: 1.44, 95% CI: 1.00-2.07).

Conclusion: Residing in neighborhoods with high deprivation may adversely influence lung cancer risk, with greater risk observed among AA women and EA men. Findings suggest that changing neighborhood environments may have important implications for lung cancer disparities. Tailoring neighborhood interventions to address cancer risk for AA women and EA men living in neighborhoods with greater socioeconomic deprivation is of importance.

National Cancer Institute - Division of Cancer Epidemiology and Genetics

66. Quiera Booker, PhD, MPH

Motherhood and receipt of chemotherapy: a population-based study of young women with breast cancer
Mentor: **Dr. Jonine Figueroa**
Study Section: **Epidemiology/Biostatistics – General**

Background. Young mothers (aged 20–39 years) with breast cancer must balance cancer health management with caregiving responsibilities, potentially affecting treatment. Having multiple or young children can add to these challenges. Treatment patterns for these mothers remain unclear, as racially homogenous qualitative studies report conflicting views on chemotherapy.
Methods. The Texas Cancer Registry (2013–2016), a multicultural SEER registry comprising 99% of cancer cases in Texas, was linked to live birth certificates (1995–2016). Among 2,133 young women diagnosed with invasive breast cancer who had complete staging and treatment data, 1,368 were mothers, and 785 were young women without children. Logistic regression (adjusted odds ratio [aOR] and 95% confidence interval [CI]) examined the association between motherhood status and offspring characteristics (number and age of children, number of young children [age ≤ 13 years]) with receipt of chemotherapy (yes/no). We assessed whether age at diagnosis, stage, or race/ethnicity (Non-Hispanic Black [NHB], Non-Hispanic White [NHW], Hispanic [any race]) modified these relationships on additive (single-referent) and multiplicative (stratified) scales.
Results. Compared to young women without children, mothers were disproportionately impacted by health-related social needs: Medicaid-insured, rural residency, and living in low-income neighborhoods. Fortunately, receipt of chemotherapy did not differ based on motherhood status or offspring characteristics. However, the association between motherhood status and receipt of chemotherapy varied by race: NHB women were 43% less likely (stratified 95% CI [0.369, 0.888]) and NHW mothers were approximately 30% less likely (single-referent 95% CI [0.513, 0.928]; stratified 95% CI [0.530, 0.951]) to receive chemotherapy than NHW women without children. Receipt of chemotherapy also differed for offspring characteristics by age: mothers diagnosed with breast cancer in their late thirties (ages 36–39 years) with ≥2 young children were half as likely to receive chemotherapy than those without young children at diagnosis (aOR = 0.45, 95% CI [0.206, 0.975]).
Conclusion. Our novel findings suggest mothers with breast cancer may experience barriers to chemotherapy owing to disadvantages and competing demands. Future research needs to identify sociocultural determinants of treatment for these women to inform tailored and culturally appropriate family support services.

67. Vicky Chang, PhD, MPH

Farm animal exposures and the oral microbiome in the Agricultural Health Study
Mentor: **Dr. Jonathan N Hofmann**
Study Section: **Epidemiology/Biostatistics – General**

Raising farm animals involves various exposures (e.g., microbes, bioaerosols, pesticides) that may influence the human microbiome. Exposure to farm animals has also been associated with some cancers including lower risk of lung cancer and elevated risks of certain hematologic malignancies. Epidemiologic evidence suggests that changes in the oral microbiome contribute to cancer development. Few human studies have investigated the potential influence of farm animal exposures on the oral microbiome. We investigated associations between farm animal exposures and the oral microbiome in 1245 farmers and spouses in the Agricultural Health Study. Participants provided information on types and numbers of animals raised at the time of oral wash sample collection. Buccal cell DNA was extracted and analyzed using 16S rRNA gene (V4 region) sequencing. We evaluated associations between farm animal exposures and alpha diversity metrics [e.g., observed amplicon sequence variants (ASVs)] using linear regression and assessed differences in beta diversity metrics using permutational multivariate analysis of variance. We evaluated associations with the presence and relative abundance of specific genera using zero-inflated negative binomial regression. All analyses adjusted for potential confounders (e.g., sex, age, smoking, alcohol intake). Overall, 63% of participants reported raising any animals, most commonly cattle (46%) and hogs (20%). Raising sheep/goats was associated with lower alpha diversity [18 (95% CI: 3-33) fewer observed ASVs vs. no sheep/goats], with an exposure-response trend for increasing number of sheep/goats (Ptrend=0.048). Increasing numbers of hogs and poultry were associated with higher and lower alpha diversity, respectively. Based on beta diversity analyses, we did not observe statistically significant differences in microbial compositions between participants with and without farm animals. After adjusting for multiple testing, having any farm animal was associated with an increased relative abundance of Porphyromonas, and several genera were more likely to be absent with specific animal exposures (e.g., Capnocytophaga for cattle and sheep/goats; Corynebacterium for sheep/goats and poultry). This was the largest investigation of farm animal exposures and the human microbiome to date. Our findings suggest that animal farming may alter the oral microbiome and provide new insights into the potential role of animal exposures in cancer etiology.

68. Azhar Khandekar, Bioinformatics

Extrachromosomal DNA in lung cancer from never smokers
Mentor: **Dr. Maria T Landi**
Study Section: **Epidemiology/Biostatistics – Etiology**

Somatic copy number amplifications (SCNAs) can be key drivers of cancer initiation, progression, and relapse. For decades, SCNAs were thought to be contained primarily on the linear chromosomes. However, recently there has been a renewed interest in extrachromosomal DNA (ecDNA) as a mode of focal amplification. ecDNA, circular DNA that resides outside of the linear chromosome, can amplify oncogenes to a greater degree than that of most other SCNAs. The structure and properties of ecDNA are markedly

different from those of linear DNA, characterized by features like non-Mendelian inheritance and an altered epigenetic landscape. Moreover, its unique characteristics also make it a promising target for therapy. Recently, efforts have intensified to profile ecDNA across many cancer types, yet comprehensive multi-omic studies of ecDNA in lung cancer in never smokers (LCINS) is lacking. Given that LCINS is partially driven by SCNAs, we hypothesized that ecDNA-driven focal amplifications significantly influence LCINS tumor evolution. To investigate this hypothesis, we comprehensively profiled 871 treatment-naive never-smokers (current data freeze) using a robust custom computational pipeline that refined ecDNA detection by accounting for tumor purity and ploidy. This highly sensitive pipeline detected additional ecDNA that conventional pipelines would have missed, and showed that ecDNA was present in 158 (18.1%) of tumor samples, with a third of ecDNA-positive samples harboring more than one distinct ecDNA amplicon. Notably, the MDM2 locus was a hotspot for recurrent ecDNA amplification and most MDM2 amplifications (36/45) were on ecDNA. MDM2-ecDNA showed higher expression levels compared to non-amplified MDM2 or MDM2 amplified via other means. A multivariate logistic regression model including age, sex, ancestry, histology, and tumor purity as covariates showed that whole-genome doubling, APOBEC mutational signature SBS2, and shorter telomere length were the most significant genomic events enriched in ecDNA+ tumors. The strong link between whole-genome doubling and ecDNA was further replicated in a pan-cancer analysis in an external cohort. Importantly, tumor purity and ploidy were not significant confounding factors in our analysis, illustrating the utility of our modified pipeline for ecDNA detection. The unprecedented size of our cohort and the integration of multi-omics data allowed us to perform the first in-depth characterization of ecDNA in LCINS.

69. Maya Spaur, PhD

Exposures to drinking water contaminants below regulatory limits and incident ovarian cancer in the California Teachers Study cohort
Mentor: **Dr. Mary H Ward**
Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Background: Though many drinking water contaminants are endocrine disruptors, limited evidence is available on drinking water exposures and ovarian cancer. We evaluated associations between regulated contaminants in community water systems (CWS) and ovarian cancer risk in the California Teachers Study (CTS), a prospective cohort of female California educators. Methods: Participants were cancer-free, no bilateral oophorectomy, living in California at baseline (1995-1996) with an enrollment address linked to a CWS (N=91,855, 92%); follow-up was through 2020 (mean=18.3 years). We estimated exposure by linking geocoded addresses at enrollment to CWS monitoring data. Among women with a residential duration at their enrollment address >=10 years (N=61,903), we computed long-term average (1990-2015) exposures for arsenic, gross alpha, nitrate, total trihalomethanes (TTHM), trichloroethylene (TCE), and tetrachloroethylene (PCE) (N=60,323), and uranium (N=58,526) and haloacetic acids (HAA5, N=52,651). We estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for incident epithelial (n=440) and high-grade serous (n=211) ovarian cancer per log2 increase in exposures, using Cox proportional hazards regression with follow-up time as the time-scale. We adjusted for age, body mass index, menopause status, parity, and oral conceptive use. We evaluated the joint effects of inorganic/radionuclide contaminants (arsenic/uranium/gross alpha/nitrate) adjusted for the other chemical contaminants (HAA5/TTHM/TCE/PCE), using quantile g-computation. Results: Almost all women (>98%) had average exposure levels below regulatory limits for all contaminants. A doubling in uranium and gross alpha concentrations was statistically significantly associated with epithelial ovarian cancer (HR=1.10, CI 1.03-1.17 and 1.13, CI 1.03-1.25, respectively); uranium and nitrate were associated with high-grade serous ovarian cancer (HR=1.10, CI 1.01-1.21, and 1.10, CI 1.02-1.18, respectively). Using quantile g-computation, HRs per quartile change in all exposures (N=51,486 participants with complete data) were 1.14 (CI 0.98-1.32) and 1.33 (CI 1.07-1.64), for epithelial and high-grade serous ovarian cancer, respectively. Uranium and nitrate were the major contributors to the joint effect of the drinking water mixture on ovarian cancer risk. Conclusions: Novel associations between drinking water contaminants and ovarian cancer risk at levels below regulatory limits warrant follow-up.

70. Jacob Williams, PhD

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Mentor: **Dr. Haoyu Zhang**
Study Section: **Epidemiology/Biostatistics – General**

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71. Shahriar A Zamani, PhD

Germline variants in cancer susceptibility genes and subsequent neoplasm risks after childhood cancer: A pooled analysis of two large-scale cohorts
Mentor: **Dr. Lindsay M Morton**
Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Background: Germline genetic susceptibility may increase the risk of developing a subsequent neoplasm (SN)—a major cause of morbidity and mortality—among childhood cancer survivors. We pooled two large-scale cohorts of childhood cancer survivors, the Childhood Cancer Survivor Study and St. Jude Lifetime Cohort, to characterize overall and specific SN risks among individuals carrying

germline variants in 88 known cancer susceptibility genes with an autosomal dominant inheritance pattern. Methods: Using whole-genome or whole-exome sequencing data, we identified rare, potentially protein-damaging germline variants with SnpEff/ClinVar. Conditional logistic regression evaluated overall SN and SN-specific risk, with up to 100 SN-free controls matched to cases by age, sex, primary childhood cancer type, SN type, radiation dose, chemotherapy, study, and follow-up time. Results: Among 11840 individuals (50.6% female) who survived childhood cancer, 2162 (18.3%) developed at least one SN. The risk of developing any SN was elevated among 288 survivors carrying rare, potentially protein-damaging germline variants in an autosomal dominant cancer susceptibility gene (cases=13.3%; controls=9.6%; odds ratio [OR], 1.48; 95% confidence interval [CI], 1.30-1.70; P=5.12x10-5). A similar proportion of carriers was observed among those who developed common SNs such as basal cell carcinoma (N=107/882, 12.1%), meningioma (N=52/373, 13.9%), breast cancer (N=49/353, 13.9%), and thyroid cancer (N=32/243, 13.2%). Higher frequencies of carriers were observed in less common SNs, specifically sarcoma (N=24/151, 15.9%), colorectal cancer (N=15/72, 20.8%), and glioma (N=16/64, 25.0%). Gene-specific analyses revealed that the risk of developing any SN was most pronounced among individuals carrying variants in TP53 (cases=0.6%; controls=0.04%; OR, 9.93; 95% CI, 4.62-21.34; P=0.001) and FANCM (cases=0.9%; controls=0.5%; OR, 2.45; 95% CI, 1.48-4.03; P=0.018). Conclusion: In large-scale cohorts of childhood cancer survivors with long-term follow-up, individuals carrying potentially protein-damaging germline variants in known autosomal dominant cancer susceptibility genes have substantially increased SN risk, emphasizing the importance of genetic testing and consideration of genetic risk in follow-up guidelines. However, these individuals account for a small fraction of all SN cases, warranting future research on underlying risk factors in this population.

National Center for Advancing Translational Sciences (NCATS)

72. Lindsey M Kirk, PhD

Hepatic Spheroid Models for Predictive Computational Models of Human Hepatoxicity
Mentor: **Dr. Marc Ferrer Alegre**
Study Section: **Biophysics and Biomedical Engineering**

Drug induced liver injury (DILI) causes 20% of drug failures in clinical trials and 30% of market drug withdrawals, highlighting the limitations of cell and animal models in predicting human hepatotoxicity. The US Tox21 is a collaboration that aims to advance methods for evaluating the safety of commercial chemicals rapidly and efficiently in humans. NCATS personnel used biological data generated by Tox 21 screens and drug structures to build computational prediction models for DILI, but models based on in vitro data showed only marginal improvement over random. Most of the in vitro data used for in silico models is obtained from 2D cellular assays that do not mimic human physiology. Additionally, the cell lines used do not capture the diversity of human factors like gender, race, age, and disease. To address these limitations, we utilized tissue engineering methods to culture 3D models of the human liver to improve the relevancy of DILI outputs, and therefore improve associated predictive models. We cultured liver spheroids with increasing physiological complexity: primary human hepatocyte (PHH) monocultures, PHH co-cultured with hepatic stellate cells (HSCs), PHH co-cultured with liver endothelial cells (LEC) and Kupffer cells (KC), or PHH, LEC, HSC, and KC quad-cultures. We exposed the spheroids to a 247-compound subset of the Tox21 library and evaluated for select indicators of DILI. By screening in increasingly complex spheroids, we aimed to determine the necessity of model complexity in accurate representation of DILI in vitro. The panel was administered after 21 days of culture, in single doses selected based on the Cmax of each drug. Spheroids were exposed to compounds for 48 hours, and assessed for cell viability, accumulation of lipids, and formation of reactive oxygen species (ROS). Initial data shows that viability increased significantly in DMSO controls with increasing cell types included in the model ($p<0.0001$), confirming that non-parenchymal cells extend PHH viability. While ROS levels remained consistent between PHH and PHH:LEC:KC models, the inclusion of LECs and KCs increased lipid accumulation in test compounds compared to controls. Additional analysis is underway, and we anticipate that more complex human liver spheroid models will provide accurate indicators of DILI, therefore improving the predictive capabilities of associated in silico models.

73. XINH-XINH M Nguyen, Ph.D.

Developing 3D Immunocompetent Skin Model of Type 1 Diabetes Mellitus for Preclinical Studies
Mentor: **Dr. Marc Ferrer Alegre**
Study Section: **Immunology – Autoimmune**

Diabetes mellitus is a complex metabolic disorder that leads to many health complications. Type 1 diabetes mellitus (T1DM) is an autoimmune-mediated destruction of pancreatic beta cells resulting in the absence of insulin. It is mediated by IFN-γ–producing Th1 cells. Long term diabetic patients have hyperglycemia and poor blood circulation that can lead to impair cellular functions and delay in wound healing. We have developed a protocol to fabricate a normal and T1DM-like 3D vascularized full thickness skin (VFTS) model. T1DM skin model is induced with IFN-γ to mimic T1DM-induced by Th1 cells. We test pharmacology and clinically tested drugs on the 3D-T1DM skin model using ruxolitinib. The IFN-γ induced T1DM skin models have lower TEER values compared to control tissues, suggesting the barrier function of the skin is impaired. Histological image shows un-differentiated epidermal in tissues with IFN-γ treatment compared to control tissues. Ruxolitinib rescues the skin barrier and restores the vascular network. To increase the physiological complexity of the T1DM-skin model, we incorporate Th1 cells into the fabricated skin tissues. Th1-incorporated skin tissue

phenotypes are assessed by histological analysis, loss of barrier function, glucose uptake, cytokine secretion, oxygen levels and quantification of angiogenesis and vascular network for wound healing ability. Our preliminary data show that Th1 cells are viable in cultured skin tissues until Day 20 of the experiment. Additionally, tissue digestion shows that 46.5% of IFN-γ levels detected in Th1-incorporated skin tissues, suggesting Th1 can maintain their phenotype in culture. Th1-incorporated skin tissues exhibit T1DM-like phenotypes including the loss of barrier function. Additional treatment of IFN-γ in the Th1-incorporated skin tissues worsens the T1DM phenotypes by disruption of skin barrier functions and inhibition of vasculogenesis, suggesting synergistic effects between Th1 cells and cytokine. Ruxolitinib rescues the barrier and restores the vascular network in Th1-incorporated skin tissues. As our ongoing effort, we plan to utilize the model for high throughput drug screening using NCATS compound libraries and combination studies with biologics. Taken together, our auto-immunocompetent 3D skin model provides a platform to study complex autoimmune driven skin diseases, while also providing testing platforms for different therapeutic approaches, including small molecules and biologics.

74. Justine Noel, PhD

Unraveling Placental Immunity: Engineered 3D human placental barrier models for in vitro assessment of immunological responses to drugs and cellular therapies.
Mentor: **Dr. Marc Ferrer Alegre**
Study Section: **Methods/Assay Development**

Placental dysfunction in response to inflammatory mediators like TNF-α have been implicated in various prenatal diseases such as gestational diabetes (GDM), preeclampsia, and intrauterine growth restriction (IUGR). There is a critical need to understand the molecular and cellular mechanisms underlying placental dysregulation for the development of treatments for these prenatal diseases and to improve maternal-fetal health. The goal of this work is to develop and validate advanced in vitro cellular models of the placenta barrier to investigate inflammatory responses and use as assay platforms for therapeutic development. We are using 3D bioprinting techniques to bio-fabricate early and late trimester human vascularized placental barrier tissues composed of human primary trophoblast, Human Umbilical Vein Endothelial Cells (HUVECs), and primary human placental fibroblasts and pericytes. Following a 72hr exposure of 10-100ng/mL of TNFα, we examined changes in immune markers, vascular angiogenesis, cytokine/hormone profiles, and overall barrier integrity using high-content imaging, flow cytometry-based multiplex immunoassays, and transepithelial electrical resistance measurements. Additional functionality assays were conducted to monitor ABC transporter expression and activity, nutrient absorption and IgG transcytosis dynamics in real time. Our results reveal a late trimester specific effect of TNF-α at the placental barrier interface in altering glucose metabolism, reducing human chorionic gonadotropin (hCG) hormone production and reducing IgG transcytosis, highlighting how disruptions in placental metabolism can directly alter maternal-fetal communication influencing passive immunity. Future research with this biofabricated placental barrier model will focus on the effects of drug and Mesenchymal Stem Cell (MSC) based therapies on placental functionality. Additionally, we aim to incorporate immune cells such as macrophages and natural killer (NK) cells into our models to investigate interactions within placental barrier microenvironment and it's influence on immune programming. Our comprehensive approach will provide a better understanding of the complex crosstalk between the environment, immune cells at the placenta barrier to develop therapeutic interventions that can improve maternal and child health outcomes.

75. Masato Ooka, PhD

Use of high throughput in vitro assays in combination with virtual screening to identify small molecules that have potential to treat opioid use disorder
Mentor: **Dr. Menghang Xia**
Study Section: **Clinical and Translational Research - Drug Discovery**

Opioids are commonly used to treat severe pain on various occasions, such as chronic cancer pain or labor. Although they are effective analgesics, they often cause physical dependency and addiction after long-term use. According to the Centers for Disease Control and Prevention, over 75% of overdose-related deaths involved opioid usage. This number is still increasing each year. There are several cellular mechanisms involved in opioid withdrawal, which triggers dependence and addiction. First, opioid withdrawal induces a sudden induction of cAMP. This phenomenon is referred to as "cAMP overshoot." Second, opioid withdrawal increases dopamine release, which highly correlates with addiction. The aim of this study is to identify small molecules that can simultaneously inhibit morphine-induced cAMP overshoot and the dopamine receptor. To identify such compounds, we applied a machine learning (ML) model to virtually screen inhibitors for opioid receptors (OPRs) based on the chemical structures. This ML model was developed based on in vitro screening data against 2805 drugs for their inhibitory effect against OPRs. The virtual screening identified 631 compounds out of 49,018 in-house compound collection for further studies. We conducted a morphine-induced cAMP overshoot and dopamine receptor D2 (DRD2) inhibition assays. The screenings identified 256 potential candidate compounds. To test their specificity and toxicity, various cell-based assays were conducted against 256 compounds for their effect on mu-OPR, delta-OPR, kappa-OPR, DRD2, DRD1, DRD3, neurotoxicity, and the human Ether-à-go-go-Related Gene potassium channel. Based on the screening results and novelty, 24 compounds were chosen for the enzyme binding assay against mu-OPR and DRD2. We identified 8 compounds, including AMG-47a and MT19, that had a direct binding to both mu-OPR and DRD2. These compounds include some compounds that are already in clinical trials (e.g., XL-888). They can be repurposed as potential drugs for opioid use disorder. Drug repurposing can save a lot of time and money that are needed in the drug development process. From this study, we successfully identified 8 compounds from 49,018

compounds using virtual screening, cell-based assays, and enzyme-based assays. These compounds can be potential problem solvers for the ongoing opioid crisis.

76. Monika Rajput, Ph.D

3D In Vitro Brain Perfusable Vascularized Model to Study Flavivirus NS1-Induced Endothelial Dysfunction
Mentor: **Dr. Emily M Lee**
Study Section: **Virology - Pathogenesis/Therapeutics**

The flavivirus Japanese encephalitis virus (JEV) can invade the peripheral and central nervous system and lead to severe encephalitic and hemorrhagic diseases, including blood-brain barrier disruption, vascular damage, excessive immune cell activation, and death. Although an effective JEV vaccine is approved for use, JEV remains the leading etiological agent of vaccine-preventable encephalitis in both Asia and Western Pacific countries. No direct-acting antivirals are available, and vaccine adoption in highly affected areas remains poor. During severe infection, JEV non-structural protein NS1 levels are secreted and circulated in the bloodstream. Given the known role of NS1 in flavivirus-induced pathogenesis, JEV NS1 may be a promising therapeutic candidate against severe JEV disease. To develop the anti-JEV NS1 therapeutics, we aimed to model neurotropic flavivirus recombinant NS1-induced pathogenesis using a hydrogel-based vascularized model to screen potential therapeutics, with a focus on JEV NS1. We developed a reproducible, perfusable brain vascular on-chip model (3DhBVas) by combining different cell types: GFP-human primary brain microvascular endothelial cells and brain pericytes in fibrinogen hydrogel. Then, we exposed the 3DhBVas to recombinant NS1 proteins from JEV, WNV, and DENV to represent a range of disease severity in a dose-dependent and time-dependent manner and documented vascular damage using various assay readouts. We observed that among all NS1 proteins, JEV NS1 induced the most significant vascular damage, followed by WNV and DENV2. Quantitative analysis showed changes in morphometric parameters like decreased vessel density, branching density, and vessel intensity in all the NS1 exposed models. The observation was further validated using Luminex assay, qRTPCR, and immunostaining. We observed that all the assays showed the decreased expression of endothelial glycocalyx components (heparan sulfate, CD31, and e-selectin) and increased expression of inflammation (IL6, VCAM-1, ICAM-1) and thrombosis markers (vWF) in JEV NS1>WNV NS1>DENV NS1 exposed models. From the results, we hypothesized that the 3DhBVas model has the potential to mimic the different stages (early: WNV and DENV and late: JEV) of virus pathogenesis. Thus, the 3DhBVas model can be used as a robust platform to investigate the early and late virus infection induced by secreted viral protein and to investigate potential targets for antiviral therapies.

77. Seungmi Ryu, PhD

Development of Adeno-Associated Virus (AAV)-Mediated Gene Therapeutics Using Human Induced Pluripotent Stem Cells (iPSCs)-Derived Cerebellar Organoids as a Test Model for Friedreich's Ataxia (FRDA) Treatment
Mentor: **Dr. Carlost Tristan**
Study Section: **Neuroscience - Therapeutics and Translational Research**

FRDA is a debilitating neurodegenerative disease caused by a genetic mutation in the FXN gene, leading to severe changes within the cerebellum. Presently, there is no effective treatment or cure that could repair FXN gene expression in FRDA. In this study, we developed gene therapeutics that promote FXN expression, tested in iPSC-derived cerebellar organoid models from FRDA patients. We employed two different strategies to promote FXN gene expression in the FRDA cerebellar model: episomal delivery of the FXN transgene or a CRISPR-Cas9 system to repair the mutation in the FXN gene. The former approach is designed for the transduced cells to transiently express FXN, acting as a temporal treatment for the disease. The latter approach is designed to permanently excise the mutated region in the FXN gene of the transduced cells, which is harder to achieve compared to the former approach but could serve as a cure for FRDA when performed successfully. As a delivery method, AAV was engineered to contain either FXN or sets of guide RNAs and Cas9 sequences, enabling safe episomal gene delivery for FXN expression. Among four different AAV serotype tested, a specific serotype shows the highest transduction rate into the cerebellar neurons affected in FRDA, confirmed through immunostaining and flow cytometry. With AAV FXN transgene delivery, western blot analyses confirmed successful expression of FXN and a decrease in activation of caspase 3, a cell death marker elevated in the FRDA cerebellum. Remarkably, FRDA cerebellar organoids show enhanced functional activity upon FXN transgene delivery upon electrophysiological interrogation via microelectrode arrays. On the other hand, using the AAV CRISPR-Cas9 system, the designed guide RNAs were able to successfully excise the mutated region in the FRDA cell lines, confirmed through PCR assays. This system was able to repair the mutated region, allowing FXN gene expression, as shown through RNA In Situ Hybridization staining against Cas9 and the FXN gene. Currently, we are evaluating the efficacy of this gene therapeutic through spatial transcriptomics, aiming to advance our understanding of how these approaches modulate FRDA associated phenotypes. In summary, we have successfully developed two gene therapeutic approaches that could act as either a temporal treatment or a cure for FRDA. Using human advanced in vitro 3D models, this study explores innovative therapeutic approaches to treat a disease with clinical unmet needs.

78. Alex J Vendola, PhD

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Mentor: **Dr. Khalida Shamim**
Study Section: **Chemistry**

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National Center for Complementary and Integrative Health

79. Donald Iain Macdonald, PhD

Defining the role of neuropeptide signaling in pain
Mentor: **Dr. Alexander T Chesler**
Study Section: **Neuroscience – General**

Over a hundred neuropeptides have been discovered in the mammalian nervous system. These molecules are prominently expressed in ascending pain pathways from periphery to brain and their release is thought to modulate diverse aspects of pain and inflammation. However, neuropeptides are almost always co-expressed with fast transmitters like glutamate, and how exactly slow peptidergic transmission contributes to pain remains unclear. To investigate the role of neuropeptides in pain, I have developed a genetic strategy to broadly and selectively block neuropeptide signaling in pain-sensing neurons, while sparing fast glutamate transmission. I generated mice where the gene encoding the neuropeptide processing enzyme peptidyl-glycine alpha-amidating monooxygenase (PAM) was knocked out in pain-sensing neurons. The PAM enzyme is essential for C-terminal amidation – the final processing step of many pain-related neuropeptides. Immunohistochemical staining of the PAM knockout mice showed mature neuropeptides were completely absent from their pain-sensing neurons. Using a cell-based biosensor to image neuropeptide release from these neurons in real-time, I observed a corresponding reduction in neuropeptide signaling. Altogether, knockout of PAM results in the loss of neuropeptide transmission in pain pathways. Now through behavioural and physiological studies, I am using this genetic approach to explore how neuropeptide secretion controls neuronal excitability, pain and inflammation, with the ultimate goal of illuminating how this unique form of signalling orchestrates the complex chemical interplay of brain, body and behaviour.

80. Nisa Roy, PhD

Amygdala cells and circuits at the intersection of pain and stress
Mentor: **Dr. Yarimar Carrasquillo**
Study Section: **Neuroscience - Cellular and Synaptic**

Pain research traditionally isolates neural correlates, often neglecting the intricate interplay of systems shaping pain behaviors. Acute stress, for example, induces robust analgesia in rodents and humans. Despite enormous contribution of a forebrain limbic structure, the central amygdala (CeA), in pain processing, we hardly know how stress-induced changes in CeA cells and circuits contribute to analgesia. CeA, housing heterogeneous groups of neurons, includes somatostatin-expressing cells (CeA-Som) that are inhibited by nerve injury, and their activation drives analgesia. CeA-Som neurons are also activated by negative affective states, driving passive defensive behaviors. CeA projects to periaqueductal gray (PAG), which is involved in analgesia and stress. Within the PAG, GABAergic and glutamatergic neurons have opposite function in pain regulation. We hypothesized that stress increases excitability in CeA-Som and PAG-projecting CeA neurons, enhancing inhibitory synaptic inputs into PAG and leading to stress-induced analgesia (SIA). We address this hypothesis with mouse behavioral assays, optogenetic-assisted circuit mapping, in-vivo fiber photometry, and chemogenetics in freely behaving mice. Forced swim (to induce stress) prior to the formalin model of inflammatory pain reduced formalin-induced nociceptive behaviors, confirming SIA. Viral-mediated channelrhodopsin (ChR2) expression in CeA labels terminals within PAG, confirming CeA inputs into PAG. Whole-cell patch-clamp electrophysiology in acute brain slices showed optically evoked currents and action potential firing in transduced CeA neurons, confirming functional ChR2 expression. Proof-of-principle experiments (agnostic to cell type) further demonstrated robust increase in frequency and amplitude of calcium events in CeA neurons post formalin injection, confirming feasibility of photometry experiments. Ongoing experiments assess SIA-induced changes in inhibitory synaptic strength and transmitter release probability in GABAergic and glutamatergic PAG neurons in response to photostimulation of CeA terminals. Parallel experiments use fiber photometry to measure in-vivo calcium dynamics of CeA-Som neurons during SIA and in-vivo chemogenetics to establish a causal link between CeA-Som neuron activation and CeA-mediated PAG inhibition with SIA. Together our results will provide a new avenue for comprehending neural circuitry associated with behaviors that lie at the intersection of stress and pain.

National Eye Institute

81. Andrea Barabino, Ph.D.

Advancing Retinal Tissue Engineering: A Novel iPSC-Derived 3D Model for Studying Choroid-RPE-Photoreceptor Interactions
Mentor: **Dr. Kapil Bharti**
Study Section: **Stem Cells - General and Cancer**

Our study represents a breakthrough in retinal tissue engineering, leveraging induced pluripotent stem cells (iPSCs) to create a complex in vitro model of the retina. By combining recent advancements in 3D-bioprinting and synthetic peptide technology, we have successfully generated a physiologically relevant RPE-photoreceptor-choroid complex derived solely from a single iPSC line. Previous attempts at recreating this intricate cellular interaction have been limited by the lack of suitable in vitro models. Our model overcomes this challenge by faithfully replicating the structure and function of the photoreceptors-RPE-choroid complex, providing a versatile platform for studying retinal physiology, disease mechanisms, and therapeutic interventions. Key components of our model include iPSC-derived RPE, photoreceptors, endothelial cells, pericytes, and fibroblasts, all integrated into a 3D-bioprinted choroid tissue. Synthetic coiled-coil peptides, known as HelixCAMs, are utilized to drive specific interactions between RPE and photoreceptors, a milestone never previously attained. Functional validation demonstrates the physiological relevance of our model, with iPSC-RPE monolayers exhibiting trans-epithelial resistance and phagocytic ability, and choroid/RPE complexes displaying superior properties compared to primary cell sources. Moreover, our model enables the investigation of immune cell reactions within the RPE/choroid microenvironment, offering insights into complex retinal diseases such as age-related macular degeneration (AMD). The versatility of our platform extends beyond basic research to therapeutic applications, providing a foundation for the development of personalized medicine for ocular disorders. In conclusion, our study represents a significant advancement in retinal research, offering a powerful tool for studying RPE-photoreceptor interactions, elucidating disease mechanisms, and developing targeted therapies. By bridging the gap between basic science and clinical application, our model has the potential to revolutionize the field of ophthalmology and improve patient outcomes for retinal diseases.

82. Vineeta Das, Ph.D.

Accelerating Visualization of Retinal Cells in Patients through Artificial Intelligence Assisted Imaging
Mentor: **Dr. Johnny Tam**
Study Section: **Artificial Intelligence - Machine Learning**

In the pursuit of unraveling the complex organization of cells in the eyes, researchers continually strive to develop advanced imaging modalities capable of elucidating their intricate structures. Among these, the adaptive optics optical coherence tomography (AOOCT) imaging stands out for its potential to provide cellular resolution and 3D visualization of retinal cells directly in the living human eye. Despite our state-of-the-art custom-built AOOCT imager (3MHz) already being approximately 30 times faster than typical commercial systems, it is still constrained by limitations in light source hardware, restricting image acquisition to 13 frames per second, resulting in prolonged imaging sessions when exploring various retinal locations during clinical diagnosis in eye clinics. Operating beyond this threshold compromises the ideal sampling necessary for resolving microscopic retinal cells. We developed an artificial intelligence (AI) assisted imaging framework where AI works together with AOOCT to accelerate imaging speed by overcoming the fundamental hardware limitation and restoring cellular details from under-sampled images. This integration of AI into the image acquisition process represents a novel approach, diverging from the traditional post-acquisition applications of AI. The model effectively combines two types of neural networks, namely the convolutional neural network that can model local spatial features in images and the transformer network capable of modeling global features across images to improve cell visualization. Trained and validated on over 10,000 images, the AI method effectively facilitates clear visualization of cone photoreceptors and hexagonally shaped retinal pigment epithelial cells in patients. Furthermore, the AI-restored images exhibit a high peak signal-to-noise ratio score of 26 +/- 1.9 dB, surpassing the under-sampled images by 6 dB (2X improvement in signal quality), indicating high visual similarity to the ground truth images. Finally, to demonstrate real world applicability, cell spacing in the restored images estimated using power spectra is also consistent with expected histologic values, ensuring accuracy and reliability of AI-assisted imaging in clinical settings. The AI-assisted AOOCT imaging provided a strategy to circumvent hardware limitations, achieving ~120X faster speed than commercial systems while maintaining signal quality, and promising accelerated and enhanced imaging for speed-constrained systems.

83. Maxwell K Foote, BA Neuroscience

Non-synaptic mechanism of ocular dominance plasticity
Mentor: **Dr. Wei Li**
Study Section: **Neuroscience – Sensory**

Synaptic plasticity is a core attribute of the brain and nervous system, manifesting the capacity for changes in neural communication over time due to variations in activity levels. This adaptability is essential for various functions, including learning and memory, neural development, information processing, and recovery post-injury. Traditionally, experiments involving monocular and binocular deprivation have laid the groundwork for our understanding of synaptic plasticity, often presupposing that neural impulses from both eyes remain constant and unaffected by visual experiences in adulthood. However, our research challenges this notion, proposing that monocular deprivation and inhibition may induce visual experience-dependent modifications in axonal conductance of the visual pathway, specifically by altering the structure of the node of Ranvier (NOR), thereby introducing a new dimension of neural plasticity in adult animals. In our study, we induced monocular inhibition (MI) in the retinal ganglion cells of Vglut2 CRE mice through the overexpression

of Kir2.1 (p40-p100; N=6). In addition, we subjected WT mice to monocular deprivation (MD) by suturing one eye (p40-p100; N=3). Subsequently, we harvested optic nerves and optic tracts at p100 for immunohistochemical analysis and measured the NOR gap lengths. We discovered that both MD and MI induce activity-dependent alterations in the NOR morphology of adult mice along the visual pathway. This suggests that axon myelination remains a dynamic process in adult animals, unveiling a previously unrecognized non-synaptic form of plasticity within the visual system. Given the critical role of myelination in determining axonal conduction velocities, spike-time arrival, and neural synchrony, it's plausible that activity-dependent myelination dynamically influences ocular dominance plasticity, adding a new layer to the synaptic plasticity paradigm initially described by Hubel and Wiesel. This novel aspect of activity-dependent neural plasticity may extend its relevance beyond the visual system, potentially impacting other areas of the nervous system during periods of development, learning, and repair in response to changes in neural activity.

84. Jaanam Gopalakrishnan, PhD in Pathology

Single cell sequencing reveals a disease associated T cell signature in Alzheimer's disease model
Mentor: **Dr. Han-Yu Shih**
Study Section: **Neuroscience - Neurological and Neurodegenerative Disorders and Injury**

Alzheimerâ€™s disease (AD) is the leading cause of dementia worldwide. Accumulation of amyloid beta plaques and neurofibrillary tau tangles in the brains results in neurodegeneration and cognitive decline. Microglia, the tissue-resident macrophages in the brains, have been recognized as a major contributor of neuroinflammation in AD progression. However, the role of non-microglial immune cells is less explored. In our study, we used 5X FAD transgenic mice harboring human familial mutations in the App and Psen1 genes that recapitulate human AD pathology, to address this question. Leveraging high dimensional flowcytometry and multi-omics approaches including single-cell RNA sequencing (scRNAseq) and single-cell assay for transposase-accessible chromatin sequencing (scATACseq) we investigated the involvement of different immune cells in neuroinflammation. Unsupervised clustering revealed a diverse array of non-microglial immune cells with selective enrichment of alpha-beta T cells in the brains of 5xFAD mice compared to the wild-type B6 mice at 6 and 12 months of age. Within the CD8+ T cell subsets, we identified a Type I Interferon responsive CD8+ T cell subset, termed disease associated CD8+ T cells (DATs), significantly enriched in 5xFAD brains. Immunohistochemistry and flow cytometry confirmed that DATs were tissue-resident memory cells primarily localized within the hippocampus and cerebral cortex of 5XFAD brains and distal from vasculature. Remarkably, DATs exhibited unique chromatin accessibility features in their interferon responsive motifs, reshaping their regulome architecture into distinct subset within the CD8+ T population. Furthermore, depletion of CD8+T cells in 5XFAD/B2M (beta-2 microglobulin) knockout mice resulted in reduced amyloid beta plaque deposition and ameliorated memory decline, highlighting the therapeutic potential of targeting DATs in AD. Future studies will entail employing anti-CD8 monoclonal antibody treatment or blocking interferon signaling specifically in CD8+ T cells in 5XFAD to validate the physiological relevance of DATs in compacting AD pathology. In summary, our study sheds light on the previously overlooked role of non-microglial immune cells, particularly DATs, in driving neuroinflammation in AD. By elucidating their distinct molecular and functional attributes, we uncover potential avenues for therapeutic intervention aimed at mitigating AD progression.

85. Ting-Yi Lin, MD

Genetic and Epigenetic Insights into the Aging of the Human Retina
Mentor: **Dr. Anand Swaroop**
Study Section: **Gene Expression - Transcriptional Regulation**

Genome-wide association studies of age-related diseases have identified genetic variants implicated in pathophysiology. However, how changes in aging patterns and genetic variations impact gene expression (GE) and aging-associated phenotypes has yet to be explored. We introduce an innovative framework that longitudinally characterizes the physiological aging of the retina and quantifies the relative contributions of advanced age and genetics to GE and DNA methylation (DNAm). We have performed global GE and DNAm profiling of at least 262 postmortem human control retinal samples spanning the nine decades of lifespan. We incorporate transcriptomic and DNAm dysregulations by modeling age-related changes using linear and non-linear regression models. Leveraging quantitative trait loci (QTL) findings, we aim to investigate the influence of GE and DNAm on aging by analyzing differential patterns across the lifespan and discern the role of the epigenome in the aging transcriptome by integrating GE and DNAm to identify the expression of quantitative trait methylation (eQTM), considering the interaction with age and the inherent variability of genetic polymorphism on these traits. Significant heterogeneity is evident in postmortem transcriptome data of the retina samples. Principal component analysis and surrogate variable analysis reveal that correcting for technical factors through batch correction effectively captures the majority of variance while highlighting age-sex products, substantiating the rationale for their inclusion. Differential GE between males and females between old (70-90) and young (20-40) age groups (in 20-year bins) indicates that most genes exhibit consistent expression (logFC = 1) and linear trends (R2 = 1) throughout the lifespan between the sexes. Upon separate analysis of DGE in males and females, it becomes evident that specific genes (BTG2, DUSP, USH2A, SERPINA3, GFAP, VTN ¾ many implicated in neurogenerative disease) display varying magnitudes of expression changes and trajectories with age in particular males. Our study identified genes whose expression changes in the retina with age between the sexes and will investigate the relationship between GE, DNAm, and genetic regulation in the aging retina. Integrating colocalization analyses and other methods with age-dependent eQTL and mQTL can help identify additional causal genes and pathways associated with aging and various eye diseases, including AMD and glaucoma.

86. Davide Ortolan, PhD

An integrated morphometric 3D map of retinal pigment epithelium subcellular structures provides a holistic view of cell states transitions during the establishment of apical/basal polarity
Mentor: **Dr. Kapil Bharti**
Study Section: **Stem Cells - General and Cancer**

The retinal pigment epithelium (RPE) is a highly specialized monolayer that forms a selective barrier between the subretinal and the choroidal space. During development RPE cells polarize perpendicularly to the plane of the monolayer and develop specialized structures to perform different functions on each side. When RPE polarity is disrupted, the homeostasis of the outer retina is altered leading to retinal degeneration. The mechanisms that regulate RPE polarity are still unclear, however understanding how this process unfolds can help develop treatment strategies for retinal degenerations. Using high-content imaging, AI-based segmentation, and phenotypic quantification techniques, we generated quantitative and statistically robust three-dimensional (3D) maps of RPE subcellular structures to understand cell state transitions occurring during the establishment of apical/basal polarity. Sixteen different iPSC lines expressing fluorescently tagged proteins were imaged separately across the 4 weeks of cell polarization and the structures were geometrically aligned, measured, and statistically characterized to generate integrated reference maps. A condition where the acquisition of apical-basal polarity was prevented was included as control. We found that the average number of desmosomes in polarized cells increased by 75% compared to week 1, while the number of gap junctions decreased by 40%. Surprisingly, we found that both the endoplasmic reticulum (ER) and mitochondria underwent fusion during polarization, as their volume increased by 30% and 15% compared to week 1, while their number decreased by 61% and 47%, respectively. This result indicates that the formation of a stable ER network and a metabolic shift contribute to RPE maturity. The average lysosome location shifted apically in polarized cells, highlighting the importance of subcellular location for cell functionality. In addition, we discovered that the nuclear envelope developed invaginations in almost all maturing cells, suggesting a connection between gene expression and changes in cell shape. These holistic morphometric maps provide statistically accurate representations of cell state transitions that can be used as reference to discover structural abnormalities in conditions where RPE maturation is disrupted. Future studies will focus on using these morphometric maps to discover defects linked to specific retinal diseases to help develop new treatment strategies for retinal degenerations.

87. Zixuan Peng, MBSS

A Single-cell View of Retina-Specific T Cells in a Model of Ocular Immune Privilege Reveals Unique Regulatory and Anergic Signatures
Mentor: **Dr. Rachel R Caspi**
Study Section: **Immunology – Autoimmune**

The healthy eye limits destructive inflammation through the phenomenon known as immune privilege, but despite that, it is subject to autoimmune uveitis driven by retina-specific effector T cells. Using an in vivo immune privilege model based on introducing allotypically marked, TCR-transgenic naive retina-specific T cells into eyes of healthy mice, we found that after one week all the injected cells became primed within the eye. Among them, about 30% had converted to functional Foxp3+ regulatory T cells (Tregs), whereas the remaining ~70% did not convert to Tregs, but nevertheless failed to elicit pathology. It has long been known that ocular fluids in vitro, as well as the ocular environment in vivo, support conversion of T cells to Tregs. However, in-depth investigation of the cell fate of the non-Foxp3-converted T cells, and why they fail to elicit pathology, was hindered by the limited number of cells retrievable from mouse eyes. Therefore, to gain insights into this, we tracked changes in gene expression of retina-specific T cells responding to their cognate antigen in the living eye, at the single-cell level. Our data revealed unique transcriptional signatures not only in the Foxp3+ Tregs, but also in the non-Foxp3-converted T cell populations. Of particular interest, most non-Foxp3-converted T cell populations appeared phenotypically anergic with high levels of inhibitory regulators (Ctla4, Lag3, Pdcd1, Cblb, and Dgkz), and expressed higher levels of some immunosuppressive genes, e.g. Tgfb1, than the converted Foxp3+ Tregs. Moreover, trajectory analyses suggested a branched pattern showing that the Tregs and anergic T cells differentiate simultaneously, rather than in tandem, from a common proliferative precursor. Our data do not exclude the possibility that some non-converted T cells could express residual effector function but are controlled by the Foxp3+ Tregs. Our findings offer novel insights into ocular immune privilege and its complex underlying network of inhibitory mechanisms and could suggest therapeutic approaches to ocular inflammatory diseases.

National Heart, Lung, and Blood Institute

88. Marcus Andrews, PhD

Historic Redlining and Contemporary Disparities in Obesity Prevalence in New York City
Mentor: **Dr. Tiffany Powell-Wiley**
Study Section: **Cultural, Social and Behavioral Sciences**

Background: Obesity is a significant public health concern and contributes to chronic disease risk and worsening life expectancy in the US. Evidence supports adverse neighborhood conditions as contributing to and increasing the risk of having obesity. However, the impact of historic redlining as a structural marker of neighborhood environment on obesity prevalence remains understudied.

Methods: 500 Cities Project data on obesity prevalence (measured as the percentage of adults having obesity) within New York City were merged with historic redlining data from the Mapping Inequality Project and census tract-level American Community Survey demographic data. We used linear regressions to examine relationships between historic redlining scores and census tract-level obesity prevalence. We also tested whether these associations were mediated by neighborhood segregation.

Results: 1,934 census tracts in New York City were analyzed. Compared to formerly greenlined neighborhoods, blue-, yellow-, and redlined neighborhoods had an increased prevalence of obesity ($\beta=4.34$, $p=0.01$; $\beta=4.65$, $p=0.00$; $\beta=7.28$, $p=<0.001$, respectively). There was a similar pattern in results when modeling the percentage of a neighborhood that is Black/African American as a mediator. Compared to formerly greenlined neighborhoods, there was a positive association between redlining score and the percentage of a neighborhood that is Black/African American (bluelined: $\beta=17.11$, $p=<0.001$; yellowlined: $\beta=15.57$, $p=<0.001$; redlined: $\beta=24.40$, $p=<0.001$). The percentage of a neighborhood that is Black/African American was positively associated with obesity ($\beta=0.13$, $p=<0.001$). Indirect effects for each redlining category were significant, suggesting that the percentage of a neighborhood that is Black/African American is a partial mediator of associations between redlining and obesity prevalence in New York City.

Conclusions: Neighborhood racial composition partially mediates associations between historic redlining and contemporary obesity prevalence. This is one of the first studies to examine such associations. These findings can support the strategic investment in housing conditions and opportunities within historically redlined and formally marginalized communities in New York City.

89. Satish Bodakuntla, PhD

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Mentor: **Dr. Naoko Mizuno**
Study Section: **Protein Structure/Structural Biology**

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90. Ayden Case, BS

The effect of low-dose interleukin-2 on the T cell receptor landscape in patients with acute myocardial infarction
Mentor: **Dr. Claudia Kemper**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

Background and Aims
Atherosclerosis is characterized by chronic inflammation in plaques, with targeted treatments being limited. Previously, we have shown low-dose interleukin 2 (IL-2) can selectively augment anti-inflammatory regulatory T cell (Treg) numbers. However, deeper insight into human T cell biology following immune modulation is needed for clinical translation. We therefore aim to leverage the T cell receptor (TCR) to explore the functions of T lymphocyte biology in atherosclerosis following immune modulation with low-dose IL-2 (ld-IL-2).
Methodology
The Low-Dose IL-2 in Patients With Stable Ischemic Heart Disease and Acute Coronary Syndromes (LILACS) study successfully used ld-IL-2 to increase Treg numbers. Here, we use 5' scRNA and TCR sequencing on peripheral blood mononuclear cells taken from patients with myocardial infarction (MI) before and after three dosing conditions (Placebo, 1.5MIU, 2.5MIU) to facilitate a detailed analysis of ld-IL-2's impact on the TCR landscape within the LILACS trial patients.
Results
Ld-IL-2 increases the number of Tregs and the size of Treg clonotypes, while decreasing Treg clonotype diversity. We developed a bioinformatic technique using the TCR as a "barcode" to track T cell subsets across timepoints, finding that acute MI results in redistribution of Treg clonotypes towards effector phenotypes, with ld-IL-2 reversing this trend. The clonally expanded Tregs were activated relative to non-expanded counterparts, specifically along Treg functional and antigen presentation pathways. We explored antigen specificity via TCR clustering; clonally expanded Tregs expressed shifted recognition motifs, which can be linked to viral and allergy-related antigens. Furthermore, the expanded Tregs demonstrate distinct antigen-receptor interactions with other leukocytes, highlighting possible anti-inflammatory pathways.
Conclusions
Ld-IL-2 impacts the subset composition of T cells at the clonotype level, with modulation of immune ligand-receptor interactions and antigen specificity. Clonally expanded Tregs resulting from IL-2 treatment are phenotypically distinct compared to nonexpanded Tregs, mediating immunosuppressive functions with therapeutic implications for acute MI.

91. Adam Fineberg, DPhil

Myosin-5 varies its step length to carry cargo straight along the irregular F-actin track

Mentor: **Dr. Keir C Neuman**
Study Section: **Biochemistry - Proteins, and Lipids**

Molecular motors employ chemical energy to generate unidirectional mechanical motion against a track. Myosin-5a, a prototypical molecular motor, is a highly expressed motor protein in neurons that is important for intracellular vesicle transport. Consequently, defects in myosin-5a can lead to locomotor dysfunction and the progression of neurodegeneration. To function, the protein must navigate a chaotic cellular environment and irregularity of available binding sites on the F-actin track, whilst moving against Brownian motion. Nevertheless, decades of nanometer-precise optical studies suggest that myosin-5a takes uniform steps spanning 13 subunits (36 nm) along its F-actin track. Using high-resolution interferometric scattering (iSCAT) microscopy, we found that myosin-5a takes strides spanning 22 to 34 actin subunits, despite walking straight along the helical actin filament. Negative stain- and cryo- electron microscopy images of myosin-5a on F-actin also indicate a variable step length. This variable step length can be accounted for by cumulative angular disorder (CAD) in F-actin. CAD within a single actin filament causes random variation in the left-handed rotation between successive subunits, while the axial separation remains fixed. Thus, the actin binding sites available to a myosin molecule is akin to crossing a river on variably-spaced stepping stones. Whilst previous data has identified CAD in static electron microscopy images of F-actin, our data represent the first dynamic measurement of CAD. To test for the presence of CAD, we extended and developed a methodology for identifying CAD in electron microscopy images of F-actin and applied it to publicly available electron micrographs. Our results suggest that CAD is a common and overlooked feature of F-actin that has the potential to be an important factor in determining the function of its interacting proteins. More generally, our results represent an example of the adaptability of motor proteins to accomplish their biological functions in a complex cellular environment.

92. Anjelika Gasilina, PhD

Myosin 10 promotes the physical coherence of migrating dendritic cells by regulating myosin 2 localization and MTOC position
Mentor: **Dr. John Hammer**
Study Section: **Cell Biology – General**

Dendritic cells (DCs) are immune sentinels that orchestrate adaptive immune responses by delivering antigens from peripheral tissues to draining lymph nodes for presentation to T cells. During this process, DCs migrate through environments of widely varying composition and topology, all while maintaining speed and directionality. Consistently, defects in DC migration are syndromic features of immunodeficiencies. DCs migrate using their actin cytoskeleton to explore space, their nucleus to gauge pore size, and their microtubule cytoskeleton to sense cell shape and prevent cell entanglement. How these cytoskeletal networks are integrated and coordinated remains largely unexplored. Here we show that myosin-10 (Myo10), which interacts with microtubules as well as with actin, is required for such integration and coordination. Live cell microscopy showed that, compared to wild type DCs, Myo10 knockout (KO) DCs exhibit multiple leading edges and impaired directionality and persistence when undergoing directed migration within microfluidic devices. In geometrically-complex maze-like microfluidic devices, Myo10 KO DCs additionally fail to correctly position their MTOC and nucleus for optimal pore size selection, remodel their microtubule network, resolve competing leading edges, and retract their trailing edge. Together, these defects lead to cell entanglement and fragmentation. Given that trailing edge retraction and migration through small pores requires myosin 2-dependent contraction at the cell rear, we asked if myosin 2 is mislocalized in the absence of Myo10 by depleting Myo10 in GFP-myosin 2 knock-in DCs using CRISPR/Cas9 or shRNA. Indeed, myosin 2 is no longer localized at the cell rear when these cells are migrating. Consistent with our in vitro observations, we show via whole mount immunostaining and live imaging of ear skin explants that Myo10 KO DCs become entangled within the collagen-rich dermis and struggle to enter lymphatic vessels. Likewise, Myo10 KO DCs injected into the footpads of recipient mice fail to reach the draining lymph nodes. Ongoing studies address the mechanisms by which Myo10 regulates myosin 2 positioning, competing edge resolution and pore size selection. Together our work demonstrates that Myo10 is important for driving the directed migration of dendritic cells and highlights the importance of cytoskeletal crosstalk in the molecular mechanisms required for mounting an adaptive immune response.

93. Poonam Ghosh, Phd

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Mentor: **Dr. J. Robert Hogg**
Study Section: **Molecular Biology – Eukaryotic**

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94. Yuta Koui, Ph.D.

Keratinocyte-derived NGF induces pain in obesity-associated small fiber sensory neuropathy
Mentor: **Dr. Yosuke Mukoyama**
Study Section: **Neuroscience – Sensory**

Small fiber sensory neuropathy often develops in obesity and pre-diabetes. Patients with small fiber sensory neuropathy often experience a sensation of burning pain in the peripheral skin due to damage to sensory nerves and capillaries. However, the therapeutic strategies and signaling mechanisms underlying disease development remain elusive, due to the absence of appropriate in vivo mouse models capable of examining the functional and morphological changes of sensory nerves, as well as the local signaling mechanisms associated with small fiber sensory neuropathy in the skin. Utilizing a novel dermal pain behavior assay involving the TRPV1 agonist capsaicin, ex vivo live calcium imaging with sensory neuron-specific calcium indicator mice, and comprehensive high-resolution immunohistochemical analysis, we have shown that obese mice develop painful behaviors accompanied by sensory hypersensitivity in response to capsaicin stimulation. Interestingly, these painful behaviors and sensory hypersensitivities diminish in the advanced stages of obesity as a result of sensory axon degeneration. At the mechanistic level, the enhanced expression of NGF, previously identified to sensitize sensory neurons in vitro, is observed in the epidermis of obese mice upon the onset of painful behaviors and sensory hypersensitivities. The treatment of skin explants from obese mice with an anti-NGF neutralized antibody or PI3K inhibitor Wortmannin suppresses sensory hypersensitivity. This suggests that NGF-mediated signaling (NGF-TrkA-PI3K signaling) contributes to obesity-associated sensory hypersensitivity. Considering that enhanced capillary permeability is also observed in the dermis of obese mice upon the onset of painful behaviors and sensory hypersensitivities, it could potentially lead to an augmented distribution of insulin and glucose within the epidermis. Indeed, primary mouse keratinocytes enhance NGF expression in culture in response to high levels of insulin, but not glucose. Collectively, these studies suggest that obesity-associated local signals, such as keratinocyte-derived NGF, induce pain in small fiber sensory neuropathy, thereby suggesting a potential therapeutic target for this condition.

95. Nathalia Rocco Machado, PhD

A single amino acid mutation prevents the oxidation of calcium/calmodulin-dependent kinase II (CaMKIId) and confers protection against myocardial ischemia-reperfusion injury in an ex vivo model
Mentor: **Dr. Rodney Levine**
Study Section: **Biochemistry - Proteins, and Lipids**

The Ca2+/calmodulin dependent protein kinase II delta (CaMKIId) is expressed mainly in the heart. It couples an increase in calcium to the activation of ion channels and gene transcription and thus has a pivotal role in cardiac signaling. CAMKII has a regulatory domain that autoinhibits its kinase activity under resting conditions. Binding of Ca2+/CAM relieves the inhibition, and inhibition is restored as Ca2+ undergoes reuptake. However, oxidation of CaMKII cause a constitutive activation that can lead to arrythmias, atrial fibrillation, and heart failure. Our group showed that the autonomy-inducing oxidative modification of CAMKIId is the formation of a disulfide linkage between two cysteines present in the regulatory domain: Cys273 and Cys290. We determined the apparent pKa of those cysteines and found that Cys273 had a low pKa while that of Cys290 was elevated. The low pKa of Cys273 facilitates oxidation of its thiol to the sulfenic acid at physiological pH. The reactive sulfenic acid then attacks the thiol of Cys290 to form the disulfide. Based on these results, we utilized CRISPR to generate a transgenic mouse in which Cys273 was mutated to serine. We evaluated the hearts from wild type and transgenic mice by imaging the heart before and after dobutamine infusion. Dobutamine is a Î²-adrenergic agonist that increases cardiac demand, simulating exercise. No differences were observed between the two groups, indicating that the mutation did not affect cardiac function under physiological conditions. To test our hypothesis that the transgenic mice would be protected from cardiomegaly and arrythmias induced by oxidative stress, we isolated the hearts and studied them with the Langendorff model of ischemia-reperfusion (I/R). It is well established that I/R leads to an increase in reactive oxygen species and, consequently, CaMKII oxidation. After I/R, the WT heart ejection fraction was around 40%, indicating a substantial decrease in the efficiency of heart contractions. In the transgenic mice heart, the ejection fraction was around 65%, which is within normal limits. Our next step will be to analyze the heart tissue and determine the infarct area and the amount of total and oxidized CaMKII before and after I/R. CaMKIId is an important therapeutic target and the discovery of this new CaMKII modification by oxidation and its impact on the I/R injury will provide a better understating of CaMKII behavior under oxidative stress.

96. Suyasha Roy, PhD

Blimp-1 negatively regulates IL-2 signaling in T cells
Mentor: **Dr. Warren J Leonard**
Study Section: **Immunology – General**

Interleukin-2 (IL-2) is a pleiotropic cytokine with immunomodulatory effects on T cells. IL-2 binds to its high-affinity IL-2 receptor (IL-2R) which comprises three subunits, CD25 (Il2ra), CD122 (Il2rb) and the common cytokine receptor gamma-chain CD132 (Il2rg). Binding of IL-2 to its high-affinity IL-2 receptor (IL-2R) initiates downstream signal transduction through the activation of JAK-STAT, PI3K-AKT and MAPK-ERK1/2 kinase pathways. IL-2 signaling regulates tolerance and immunity, with both Il2ra-/- and Il2rb-/- mice developing autoimmunity. This reflects the importance of IL-2 signaling in immune homeostasis which intrigued us to study and explore its regulation in T cells. To identify potential regulators of IL-2 signaling, we performed genome-wide CRISPR knockout screening in IL-2-dependent cells derived from a patient with Adult T-cell Leukemia (ATL). ATL cells are CD4+CD25+ with regulatory T cells (Treg)-like phenotype and constitutively active JAK-STAT signaling. Our CRISPR knockout screening showed enrichment of sgRNAs targeting PRDM1, which encodes Blimp-1, a known inhibitor of IL-2 production in T cells; however, the role of Blimp-1 in IL-2 signaling remains unknown. Conditional knockout (CKO) of Prdm1 in mouse CD4+ T cells and Tregs resulted in increased expression of IL-2R subunits

and pSTAT5. This was further validated in mice infected with influenza virus where IL-2 signaling was enhanced in Blimp-1-deficient T follicular helper (TFH) and T follicular regulatory (TFR) cells. Moreover, adoptive transfer of Prdm1-conditional knockout Tregs into Rag2-/- mice resulted in augmented IL-2 signaling, with attenuated suppression of inflammation in T-cell induced colitis, as compared to wild-type Tregs. Consistent with this, CRISPR/Cas9-mediated deletion of PRDM1 in human CD4+ T cells and natural Tregs led to an enhanced IL-2 signaling, and ChIP-Seq in human Tregs demonstrated direct binding of Blimp-1 to key gene regulatory elements of genes involved in IL-2 signaling. Finally, single-cell RNA-Seq analysis of cells from patients with acute ATL showed a lower percentage of PRDM1+ cells, and this correlated with aberrant constitutively-activated JAK-STAT signaling. Thus, Blimp-1 is a pivotal regulatory node in IL-2 signaling that can be modulated to control T cell responses in human diseases such as ATL.

97. Rachel Zajdel, Ph.D.

The Mental Health of Incarcerated Immigrants
Mentor: **Dr. Eliseo Perez-Stable**
Study Section: **Cultural, Social and Behavioral Sciences**

Background: Immigrants tend to exhibit better mental health compared to their US-born counterparts. Research suggests this immigrant health advantage (IHA) is bolstered by social support embedded in migrant networks. However, the IHA has not been tested among incarcerated individuals, a population which is forcibly separated from family and friends. The removal of this social support may be especially impactful for Black and Latino immigrants due to existing racial and ethnic inequities in incarceration. Therefore, the present study examines if the IHA in mental health extends to incarcerated persons and if there are differences by race and ethnicity. Methods: Data came from the 2016 Survey of Prison Inmates, a nationally representative sample of 20,226 adults in state and federal prisons. Respondents were Black (n=7,104), Latino (n=5,029), and White (n=8,093), and 10% were immigrants. We assessed three facets of mental health. Psychological distress was measured as past 30-day frequency using the Kessler 6-item scale (range:0-24). Depression and anxiety were self-reported binary measures of ever having a physician diagnosis (1=yes). We estimated overall and race and ethnicity-stratified multivariable linear and logistic regressions, while adjusting for age, education, marital status, health insurance, and crime type. Beta coefficients for linear regressions and odds ratios for logistic regressions, both with 95% confidence intervals, are presented. Results: As predicted by the IHA, incarcerated US-born individuals reported significantly more symptoms of psychological distress (B=0.89; C.I.=0.46,1.32) and higher odds of depression (OR=2.03; C.I.=1.64,2.50) and anxiety (OR=2.30; C.I.=1.82,2.90) diagnosis compared to incarcerated immigrants. When stratified by race and ethnicity, the same pattern emerged for incarcerated Latino individuals by immigration status. In contrast, incarcerated US-born Black individuals had lower levels of psychological distress (B=-1.64; C.I.=-2.99,-0.29) compared to incarcerated Black immigrants. There was no relationship between immigration status and mental health among incarcerated White individuals. Conclusion: This is the first study to show that the IHA varies by race and ethnicity within the imprisoned population. The IHA extended to incarcerated Latino immigrants, but it did not hold for incarcerated Black or White immigrants. Black immigrants are particularly at-risk for psychological distress while incarcerated.

98. Cheng Zhang, PhD

Distinguish single nucleotide polymorphism on mtDNA in tissues
Mentor: **Dr. Hong Xu**
Study Section: **Genetics – Diseases**

Mitochondria are transmitted through maternal lineage exclusively in most metazoan, and hence female germline is tasked to limit the transmission of deleterious mtDNA mutations. Previous studies demonstrated that mtDNA replication in female germline depends on active mitochondrial respiration, supporting a model of replication competition, which predicts that wild type mtDNA in healthy mitochondrial would replicate more vigorously than deleterious variants in de-energized mitochondria, and consequently limit the transmission of these mutations to the next generation. While the replication-competition model is logically compelling, it has not yet been demonstrated that harmful mtDNA variants indeed replicate less than wild-type genome within a same heteroplasmic germ cell, due to a lack of effective method to distinguish mtDNA variants in-situ. We developed a method, termed “CARL” (Circular DNA Amplification at Restricted Loci) that can effectively and selectively amplify a circular DNA primer at a specific restriction enzyme site on mitochondrial genome. In this assay, the endogenous mitochondrial DNA were digested with a restriction enzyme and subsequently treated with the exonuclease, to generate a stretch of single strand DNA with free 3’ end. A circular DNA containing a stretch of complementary sequence was then annealed to the ssDNA, which subsequently initiated the rolling circle amplification. This reaction generated a ultralong, periodic single strand DNA that was detected by fluorescence in-situ hybridization and super-resolution microscopy. As a test of principle, we created heteroplasmic lines of Drosophila melanogaster carrying a healthy mutation, abolishing the single Bgl II site on mtDNA (BglII-) and the temperature-sensitive mutation (ts) that disrupts the single Xho I site. We effectively detected both genomes in ovaries and accurately deduced their proportions that was determined by PCR-RFLP analyses. We also confirmed that the ts genome was restricted from transmission in female germline of heteroplasmic flies. By visualizing both healthy and deleterious genomes, we will be able to directly evaluate the replication-competition model. To further expand the application of CARL, we are testing CRISPR-Cas9, other than restriction enzymes, to cleave DNA for visualizing mtDNA SNPs. We anticipate the improved method will greatly advance studies on mitochondrial diseases that are often caused by heteroplasmic point mutations on mtDNA.

National Human Genome Research Institute

99. Mudabir Abdullah, PhD

Unveiling the spectrum of potential hosts for SAR-CoV-2 using a high-throughput yeast display screen.
Mentor: **Dr. Meru J Sadhu**
Study Section: **COVID-19**

The global impact of SAR-CoV-2 extends beyond humans, raising concerns about potential reservoirs and broader implications for disease transmission in various animal species. Understanding the virus's behavior necessitates a comprehensive assessment of its potential host range. However, due to the enormous number of animal species, it is difficult to screen all the species in the laboratory. Central to this inquiry is the angiotensin converting enzyme 2 (ACE2), the primary attachment site for SARS-CoV-2 spike protein, which exhibits substantial variability among species. The ability of the spike protein to interact with ACE2 receptors in potential animal hosts emerges as a pivotal determinant for host compatibility. In this study we use a high-throughput assessment of ACE2 compatibility with coronavirus spike proteins across hundreds of diverse animal species. The interface of ACE2 with the spike protein exhibits high amino acid diversity between species, influencing the potential binding of spike orthologs. Previously it was shown that the main spike-interacting region on the surface of ACE2 involves amino acids from 24-83 and 329-363. To elucidate these interactions, we constructed a library of chimeric ACE2 receptors representing various species' orthologs in the 24-83 ACE2 region crucial for interfacing with SAR-CoV-2 spike protein. We printed an oligo array of DNA molecules containing these segments from ACE2 orthologs representing more than 800 species and cloned them into a backbone of the human ACE2 gene.
The chimeric ACE2 library was transformed into yeast to express the ACE2 protein on the cell surface via yeast surface display, and subsequently incubated with fluorescently labeled SAR-CoV-2 spike proteins. The spike-binding ACE2 chimeras are enriched through fluorescence-activated cell sorting (FACS) and identified through high-throughput Illumina sequencing to identify which animals' ACE2 orthologs are likely to support SARS-CoV-2 infection. On exciting extension of this approach will be to explore whether SARS-CoV-2 variants have altered host range, offering insights into the future dynamics of SAR-CoV-2 host compatibility. This investigation will be pivotal in discerning whether the SARS-CoV-2 host range will remain consistent or evolve into a subset of the original potential hosts.

100. Sara Bang-Christensen, PhD

Combining Methylation Markers for Efficient, Blood-Based Multi Cancer Detection
Mentor: **Dr. Laura Elnitski**
Study Section: **Clinical and Translational Research – General**

Early detection of cancer is of key importance to effective treatment. Since FDA screening programs currently target only single cancer types, the potential to revolutionize oncology screening lies in the ability to detect multiple cancer types using a generalized, pre-symptomatic, non-invasive test. Tumors are known to shed DNA into the bloodstream, manifesting aberrant DNA methylation patterns at CpG dinucleotide sites. We hypothesized that a delimited set of DNA methylation markers could differentiate tumor DNA from multiple cancer types in a simple blood sample. In an analysis encompassing >4,000 tumor samples from the Cancer Genome Atlas spanning 14 cancer types and 2,700 healthy samples we screened for CpG sites with extreme and opposite methylation values between tumors and controls and identified a panel of 8 targeted CpG sites. This panel robustly differentiated tumors across all 14 cancer types with an overall sensitivity of 91.4%. To evaluate the performance of the 8-marker panel in plasma samples from various cancer types we applied targeted methylation sequencing. By incorporating neighboring CpG sites surrounding the original target site, we were able to enhance the sensitivity of detecting tumor signal despite its very low DNA concentration in blood. We developed a computational pipeline to assess the degree of methylation and quantify the fraction of methylated DNA reads. Samples were classified as positive for cancer if the methylation level in at least one of the 8 markers surpassed the signal from all healthy controls, ensuring specificity of 100%. We assessed 38 plasma samples from patients with liver (n=12) and pancreatic (n=13) cancer as well as healthy individuals (n=13). A positive signal was detected in 10 out of 12 liver cancer plasma samples (sensitivity = 83%), and 11 out of 13 of the pancreatic cancer plasma samples (sensitivity = 85%). The best performing marker showed an AUC value of >0.88 in liver and pancreatic cancer types. Additionally, tumor samples displayed a significantly higher fraction of methylated reads compared to healthy controls ($p = 0.001$, Wilcoxon rank sum test with continuity correction). These performance characteristics match state-of-the-art multi-cancer detection platforms analyzing >100,000 regions, making this the first study to describe an economically feasible, low-complexity screening assay with a high potential for application in clinical settings.

101. Braveen B Joseph, PhD

Functional analysis of Ultaconserved sequences in the zebrafish
Mentor: **Dr. Shawn Burgess**
Study Section: **Developmental Biology - Early Development/Embryology**

Approximately 5% of human genome DNA sequences exhibit conservation with the mouse genome. Notably, only 1% of these sequences encode for proteins, suggesting the vertebrate genomes comprises positively selected non-coding regions. The term, “Ultraconserved sequences” (UCs) was coined in 2004 for contiguous DNA Sequences that are at least 200 bp long and exhibit 100% conservation (no deletions, insertions, or single nucleotide polymorphisms), among the human, rat and mouse genomes. There are 481 human-mouse-rat ultraconserved sequences identified. A large proportion of ultraconserved sequences overlap the non-coding regions (53% ; 256/481) in the human genome. Studies in mice using the LacZ transgenic reporter assays has showed that these regions function as regulatory elements. Mutagenesis of a few of these non-coding regions in mice neither dramatically altered their regulatory functions nor elicited any abnormal phenotypes at early developmental time points. Larger-scale analysis of the all the UCs in vivo would give a more complete picture of the function of these regions. Notably, multispecies sequence alignments of UCs exhibit strong conservation across all vertebrates. The model organism zebrafish (Danio rerio) presents as an ideal candidate for in vivo testing the roles of UCs, due to its low cost and well-established CRISPR/Cas9 mutagenesis and genetic strategies.

First, we identified regions within the zebrafish genome exhibiting high sequence similarity to known UCs through sequence alignments. Next, we designed guide RNAs (gRNAs) targeting these non-coding UC regions. We synthesized gRNA templates as oligo pools, then amplified and transcribed individual gRNAs for each locus. We used a previously described high throughput, in vivo knockout technique, Multiplexed Intermixed CRISPR Droplets (MIC-Drop) to mutagenize these regions. Briefly, this approach involves injecting single-cell embryos with droplets containing a gRNA-Cas9 mix targeting a specific locus and a unique barcode. Healthy-looking larvae at 5 days post-fertilization (dpf) are collected, and the barcodes are sequenced. This analysis aims to identify under-represented barcodes, indicating potential essential sequences for early development disrupted by the CRISPR mutagenesis. After optimization and pilot screens, we are now poised to perform a comprehensive screen targeting all UCs in the zebrafish genome.

102. Gustavo Nieto-Alamilla, Doctorate

Hermansky-Pudlak Syndrome: Gene Therapy Assessment for HPS-1-Associated Pulmonary Fibrosis in a Novel Hps1 Knockout Mouse Model

Mentor: **Dr. May Christine V Malicdan**

Study Section: **Clinical and Translational Research - Animal Models**

Hermansky-Pudlak Syndrome type 1 (HPS-1) is an autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding diathesis, and a progressive, often fatal, form of pulmonary fibrosis (HPS-PF). The absence of effective treatments for HPS-PF and the lack of models that accurately mimic the human HPS1 genotype and phenotype have impeded therapeutic advancement. Our research aims to bridge this gap by introducing a novel gene therapy approach using adeno-associated virus (AAV) to deliver the Hps1 gene in a novel HPS1 murine model.

We engineered a new Hps1 knockout mouse model (Hps1-KO) using CRISPR-Cas9 technology, recapitulating the HPS-1 phenotype, including reduced pigmentation, a bleeding diathesis, the hallmark enlarged and foamy type 2 alveolar cells (AEC2), altered pulmonary function, and measurable pulmonary fibrosis as ascertained by LS-MS/MS quantification of hydroxyproline in lungs. We tested AAV serotypes AAV5 and AAV8, known for their lung cell tropism, to deliver the Hps1 gene to the lungs.

To confirm the delivery capabilities of these vectors, we used AAV5-eGFP and AAV8-eGFP and verified the tropism of both vectors after a month of transduction. We then injected AAV5-Hps1 AAV8-Hps1 systemically via facia vein in neonatal mice. Six months following systemic injection of both vectors, we observed an approximate 80% increase in Hps1 mRNA expression in lung tissue. The size of AEC2s was reduced significantly in AAV8-Hps1 treated Hps1-KO mice compared to those given saline.

Of note, AAV8-Hps1 treated mice demonstrated reduced pulmonary fibrosis, and a significant amelioration in lung function parameters, such as tissue stiffness, elastance, compliance, and forced vital capacity. Collectively, these results indicate a marked deceleration in pulmonary function deterioration. Importantly, no adverse effects were associated with the gene augmentation strategy.

The development of the Hps1-KO mouse and subsequent gene therapy experiments provide not only a valuable resource for understanding HPS-1 but also demonstrate the transformative potential of gene therapy as a treatment modality. Our findings lay the groundwork for future research and potential translation into clinical therapy. Ongoing studies are vital for optimizing this therapeutic strategy, ensuring safety, and assessing long-term outcomes, thereby offering new hope for patients afflicted with HPS1.

103. Marya Sabir, BSc

At the intersection of lipid metabolism dysregulation and neurodegeneration: Pathophysiological insights from a novel mouse model of lysosomal Free Sialic Acid Storage Disorder

Mentor: **Dr. May Christine V Malicdan**

Study Section: **Clinical and Translational Research – General**

Free sialic acid storage disorder (FSASD) is an ultra-orphan, rare disease caused by biallelic mutations in SLC17A5, which has also been linked to Parkinson’s disease. Mutations in sialin, the lysosomal sialic acid exporter encoded by SLC17A5, leads to the excessive accumulation of free sialic acid within lysosomes. Patients with FSASD exhibit a broad clinical spectrum, ranging from childhood lethal disease to a slowly progressive neurodegenerative disease course. To study neurodegeneration in FSASD, we generated a knock-in mouse model carrying the prevalent p.R39C variant, which recapitulates key features observed in patients, including increased urinary free sialic acid and progressive ataxia. Histopathological and immunohistochemical analyses demonstrate central hypomyelination, mirroring patient findings. Bulk transcriptomic studies unveiled a significant dysregulation of genes involved in myelination processes,

particularly those governing the development and differentiation of oligodendrocytes. Notably, the knock-in mouse cerebellum exhibited the most pronounced dysregulation, including heightened expression of immune-related genes associated with neuroinflammation (e.g., microgliosis). Furthermore, we investigated the role of lipid metabolism dysregulation in FSASD through targeted transcriptomic profiling, untargeted lipidomics, and high-performance liquid chromatography (HPLC) assays for glycosphingolipids (GSLs) in mouse brains. Transcriptomic studies revealed significantly increased expression of GSL catabolism genes, while untargeted lipidomics highlighted a significant dysregulation of lipids, particularly in the cerebellum, indicating regional vulnerability. Network analyses pinpointed sphingolipid and GSL metabolism pathways as central hubs of dysregulation. Additionally, HPLC-based assays demonstrated accumulation of several GSL species in both the forebrain and cerebellum, with the cerebellum showing the greatest degree of accrual. Our results suggest defects in glycolipid sialylation downstream of lysosomal sequestration of free sialic acid. Collectively, these findings shed light on the dysregulation of lipid metabolism as a prominent disease phenotype in FSASD and paves new paths for therapeutic intervention in FSASD patients and other SLC17A5-linked common diseases.

104. Dennis L Taylor, PhD

Single-cell transcriptomic profiling of human pancreatic islets reveals genes responsive to glucose exposure over 24 hours
Mentor: **Dr. Francis Collins**
Study Section: **Omics - Genomics/Transcriptomics**

Aims/hypothesis: Disruption of pancreatic islet function and glucose homeostasis can lead to the development of sustained hyperglycemia, beta cell glucotoxicity, and subsequently type 2 diabetes. In this study, we explored the effects of in vitro hyperglycemic conditions on human pancreatic islet gene expression across 24 hours in six pancreatic cell types: alpha, beta, gamma, delta, ductal, and acinar cells. We hypothesized that genes associated with hyperglycemic conditions may be relevant to the onset and progression of diabetes.

Methods: We exposed human pancreatic islets from two donors to low (2.8mM) and high (15.0mM) glucose concentrations over 24 hours in vitro. To assess the transcriptome, we performed single-cell RNA sequencing (scRNA-seq) at seven time points. We modeled time as both a discrete and continuous variable to determine momentary and longitudinal changes in transcription associated with islet time in culture or glucose exposure. Additionally, we integrated genomic features and genetic summary statistics to nominate candidate effector genes. For three of these genes, we functionally characterized the effect on insulin production and secretion using CRISPR interference to knockdown gene expression in EndoC-βH1 cells, followed by a glucose-stimulated insulin secretion assay.

Results: Across all cell types, we identified 1,447 genes associated with time, 680 genes associated with glucose exposure, and 418 genes associated with interaction effects between time and glucose. By integrating these expression profiles with summary statistics from genetic association studies, we identified 2,449 candidate effector genes for type 2 diabetes, HbA1c, random blood glucose, and fasting blood glucose. Of these candidate effector genes, we showed that three—ERO1B, HNRNPA2B1, and RHOBTB3—exhibited an effect on glucose-stimulated insulin secretion and production.

Conclusions/interpretation: The findings of our study provide an in-depth characterization of the 24 hour transcriptomic response of human pancreatic islets to glucose exposure at a single-cell resolution. By integrating differentially expressed genes with genetic signals for type 2 diabetes and glucose-related traits, we provide insights into the molecular mechanisms underlying glucose homeostasis. Finally, we provide functional evidence to support the role of three candidate effector genes in insulin secretion and production.

National Institute of Allergy and Infectious Diseases

105. Parvez Alam, Ph.D

Development of RT-QuIC assay for detection of frontotemporal temporal dementia-TDP 43 (FLTD- TDP)
Mentor: **Dr. Byron Caughey**
Study Section: **Biochemistry - Proteins, and Lipids**

FTLD-TDP is the second most common cause of dementia after Alzheimer’s disease, and it is caused by the aggregation of TDP-43 (Tar DNA binding protein 43). As a multifunctional DNA/RNA binding protein, TDP-43 plays critical roles in nucleus-cytoplasmic shuttling, stress granule formation, transcription, translation, mRNA splicing, mRNA stability, and microRNA biogenesis. TDP-43 is mainly localized in the nucleus and moves between the nucleus and cytoplasm. Under pathological conditions, there is an increase in the concentration of TDP-43, leading to the formation of inclusions, resulting in loss of function and gain of toxic function of TDP-43. So far, there is no clear diagnostic test for the detection of FTLD-TDP, and definitive diagnoses can only be made after death.
Real-Time Quaking Induced Conversion (RT-QuIC) assays exploit the self-propagating potential of amyloids or pre-amyloid aggregates to act as seeds that can grow by incorporating recombinant monomers of their constituent proteins through seeded polymerization. Seeding activities in biospecimens can be amplified by many orders of magnitude in vitro using recombinant protein monomers as

substrate. ThT (Thioflavin T), an amyloid-specific dye, is used to detect the formation of amyloids; it will only fluoresce to detectable levels when there is massive amplification of any amyloid seeds in the sample. Here, we developed a novel TDP-43 RT-QuIC assay for the detection of FTLD-TDP in humans. We can detect seeding activity in a 10-3 dilution of brain homogenates from FTLD-TDP patients with 100% specificity (8/8) and (0/6) in case of control brains. We found that the lag time for our TDP-43 positive cases is somewhere between 15-25 hours, and our negative controls remain flat even up to 48 hours. Our electron microscopy analysis of RT-QuIC end products showed the formation of fibrillar aggregates in TDP-43 positive seeded brains compared to no fibrils in the case of controls. Furthermore, our assay can detect both genetic and sporadic cases of FTLD-TDP with 100% specificity. Our overall goal is to develop a diagnostic assay for FTLD-TDP that can be used in clinics. We are currently in the process of implementing this TDP- 43 RT-QuIC assay with some modifications to detect TDP-43 seeding activity in patients' cerebrospinal fluid and nasal brushings. Overall TDP- 43 RT-QuIC assay has huge potential for early diagnosis of FTLD- TDP.

106. Pedro Amado Cecilio, PhD

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Mentor: **Dr. Jose Ribeiro**
Study Section: **Microbiota**

Removed at request of author

107. Nick Berg, MS

Evaluating the Assembly Kinetics of DNA Origami for Development of Antivirals
Mentor: **Dr. Tijana Ivanovic**
Study Section: **Methods/Assay Development**

DNA origami enables the creation of tunable structures at molecular scale. Prior work has yielded self-assembling DNA origami constructs conjugated to antibodies that bind virus particles. These molecules form neutralizing 'cages' around their targets, a cooperative process which supersedes the efficiency of independent binding and neutralization by antibodies. Although encapsidation by DNA origami presents a promising avenue for antivirals, it has thus far failed to achieve complete capture of viruses beyond those with a defined structure. We aim to design a universal origami construct that self-assembles via flexible linker regions to allow for capture of virus particles of all shapes and sizes. However, designing new origami molecules and evaluating their functional performance remains a challenge. To address this limitation, we developed a TIRF microscopy system for visualizing the behaviors of self-assembling DNA origami on a lipid bilayer surface. We demonstrate the capabilities of the system in tracking origami as it diffuses on a model envelope and capture evidence of binding events between monomers. Our approach, combined with single molecule tracking software and an accompanying analysis workflow, automatically extracts interaction kinetics from movies of origami assembly. Using this system, we observed the dynamic formation and dissolution of 2-dimensional origami sheet assemblies on a bilayer surface, confirming the viability of our flexible design in large-scale assembly. Our TIRF system also allows for studying interactions among multiple distinct origami species by way of labeling constructs with different dyes. In this way, we open avenues for studying more complex assembly systems composed of more than one origami design. We next plan to employ these tools in observing origami assembly on the surfaces of pleomorphic influenza virions. Our system allows us to experiment with novel origami constructs, test new approaches for virus recognition and binding, and determine effectiveness of assembly under varying environmental conditions with high efficiency.

108. Cherrelle Dacon, DPhil/PhD

Protective antibodies target a novel, cryptic epitope revealed by cleavage of malaria circumsporozoite protein
Mentor: **Dr. Joshua Tan**
Study Section: **Immunology – General**

In 2022 alone, 249 million cases of malaria and over 600,000 deaths were estimated globally with children under 5 years accounting for 78.1% of deaths within the sub-Saharan African region. Most cases are attributed to the Plasmodium falciparum (Pf) parasite, which initiates infection when the sporozoite form is injected into the skin during the blood meal of a female Anopheles mosquito. The most promising malaria vaccines and monoclonal antibody (mAb) candidates to date target the Pf circumsporozoite protein (PfCSP), which coats the surface of sporozoites. However, the antigenic diversity of the sporozoite surface remains largely uncharacterized. Thus, we have applied an antigen-agnostic approach to isolate and characterize functional mAbs that target novel epitopes on the Pf sporozoite surface. Our study focuses on cohorts of naturally exposed individuals in Mali and US vaccinees (n = 941) who received large doses of radiation-attenuated sporozoites. We screened plasma samples by high-throughput flow cytometry and identified rare donors who retain high reactivity to sporozoites after blocking of immunodominant PfCSP-specific antibodies. We also developed a novel, optofluidics-based Beacon assay which allows for screening of individual B cells for reactivity towards whole sporozoites and recombinant PfCSP in tandem. Using this approach, we isolated 11 mAbs that bind to sporozoites, but not to any form of recombinant PfCSP. Subsequent analysis revealed that these mAbs bind to a cryptic PfCSP epitope on the surface of sporozoites. Western blot and peptide scanning analyses confirm that the mAbs recognize a proteolytically cleaved form of PfCSP that presents an unusual

pyroglutamate residue at its N-terminus. In an in vivo mouse model of infection, the most potent mAb (MAD21-101) reduced liver parasite load by more than 90%. When further tested in an in vivo humanized-liver mouse model, MAD21-101 conferred similar protection from parasitemia versus CIS43, a mAb currently being evaluated in clinical trials. Altogether, our study reveals a novel, protective epitope generated by parasite-mediated, post-translational modification of PfCSP. Notably, the epitope is different from the target of previously discovered anti-PfCSP mAbs. Thus, we propose that this class of mAbs can form the basis of dual-epitope targeting strategies and will enhance the cache of biological tools available for malaria prophylaxis.

109. Katelin L Davis, DVM

Atopic dermatitis promotes food allergy through intestinal remodeling and metabolic perturbances
Mentor: **Dr. Pamela Guerrerio**
Study Section: **Immunology - Mucosal Immunity**

Food allergy (FA) is often a life-long disease with no treatment. The list of risk factors associated with an increased incidence of FA is long and includes a wide array of genetic, environmental, and life-style factors. One of the strongest risk factors is childhood atopic dermatitis (AD). Nearly half of children with AD will go on to receive a FA diagnosis; an epidemiologic phenomenon known as the atopic march. Current literature supports the hypothesis that food antigen exposure through the disrupted skin barrier of AD lesions leads to food antigen specific IgE production and FA sensitization. However, beyond a role in sensitization, little is known about how AD predisposes the immune system to allergic disease at distant organ sites. I addressed this knowledge gap related to the skin-gut axis using a mouse model of the atopic march. MC903 or vehicle control was applied to the ear of mice to induce AD lesions followed by ovalbumin to promote food antigen sensitization. MC903 induced gross and histologic lesions that mimicked human AD. Mice developed ovalbumin-specific IgE antibodies and anaphylaxed to ovalbumin upon challenge, similar to infants with FA. Thus, I validated this as a model of the atopic march. AD, alone, was sufficient to induce major changes in the intestinal cellular composition and metabolome. Histology, immunohistochemistry, and flow cytometry demonstrated that AD mice had increased numbers of small intestinal goblet cells, tuft cells, and intraepithelial mucosal mast cells. Within the lamina propria there were increased numbers of GATA3+ Tregs and less IL-10 producing macrophages. T lymphocytes within the mesenteric lymph nodes produced more IL-4 upon stimulation. Liquid chromatography-mass spectrometry analysis of the plasma, intestine, and feces identified multiple metabolic perturbance. AD mice had increased levels of unconjugated bile acids in their feces, decreased levels of tryptophan and cyclooxygenase dependent metabolites in their intestine, and alterations in circulating and intestinal neurotransmitters. These findings indicate that AD induces a proinflammatory, type 2 skewed environment in the small intestine which may interfere with oral tolerance and predispose AD patients to FA. Several of the metabolic alterations observed in my mice were consistent with an ongoing study of food allergic children in my lab, emphasizing the translational potential of these findings.

110. Francis De Souza Saraiva, PhD

The protective role of mammal hemopexin for hematophagous insects and their transmitted parasites
Mentor: **Dr. Joel Vega Rodriguez**
Study Section: **Stress, Aging, and Oxidative Stress/Free Radical Research**

Hematophagous insects and the parasites they transmit evolved to mitigate the toxicity resulting from blood digestion, including heme release. Mosquitoes rely on the peritrophic matrix to sequester heme crystals, while Plasmodium parasites employ antioxidant enzymes to counteract damage from reactive oxygen species. Proteomic analysis of mosquito hemolymph revealed that Plasmodium infection enriches mammal blood proteins in Anopheles hemolymph. Among these, 8 proteins notably associated with iron metabolism and transport showed significant enrichment in infected hemolymph. These include hemoglobin, albumin, transferrin, hemopexin, catalase, ceruloplasmin, and peroxiredoxin-2. In humans, hemopexin binds free heme and protects from heme-induced damage. Further investigation into hemopexin elucidated its key role in protecting hematophagous insects and their parasites from free heme toxicity. We observed that hemopexin enrichment in the hemolymph is contingent upon parasite invasion of the midgut epithelium and immunofluorescence assays shows hemopexin uptake by both infected and non-infected midgut cells. Utilizing hemopexin knockout mice, we demonstrated that hemopexin facilitates P. berghei and P. falciparum infection in mosquitoes. Mosquitoes feeding on hemopexin knockout mice had increased hemolymph hydrogen peroxide, lipid peroxidation and protein nitration, impacting the mosquito physiology. A. gambiae survival, egg oviposition and egg hatching decreased when feeding on hemopexin knockout mice, also the peritrophic matrix integrity was compromised. Parasite infection and oxidative stress returned to normal levels upon supplementation of blood with recombinant wild-type hemopexin, whereas mutated hemopexin lacking heme-binding amino acids failed to rescue the effects. Notably, mammal hemopexin is important for others vector-borne diseases. Hemopexin enhances A. aegypti and Lutzomia longipalpis survival as well as Leishmania donovani parasites transmission. These results underscore the crucial role of mammal hemopexin for hematophagous vectors and their associated parasites, suggesting a co-evolutionary strategy where they exploit mammal blood molecules to enhance their survival and counteract blood digestion. This host-parasite-vector interaction can be targeted with novel interventions to prevent the transmission of multiple vector-borne diseases.

111. Johannes S Doehl, Ph.D.

The itch/scratch response is an immune-induced defense mechanism against ectoparasites

Mentor: **Dr. Jesus G Valenzuela**
Study Section: **Bioinformatics - algorithms, packages and tools**

Itch [or pruritus] was defined in 1660 as an unpleasant cutaneous sensation which provokes the desire to scratch; a definition largely unchanged to this day. Itch is generally regarded as a nuisance sensation and chronic itch has been frequently associated with detrimental dermatological pathologies. Yet, scratching an itch is a highly rewarding sensation that stimulates the same pleasure centers in the brain as an orgasm. Considering that virtually all higher vertebrates from mammals to fish itch and scratch, it raises the question what the benefits of the itch/scratch response are. In humans, reports of acute itch at tick-bite sites have been associated with significant reduction in Lyme disease incidence. However, experimental evidence is lacking. Like humans, guinea pigs (Cavia porcellus) are known to develop an anti-tick response to repeated tick infestations. Interestingly, this anti-tick response has never been experimentally linked to an itch/scratch response; largely because capsules have been used to evade scratching. By abolishing such restrains, we showed that sensitized guinea pigs actively removed ticks with increasing efficiency with every subsequent infestation within hours after tick placement, which correlated with increased targeted scratch bouts to the site of tick infestation. Restricting physical access to tick-bite sites, e.g., by Elizabethan collar placement, abolished tick removal in sensitized guinea pigs, indicating scratching as the removal mechanism. As scratching is used as an indirect measure for itch and itch intensity, tick location is reveled to the sensitized guinea pig by a very local itch at the tick-bite site, which correlated with the intensity of local inflammation, that increased with every subsequent infestation, too. Immunosuppression by FTY720 treatment, which removed lymphocytes from circulation, prevented acquisition of the tick removal response, suggesting that the itch/scratch response was immune induced and dependent on an acquired immune recall response. Interestingly, detection of host anti-tick antibodies did not occur until ~3 weeks after the initial infestation, while the itch/scratch response was apparent within 5-6 days after the initial infestation. Collectively, this study represents the first formal demonstration that the itch/scratch response is an immune-induced defense mechanism against ectoparasites, like ticks, effectively interfering with vector-borne disease transmission.

112. Paige Fletcher, PhD

Mucosal infection of Taï Forest virus causes disease in ferrets and elicits cross-protective responses
Mentor: **Dr. Andrea Marzi**
Study Section: **Virology – General**

Filoviruses are negative-sense, single-stranded RNA viruses that can cause fatal hemorrhagic disease in humans and nonhuman primates (NHPs). An understudied filovirus, Taï Forest virus (TAFV), has caused only a single human case of infection originating from a TAFV disease NHP outbreak demonstrating that transmission to humans can occur. Existing animal disease models use intramuscular (IM) challenge, however, natural TAFV infection occurs mucosally. We aimed to develop an animal disease model mimicking this natural route of infection. Ferrets are an established model for many mucosal viral infections including filoviruses and develop disease including death after mucosal exposure. Therefore, ferrets were infected with TAFV by IM, intranasal (IN), or aerosol exposure. The IM group showed minimal signs of disease and 100% survival even though TAFV replication was detected in these ferrets. In contrast, mucosal infection resulted in 83% (IN) and 50% (aerosol) survival. Radiograph scores showed increased lung damage in the mucosally-infected groups and preliminary histopathology results confirm this finding. The ferrets that developed severe disease were euthanized 9-10 days post-infection (dpi) presenting with fever, thrombocytopenia, viral shedding, high-titer viremia, and systemic viral spread. Ferrets surviving TAFV infection developed both TAFV- and Ebola virus (EBOV)-specific IgG responses. The current dogma in the field is that there is no to limited cross-protection between filovirus species after IM infection, however, mucosal routes have never been investigated. Therefore, the surviving ferrets from the IM and IN groups were re-challenged IM or IN with EBOV. Only ferrets infected IN with TAFV and IN with EBOV were uniformly protected from disease and EBOV replication. All other ferrets succumbed to EBOV infection 5-7 dpi presenting with filovirus disease.
This data shows that ferrets are a feasible model to assess TAFV pathogenicity by natural, mucosal exposure routes. More importantly, we are the first to demonstrate cross-protection between TAFV and EBOV in ferrets after IN infection. These results challenge the existing dogma in the field as cross-protection can clearly be achieved after mucosal infection in ferrets and very likely occurs in the human population since the natural filovirus infection route is mucosal. Further studies will investigate the mechanism mediating cross-protection in ferrets and the impacts for human disease.

113. Kerry Goldin, DVM, DACVP

Nipah virus replication kinetics and host responses in young versus mature human cerebral organoids
Mentor: **Dr. Emmie de Wit**
Study Section: **Virology - Pathogenesis/Therapeutics**

Nipah virus (NiV) causes severe respiratory and neurologic disease. Outbreaks occur throughout SE Asia, and mortality is typically high (~70%). Nipah virus neuropathology is poorly understood, as human data are lacking, and current animal models skew towards respiratory disease. While neuronal cell monoculture can be helpful in studying NiV, it does not adequately capture the complex environment of the brain or allow for the study of crosstalk between neuronal cell types. Cerebral organoids (COs) are 3D, self-organizing tissue-like structures derived from human induced pluripotent stem cells. COs undergo neuronal development and can be used as models of embryonal-like to adult-like brains. In the embryonal state (~2-months-old (2mo)), COs are composed of neuronal

progenitor cells, low numbers of neurons, and astrocytes. In a more developed state (~6-months-old (6mo)), COs are composed of fewer neuronal progenitors and a higher proportion of neurons, astrocytes, and oligodendrocytes. In addition to more closely recapitulating the complex neuroenvironment than cell monoculture, COs allow us to study the human host response to NiV infection, and reduce the use of animals in research. We compared NiV infection in both 2mo and 6mo CO over a 14-day period. Supernatant was analyzed daily for the presence of viral RNA, infectious virus, and cytotoxicity and whole COs were collected regularly for host gene expression analysis. We found that NiV replicated continuously over a 14-day period in both groups, with replication slightly more efficient in the 6mo COs. Interestingly, cytotoxicity was only mildly increased compared to mock-inoculated COs. This is in contrast to neuronal monoculture, where cells die rapidly after NiV infection. H&E-stained slides revealed little cell death or pathology in either group and on IHC, NiV antigen was abundant. We assessed the interferon response by measuring RNA levels of interferon-stimulated genes (ISGs). We found that only the 6mo NiV-infected COs mounted an effective interferon response. This is likely due to the different cellular composition of the 6mo versus the 2mo COs. We will use single cell RNA sequencing and immunofluorescence analysis to determine which cell types are infected, and which cell types drive the interferon response. Eventually, we will use this model to characterize the host response to NiV in different cell types in the brain and potentially identify targets for Nipah virus encephalitis therapeutics.

114. Julia Gross, BS

Bactericidal Antibiotics Induce Inflammation through TLR9-dependent Sensing of Bacterial DNA
Mentor: **Dr. Iain Fraser**
Study Section: **Microbiology and Antimicrobials**

Despite extensive clinical use of both bactericidal antibiotics (those that directly kill bacteria) and bacteriostatic antibiotics (those that arrest bacterial growth), the immunologic consequences of treating bacteria with antibiotics are not clear. To address that gap we assessed how bacteria treated with the different classes of antibiotics impact innate inflammatory responses. We found that cidal antibiotic treatments that kill bacteria induce greater cytokine responses than static antibiotic treatments that simply halt bacterial growth. We observed this phenotype across multiple cytokine readouts, using several clinical Gram-negative bacterial isolates, and in the serum of infected mice. Bacterial death was sufficient to drive the phenotype; when we treated with artificially high doses of the normally static drug tetracycline to induce bacterial death, we saw increased TNF from infected macrophages that directly tracked the percentage of killed bacteria. Further mechanistic investigation demonstrated that this effect was dependent on TLR9 sensing of liberated bacterial DNA. Imaging studies revealed that only cidal drug treatments liberated measurable quantities of DNA from the bacteria. Furthermore, the enhanced macrophage response to cidal-treated bacteria was lost either in the presence of DNase or the absence of TLR9. Finally, we show that this effect of cidal antibiotics impacts survival in an in vivo murine peritonitis model. Wild-type infected mice that received cidal antibiotic showed increased mortality compared to mice that received static treatments. However, in TLR9-deficient mice, both types of antibiotic treatment supported high survival rates. This demonstrates that the type of antibiotic selected has meaningful consequences for downstream inflammatory effects, and that TLR9-dependent sensing of liberated bacterial DNA can drive harmful inflammation when cidal antibiotics are employed. In summary, we have uncovered a novel link between antibiotic mechanism and its downstream impact on host innate inflammatory responses. A better understanding of the link between antibiotic mechanism and inflammation may allow clinicians to tailor therapy to simultaneously control infections and modulate immune responses in ways that are beneficial to the patient.

115. Adam Hage, PhD

The cellular RNA-binding protein NONO is critically required for replication of pathogenic coronaviruses.
Mentor: **Dr. Sonja Best**
Study Section: **COVID-19**

As demonstrated during the COVID-19 pandemic, coronaviruses (CoVs) represent a threat to public health due to their potential to emerge from zoonotic sources and rapidly spread in human populations on a global scale. Members of the Coronaviridae family are single-stranded, positive-sense RNA viruses with a genome length of approximately 30 kb. Due to this limited genome size, CoVs are unable to encode for the number of proteins necessary for viral replication. To overcome this, CoVs have evolved to “hijack” host RNA-binding proteins (RBPs) and repurpose them as their own replication machinery. Recent published work identifying RNA-binding proteins by mass spectrometry identified the Non-POU Domain Containing Octamer Binding (NONO) protein as a host factor interacting with CoV vRNA. NONO is ubiquitously expressed in tissues and resides in the nucleus where it participates in every step of gene regulation including transcription, pre-mRNA splicing, and RNA transport. However, a role for NONO in CoV replication has not been characterized. Utilizing NONO CRISPR knockout (KO) cells or siRNA-mediated knockdown of NONO mRNA, we identified NONO as a host RBP required for CoV replication. Loss of NONO reduced SARS-CoV-2 replication by 10-fold, while replication of Middle East respiratory syndrome CoV (MERS-CoV) was crippled in the absence of NONO by a 1000-fold reduction in release of infectious virus. Importantly, expression of type-I and III interferons (IFNs) and IFN-stimulated genes were not altered in NONO KO cells following infection compared to wild type controls, and use of the JAK-STAT inhibitor Ruxolitinib did not recover MERS-CoV titers in NONO KO cells. Thus, the requirement for NONO is direct and not due to enhanced IFN signaling. Interestingly, NONO KO cells afforded only a single round of MERS-CoV replication during viral kinetic studies. Mechanistically, viral entry was not affected in NONO KO cells as measured by expression of luciferase delivered by CoV-like particles, while expression of both genomic vRNA and subgenomic mRNA

was halted in infected NONO KO cells. Taken together, our study identifies a role for NONO as a proviral host factor co-opted by pathogenic CoVs supplementing their need for RNA replication machinery by aiding transcription of vRNA. Understanding the proviral role of NONO will be critical for the development of pan-CoV therapeutics and will enhance our understanding of the roles RBPs play during infection.

116. Chang Huang, Ph.D.

Infected Pulmonary Epithelial Cells Mediate COVID-19 Associated Fibrin Deposition in the Lung
Mentor: **Dr. Peter D Sun**
Study Section: **COVID-19**

COVID-19 associated fibrin deposition in the lung is one form of coagulopathy and is prevalent in severe COVID-19 patients. Routine anticoagulation regimens, e.g. enoxaparin and heparin, are empirically introduced to mitigate COVID-19 associated coagulopathy, however, randomized controlled trials indicate patients who are hospitalized and critically ill do not benefit from treatment-dose anticoagulation. Therefore, a deeper understanding of how COVID-19 induces lung fibrin deposition is imperative to therapeutic treatments that reduce the COVID-19 associated deaths. Our proteomic analysis of bronchoalveolar lavage (BAL) fluid indicated plasma leakage into the lung airspace in acute COVID-19 patients, suggesting the need to study how lung epithelial cells mediate COVID-19 associated fibrin deposition. First, we established a cell infection model using primary lung epithelial cells to study lung coagulopathy by SARS-CoV-2 infection. Turbidity assay, Z-stacked imaging, and scanning electron microscope (SEM) validated that SARS-CoV-2 infection indeed induced fibrin clot formation in this model. In a more biologically relevant condition in which we added BAL fluid to the infected lung epithelial cells, we found the fibrin clotting occurred in majority of BAL fluid from acute COVID-19 patients but not in BAL fluid from recovered COVID-19 patients or from healthy donors. We found that these fibrin clots formed in the cell infection model had indistinguishable architecture from blood clots and their formation was dependent on thrombin activation as venous coagulation is. On the other hand, our results showed the primary lung epithelia cell-mediated fibrin clotting was independent on tissue factor which initiates extrinsic pathway of venous coagulation. Furthermore, we found viral infection shed a transmembrane serine protease, matriptase from the lung epithelial cells. Through enzymatic assays, we found recombinant matriptase cleaved prothrombin and in turn induced fibrin clotting, suggesting SARS-CoV-2 infection cleaves transmembrane serine proteases in the primary lung epithelial cells to directly activate prothrombin for fibrin clotting. Our findings revealed a lung epithelial cell-mediated fibrin deposition induced by SARS-CoV-2 infection which differed from venous coagulation, suggesting the need for focusing therapeutic treatments on lung epithelial cells rather than on blood vessels to mitigate COVID-19 induced lung fibrin deposition.

117. Eva Iniguez, Ph.D.

A toolkit based on blood fed sand flies identifies infection reservoirs towards interruption of leishmaniasis transmission
Mentor: **Dr. Shaden Kamhawi**
Study Section: **Methods/Assay Development**

Leishmaniasis is a neglected tropical disease caused by protozoan Leishmania parasites and transmitted by phlebotomine sand flies. The main clinical forms are cutaneous (CL) and visceral (VL) leishmaniasis. CL affects a million people annually, while VL is responsible for up to 90,000 cases per year and is 95% fatal without treatment. Leishmaniasis is mostly a zoonosis, involving animals whose identity is dependent on the nature and biodiversity of disease foci. Unidentified infection reservoirs represent one of the most significant knowledge gaps in CL or VL foci worldwide, hampering development of efficacious vector control strategies to interrupt disease transmission. In this project, we developed an innovative field-applicable toolkit focused on in-depth analysis of individual blood fed sand flies (IBF-SF). Naturally, and without manipulation, the toolkit will reliably identify infection reservoirs and recently infected mammals by linking recurrent feeding behavior of sand flies to parasite development and blood meal source. The toolkit will also reveal focality and intensity of transmission. To build the toolkit, we fed sand flies on Leishmania-infected animals, followed by 2 uninfected blood meals given every 6 days, and processed them at discrete timepoints post-infection. We optimized DNA (mean of 848 ng ±SD 226) and RNA (mean 1099 ng ±SD 252) co-extraction from IBF-SF midguts, enough to run several assays. We used a sensitive Taq-Man probe-based qPCR targeting kinetoplast DNA to quantify the number of Leishmania in early (64 parasites per midgut) compared to mature (59,054 parasites per midgut) infections. We also developed a multiplex PCR panel based on mitochondrial cytochrome b, cytochrome c oxidase subunit I (COI) and d-loop vertebrate-specific gene regions that identified the meals taken by IBF-SF fed on blood from humans and several domestic and sylvatic animals prevalent in disease foci. Lastly, using an RNAseq approach, we identified novel early and late stage-enriched target genes expressed by gut-residing Leishmania parasites. These are being validated by RT-qPCR using IBF-SF. Protocols will be combined to screen IBF-SF collected from VL foci in East Africa and India. Once validated in a field setting, our toolkit has the potential to revolutionize epidemiological investigations of leishmaniasis foci globally, facilitating a targeted approach to vector control in endemic or emerging foci of leishmaniasis towards better global health.

118. Asher Kantor, PhD

Interaction of Dengue virus with a putative Aedes aegypti midgut receptor
Mentor: **Dr. Carolina Barillas-Mury**
Study Section: **Virology – General**

Dengue virus (DENV) is primarily transmitted by Aedes aegypti and causes an estimated 100 million cases of dengue fever and 22,000 deaths annually according to the CDC. There are four serotypes of DENV in circulation and although infection with one strain will lead to long-term protection against the infecting serotype, a secondary infection with a different strain promotes greater risk for dengue hemorrhagic fever through antibody dependent enhancement of disease. The mosquito midgut is the first tissue infected following a bloodmeal from an DENV positive host, and cell entry is thought to be driven by receptor-mediated endocytosis. However, a definitive receptor for DENV in the midgut has yet to be identified. We used a virus overlay protein binding assay (VOPBA) to detect direct interactions between midgut proteins and DENV particles. To simulate the proteolytic processing that occurs during bloodmeal digestion in the midgut, DENV particles were pre-treated in vitro with trypsin and interacted with a single 31 kDa protein that was identified as AAEL01180 by protein mass spectroscopy. This putative midgut receptor (pDENVRec) is a highly conserved protein and is the ortholog of the recently characterized An. gambiae p47 midgut receptor, which allows P. falciparum to evade the mosquito immune system and establish infection of the midgut. We confirmed that silencing the expression of pDENVRec abolished the interaction between DENV and midgut homogenates and showed that recombinant pDENVRec also binds DENV in VOPBAs and ELISAs. Additionally, recombinant DENV surface E glycoprotein binds to recombinant pDENVRec with high affinity (38.2nM) in binding kinetic analysis with surface plasmon resonance. Immunofluorescence staining of midguts establishes that pDENVRec localizes directly under the microvilli of the midgut lumen and could interact with DENV particles during the early stages of viral cell entry. To further study the role of pDENVRec during DENV infection, pDENVRec was disrupted in Ae. aegypti with CRISPR/Cas9, but it proves to be an essential gene only permitting heterozygous knockout (HzKo) individuals. dsRNA injections into HzKo females further reduced mRNA expression of pDENVRec and led to decreased prevalence of DENV infection, demonstrating the importance of the interaction between DENV and pDENVRec for efficient infection of Ae. aegypti.

119. Zenia Kaul, Ph.D.

Itk is essential for metabolic reprogramming, protein translation and effector functions in CD4+ T lymphocytes
Mentor: **Dr. Pamela Schwartzberg**
Study Section: **Cell Biology - Cell Cycle and Metabolism**

The Tec family non-receptor tyrosine kinase, IL-2 inducible T cell kinase (Itk), is a critical component of T cell receptor (TCR) signaling, required for full activation of phospholipase-Cg and effective TCR signaling. However, downstream consequences of loss of Itk are still not fully understood. Activation of T cells is characterized by promotion of effector differentiation, IL-2 signaling, nutrient uptake and reprogramming of metabolism. We previously reported that Itk-/- CD4 T cells have impaired differentiation to multiple T helper lineages with the most severe defects in Th9 cells. We linked this defect to impaired production of IL-2, a cytokine having immune activation functions. Gene set enrichment analysis (GSEA) of RNA-Seq data revealed that genes poorly induced in Itk-/- cells were enriched in metabolic and biosynthetic pathways, including 'biosynthesis of amino acids' and 'glycolysis'—these findings corresponded to impaired induction of enzymes that regulated these metabolic and biosynthetic pathways in the absence of Itk. Furthermore, whereas WT Th9 cells were primarily glycolytic, Itk-/- T cells exhibited marked defects in protein synthesis and glycolysis and were more dependent on mitochondrial respiration. Itk-/- Th9 cells exhibited decreased expression of Myc and multiple downstream nutrient transporters. In-vivo allergic papain experiment also depicted less effector functions of Itk-/- T cells which were also metabolically less synthetic than their WT counterparts. IL-2 addition improved expression of key metabolic genes and transporters in Itk-/- T cells, which in turn was associated with increased glycolysis and improved protein translation. To dissect these phenotypes, we expressed two IL-2-induced transcription factors, ca-STAT5 and c-Myc in Itk-/- cells. Although both could rescue metabolic defects, only ca-STAT5 could rescue IL-9 expression in Itk-/- cells, whereas Myc increased expression of nutrient transporters to a greater extent. Our results suggest that IL-2 integrates with Itk-mediated TCR signaling both to provide a metabolic checkpoint by regulating nutrient transport and metabolism, and to promote effector T cell function, providing insight into the underlying cellular and metabolic programs that regulate T cell activation that may be key for developing novel approaches to modulate T cell responses in disease. We are currently looking further at mechanisms of rescue by IL-2 to understand the pleiotropic effects of IL-2.

120. Shanna S Leventhal, B.S./B.A.

Antibodies Targeting the CCHFV Nucleocapsid Protein Require TRIM21 for Protection
Mentor: **Dr. Heinrich Feldmann**
Study Section: **Immunology - Infectious Disease**

Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a negative-sense, RNA bunyavirus prevalent across Europe, Asia, and Africa. Commonly transmitted by Hyalomma genus ticks, CCHFV causes severe hemorrhagic disease with case fatality rates ranging from 5-70%. There is a lack of approved interventions and correlates of protection for vaccines are not understood. Recently, we reported an alphavirus-based, self-replicating RNA vaccine expressing the CCHFV nucleocapsid protein (repNP) which induces high titers of non-neutralizing anti-NP antibodies. This vaccine protects both mice and non-human primates (NHPs) from CCHFV infection, and protection correlates with anti-NP titers. However, it remains unclear how non-neutralizing antibodies against the intracellular NP confer protection.

To investigate these antibodies in vivo, we first vaccinated mice deficient in Fc effector functions with repNP. Interestingly, FcγR KO, C3 KO, and mice depleted of NK cells were protected from lethal CCHFV infection, indicating that anti-NP antibodies protect independently

of these pathways. However, mice deficient in the intracellular Fc-receptor tripartite motif containing 21 (TRIM21) vaccinated with repNP were not protected from CCHFV challenge. Vaccinated TRIM21 KO mice experienced lethal disease indistinguishable from sham vaccinated mice. Further, passive transfer of sera from WT mice vaccinated with repNP protected naïve WT but not TRIM21 KO mice from CCHFV disease. These data indicate an essential role for TRIM21 in anti-NP mediated protection. We also found that NP-specific immunity does not require T-cells for protection, arguing against TRIM21-dependent priming of cellular immunity. Further, although anti-NP antibodies are non-neutralizing, delivery of NP-specific antibody to the cytoplasm of cells before or after infection could inhibit CCHFV replication, indicating that these antibodies can inhibit CCHFV. This inhibition was shown for NP-specific antibody from both vaccinated mice and NHPs. Currently, we are investigating how anti-NP antibodies access intracellular NP and TRIM21 to control CCHFV infection and in what cell types this mechanism occurs in vivo. Overall, our findings identify how antibodies against CCHFV NP confer protection, redefine our understanding of "non-neutralizing" antibodies in antiviral immunity and support countermeasure development against CCHFV. This work was funded by the NIH Intramural Research Program.

121. Sandra Mon, MSPH

Longitudinal dynamics of HIV infection into and between stable sexual couples during antiretroviral therapy scale-up in Uganda: a Bayesian population-based approach
Mentor: **Dr. Steven J Reynolds**
Study Section: **HIV and AIDS Research**

BACKGROUND: Sexual HIV transmission remains a priority for HIV pandemic control. Despite unequivocal evidence that HIV antiretroviral therapy (ART) eliminates onward HIV transmission in sexual couples, post-scale-up reductions in population-level HIV incidence have not been commensurate. In sub-Saharan Africa, where stable couples contribute nearly two-thirds of new HIV infections annually, widespread ART uptake has increased population HIV viral load suppression yet HIV transmission persists regionwide. Disentangling spatiotemporal drivers of HIV infection between stable sexual partners (within-couple) versus from external sources (extra-couple) is key to uncovering remaining gaps in population-level ART effectiveness.
METHODS: Longitudinal dynamics of sexual HIV transmission were estimated for 12,468 retrospectively-identified stable, heterosexual couples between 1994-2021 in a population-based open cohort in south-central Uganda. A Bayesian proportional hazards modelling framework with frailty was used to disaggregate relative contributions of within- and extra-couple transmissions to overall HIV incidence. Analyses were conducted with respect to three epochs of ART rollout in Uganda: pre-ART, early ART, and mature ART/Universal Test and Treat (UTT).
RESULTS: 736 couples had incident HIV infection in one or both partners while 11,733 couples did not seroconvert. Overall, within-couple HIV incidence was higher than extra-couple, with no difference by gender. However, men had higher rates of extra-couple infection compared to women. Early ART scale-up had no significant effect on within- nor extra-couple incidence, but UTT expansion more than halved the rates of HIV incidence via both routes, relative to pre-ART rates.
CONCLUSION: Our initial findings support that population-level reductions in HIV incidence are associated with ART scale-up in Uganda. However, this relationship is non-linear, particularly for within-couple transmissions, which suggests that additional factors may be sustaining HIV incidence in stable sexual couples. Next steps will identify and quantify the salient individual- and population-level drivers of HIV risk in this cohort through ART scale-up.
IMPACT: This study will outline the overall public health impact of current combination prevention interventions on HIV incidence in stable sexual couples, and identify important route-specific targets of HIV risk to inform effective and equitable HIV interventions.

122. Palak N Patel, Ph.D.

Strain-transcending anti-AMA1 human monoclonal antibodies neutralize malaria parasites independent of direct RON2 receptor blockade
Mentor: **Dr. Niraj H Tolia**
Study Section: **Immunology - Infectious Disease**

Malaria remains one of the most fatal and prevalent infectious diseases globally. Apical membrane antigen 1 (AMA1) is a promising vaccine and therapeutic antibody target to prevent blood-stage malaria infection. However, AMA1 alleles in endemic areas are highly polymorphic. These polymorphisms serve as an immune evasion strategy to circumvent strain-transcending protection, preventing the development of effective strain-transcending vaccines based on AMA1. AMA1 interacts with rhoptry neck protein 2 (RON2) during merozoite invasion into red blood cells, and this essential interaction is conserved among apicomplexan parasites. While extensive research has focused on antibodies that neutralize parasite growth by disrupting the direct interaction between AMA1 and RON2, no monoclonal antibodies have yet been identified that neutralize parasites through alternative mechanisms. In this study, we investigated a panel of naturally acquired human monoclonal antibodies (hmAbs) specific to AMA1, derived from individuals living in malaria-endemic areas. Our approach utilized structural biology and biophysical tools, along with parasite growth inhibition assays. We evaluated the specificity and binding kinetics of human antibodies using enzyme-linked immunosorbent assay and biolayer interferometry. Epitope binning experiments revealed that two potent neutralizing hmAbs engage AMA1 without disrupting RON2 binding. Co-crystal structures of AMA1 with these neutralizing hmAbs were determined, revealing novel and distinct conformational epitopes. Both hmAbs neutralized diverse parasite strains, and the combination of these hmAbs showed synergistic enhancement of parasite neutralizing activity. These findings highlight a novel, strain-transcending surface targeted by naturally acquired human

antibodies that hold promise for the development of broadly protective vaccines and therapeutics. Importantly, this work underscores the significance of neutralization mechanisms for AMA1 hmAbs that are independent of RON2 blockade. These new paradigms are currently being leveraged to develop therapeutic antibodies and design potent and durable vaccines for malaria and other diseases caused by apicomplexan parasites.

123. Annika Pfeiffer Daniels, PhD

IgE sensitized mast cells secrete extracellular vesicles presenting IgE with potential implications for allergic disease
Mentor: **Dr. Ana Olivera**
Study Section: **Immunology - Innate and Cell-mediated Host Defenses**

Elevated IgE levels are commonly associated with allergic diseases. IgE bound to the high affinity IgE receptor (FceRI) can be crosslinked by antigen, stimulating mast cells (MCs) to degranulate and release inflammatory mediators responsible for allergic symptoms. We and others have shown that MC degranulation is accompanied by the secretion of extracellular vesicles (EVs). EVs are nanosized lipid-bound particles that carry cargo such as proteins, nucleic acids, or metabolites and function as intercellular messengers by interacting and transferring their cargo to recipient cells. While IgE/antigen mediated activation of MCs has been broadly studied, less is known about the consequences of IgE sensitization alone. We found that IgE sensitization with various antigen specific IgEs increased EV secretion from a human MC line (LAD2) and from primary human MC cultures. In contrast, MC exposure to IgG did not alter EV secretion, suggesting IgE-specific events. Interestingly, secreted EVs carried IgE bound to FceRI on their surface that could bind antigen, implying that EVs disseminate FceRI/IgE complexes from sensitized MCs. A lipidomic analysis revealed that EVs released from IgE sensitized MCs had a unique phospholipid signature compared to EVs from non-sensitized cells, supporting that IgE sensitization alone has cell intrinsic consequences. In comparison, EVs co-secreted during IgE/antigen mediated activation differed in lipid and protein composition, and carried significantly reduced IgE, suggesting specific biological roles of IgE presenting EVs distributed by sensitized MCs. Thus, while IgE sensitization is mostly considered to be a passive event, we reveal that sensitized MCs actively disseminate IgE complexed with FceRI on EVs, granting the hypotheses that this process may alter the MC activation threshold as well as that circulating IgE presenting EVs may capture antigen to present it to other immune cells and/or to prevent overactivation of MCs and detrimental consequences to the organism. In addition to testing these hypotheses, we are examining clinical samples from pediatric patients with high total IgE and food allergies to investigate potential correlations between IgE levels, concentration of plasma EVs and the amount of IgE carried by circulating EVs.

124. Steven D Planitzer, B.S. Physics

Dissecting the functional interplay between receptor binding and membrane fusion of single influenza A virions
Mentor: **Dr. Tijana Ivanovic**
Study Section: **Virology – General**

Pandemic outbreaks of influenza A virus (IAV) can arise when zoonotic transmission events occur, requiring viral adaptation to human receptors. Binding of IAV to host receptors determines specificity, with α2,6- and α2,3-sialic acids serving as receptors on human and avian cells, respectively. Understanding the effect of changes in receptor binding at each step of the viral lifecycle may underlie future clinical strategies. Receptor binding allows virion-cell attachment and internalization into endosomes which is followed by membrane fusion and genome release. Whether receptor binding contributes to fusion beyond serving as a physical linker is debated in prior literature, and in vitro investigations have been frustrated by lack of control over the displayed receptors. Here, we augment an existing single-particle fusion assay with a novel, modular and programmable receptor-display platform which allows for a precisely defined receptor environment. To accomplish this, we generated supported planar lipid bilayers presenting single-stranded DNA oligonucleotides (ssDNAs) to which we can attach a receptor (e.g., an α2,6- or α2,3-sialic acid-containing glycan) by conjugation to complementary ssDNA. In this way, we control all aspects of the receptor environment including structure, surface density, multivalency, and distance from the bilayer. We demonstrate that binding and fusion of virions to these bilayers are dependent on receptor presence and explore co-varying surface receptor concentration with parameters which affect fusion kinetics such as pH, fusion inhibitors, or virion size. Moreover, this assay probes for binding avidity since binding and lateral diffusion rate on the bilayer can be extracted from time-lapse data. Using this approach, we aim to determine the contribution of receptor binding to the kinetics and efficiency of fusion. Although we focus here on the IAV fusion mechanism, this technique can be readily extended to other enveloped viruses that use sugar receptors for attachment or membrane fusion.

125. Deepashri Rao, PhD

Implications of using anti-TNF therapies for Crimean-Congo hemorrhagic fever- a cautionary tale
Mentor: **Dr. Heinrich Feldmann**
Study Section: **Virology - Pathogenesis/Therapeutics**

Crimean-Congo hemorrhagic fever (CCHF) is a severe febrile illness in humans caused by the tickborne CCHF virus (CCHFV). The Hyalomma tick vector is distributed widely across the globe with an expanding geographic range due to climate change. CCHF can have case fatality rates ranging from 5-30% or even higher. This is compounded by the issue of non-availability of approved vaccines or

therapies for CCHF, leading to CCHFV being classified as a high-priority pathogen by the WHO. Patients often only present to health care systems in the later stages of disease, further limiting treatment options. A dysregulated inflammatory response is a key feature of CCHF disease, with a proinflammatory cytokine milieu in infected patients. Broadly immunosuppressive corticosteroids have been beneficial in treating severe cases of CCHF, albeit in studies with limited cohort sizes. In severe CCHF cases, multiple inflammatory cytokines including TNF alpha are upregulated and due to the availability of clinically approved anti-TNF alpha therapeutics, we hypothesized that blocking TNF alpha signaling would improve CCHF disease. We tested TNF alpha neutralization in our immunocompetent mouse model of CCHF using a mouse-adapted strain of CCHFV (MA-CCHFV). Surprisingly, mice treated with an anti-TNF alpha antibody displayed worsened clinical disease than their isotype control treated counterparts including increased mortality. At peak disease, significantly higher viral loads in the blood and spleens, as well as elevated levels of several inflammatory cytokines were observed in anti-TNF-treated mice compared to isotype control-treated mice. Recently, the use of therapies targeting the two TNF alpha receptors (TNFR), TNFR1 and TNFR2, has gained attention, and it has been speculated that selective targeting of TNFRs is better than global TNF alpha blockade. However, we found that transient blockade of TNF receptor 1 (TNFR1) resulted in uniform lethality in MA-CCHFV infected mice, demonstrating for the first time, that signaling through TNFR1 is required for survival in CCHFV infection. Together our results suggest that proinflammatory cytokines such as TNF alpha may contribute to protection from CCHFV. Further, our results underscore the need to characterize host contributions to the pathogenesis of CCHFV, in order to carefully weigh the risks and benefits of immunomodulatory therapies for treatment of CCHF. This research was supported by the Intramural Research Program of the NIH.

126. Banhisikha Saha, PhD

Role of immune signaling cascaded in mosquito hemocyte differentiation
Mentor: **Dr. Carolina Barillas-Mury**
Study Section: **Immunology - Innate and Cell-mediated Host Defenses**

Anopheline mosquitoes transmit human malarias, causing more than 600,000 deaths annually. Our group has shown that microvesicle release by mosquito hemocytes (immune cells) is necessary for effective activation of the Anopheles gambiae complement-like immune system, which eliminates most of the parasites during early stages of infection. Morphologically there are 3 types of hemocytes: prohemocytes are precursor cells; oenocytoids are involved in pathogen melanisation; and granulocytes are phagocytic cells. However, single-cell transcriptomic analysis revealed novel prohemocyte and granulocyte subpopulations with distinct molecular markers. The Toll, JNK, IMD and STAT signaling cascades are important modulators of mosquito immunity and of hematopoiesis in Drosophila larvae. However, their role in hemocyte differentiation in adult female mosquitoes has not been well established. We investigated the effect of modulating specific immune pathways on hemocyte gene expression by bulk RNAseq, and on cell differentiation by single-cell RNAseq (scRNAseq) transcriptomics. Through an in-depth sequencing and annotation of hemocytes transcripts using long (PacBio) and short sequencing reads (Illumina), we identified 4,020 transcripts from novel An. gambiae genes and 20,008 novel isoforms resulting from differential splicing of transcripts from previously annotated genes. Differential expression analysis of bulk RNA sequencing showed that activation of some immune signaling pathways enhanced expression of cytoskeleton, vesicle-transport and secretion-related genes, as well as markers of specfic hemocyte subpopulations. A protocol for scRNAseq was optimized that allowed for stabilization and storage hemocytes prior to sequencing. This resulted in high target cell recovery, enabling us to investigate hemocyte population dynamics at high resolution. A novel pipeline, suitable for the analysis of mosquito hemocyte scRNAseq data, was established, and we are currently analyzing a scRNAseq data set from more than 64,000 hemocytes collected from mosquitoes under different experimental conditions. These studies are providing new insights into how immune signaling cascades modulate hemocyte differentiation.

127. Wanjing Shang, PhD

Unlocking the Therapeutic Potential of HPK1: A Comprehensive Investigation Through High-multiplex Imaging Techniques and Computational Analysis
Mentor: **Dr. Ronald Germain**
Study Section: **Immunology - Tumor Immunology**

Anopheline mosquitoes transmit human malarias, causing more than 600,000 deaths annually. Our group has shown that microvesicle release by mosquito hemocytes (immune cells) is necessary for effective activation of the Anopheles gambiae complement-like immune system, which eliminates most of the parasites during early stages of infection. Morphologically there are 3 types of hemocytes: prohemocytes are precursor cells; oenocytoids are involved in pathogen melanisation; and granulocytes are phagocytic cells. However, single-cell transcriptomic analysis revealed novel prohemocyte and granulocyte subpopulations with distinct molecular markers. The Toll, JNK, IMD and STAT signaling cascades are important modulators of mosquito immunity and of hematopoiesis in Drosophila larvae. However, their role in hemocyte differentiation in adult female mosquitoes has not been well established. We investigated the effect of modulating specific immune pathways on hemocyte gene expression by bulk RNAseq, and on cell differentiation by single-cell RNAseq (scRNAseq) transcriptomics. Through an in-depth sequencing and annotation of hemocytes transcripts using long (PacBio) and short sequencing reads (Illumina), we identified 4,020 transcripts from novel An. gambiae genes and 20,008 novel isoforms resulting from differential splicing of transcripts from previously annotated genes. Differential expression analysis of bulk RNA sequencing showed that activation of some immune signaling pathways enhanced expression of cytoskeleton, vesicle-transport and secretion-related genes, as well as markers of specfic hemocyte subpopulations. A protocol for scRNAseq was optimized that allowed for stabilization and storage

hemocytes prior to sequencing. This resulted in high target cell recovery, enabling us to investigate hemocyte population dynamics at high resolution. A novel pipeline, suitable for the analysis of mosquito hemocyte scRNAseq data, was established, and we are currently analyzing a scRNAseq data set from more than 64,000 hemocytes collected from mosquitoes under different experimental conditions. These studies are providing new insights into how immune signaling cascades modulate hemocyte differentiation.

128. Mansoor Azeem Siddiqui, PhD

Single-cell studies uncover a large-conductance ion channel on malaria parasite merozoites
Mentor: **Dr. Sanjay A Desai**
Study Section: **Microbiology and Antimicrobials**

Plasmodium falciparum, the most virulent human malaria parasite, invade and replicate within red blood cells to evade host immunity and consume hemoglobin. Invasion by the small extracellular merozoite requires transport of Ca++, K+, and possibly other ions, but the responsible mechanisms remain unexplored because merozoites are short-lived and cannot be readily purified from other parasite stages in culture. We have now addressed these problems through single-cell patch-clamp of healthy merozoites after mechanical release from mature infected cells. While generally reserved for much larger cells, we obtained high-resistance patch-clamp seals on the small merozoite (~ 1 μm diameter) by manufacturing small-bore pipettes with suitable resistance-capacitance properties. We confirmed merozoite capture by using an engineered P. falciparum line with tandem GFP-derivative tagging of CLAG3 and RhopH2, two parasite proteins packaged in apical organelles of viable merozoites. Using patch-clamp and a near-physiological buffer containing NaCl and KCl, the primary activity detected on merozoites was a 358 picosiemen ion channel. This channel exhibited complex gating with a bell-shaped voltage-dependence and was open nearly 100% of the time at the cell's resting membrane potential. A smaller conductance channel was also occasionally seen. Treatment of parasite cultures with ML10, a protein kinase G inhibitor that prevents discharge of microneme organelles from invasive merozoites, did not abolish channel activity, suggesting one or more channel proteins resident on the merozoite plasma membrane prior to escape from host erythrocytes. The ion channel was detected on two P. falciparum lines originally harvested from patients on two separate continents, suggesting a conserved channel required by invasive merozoites. Experiments with different recording solutions, conditional knockout lines, and the distantly-related P. knowlesi human & primate malaria parasite are underway to determine which ions are permeant through this new channel, its molecular basis, and degree of conservation in the Plasmodium genus. These experiments provide the first direct measurement of ion transport at the surface of merozoites and define an ion channel mechanism. Because infection cannot develop without merozoite signaling and invasion, our findings also lay the foundation for novel antimalarial therapies that prevent the parasite's replicative cycle and onset of clinical disease.

129. Brendan D Snarr, PhD

CARD9-expressing monocytes are critical in defending against fungal encephalitis.
Mentor: **Dr. Michail Lionakis**
Study Section: **Immunology - Infectious Disease**

Patients with deleterious variants in caspase recruitment domain-containing protein 9 (CARD9), which relays signals from C-type lectin receptors, are selectively susceptible to fungal infections of the brain. Mechanistically, CARD9 promotes protective neutrophil (PMN) recruitment to the Candida-infected brain by activating resident microglia to sequentially produce IL1b and CXCL1. However, mice with microglia-specific CARD9 deletion only partially phenocopy CARD9-null mice regarding defective PMN recruitment and impaired control of brain fungal burden, indicating that other non-microglial cell types must also require CARD9 to protect against brain fungal invasion. Here, we investigated what other CARD9+ cells are involved and the mechanisms by which they protect against fungal encephalitis. Candida infection of bone marrow chimera mice showed that CARD9 expression in the radiosensitive hematopoietic compartment confers greater antifungal protection relative to CARD9 expression in the microglia-containing radioresistant compartment. To define which cells express CARD9 during Candida encephalitis, we generated a CARD9-reporter mouse and found CARD9 to be widely expressed across myeloid cells, with the highest expression in PMNs and CCR2+ inflammatory monocytes (IMs). Notably, PMN-specific deletion of CARD9 did not impair susceptibility to candidiasis. By contrast, conditional CARD9 deletion of the monocytic lineage in CCR2creCARD9flox mice, which had intact CARD9 expression in microglia, decreased survival and increased brain fungal burden compared to littermate control mice. While CCR2creCARD9flox mice had similar PMN accumulation in the infected brain compared to littermates, they exhibited markedly decreased accumulation of IMs and monocyte-derived dendritic cells. Decreased IM accumulation was not due to decreased cell survival or reduced hematopoiesis, pointing to an IM recruitment defect. Mixed bone marrow chimera mice had similar accumulation of wild-type and CARD9-deficient IMs in the infected brain, indicating that CARD9 deficiency does not confer cell-intrinsic chemotaxis defects in IMs and pointing to defective chemoattractant production, which is under investigation. Moreover, whether CARD9 expression in IMs mediates IM antifungal effector functions and/or primes recruited PMNs to effectively clear the fungus are under study. In summary, our data reveal a critical, CARD9-dependent role of CCR2+ monocytes in defense against Candida encephalitis.

130. Monica A Valtierra Alvarado, PhD

Lung intrinsic resistance to bacterial infection in obese mice.

Mentor: **Dr. Catharine M Bosio**
Study Section: **Immunology - Innate and Cell-mediated Host Defenses**

Obesity represents a serious and growing health problem that has been reported to predispose individuals to an increased risk of developing more severe infections. While the increased sensitivity to viral pulmonary infections is well documented in the obese host, the outcomes of bacterial infections originating in the lung are less well described. Utilizing a diet-induced obesity (DIO) mouse model, we previously demonstrated that obese mice were more resistant to pulmonary infection mediated by the virulent bacterium Francisella tularensis (Ft) SchuS4 in contrast to DIO mice infected with SARS-CoV-2. However, we did not determine if this improved outcome was specific to Francisella tularensis and/or if resistance to infection could also be observed following infection initiated in the periphery. In this study, DIO and regular weight (RW) mice were intranasally (i.n.) challenged with Ft SchuS4, the attenuated Ft LVS, and Bordetella pertussis, or intradermally (i.d.) infected with Ft SchuS4 and LVS to determine differences in survival. Similar to our previous results, DIO mice were more resistant to intranasal infection regardless of the infecting bacterial pathogen. However, there were differences in the lipid mediator profile associated with improved outcomes depending on the infecting agent. Further, improved control of inflammation in obese lungs following pulmonary infection was associated with decreased cell death as reflected by flow cytometric analysis of dead cells, changes in efferocytic markers, and functional efferocytosis among DIO mice compared to RW controls. Interestingly, the positive contribution obesity made to pulmonary infection was not preserved following intradermal inoculation with either no differences observed among RW and DIO mice or (depending on the infecting agent) DIO mice exhibiting increased susceptibility to i.d. infection. Together these data demonstrate that there are organ-specific differences that develop during obesity that have important contributions to the relative susceptibility of the host to bacterial infection.

131. Laura Willen, PhD

Aedes aegypti mosquito saliva inhibits human T cell proliferation: implications for arboviral disease outcome?
Mentor: **Dr. Fabiano Oliveira**
Study Section: **Virology - Pathogenesis/Therapeutics**

Dengue fever is one of the most rapidly spreading mosquito-borne viral diseases in the world. Current outbreaks in South America demonstrate the urgent need for effective preventative measures. The disease is caused by the bite of an infectious Aedes mosquito, the most prolific mosquito vector in the world, found on every continent. When the mosquito takes a bloodmeal it salivates into the host skin. Mosquito salivary proteins are known to facilitate blood feeding by limiting the host vasoconstriction, inhibiting coagulation processes, and suppressing pain receptors. Saliva of Ae. aegypti mosquitoes contains about 100 salivary proteins implicated in enhanced viral dissemination and increased pathogenesis of viral infections. Our team previously established an association with the level of antibody (Ab) responses elicited in humans against saliva of Ae. aegypti mosquitoes and dengue outcome. Interestingly, people who elicited higher Ab responses had a higher risk of developing asymptomatic disease than symptomatic disease, suggesting a protective role of these anti-saliva Abs. To better understand how the immune response against Ae. aegypti saliva influences dengue disease outcome, we investigate the response of peripheral blood mononuclear cells (PBMCs) from highly Ae. exposed and unexposed individuals to these salivary antigens. We discovered Ae. salivary proteins inhibit T cell proliferation and cytokine secretion. More specifically, preincubating human PBMCs with salivary proteins of Ae. for three hours prior to being stimulated with Concanavalin A, a lymphocyte mitogen, inhibited CD3+ T cells proliferation by 30.8%. Repeating this experiment with either denatured (30min at 70C) or digested (proteinase K) salivary proteins, abrogated the phenotype (7.1 and 3.6% inhibition, respectively), supporting the hypothesis that saliva has a direct effect on T cell proliferation. Although previous murine studies have shown a saliva mediated increased pathogenesis and viral dissemination, this is the first time that Ae. saliva is shown to have an inhibitory effect on human lymphocytes. Since no vaccines are widely available nor any antiviral treatment exists against any Ae.-borne arboviral disease, it is imperative for future mechanistic studies to uncover the implicated salivary components, characterize whether host differences in immune inhibition are associated with disease outcome, and identify the role that Abs might play in all this.

National Institute of Arthritis and Musculoskeletal and Skin Diseases

132. William G Ambler, MD

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Mentor: **Dr. Kaplan J Kaplan**
Study Section: **Immunology – Autoimmune**

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133. Urvashi Kaundal, Ph.D.

Gain of Function NOTCH4 Variants Discovered in the African American Population Disrupt Angiogenesis and provide therapeutic targeting of NOTCH4 pathway in Systemic Sclerosis

Mentor: **Dr. Pravitt Gourh**
Study Section: **Clinical and Translational Research – General**

Systemic Sclerosis (SSc) is a rare, autoimmune disorder and vasculopathy is central to its pathogenesis. African American (AA) patients with SSc exhibit a more severe vascular phenotype and poorer outcomes than European Americans. We hypothesized that the higher prevalence and severe phenotype of SSc in AA patients is due to the genetic differences in the two ancestral populations (African and European) and the African ancestry variants will explain the increased frequency and severity of SSc and higher mortality in the AA population. Exome and targeted sequencing were performed in discovery and replication cohorts comprising 969 AA SSc patients with SSc and 771 AA controls. Gene-based testing identified NOTCH4 association with SSc (P=1.6x10-7); this association was strongest among AAs with severe vascular disease (P=3.5x10-7). The risk haplotype “TA”, defined by the missense (c.2824C>T) and the promoter (c.-117G>A) variants, was enriched in AAs with SSc (11%) compared to controls, and increased risk of manifesting the combination of severe Raynaud’s, scleroderma renal crisis and pulmonary arterial hypertension (OR=10.6, 95% CI 2-56). The c.-117A allele resides in the glucocorticoid receptor binding site and was associated with increased NOTCH4 transcripts as well as protein expression. The c.2824T allele upregulated downstream NOTCH4 signaling in lymphoblastoid cell lines (LCLs). SSc skin single-cell RNA sequencing identified NOTCH4 expression principally in endothelial cells (ECs), associated with dysregulated angiogenesis and endothelial-to-mesenchymal (EndMT) transition. Stimulation of NOTCH4 in an EC line and in SSc primary ECs carrying the c.-117A allele led to increase in EndMT transcription factors (TFs) and decreased tube formation. Inhibiting the NOTCH4 pathway using a NOTCH4-specific blocking antibody or nirogacestat, FDA approved, oral, selective gamma-secretase inhibitor normalized EndMT TFs and rescued normal tube formation. NOTCH4 variants implicate this gene in SSc pathogenesis and the associated vasculopathy, and in part explain the increased prevalence of SSc in AAs. Blocking NOTCH4 signaling restored angiogenesis, providing a molecular basis for therapeutic targeting of EC dysfunction in SSc and other vasculopathies. Our study illustrates the value of studying disease in underrepresented minorities not only as a way of understanding human biology more broadly, but as a first step towards personalized medicine for us all.

134. Masataka Suzawa, PhD

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Mentor: **Dr. Markus Hafner**
Study Section: **Gene Expression - Postranscriptional Regulation**

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135. Kang Yu, PhD

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Mentor: **Dr. John O'Shea**
Study Section: **Immunology - Lymphocyte Development and Activation**

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National Institute of Biomedical Imaging and BioEngineering

136. Andrew Massey, PhD

Sialic acid perturbation leads to profound remodeling of glycocalyx architecture and cellular mechanics in pancreatic cancer cells
Mentor: **Dr. Alexander Cartagena-Rivera**
Study Section: **Biophysics and Biomedical Engineering**

Pancreatic cancer remains one of the most lethal malignancies due in part to issues with early detection and high levels of drug resistance. Partly to blame for these factors is the glycocalyx, an extracellular structure found on most cells that is aberrantly glycosylated and has overexpressed biopolymers in cancerous cells. In pancreatic cancer, this includes higher levels of mucin expression, increased sialylation, and more hyaluronic acid production. Although there is considerable work detailing the biochemical role of these glycocalyx modifications, we sought to understand their role on the architecture and mechanical properties of the cell. We enzymatically degraded different components of the glycocalyx commonly found to be aberrantly expressed in pancreatic cancer (e.g., N-glycans, sialic acid, mucins, and hyaluronic acid) and visualized changes in the structure of the membrane via atomic force microscopy, confocal staining and scanning electron microscopy. We observed a profound reduction in microvilli density that was the most consistent with sialic acid removal across all three cell lines investigated, as well as a significant reduction in the viscoelastic properties (elastic storage and viscous loss moduli) with this treatment. This observation suggests that the cell surface fluidizes in response to desialylation. Future studies will attempt to link in vitro effects of de-glycosylation with patient tissue viscoelastic data to highlight the role of glycocalyx modulation at an intratumoral level to better understand chemo and immune therapy resistance.

National Institute of Child Health and Human Development

137. Melania Bruno, PhD

Adding the missing tiles to the puzzle: sequence reconstruction of KRAB-ZFP gene clusters highlights their rapid evolution in mice
Mentor: **Dr. Todd S Macfarlan**
Study Section: **Omics - Genomics/Transcriptomics**

KRAB-zinc finger protein (KZFP) genes are an abundant and fast evolving family of transcription factors, numbering in the hundreds in most mammalian genomes. Many evolutionary young and clade- or species-specific KZFPs bind to and repress endogenous retroviruses (ERVs) and often share a similar evolutionary age with the ERVs they repress. ERVs establish themselves in genomes as remnants of exogenous retroviral infections and increase their copy number by retro-transposition. However, how new KZFP genes emerge to repress new ERVs is currently unknown.
The mouse is a great model to study the evolution of new KZFPs. The mouse germ line has been recently colonized by numerous retroviruses that have established ERV families unique to mice. The KZFP gene family has also expanded in the mouse lineage, with parallel evolution in different mouse strains. KZFP genes are organized in highly repetitive genomic clusters which often present sequence gaps in the available genome assemblies, preventing a thorough reconstruction of their evolution.

We have generated de novo assemblies and filled the sequence gaps in all KZFP gene clusters for the C57BL/6J and 129S1/SvImJ mouse strains using PacBio HiFi and Nanopore sequencing. We found that some KZFP gene clusters are notably larger than previously thought, harboring many previously unknown KZFP genes.
One particular KZFP gene cluster, which presented several gaps in the current mouse reference assembly and was expected to be 2.5Mb, is actually 5.4Mb and 6.9Mb large in C57BL/6J and 129S1/SvImJ mice, respectively, harboring dozens of new coding KZFP genes. De novo assembly of this locus in Mus spretus (which diverged from Mus musculus only 1.7 million years ago) revealed a cluster size of 2.4Mb, suggesting that this cluster dramatically expanded in Mus musculus very recently. Repeat annotation showed that this KZFP gene cluster is also enriched with mouse specific ERVs, suggesting that the acquisition of new ERVs may have increased the recombinogenic potential of this locus. Finally, we observed two different modes of cluster expansion: the C57BL/6J cluster displays broad duplications with a large number of coding KZFP genes, while the larger 129S1/SvImJ cluster displays more fragmented duplications and disruptions to coding potential. Our findings explain strain differences in the regulation of some ERVs in these two strains and suggest likely differences in the maintenance and evolution of some KZFP gene clusters.

138. Amara Channell Doig, PhD, MPH

Profiles of Maternal Feeding Practices and their Association with Parental Feeding Styles, Child Diet Quality, and the Home Food Environment
Mentor: **Dr. Tonja Nansel**
Study Section: **Cultural, Social and Behavioral Sciences**

Background: Early nutrition is critical for development and health, yet many children in the U.S. do not meet dietary recommendations. The Comprehensive Feeding Practices Questionnaire (CFPQ) is a validated measure of 12 feeding practices (e.g., restricting, pressuring, modeling) that influence child diet. Although feeding practices are used in conjunction, no studies have examined these practices simultaneously. Our objective was to examine latent profiles of CFPQ subscales (i.e., patterns of individual responses across all subscales) and test relations with parent feeding styles (i.e., authoritative, authoritarian, indulgent, uninvolved), child diet quality, and the home food environment. Methods: Latent Profile Analysis was used to examine profiles of the 12 CFPQ subscales using data from 118 maternal-child dyads in North Carolina assessed at child ages 3 and 5 years. Multinomial regression estimated associations of CFPQ latent profiles with feeding styles, measured by the Caregiver Feeding Styles Questionnaire. Linear regression examined associations of profile membership with child Healthy Eating Index 2020 (HEI; calculated from multiple parent-proxy 24-hour recalls) overall and separately for adequacy (e.g., fruit, vegetables, whole grains, HEI-adq) and moderation (e.g., refined grains, sodium, HEI-mod) components (higher scores indicate greater conformance to dietary guidelines). Associations of the Home Food Inventory obesogenic (HFI-OB) and fruit/vegetable (HFI-FV) scores with CFPQ latent profiles were estimated using ANOVA. Results: Fit indices (BIC and ICL) supported a three-profile model: 1-high supportive and low controlling practices, 2-high supportive and high controlling practices, and 3-low supportive and moderate controlling practices. Feeding styles did not predict CFPQ latent profile membership. Total HEI, HEI-adq and HEI-mod were lower in profile 3 than profile 1 (Total HEI β=-5.85, SE=2.98; HEI-adq β=-3.65, SE=1.96; HEI-mod β=-2.19, SE=1.34). HFI-OB was lower in profile 1 than profile 2 (mean difference= -4.85, 95%CI= -9.00, 0.70) and profile 3 (mean difference= -4.68, 95%CI= -8.40, -0.97), while HFI-FV was higher for profile 1 than profile 3 (mean difference= 3.30, 95%CI= 0.10, 6.49). Conclusion: A profile representing high supportive and low controlling feeding practices was associated with better diet quality and home food environment compared to parents with moderate controlling and low supportive practices.

139. Saikat Ghosh, PhD

Lysosome-coupled mRNA transport maintains axonal homeostasis.
Mentor: **Dr. Juan Bonifacino**
Study Section: **Cell Biology - Intracellular Trafficking and Cell Signaling**

Lysosomes were classically defined as cytoplasmic organelles that function to degrade biomacromolecules in the endomembrane system of eukaryotic cells. More recently, lysosomes were found to play multifaceted roles in nutrient sensing, regulation of gene expression, plasma membrane repair, immunity and cholesterol transport. Previous work from our laboratory showed that these functions are influenced by the positioning and motility of lysosomes within the cytoplasm. In particular, we discovered a lysosome-associated, hetero-octameric complex named BORC that sequentially recruits the small GTPase ARL8 and kinesin motors for anterograde movement along microtubules. Interference with this machinery causes redistribution of lysosomes towards the cell center in non-neuronal cells and depletion of lysosomes from the axon in neurons. Based on recent work showing that RNA granules hitchhike on lysosomes for transport into the axon, we examined the effect of knocking out BORC subunits on axonal mRNA transport in human iPSC-derived neurons grown in microfluidic devices. We observed that BORC KO caused a dramatic depletion of many mRNAs encoding components of mitochondria and ribosomes. The depleted axonal mRNAs were common with those involved in pathways of neurodegeneration in Parkinson's, Alzheimer's, Huntington's and prion diseases, as well as amyotrophic lateral sclerosis (ALS). A puromycin-proximity ligation assay (Puro-PLA) revealed decreased synthesis of mitochondrial and ribosomal proteins in the axon of BORC-KO neurons. Moreover, immunoblot analyses of BORC-KO axons showed reduced the levels of components of the mitochondrial electron transport chain (ETC) and the mitochondrial contact site and cristae organizing system (MICOS). In addition, we observed that axonal mitochondria were smaller, had disorganized cristae and reduced membrane potential, and were targeted for mitophagy in BORC-KO axons. Finally, we found that BORC-KO axons developed swellings filled with mitochondria, autophagosomes and Tau aggregates, and eventually degenerated. These findings thus demonstrated a critical role of lysosome-coupled mRNA transport into the axon for the maintenance of mitochondrial and ribosomal homeostasis. Failure of this mechanism could explain the pathogenesis of neurodevelopmental disorders caused by mutations in BORC subunits, and, more generally, of neurodegenerative disorders characterized by defective lysosomal transport and mitochondrial function.

140. Ritu Gupta, PhD

Unveiling the Molecular Dance of Gcn2 Activation during stress
Mentor: **Dr. Alan Hinnebusch**
Study Section: **Molecular Biology – Eukaryotic**

Ribosomes are central to protein synthesis and frequently stall during translation in stressed cells, activating the conserved integrated stress response (ISR). In yeast, this response is known as general amino acid control and involves activation of the conserved protein kinase Gcn2. Defects in ISR activation are linked with metabolic and neurodegenerative diseases, highlighting the importance of understanding Gcn2 activation. Activated Gcn2 phosphorylates eukaryotic initiation factor 2, impairing global protein synthesis while activating translation of GCN4 mRNA, encoding a master regulator of amino acid biosynthetic genes. It has been proposed that Gcn2 is activated by binding of uncharged tRNAs to its histidyl-tRNA synthetase (HisRS)-like domain, which accumulate during amino acid starvation. It was shown that mammalian Gcn2 can be activated by ribosomes stalled with an empty A-site independent of uncharged tRNAs. We exploited the yeast system to address the molecular requirements of this starvation-independent (SI) ribosome-stalling pathway. We identified three regimes that activate yeast Gcn2 by SI pathway: tigecycline treatment, eliminating the sole gene encoding arginyl-tRNA and depleting peptide termination factor eRF1. Interestingly, the HisRS-like domain, ribosome-binding domain of Gcn2, Gcn1 and Gcn20—all necessary for starvation pathway—are likewise required by the SI pathway. Furthermore, the ribosomal P-stalk complex comprised of uL10 protein, P1A/P2B and P1B/P2A heterodimers, is required for the SI pathway but not for starvation pathway. Using CRISPR-Cas9 gene editing, we uncovered that P1A/P2B heterodimer alone is sufficient by the SI pathway. Our findings reveal distinct pathways for Gcn2 activation with different requirements for the P-stalk. We propose that uncharged tRNAs substitute for the P-stalk in binding to the HisRS-like domain for Gcn2 activation in amino acid-starved cells. Binding of uncharged tRNAs to Gcn2 has not been demonstrated in starved yeast cells, nor is it known whether Gcn2 activation by tRNA occurs exclusively on collided ribosomes. We are addressing these by immunoprecipitating Gcn2 and associated proteins and RNAs from polysomes and monosomes fractions from starved cells. Mass-spectrometry is also being conducted to identify the complete Gcn2 interactome. These experiments should yield important new insights into molecular mechanisms of Gcn2 activation during stress.

141. Teri Hatzihristidis, Ph.D.

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Mentor: **Dr. Paul E Love**
Study Section: **Clinical and Translational Research - Animal Models**

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142. Leanne E Iannucci, PhD

Decoding Vertebrate Signaling Gradients with In Vivo Optogenetics
Mentor: **Dr. Katherine W Rogers**
Study Section: **Developmental Biology - Early Development/Embryology**

In embryogenesis, a handful of signaling pathways give rise to hundreds of cell types. In vertebrates, many pathways, such as the Bone Morphogenetic Protein (BMP), form stereotyped spatial signaling gradients. Dysregulation of the BMP signaling distribution can lead to lethal patterning defects. It remains unclear how spatial signaling gradients convey fate to naive cell populations. Classic developmental models (e.g., "French Flag") posit that absolute thresholds of signaling define cell fate. Recent evidence suggests the gradient slope (e.g., relative differences in signaling between neighboring cells) may play a role. To tease apart these hypotheses, an ideal experiment would manipulate graded signal distributions and evaluate how cell fate and gene expression changes in vivo. The spatiotemporal control required to enable this experiment remains technically intractable via genetic and pharmacologic tools. To overcome this, we employ optogenetic signaling activators to place BMP under control of light in the transparent zebrafish model. Blue light-dimerizing proteins (light-oxygen-voltage, or LOV) are fused to BMP receptors, enabling light-mediated pathway activation. We have confirmed in vivo in zebrafish embryos that our optogenetic BMP activator, bOptoBMP, is: (1) wavelength specific (not activated by light over 495nm), (2) fast-acting and reversible (on/off in less than 10 min after light is applied/removed), (3) can be locally activated via spatially restricted light, (4) pathway specific (does not ectopically activate other developmental pathways), and (5) intensity dependent (signaling level is proportional to illumination power). bOptoBMP thus can be used to create a spatiotemporal BMP gradient in vivo using patterned blue light. We also demonstrated that bOptoBMP can be multiplexed with a photoconvertible fluorescent protein that converts at the same wavelength as bOptoBMP activation. By identifying which cells receive light via photoconversion and comparing this to which cells have BMP activation, we can optimize delivery of spatially patterned light to establish a high resolution BMP distribution. Future work will optogenetically prescribe optimized signaling gradients of differing slopes in a BMP-deficient zebrafish line and evaluate which BMP distributions can rescue normal development and cell fate patterning. These results will provide causative information about how signaling gradients encode cell fate information during development.

143. Kanako Inoue, D.D.S. Ph.D.

Studying tooth development using the zebrafish
Mentor: **Dr. Brant M Weinstein**
Study Section: **Developmental Biology – Organogenesis**

Once human teeth are lost, they do not regenerate, and their loss substantially reduces quality of life. Understanding how teeth develop is a critical step toward future tooth regeneration. Although the process of tooth development is highly conserved from fish to mammals, it is difficult to study in mice and still not well understood. I have developed the zebrafish as an efficient and powerful new model for studying tooth development. Zebrafish have 22 pharyngeal teeth that are constantly replaced every 10 days throughout life, allowing observation of multiple stages of tooth development within the same animal. Fish are also highly accessible to high-resolution optical imaging, and an enormous number of transgenic lines are already available marking different cell populations.

I have performed single cell RNA-sequencing (scRNA-seq) to uncover the cell types present in developing zebrafish teeth and their gene expression profiles. I have used transgenic lines marking some of these cell types, HCR (RNA fluorescent in situ hybridization) for cell type specific genes, and novel clearing techniques facilitating optical imaging through bone to characterize the 3-D morphology, cellular dynamics, and gene expression profiles of different tooth cell populations, including dentin-forming odontoblasts located deep inside the tooth and enamel-forming ameloblasts. By comparing my zebrafish scRNA-seq data to mouse scRNAseq data, I have also been able to identify conserved candidate genes potentially involved in initiation of tooth development across species.

One major hurdle for tooth regeneration in mammals is that ameloblasts disappear from the body once enamel is formed, making their analysis challenging. I have found that ameloblast in mammals and fish are both marked by a specific gene, FAM20A golgi associated secretory pathway pseudokinase (fam20a), also a causative gene for enamel defects in humans. I am generating Tg(fam20a:egfp-2a-rpl10a3xHA) zebrafish transgenics expressing both eGFP and 3xHA epitope-conjugated ribosomal protein subunits specifically in ameloblasts, allowing simultaneous visualization and in vivo transcriptional profiling of these cells. These lines will allow me to carry out a detailed cellular and molecular characterization of ameloblast specification and differentiation and help identify important regulators of ameloblast differentiation that will serve as targets for development of tooth regeneration strategies in the future.

144. Fnu Khushboo, PhD

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Mentor: **Dr. Forbes D Porter**
Study Section: **Omics - Metabolomics/Proteomics**

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145. Sarita Kumari, PhD

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Mentor: **Dr. Forbes D Porter**
Study Section: **Omics - Genomics/Transcriptomics**

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146. Louis Tung Faat Lai, PhD

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Mentor: **Dr. Doreen Matthies**
Study Section: **Protein Structure/Structural Biology**

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147. Tanmay Mondal, Ph.D.

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Mentor: **Dr. Anirban Banerjee**
Study Section: **Biochemistry - Proteins, and Lipids**

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148. Kiyohito Taimatsu, Ph.D.

Establishing the zebrafish as a model to study the pharynx
Mentor: **Dr. Brant M Weinstein**
Study Section: **Developmental Biology – Organogenesis**

The pharynx and associated organs have important roles in breathing, eating, drinking, digesting, speaking and protecting the body from infection. Pharyngeal dysfunction can manifest as dysphagia, persistent palatal displacement, respiratory function decline, or exercise intolerance. Secondary complications are serious and life threatening and can include aspiration pneumonia, weight loss, and death. However, pharynx development is not well understood, in large part because of the inaccessibility of the developing pharynx to observation and experimental manipulation in most model organisms. I am establishing the zebrafish as a powerful model for observation and genetic and experimental analysis of pharynx development. Although the pharynx is more accessible in zebrafish than in mammalian models, it is still difficult to observe clearly because of its deep location and surrounding opaque tissues.

I have developed a powerful new "super deep imaging" method, combined with optimized staining and Hybridization Chain Reaction (HCR) procedures, that together permit high-resolution imaging of pharyngeal tissues and cells even in older developing animals and adults. I have used these and other methods to describe developing and adult pharynx anatomy, and I have used scRNAseq to identify resident cell types of the pharynx and their gene expression signatures. My anatomical and molecular findings show the zebrafish pharynx is a complex structure with numerous specialized cell types, many of which correspond well to cell types identified in the mammalian pharynx. I have also used genetic screens to identify pharynx-specific mutants that I am currently characterizing in detail. One mutant has more ionocytes and less mucous cells in the pharynx, suggesting a hidden balancing system between these cells, and demonstrating that genetic mutants can reveal new and unknown mechanisms in pharyngeal development. By developing new methods, uncovering mutants, and compiling fundamental information on pharyngeal anatomy and resident pharyngeal cell types, I am establishing the zebrafish as a valuable new model for experimental and genetic analysis of the pharynx.

149. Marcela Teatin Latancia, PhD

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Mentor: **Dr. Roger Woodgate**
Study Section: **DNA Replication, Damage and Repair**

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150. Prabhavi Wijesiriwardhana, Ph.D.

Does reproductive experience modify maternal genetic association with fetal growth?
Mentor: **Dr. Fasil T Ayele**
Study Section: **Epidemiology/Biostatistics – Etiology**

Pregnancy is a major physiological adaptation. The immunologic and cardiometabolic changes are more pronounced during a woman's first pregnancy experience. Birthweight abnormalities are more common among women with no previous childbearing experience and are partly influenced by maternal genetic risk. However, it is unknown whether previous reproductive experience of pregnant women modulates the effect of the maternal genome on fetal growth. Here, we investigated whether the maternal genome's influence on fetal growth varies by previous childbearing experience (nulliparity vs multiparty) using two multi-ancestral pregnancy cohorts. Discovery analyses for weekly fetal growth measures (at 10-40 weeks gestation) were performed using the NICHD Fetal Growth Studies-Singletons cohort of pregnant women that included 885 nulliparas and 1059 multiparas. Replication analyses were performed using the Nulliparous Pregnancy Outcomes Study cohort of nulliparous women (n=6058). Fetal weight was calculated using femur length (FL), head and abdominal circumferences (HC and AC). Maternal genetic risk was proxied by genetic risk scores derived from 32 and 33 birthweight-reducing maternal genetic variants, in the discovery and replication cohorts, respectively. Associations of maternal genetic risk with fetal growth measures were tested using linear regression analysis adjusted for fetal sex and top 10 genetic principal components. Among nulliparas, maternal genetic risk was significantly associated with lower fetal weight from gestational week 20 onwards in the discovery cohort (β at week 40=-44.1 grams, p=0.003), and from week 23 onwards in the replication cohort (β at week 40=-22.5 grams, p=0.0001). In addition, maternal genetic risk was significantly associated ($p<0.05$) with smaller FL, HC, and AC beginning at 16, 22, 27 weeks, respectively. Among multiparas, however, no significant associations were found in either cohort, despite the much larger sample size. The findings remained consistent with additional adjustment for maternal age and body mass index. In conclusion, maternal genetic factors exhibited a more pronounced influence on fetal growth at first pregnancy but not at subsequent pregnancies. It is possible that the genetic factors target major physiological changes at first pregnancy that get less profound with childbearing experience. Consideration of reproductive experience as a biological variable may facilitate interventions for pregnancy complications.

151. Josette Wlaschin, MA, PhD in progress

How the most common pathology in ALS, loss of nuclear TDP-43, leads to motor neuron dysfunction and death
Mentor: **Dr. Claire Le Pichon**
Study Section: **Neuroscience - Therapeutics and Translational Research**

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive paralysis. 97% of ALS patients display a common pathology where the essential nucleic acid binding protein and splicing factor, TDP-43, is mislocalized outside of neuronal nuclei. Extranuclear mislocalization of TDP-43 results in the aberrant splicing of numerous transcripts, including the retention of normally repressed cryptic exons (CEs). Inclusion of these CEs leads to loss, reduced, or otherwise altered expression of numerous genes and thus has wide ranging consequences on cellular health. To understand how loss of TDP-43 affects motor neurons (MNs), we examined functional and transcriptomic alterations in a mouse model where TDP-43 is conditionally deleted from these neurons (TDP-43cKO).

TDP-43cKO mice develop progressive motor symptoms due to MN loss during 2-3 months after birth. To better understand the time course of motor neuron dysfunction in these mice, I performed weekly compound muscle action potential recordings from the tibialis anterior muscle between 4-14 weeks of age. I detected a significant reduction in MN function and nerve conduction velocity preceding MN loss and muscle denervation, suggesting there is an early phase preceding the death of MNs where their function is severely reduced.

To link the loss of TDP-43 in MNs with the dysregulation of specific genes that can explain the impairment of MN function, we performed bulk RNA sequencing of lumbar spinal cords and single nucleus RNA sequencing of MNs. So far, we have determined that the expression of several key potassium channels known to regulate motor neuron firing properties is disrupted at pre-symptomatic time points. Notably, one of these potassium channels retained a cryptic exon upon loss of TDP-43.

Having discovered mouse MN specific cryptic exons not only allows us to have a readout for loss of TDP-43 function but also for correction of TDP-43 function in this mouse model. We are currently testing viral strategies developed by a collaborator to restore TDP-43 splicing specifically in neurons that lack nuclear TDP-43. So far, using mScarlet transgene as a proof of concept, we have been successful in targeting TDP-43 deficient neurons for transgene expression. This work will be beneficial in establishing an animal model for proof-of-concept preclinical testing of new therapeutics that may benefit patients in the future.

National Institute of Dental and Craniofacial Research

152. Anastasia De Poulpiquet Du Halgouet, PhD

Unraveling the properties of Prenatal B Cells: Insights from Fate-Mapping and Functional Analyses
Mentor: **Dr. Roxane Tussiwand**
Study Section: **Immunology - Lymphocyte Development and Activation**

To date, the functional relevance and properties of prenatal lymphocytes remain elusive. Therefore, we developed a fate-mapping mouse line based on the postnatal expression of the terminal deoxynucleotidyl transferase enzyme (Dntt), that inserts random nucleotides at VDJ-junctions. In our model, we can detect prenatal B cells in any tissue at any age and following any perturbation. We first observed persistence of prenatal cells not only within B1 cells but also across all subsets including marginal zone and follicular B cells.
Initial, BCR heavy-chain usage indicated that B cells clustered based on their ontogeny rather than their subset identity. We hypothesize that prenatal B cells are selected on endogenous/maternal antigens, and may be enriched in self-reactive clones, while postnatal B cells likely result from selection on exogenous antigens, including microbiome. Protein microarray results confirmed higher binding to self-antigen for prenatal-derived antibodies. In contrast, broad antibiotic treatment reduced both the number and frequency of postnatal B cells, supporting a partial reliance on microbial-derived antigens.
Furthermore, prenatal B cells exhibited intrinsic transcriptional differences as established by scRNAseq. Interestingly, unsupervised clustering revealed a distinct cluster of prenatal cells comprising of various B cell subsets. Moreover, we established a significant difference in the responsiveness to stimulations between pre- and postnatal B cells, i.e. T-cell dependent, TLR-mediated, and BCR-crosslinking. Notably, prenatal B cells exhibited a significantly lower proliferation following TLR or T-cell dependent activation but were refractory to anti-IgM-induced apoptosis, suggesting a certain degree of anergy in this compartment, that would explain the enrichment in self-reactive clones.
Given, the persistence of an anergic prenatal pool of B cells, capable of recognizing self, we hypothesized that this subset could be regulatory by inhibiting the postnatal pool. Co-culturing of pre- and postnatal B cells revealed reduced activation of postnatal cells as measured by decreased CD69 expression, supporting a suppressive effect by prenatal B cells. This suppression appeared to be contact-independent, suggesting that they release inhibitory cytokines. Collectively we envision that upon tissue damage under inflammatory or infection conditions, prenatal B cell activation acts as a break to ensure an appropriate immune response.

153. Jenny Hsin, B.S.

Investigating Initiation Mechanisms of Neuroblastoma in Neural Crest Development via Transcriptional Profiling and 3D Organoid Modeling
Mentor: **Dr. Laura Kerosuo**
Study Section: **Oncology - Development and Metastasis**

Neuroblastoma, the most common extracranial pediatric solid tumor, is a neural crest cell (NCC) derived cancer of the peripheral nervous system with a widely varying clinical course. The prognosis for neuroblastoma ranges from 50% five-year survival for high-risk groups to 90% for low-risk groups. Understanding the exact initiation mechanism of neuroblastoma could explain the difference between these groups and inform treatment strategies. However, the exact stage of NCC development in which neuroblastoma occurs remains unclear. While most research has focused on studying neuroblastoma initiation in NCC derivatives such as sympathoadrenal progenitors, chromaffin cells, and sympathetic neurons, recent studies by us and others suggest the initiation may occur earlier in NCC development.

First, we investigated the timing of neuroblastoma initiation by searching for matching transcriptional profiles between stages of normal NCC development and published neuroblastoma patient samples with different prognosis and stage. We generated gene sets called developmental gene modules (DGMs) across NCC developmental timepoints from single cell RNA sequencing samples. We cultured in vitro human embryonic stem cell (hESC) derived pre- and post-migratory NCC that develop in ectodermal organoids to obtain early NCC profiles. For profiles of NCC derivatives, we used published human fetal adrenal gland samples. Our results show that some neuroblastoma tumors score highly for the pre-migratory NCC DGM profiles and these higher NCC DGM scores correlate with higher risk tumors and MYCN oncogene amplification, one of the genetic aberrations linked to poor prognosis.

Next, we sought to model neuroblastoma formation by differentiating hESCs with gains of chromosomes 17q and 1q (common neuroblastoma-associated chromosomal abnormalities) to 3D ectodermal organoids. Preliminary data of immunofluorescence staining in these abnormal organoids show decreased expression of neural crest markers, PAX7 and TFAP2A, but maintain normal levels of neural stem cell markers, SOX2 and PAX6. We aim to elucidate the genetic regulatory networks behind neuroblastoma formation by ongoing characterization and manipulation of these organoids.

Together, our single cell RNA sequencing data and our 3D neural crest culture model provides increasing evidence that neuroblastoma can initiate earlier in NCC development than previously thought, with earlier initiation potentially being higher risk.

154. Alexander Kiepas, PhD

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Mentor: **Dr. Kenneth Yamada**
Study Section: **Cell Biology - Cell Cycle and Metabolism**

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155. Julia Licholai, BS

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Mentor: **Dr. Nicholas J Ryba**
Study Section: **Neuroscience – General**

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156. Sasirekha Narayanasamy, PhD

Resolving the N-terminal determinants underlying STIM2 activation
Mentor: **Dr. Indu Ambudkar**
Study Section: **Biochemistry – General**

STIM proteins, STIM1 and STIM2, play important roles in the activation of store-operated calcium entry (SOCE). STIMs are ER-transmembrane proteins with the N-terminal and C-terminal localized in the ER and cytoplasm, respectively. Despite having high homology, STIMs display distinct properties and differential responses to agonist stimulation. Unlike STIM1, STIM2 senses small decreases in ER-Ca^{2+} levels (i.e., at low stimulus), and form clusters in the ER-PM junctions. Both proteins have canonical and non-canonical EF-hands (cEF and nEF, respectively) and a SAM domain in the N-terminus. Differences in their Ca^{2+} sensitivities are suggested to be defined by the cEF motif that binds Ca^{2+}. Thus, what determines STIM2 cEF-Ca^{2+}-binding affinity and the conformational changes associated with Ca2+-binding/release are largely unknown. Our present study uses biochemical, biophysical, and molecular dynamics (MD) simulation to delineate the underlying molecular changes during STIM2 activation. MD simulations show that while STIM1 undergoes major conformational changes in the absence of Ca2+, STIM2 exhibits minor conformational changes indicative of stable Ca2+-free structure. Comparing the amino acid sequence of STIM2 cEF-loop with consensus sequences found in other Ca^{2+}-binding proteins, we found that STIM2 cEF-loop have “G” and “V” instead of “T” and “F” at the 7th and 10th positions, respectively. Our results shows that double mutation (GV/TF) in the STIM2 cEF-loop causes less constitutive Ca^{2+} entry, decreased pre-clusters and lower responses to low [CCh] c.f. STIM2-WT. Nonetheless, both WT and GV/TF display robust clustering at high [CCh]. Far-UV CD studies show that the GV/TF mutant exhibits α-helical structure both in its apo (Ca2+-free) and holo (Ca2+-bound) forms. Consistent with this, MD simulations show that GV/TF mutants did not undergo major conformational changes in the absence of Ca2+. Despite this, GV/TF mutant exhibits lower thermal stability than apo-STIM2WT. MST studies indicate higher Ca2+-binding affinity of GV/TF mutant than STIM2-WT, a property reminiscent of STIM1. Together, our studies indicate that the STIM2 cEF plays an important role in the high Ca2+ sensitivity, stability, and basal regulatory function of STIM2.

157. Sajeev Thanikunnath, Ph.D.

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Mentor: **Dr. Achim Werner**
Study Section: **Developmental Biology - Early Development/Embryology**

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158. Vasileios Ionas Theofilou, DDS

Spatial atlas of human oral mucosa reveals an immune- activated epithelium actively recruiting neutrophils to police the barrier
Mentor: **Dr. Niki Moutsopoulos**
Study Section: **Immunology - Mucosal Immunity**

The oral mucosa is a unique tissue microenvironment, constantly exposed to environmental stimuli, including a rich and diverse community of commensal microbiota, second only to the small intestine. Yet, while host-microbiome interactions have been much studied in other barrier sites, how the local epithelial barrier and immune cell network maintain the balance between microbes and host is not well understood at this mucosal interface. Importantly, an imbalance between microbiota and host leads to a common, oral inflammatory disease, periodontitis.
Previously, our lab has compiled a human single cell atlas of oral mucosal tissues in health and in the setting of periodontitis, but without insights into spatial distribution of cell subsets. Yet, we expect that location-specific functions of distinct cell subsets will be important both in tissue homeostasis and in the pathogenesis of periodontitis. Hence, our current project aimed to generate a spatial atlas of human gingiva in health and periodontitis, using single cell resolution 450-plex spatial transcriptomics (10x Genomics, Xenium), and 19-plex spatial proteomics platforms (iterative bleaching extends multiplexity, IBEX), which we have optimized for oral tissues. Our work provides spatial context for the diverse epithelial, vascular, stromal and immune cell populations at this mucosa, and uncovers location-specific gene expression of the different cellular compartments. We particularly identify that a unique epithelial microenvironment is adjacent to the tooth surface and exposed to the local microbiome which displays specialized functions associated with, increased permeability and engagement of neutrophil recruitment pathways. This thin, few layer epithelial barrier called the crevicular epithelium, displays an odontogenic phenotype (ODAM and ODAPH positive) and expresses acute-phase proteins implicated

in Th17 responses (SAA1, SAA2), and neutrophil chemo-attractants (CXCL6, CSF3), even in the context of health. Consistent with the local epithelial transcriptome, at a protein level specialized niches of immune cells are visualized including an abundance of neutrophils and CD4+ T cells bordering and/or penetrating the crevicular epithelium. Collectively, our work uncovers the transcriptional landscape of oral mucosal tissues and reveals the presence of a particularly active epithelial barrier continuously promoting inflammatory responses through active neutrophil recruitment.

159. Yizhen Zhang, BA

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Mentor: **Dr. Mark Hoon**
Study Section: **Neuroscience - Neural Circuits**

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National Institute of Diabetes and Digestive and Kidney Diseases

160. Ivan C Alcantara, MS

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Mentor: **Dr. Michael J Krashes**
Study Section: **Neuroscience - Neural Circuits**

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161. Yang Chen, PhD

Deciphering the contribution of 3D interactions between cis-regulatory elements and promoters to regulate gene expression using graph neural networks
Mentor: **Dr. Elissa Lei**
Study Section: **Bioinformatics - algorithms, packages and tools**

Gene expression is regulated by various factors including histone modifications (HMs), binding of transcription factors (TFs), and interplay of diverse cis-regulatory elements (CREs) in a cooperative manner. Two previous methods (GC-MERGE and Chromoformer) have been developed to predict gene expression by constructing a promoter-centered network based on 3D physical contacts between the promoter and CREs. However, these published models lack transferability to various sizes of gene graphs and explanation of the influence of these factors on gene expression.
We present a novel deep learning architecture creGNN based on a graph transformer network. Our approach can ascertain the key factors that regulate gene expression and can identify important CREs for each gene. We pre-trained the model using data augmentation and contrastive learning. We used ~800 ChIP-seq datasets of HMs and TFs, Hi-C/micro-C, ATAC-seq and RNA-seq data from human, mouse and fly genomes. Promoter-centered graphs were built for each gene based on the regions across the genome that contact the promoter in 3D space.
We trained a model based on HMs and calculated the Pearson correlation, AUC, and AUPRC between raw gene expression and predicted gene expression in four cell lines. creGNN showed better prediction performance relative to other existing methods. Second, we trained another model based on TFs, which showed close performance relative to that using HMs. By ranking node importance, we found that TFs and HMs co-regulate gene expression by binding to different CREs. Third, we tested the effect of 3D contacts on gene expression. 3D contact frequency showed low correlation with attention scores for all genes on a global level. Our results suggest that gene expression is dictated by promoter region activation or repression by CREs and that 3D contacts are important/necessary in specific cases but provide a lower relative contribution to overall gene expression than promoters and CREs themselves.
Compared to previously developed methods, creGNN exhibited higher accuracy and good transferability. We aspire to use our method to identify novel specific 3D contacts among promoters and CREs that contribute to the regulation of specific genes. This predictive power should not only contribute to our knowledge of specific gene regulatory mechanisms on the 3D level but also allow us to design targeted strategies to modify gene expression within individual cells.

162. Henrik Elenius, M.D.

Treatment of ACTH-Dependent Cushing’s Syndrome with CRN04894, a novel MC2R antagonist
Mentor: **Dr. Lynnette K Nieman**
Study Section: **Endocrinology**

Background:

Resection of the causal tumor is the optimal treatment (Rx) for ACTH-dependent Cushing's Syndrome (A-CS). Medical Rx is often needed, eg for occult or unresectable tumors; or while awaiting irradiation effects. Current Rx options have suboptimal efficacy, tolerability and hepatotoxicity. CRN04894 (CRN) is a novel oral competitive antagonist of the melanocortin 2 receptor (MC2R) that blocks ACTH-induced cortisol (F) synthesis in the adrenal gland. In healthy subjects, CRN lowered F in a dose-dependent manner, causing temporary adrenal insufficiency (AI) in 5/5 subjects receiving 80 mg daily for 10 days. Here, we report data from the first-in-disease study of CRN.

Methods:
Subjects aged 18-75 with confirmed A-CS were eligible if they had active A-CS (high bedtime F and AM ACTH (A) >10 pg/mL) ≤14 days before study day 1 (D+1). Use of short-acting steroidogenesis inhibitors required a 14-day washout. Exclusion criteria included bilateral adrenalectomy, use of mitotane or moderate/strong CYP3A4/P-gp inducers/inhibitors, eGFR <60 mL/min and liver transaminases ≥3xULN. We monitored efficacy, AI and other adverse events (AEs) during 10 days of CRN administration (80 mg/day) and 5 days of washout.

Results:
Three subjects (all male; pituitary CS n=2, ectopic CS n=1) aged 47-55 years with baseline AM A 33-1504 pg/mL (RR 5.0-46.0), AM F 15.3-17.4 mg/dL (RR 3.7-19.4) and 24-hr urine free F (UFC) 129–271 mcg (RR 3.5-45) completed therapy. Labs at D+10: AM A 60.4-2945 pg/mL, AM F 3.6-5.5 mcg/dL, UFC 3.9-28 mcg. All developed AI (AM F <5 mcg/dL) after 2, 2 and 10 days of therapy. AI resolved (AM F >7 mcg/dL) 1, 2 and 3 days after drug cessation. AEs were mild and included transient increases of creatinine (n=2), ALT (n=2) and gamma glutamyl transferase (n=1); transient neutropenia (n=1); fatigue, headache (n=2 each); and itch, reduced appetite and nausea (n=1 each). Brain fog, concentration, insomnia (n=2 each); libido, depression, irritability and abdominal bloating (n=1 each) improved. Leukocytosis (n=2) and neutrophilia (n=1) resolved.

Conclusion:
ACTH receptor blockade is a novel approach to Rx of ACTH-CS. Short-term CRN was highly effective and safe. All subjects developed AI within 10 days of therapy, resolving ≤3 days after CRN cessation. If rapid response, safety and efficacy continue in the remainder of this cohort (n=6), treatment at a lower dose(s) and longer durations should be evaluated in A-CS.

163. Lila Gonzalez Hodar, PhD

HSD17B13 regulates hepatic lipid droplet fusion by interaction with phosphatidylinositol.
Mentor: **Dr. Yaron Rotman**
Study Section: **Cell Biology – General**

Metabolic dysfunction-associated steatotic liver disease (MASLD, previously termed NAFLD) is a highly prevalent condition worldwide with no approved treatment, characterized by the accumulation of lipid droplets (LDs) in the liver. Genetic studies in MASLD identified several hepatic LD proteins that modulate disease severity, such as 17-beta hydroxysteroid dehydrogenase 13 (HSD17B13).
HSD17B13 is an LD-associated liver enzyme in which genetic loss-of-function variants confer protection from MASLD-associated liver injury, fibrosis, cirrhosis, and hepatocellular carcinoma in humans. Our lab found Hsd17b13 knock-out (Hsd17b13-KO) to be protective in a MASLD fibrosis mouse model. Even though anti-HSD17B13 therapies are already in clinical trials, the physiological role of HSD17B13 is unknown. We aim to determine the role of HSD17B13 on hepatic LD properties.
Hsd17b13-KO and wildtype (WT) mice were fed high fat diet (HFD) for 8 weeks to induce steatosis. Liver LDs were isolated by sucrose gradient, with their lipid composition assessed by LC/MS/MS and protein levels by western blot. Primary hepatocytes (PH) from WT and Hsd17b13-KO mice were treated with oleate:palmitate (2:1) with LD size measured by confocal microscopy and LD fusion via live cell imaging.
Hsd17b13-KO did not affect body weight, liver weight, or liver triglyceride content. However, livers from Hsd17b13-KO mice showed larger LDs (p=0.017), and oleate:palmitate induced larger LDs in Hsd17b13-KO PH. LD fusion was more frequent in PH from Hsd17b13-KO and CIDEC, which mediates LD fusion, was upregulated in livers from Hsd17b13-KO mice with no change in proteins related to LD consumption (lipolysis or autophagy). Moreover, in WT PH, HSD17B13 was mainly present on small LDs. Hsd17b13-KO LD had significantly higher phosphatidylinositol (PI) concentration compared to WT. Interestingly, levels of two enzymes involved in the PI cycle, phosphatidylinositol 3-kinase and phosphatidylinositol 4-kinase, were increased in Hsd17b13-KO LDs. In a protein-lipid overlay assay, HSD17B13 interacted directly with PI and two phosphoinositide (PIP) species. Through molecular docking, we identified putative residues in HSD17B13 to interact with PI/PIPs.
Our results suggest that HSD17B13 inhibits LD fusion, possibly through its interaction with PI/PIPs on the LD membrane. Further experiments are needed to determine if this new role for HSD17B13 explains the protection from liver injury it has been associated with.

164. Harish Gopalakrishna, MD

Noninvasive Model to Predict Portal Hypertension and Varices in Patients with Non-cirrhotic Portal Hypertension
Mentor: **Dr. Theo Heller**
Study Section: **Clinical and Translational Research – General**

Background
Non-cirrhotic portal hypertension (NCPH) refers to a diverse group of disorders that affects the hepatic porto-sinusoidal vascular system resulting in portal hypertension (PH). Multiple etiologies including immunological, hematological, genetic disorders, infections, toxins, and drugs can cause NCPH. Variceal bleeding is the common initial manifestation of PH in patients with NCPH. Traditional invasive transjugular portal pressure measurement for diagnosis of PH can be unreliable in NCPH. Baveno VII criteria are a validated noninvasive model to identify PH and varices in cirrhotic patients. However, currently there are no noninvasive criteria to diagnose PH and varices in NCPH.

Methodology
Single center prospective cohort of NCPH patients who had transjugular liver biopsy, liver stiffness (LSM) measured using transient elastography (FibroScan), laboratory and imaging data were included. PH was defined by presence of one among the following - varices on endoscopy, portosystemic collaterals, or ascites on imaging. Univariate analysis was used to examine variables that can predict varices and PH. Multivariable model was constructed using a logistic regression analysis of statistically significant variables in univariable analysis. A sequential-testing algorithm was developed using the best performing model.

Results
Of the 55 patients, 35 (64%) had varices and 39 (71%) had PH. LSM was higher in patients with varices (12.2 KPa vs 5.7 KPa, $p<0.01$) and PH (11.7 KPa vs 5.5 KPa, $p<0.01$). Platelet count was lower in patients with varices (75 vs 210 x 109/L, $p<0.01$) and PH (82 vs 218 x 109/L, $p<0.01$). Multivariate analysis combining LSM, and platelet count predicts varices (AUROC 0.89 ± 0.08, $p<0.01$) and CSPH (AUROC 0.87 ± 0.07, $p<0.01$). A model with combination of LSM of 10 KPa and platelet of 80 x 109/L performed with a sensitivity of 86%, specificity of 85%, PPV of 91% and accuracy of 85% to detect varices ($p<0.001$). The same model performed with sensitivity of 79%, specificity of 88%, PPV of 94% and accuracy of 82% to detect PH ($p<0.001$).

Conclusion
We developed a simple non-invasive model by combining LSM with platelet count that can aid in identifying PH in NCPH patients. This noninvasive model can predict the risk of varices and dictate the need for endoscopic surveillance and therapeutic strategy. However, this model requires further validation in an independent cohort.

165. Dhanush Haspula Giridhar, PhD

Unraveling a key metabolic role for G12/13 signaling in POMC neurons
Mentor: **Dr. Jurgen Wess**
Study Section: **Molecular Biology – Eukaryotic**

Pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus exert a multi-tier control over glucose and energy homeostasis. Stimulation of these neurons leads to the release of α-melanocyte-stimulating hormone (α-MSH) which strongly suppresses appetite. Like other cell types, POMC neurons express many G protein-coupled receptors (GPCRs) which detect changes in hormone and metabolite levels. Several of these receptors activate G proteins of the G12/13 family, besides other classes of heterotrimeric G proteins. The potential metabolic relevance of G12/13 signaling in POMC neurons remains unknown at present. To address this question, we generated mice expressing a G12/13-coupled designer GPCR (G12/13 DREADD; G12/13D) specifically in POMC neurons (POMC-G12/13D mice).
Chronic treatment of POMC-G12/13D mice with deschloroclozapine (DCZ), a highly selective agonist for G12/13D that is otherwise pharmacologically inert, led to significant metabolic improvements including blunted weight gain and improved glucose tolerance by promoting insulin release. Furthermore, activation of G12/13 signaling in POMC neurons potentiated the effects of leptin on satiety and weight loss. In vitro studies showed that G12/13D acts synergistically with the leptin receptor in activating the JAK2-STAT3 pathway. Finally, we showed that lorcaserin, an appetite-suppressive drug that selectively activates serotonin 5-HT2C receptors, failed to reduce food intake and improve glucose homeostasis in POMC-G12/13 KO mice, indicating that the beneficial metabolic effects of lorcaserin require G12/13 signaling in POMC neurons. In vitro experiments with POMC neurons further confirmed that lorcaserin-mediated activation of the Rho-ROCK pathway resulted in a significant stimulation α-MSH release.
Collectively, our data indicate a critical role for G12/13 signaling in POMC neurons in the maintenance of glucose and energy homeostasis. Mechanistic studies further revealed that the signaling pathways stimulated by G12/13 signaling in POMC neurons are similar to those activated by leptin. Finally, we identified a novel mechanism by which 5-HT2C receptors regulate appetite and glucose homeostasis, involving the activation of G12/13 signaling in POMC neurons. We conclude that targeting endogenous G12/13-coupled receptors expressed in POMC neurons may prove useful for the treatment of obesity, type 2 diabetes, and related metabolic disorders.

166. Jakub Jankowski, PhD

Activating human mutation in the JAK/STAT signaling pathway protects male mice against acute kidney injury
Mentor: **Dr. Lothar Hennighausen**
Study Section: **Gene Expression - Transcriptional Regulation**

Development of chronic disease is often linked to single nucleotide polymorphisms (SNPs), but the extent of their impact is not well understood. Mutations within JAK/STAT pathway, which regulates immune response, frequently occur in cancer and autoimmune diseases. STAT5b SNPs changing tyrosine Y665 to phenylalanine (Y665F), or histidine (Y665H) have been found by genome sequencing of leukemia patients. They are thought to respectively increase or attenuate STAT5b activity but have not been confirmed as a disease driver. Using base editing, we developed mice mimicking the human STAT5b SNPs to investigate changes in phenotype. I observed an increased infiltration of CD3+ and CD4+ cells in the kidney tissue of the Y665F strain using immunohistochemistry, implying increased vulnerability to development of inflammation. To confirm it, I performed bilateral kidney ischemia-reperfusion surgery by restricting blood flow to the kidneys for 30 minutes and measured serum creatinine after 24 hours. Surprisingly, male Y665F mice had lower creatinine levels than controls (mean 0.66 vs 1.23 mg/dL, $p<0.001$), suggesting protection against injury. Similar effect was absent in Y665H mice, indicating protective effect of STAT5b activation in renal injury. To elucidate the involvement of JAK/STAT pathway regulation in my model, I performed extensive kidney RNA-seq analysis in control, Y665F and Y665H strains, including male and female mice, before and after kidney injury. Known genes associated with the JAK/STAT pathway like Prlr, Cox2 and Lpl are statistically significantly deregulated among 467 others in male Y665F mice compared to control at the baseline. After injury, expression of 1122 genes differs between control and Y665F mice, including and possibly driven by transcription factors linked to JAK/STAT like Sox9, Esr1 and Jun. Remarkably, this effect was limited to males, with Y665F females displaying no statistically significant difference in plasma creatinine compared to control (0.79 vs 1.04 mg/dL), and few significantly deregulated genes either before or after injury. Those results strongly indicate that STAT5b hyperactivation has a sexually dimorphic, renoprotective effect due to deregulation of immune pathways. Our work highlights the far-reaching effects of a singular SNPs and might enable more personalized medicine approach incorporating patient's genome.

167. Kyra Kerkhofs, Ph.D.

Respiratory syncytial virus (RSV) optimizes the translational landscape during infection
Mentor: **Dr. Nicholas Guydosh**
Study Section: **Molecular Biology – Eukaryotic**

Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections in children, with nearly all children infected before the age of two. During infection, viruses rely entirely on the cellular translational machinery to produce viral proteins required for replication. Generally, viral infection triggers stress-related pathways leading to activation of factors which inhibit cap-dependent translation of messenger RNAs (mRNAs). However, since RSV mRNAs mimic cap structures of host mRNAs, inhibiting cap-dependent translation would also strongly reduce viral translation. Consistent with this prediction, we found that RSV limits widespread translation inhibition, but we unexpectedly found that translation was enhanced because of increased loading of ribosomes onto cellular mRNAs. However, it remains unknown how RSV competes for ribosomes among highly abundant cellular mRNAs for translation of its own mRNAs. We used high-throughput sequencing of translating mRNAs to investigate how RSV affects host mRNA translation. Interestingly, we found that cellular mRNAs that are normally less efficiently translated become more efficient at recruiting ribosomes during infection. In addition, these transcripts have a low GC-content, similar to RSV-encoded mRNAs. Having a higher number of ribosomes translating less optimal AU-rich mRNAs increases the potential of two or more ribosomes colliding (forming "disomes") by chance and initiation of quality control pathways that help the cell respond to stress. Consistently, we found that the ribosome collision sensor ZAKalpha becomes active in infected cells, suggesting RSV infection induces ribosome collisions. Interestingly, RSV was shown to be dependent on ribosome recycling factors, which are important for removing ribosomes that terminate translation. Efficient recycling also limits the buildup of excess ribosomes on mRNA which may help the virus limit ribosome collisions. Using disome profiling and a wide range of molecular biology methods, we are further investigating how RSV utilizes host factors, such as ribosome recycling factors and ribosome collision sensors, to avoid and resolve persistent ribosome collisions that would otherwise result in activation of signaling pathways that would reduce viral replication. This work will further advance our understanding of RSV-host interactions, revealing critical aspects of viral pathogenesis and ribosome collisions as an antiviral pathway.

168. Victoria Klein, Ph.D.

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Mentor: **Dr. Carole Bewley**
Study Section: **Microbiology and Antimicrobials**

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169. Liu Liu, PhD

Crosstalk between Gs and Gq signaling is essential for proper α-cell function
Mentor: **Dr. Jurgen Wess**
Study Section: **Endocrinology**

Glucagon, a hormone released from α-cells of pancreatic islets, regulates systemic glucose homeostasis by stimulating hepatic glucose production during hypoglycemia. Dysregulated glucagon secretion is predicted to play a key role in the pathophysiology of type 1 and 2

diabetes. Despite extensive research, the molecular mechanisms governing the regulation of glucagon secretion remain incompletely understood. Like all other cell types, pancreatic α-cells express many G protein-coupled receptors (GPCRs) that are linked to different functional classes of heterotrimeric G proteins (Gs, Gq/11, Gi/o, and G12/13). To gain insight into the functional relevance of α-cell Gs and Gq/11 signaling, we generated and analyzed mice in which we ablated the α-subunits of Gs (Gαs) or Gαq/Gα11 selectively in α-cells of adult mice in an inducible fashion. We refer to the resulting mutant mice as α-Gq/11KO and α-GsKO mice, respectively. Interestingly, both α-Gq/11KO and α-GsKO mice showed significantly reduced circulating plasma glucagon levels, resulting from decreased proglucagon gene expression and islet glucagon content. Furthermore, disruption of α-cell Gs or Gq/11 signaling greatly impaired glucagon secretion in response to insulin-induced hypoglycemia or 2-deoxy-D-glucose-induced glucopenia, respectively. Surprisingly, islet perifusion studies revealed that α-GsKO islets exhibited reduced glucagon secretion not only in response to Gs-coupled receptor agonists (e.g. A2A adenosine or beta-adrenergic receptor agonists) but also to Gq/11-linked receptor agonists (e.g. V1b vasopressin receptor agonist), suggesting that Gq-mediated glucagon release requires the presence of functional Gs in α-cells. In an analogous fashion, Gs-coupled receptor agonists were unable to stimulate glucagon secretion in α-Gq/11KO islets, indicating that crosstalk between α-cell Gs and Gq/11 signaling is critical for the proper regulation of α-cell function.
In summary, our findings highlight the critical role of both α-cell Gs and Gq/11 signaling in stimulating glucagon secretion during hypoglycemic and glucopenic conditions. Moreover, we demonstrate that crosstalk between Gs and Gq/11 signaling is essential for the proper regulation of glucagon release from α-cells. These new findings are of potential clinical relevance for developing novel strategies for managing dysregulated glucagon secretion in metabolic disorders.

170. Srinivas Pittala, Ph.D.

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Mentor: **Dr. Jurgen Wess**
Study Section: **Cell Biology - Intracellular Trafficking and Cell Signaling**

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171. Misbah Rashid, PHD

Melanocortin 4 Receptor-Mediated Metabolic Effects Require Beta-Arrestin-2 Signaling
Mentor: **Dr. Jurgen Wess**
Study Section: **Neuroscience – Molecular**

Obesity and obesity-related morbidities have reached epidemic proportions worldwide. There is an unmet need for the development of novel classes of drugs that promote weight loss with high efficacy but cause minimal side effects. The melanocortin 4 receptor (MC4R) is a G protein-coupled receptor (GPCR) that plays a key role in suppressing appetite and stimulating energy expenditure by modulating hypothalamic signaling circuits. Studies in both rodents and humans have shown that inactivating MC4R mutations are the most common form of monogenetic obesity. However, little is known about how neuronal MC4R signaling is regulated at the molecular level. Previous studies have shown that ligand-activated MC4Rs couple to heterotrimeric G proteins (Gs/Gq) and recruit beta-arrestin-2 (barr2). Traditionally, barr2 is known for its role in terminating GPCR signaling via binding to activated receptors. However, many recent studies suggest that barr2, following its recruitment by activated GPCRs, can activate or inhibit numerous intracellular signaling pathways in a G protein-independent fashion, thus regulating a wide range of cellular processes.
A previous study analyzing a large number of human MC4R variants found that variants endowed with increased efficacy of barr2 recruitment to MC4R correlated with reduced body weight. Based on this observation, we hypothesized that barr2-dependent signaling may play a key role in mediating the beneficial metabolic effects resulting from the activation of central MC4Rs. To address this question, we generated and analyzed mice that lacked barr2 specifically in MC4R-expressing neurons (MC4R-barr2-KO mice). Compared to control littermates, the MC4R-barr2-KO mice showed enhanced food intake, increased adiposity, glucose intolerance, and insulin resistance. To investigate the role of barr2 in MC4R-mediated signaling in a more direct fashion, we injected MC4R-barr2-KO mice and control mice with melanotan II (MTII), a potent MC4R agonist. While MTII strongly reduced food intake in control mice, this effect was greatly reduced in MC4R-barr2-KO mice. Interestingly, selective re-expression of barr2 in the paraventricular nucleus of MC4R-barr2-KO mice restored control-like MTII activity. These data highlight the key role of barr2 in mediating the beneficial metabolic effects of MC4R signaling. MC4R agonists capable of recruiting barr2 with high efficacy may prove therapeutically useful as novel anti-obesity drugs.

172. Shubhra Saha, Ph.D.

Nanobody-based bioconjugates for targeted HIV inhibition
Mentor: **Dr. Ross W Cheloha**
Study Section: **Chemistry**

Human immunodeficiency virus (HIV) causes immune depletion, leading to AIDS and death if untreated. There is currently no cure or vaccine to prevent HIV infection, necessitating the development of new therapeutic approaches. Envelope glycoprotein found on the HIV surface binds to the immune cell surface proteins (CD4, CXCR4, CCR5) to facilitate fusion between viral and host membranes,

resulting in viral infection. Anti-HIV fusion inhibitor peptides (FIs) have been developed to block this process and treat infection; however, these approaches suffer from drawbacks such as toxicity and the emergence of drug resistance. Here, we describe a new approach for the localized delivery of antiviral FIs that relies on the binding of antibody fragments known as nanobodies (Nbs).

We link synthetic peptides that act as FIs to Nbs to generate novel, chimeric antiviral conjugates. Such conjugates are generated using a combination of synthetic peptide chemistry, recombinant antibody expression, and enzymatic conjugation chemistry. These conjugates enable delivery of FIs to cells susceptible to HIV infection by using Nbs specific for cell surface markers (CD4 and CXCR4) expressed in this context. We confirmed that Nbs maintain binding to specified cell surface proteins using flow cytometry. We assessed the efficacy of Nb-FI peptide conjugates using a cell-based HIV pseudovirus neutralization assay. Cells engineered to express CD4 and CCR5 were exposed to HIV pseudovirus (Yu-2 strain) expressing HIV fusion machinery. These experiments showed that Nb-FI conjugates outperformed FI peptides alone, with improvements in potency of nearly 1000-fold.

We hypothesize that the use of a delivery vector Nb will ensure routing of FIs to the surface of cells susceptible to viral infection. Such a delivery mechanism may allow for the use of lower doses of antiviral compounds with a concomitant reduction in side effects, a common problem with anti-HIV peptide therapeutics. Beyond simply serving as a delivery mechanism, Nbs that target surface receptors such as CD4 and CXCR4 can themselves contribute to antiviral activity. The ability to engage multiple mechanisms of antiviral activity (blockage of receptor binding, inhibition of viral protein conformational changes) provides a route towards raising the barrier to viral resistance mechanisms, a longstanding goal of HIV therapeutic development. These hypotheses will be tested using HIV strain libraries and in vivo experimentation.

173. Rima Sakhawala, B.S.

A new noncanonical biogenesis pathway generates a germline enriched miRNA family in C. elegans
Mentor: **Dr. Katherine McJunkin**
Study Section: **RNA Biology**

MicroRNAs (miRNAs) are short RNAs that post-transcriptionally regulate gene expression and play critical roles in development and differentiation. Despite their biological importance, the biogenesis of miRNAs is not completely understood. In canonical miRNA biogenesis, primary miRNAs are transcribed from intergenic loci or intronic regions by RNA polymerase II (RNAPII). These transcripts are then processed by the Microprocessor, an enzyme complex comprised of Drosha and RNA-binding protein DGCR8 (known as PASH-1 in C. elegans). Subsequent processing by Dicer produces the miRNA that is loaded into Argonaute to repress target mRNAs. However, rare noncanonical pathways also bypass the requirement for the Microprocessor and/or Dicer. Using a temperature-sensitive allele of pash-1, we discovered a unique family of PASH-1-independent miRNAs, the mir-1829 family. The mir-1829 family is a germline expressed miRNA family that resides in unusually long introns of three host genes that have no apparent overlapping functions. From 5' RACE data, we determined that the mir-1829 family is derived from independent transcripts with consistent transcription start sites. We posited that these independent transcripts were intronic RNA Polymerase III (RNAPIII) transcriptional units based on the presence of RNAPIII promoter elements and published RNAPIII ChIP-sequencing signals at the mir-1829 loci. CRISPR-mediated mutations of putative RNAPIII promoter elements abrogated mir-1829b expression. Northern blotting and qPCR show that the depletion of RNAPIII, but not RNAPII, results in the dramatic loss of mir-1829, confirming that the mir-1829 family is solely transcribed by RNAPIII. Furthermore, we have reintegrated a minimal transcriptional unit containing the identified RNAPIII promoter elements at an intergenic locus and observed similar expression of mir-1829b compared to expression from its endogenous locus. Although mir-1829 family biogenesis bypasses the Microprocessor, we have determined that it is Dicer-dependent using Northern blot and small RNA-sequencing. Thus, we have delineated a novel biogenesis pathway involving RNAPIII and Dicer, but not Microprocessor. Future work will examine the biological function of these miRNAs. Insights into noncanonical miRNA biogenesis pathways may drive innovation in therapeutics that target offending miRNAs and their role in developmental diseases and cancer by manipulating the RNA itself or its biogenesis pathway.

National Institute of Environmental Health Sciences

174. Marine Baptissart, PhD

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Mentor: **Dr. Marcos Morgan**
Study Section: **Reproductive Biology**

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175. Niketa Bhawsinghka, PhD

dGTP starvation in E. coli

Mentor: **Dr. Roel M Schaaper**
Study Section: **DNA Replication, Damage and Repair**

Cells ensure faithful replication of their DNA by several means. One way is ensuring the appropriate absolute and relative levels of the DNA precursors, i.e. the four deoxynucletotides (dNTPs) during replication. An intricate pathway comprising of several enzymes regulates the synthesis of dNTPs. In addition several dNTPases also regulate the dNTP pool by hydrolyzing the dNTPs. E. coli possess a unique class of dNTPase known as deoxyguanosine triphosphohydrolase (dGTPase) which hydrolyses dGTP into deoxyguanosine and triphosphate. Deletion of the encoding dgt gene resulted in a mutator effect consistent with a proposed fidelity role. Interestingly, a crystal structure of the Dgt enzyme showed it to contain a short stretch of single stranded DNA, subsequently shown to be an activator for the enzyme. We report here on a mutant Dgt created as part of ongoing structure-function studies. One interesting mutant recovered carried a Cys273Ser substitution and was found to be more active than the wild type, bypassing the need for ssDNA binding (i.e., it is constitutively active). Furthermore, cells harboring the mutant gene (a single copy on the chromosome replacing the WT gene) exhibited diminished growth. To further investigate this seemingly toxic effect, the C273S Dgt protein was expressed under controlled conditions from a plasmid vector in E. coli BL21-AI, a strain widely used for expression of toxic proteins. Just within 1hr after (modest) induction, the number of viable cells started to decrease, followed by a rapid decline such that after 2h over 99.9% were dead. Microscopy showed that killing was associated with loss of chromosomal DNA as seen by DAPI staining. The most logical explanation for the killing is rapid depletion of dGTP which leads to crashed replication forks and annihilation of the chromosome by nucleases. We conclude that the continuous presence of activated dGTPase is avoided in the cell but may nevertheless occur and is beneficial under certain conditions signalled by excess ssDNA (eg. viral infection), at least for a limited amount of time. Under those conditions low dGTP levels might temporarily stop DNA replication providing more time for DNA repair activities. Our studies highlight the importance of cellular dNTP control and have revealed a previously unrecognized mode of cell killing. A further detailed understanding of the killing mechanism may lead to effective therapeutics to target pathogenic and/or antibiotic resistant E. coli.

176. Dazhe Chen, PhD

Childhood and adolescent residential and farm pesticide exposures and inflammatory bowel disease incidence in a U.S. cohort of women
Mentor: **Dr. Dale Sandler**
Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Background: Evidence suggests that the human gut microbiome is an important contributor to the pathogenesis of autoimmunity and is affected by early life environmental exposures. Pesticide exposures have been associated with altered gut microbiome in animals and some autoimmune diseases in humans, but no study has examined early-life pesticide exposures in relation to inflammatory bowel disease (IBD).
Methods: We used data from the Sister Study (2003-2021) to examine self-reported residential and farm pesticide exposures during childhood or adolescence in relation to incident IBD diagnosis after study enrollment. We estimated Hazard Ratios (HR) and 95%CIs using Cox models, with age as the time scale, adjusting for race and ethnicity, attained education, smoking, and birth year.
Results: We identified 1,151 self-reported incident IBD cases among 49,263 participants without IBD at enrollment. IBD hazards were elevated among those whose childhood residence was regularly treated with pesticides (HR: 1.26, CI: 1.09, 1.45), including those who ever personally applied the pesticides (HR: 1.47, CI: 1.04, 2.08), compared to those who were never exposed during childhood. We also observed a positive association between IBD and exposure to broadcast pesticide sprays before 1975 (i.e., time of DDT ban) (≥6 times vs. never HR: 1.42, CI: 1.17, 1.74). Among participants who lived on a farm during childhood for ≥1 year (N=9,370), IBD hazards were higher among those who were in crop fields during pesticide application (HR: 1.58, CI: 1.04, 2.40) and who ever personally applied pesticides on crops (HR: 1.79, CI: 1.19, 2.68) or livestock (HR: 1.63, CI: 1.05, 2.54) during childhood or adolescence compared to those without the specified exposure. Results were similar for ulcerative colitis alone (N=945) and when using a stricter definition for IBD that incorporated IBD surgery and medication.
Conclusions: Findings provide preliminary evidence on early-life pesticide exposure as a novel risk factor for IBD.

177. Martin A Estermann, PhD

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Mentor: **Dr. Humphrey Yao**
Study Section: **Reproductive Biology**

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178. Jacob Gordon, B.S.

The SUMO protease SENP3 is allosterically regulated by the rixosome complex.
Mentor: **Dr. Robin E Stanley**
Study Section: **Biochemistry – General**

In response to stress, cells can adapt a variety of signaling pathways using 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 such as protein-protein interaction networks, enzymatic activity, and stability. SUMO targeted proteins are in a controlled equilibrium of SUMOylation and deSUMOylation, ensuring a dynamic molecular switching mechanism. Moreover, aberrant SUMOylation has been implicated in several human diseases underscoring the need to understand the mechanisms of molecular switching. SENP3 is a SUMO protease that deSUMOylates conjugated proteins. It is part of the multi-protein rixosome complex that functions in ribosome biogenesis and chromatin maintenance. Despite SENP3's essential involvement in these cellular processes, the molecular details of SENP3 deSUMOylation enzymatic function have remained elusive.

We hypothesized that association of SENP3 within the rixosome regulates its SUMO protease activity. To test this hypothesis, we reconstituted the multi-subunit rixosome complex and identified an 18 amino acid SENP3 binding patch within the PELP1 subunit. The PELP1-peptide directly bound the SENP3 protease domain, and AlphaFold predicted a high-confidence interface between the PELP1-peptide and SENP3. We next expressed and purified recombinant SENP3 protease domain +/- the PELP1-peptide to assess SENP3 protease activity in vitro. Purified substrates for these assays included precursor SUMO isoforms 1-3 to test endopeptidase function plus isoform specificity, and a biologically relevant conjugated protein SUMO2-NPM1 to test isopeptidase function. Interestingly, both endo- and isopeptidase activity of SENP3 was significantly increased when in complex with PELP1 compared to SENP3 alone. While SUMO2 is the preferred SUMO isoform of SENP3 -/+ PELP1, binding of PELP1 reprograms SENP3 to be additionally active towards the other SUMO isoform precursors 1 and 3. This work supports a model where SENP3 association within the rixosome complex allosterically increases the SUMO protease activity of SENP3 and widens its isoform substrate specificity. Signaling pathways that control chromatin accessibility are hotspots for SUMOylation and deSUMOylation during cell stress. Considering the localization of the rixosome at chromatin, we suspect that SENP3 may influence chromatin regions adapted by SUMOylation during stress.

179. jicheng Li, Ph.D

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Mentor: **Dr. Guohong Cui**
Study Section: **Neuroscience - Neurological and Neurodegenerative Disorders and Injury**

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180. Wilfred Lopez Perez, PhD

Deletion of Epithelial Membrane Protein 2 Protects from Lung Fibrosis
Mentor: **Dr. Michael B Fessler**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

Epithelial membrane protein 2 (EMP2) is a tetraspan protein highly expressed by pulmonary alveolar epithelial type 1 (AT1) cells. We have previously reported that EMP2 regulates epithelial injury during pneumonia. Epithelial injury, when severe, reportedly induces accumulation of senescent transitional alveolar epithelial cells that promote lung fibrosis. Here, we used Emp2-/- and Emp2Sftpc (lung epithelial-specific Emp2-deficient) mice in lung fibrosis models to define the role of EMP2 in lung epithelial cell fate and fibrogenesis. Compared to controls, Emp2-/- and Emp2Sftpc mice had reduced lung fibrosis (lung hydroxyproline, trichrome staining) after bleomycin inhalation, a model of severe primary epithelial injury, whereas there was no reduction in fibrosis after inhaled silica or adenoviral TGFb, models that primarily target macrophages or fibroblasts, respectively. Consistent with attenuated early epithelial injury in Emp2-deficient mice, bronchoalveolar lavage levels of AT1 membrane proteins RAGE and PDPN were reduced during the first 5 days post-bleomycin, as were levels of monocytes and neutrophils, pro-inflammatory cytokines, and TGFb. Emp2-null mice exhibited a marked reduction in accumulation of senescent KRT8+/ITGB6+ alveolar epithelial transitional cells, as well as reduced senescence of AT1 and AT2 cells. Examining early timepoints to define the impact of EMP2 on the primary response of alveolar epithelial cells to bleomycin insult, we found that Emp2-null mice had reduced gH2AX (dsDNA break marker) and attenuated induction of p53 target genes in both AT1 and AT2 cells at 24 hours post-bleomycin inhalation. Studies using BODIPY-FL-bleomycin revealed reduced internalization of bleomycin into AT1 and AT2 cells at 2 hours post-inhalation in Emp2-/- and Emp2Sftpc mice, whereas bleomycin internalization into AT1 and AT2 cells was normal in Emp2Sftpc-CreERT2 mice, in which EMP2 is selectively deleted in AT2 cells. Taken together, our findings suggest that EMP2 deletion attenuates lung fibrosis by protecting alveolar epithelial cells from DNA damage-induced senescence, and that this may arise, at least in part, from reduced bleomycin internalization. The reduced internalization by AT1 and AT2 cells in Emp2Sftpc but not Emp2Sftpc-CreERT2 mice suggests that AT1 EMP2 is required for bleomycin entry into AT1 cells and also co-regulates AT2 bleomycin levels via either contact-dependent or paracrine mechanisms.

181. Mahina Monsur, MD, Ph.D

Whole Genome Study of Nucleotide Excision Repair and Translesion Synthesis in Saccharomyces cerevisiae
Mentor: **Dr. Thomas A Kunkel**
Study Section: **DNA Replication, Damage and Repair**

DNA lesion repair is a complex process involving both multiple pathways of repair and tolerance. We used Saccharomyces cerevisiae whole-genome mutation accumulation experiments to investigate the interplay of translesion synthesis and nucleotide excision repair in Saccharomyces cerevisiae. We focused on two important components: DNA Polymerase (Pol) Zeta and Nucleotide Excision Repair Factor 1 (NEF1). Pol Zeta is a B-family DNA polymerase specialized for translesion DNA synthesis with a catalytic subunit encoded by REV3. NEF1 recognizes and binds damaged DNA during nucleotide excision repair (NER). An essential subunit of NEF1 is encoded by RAD14. Previous studies have explored Pol Zeta mutational patterns through in vitro methods and yeast reporter genes, revealing distinct patterns of complex and simple mutations. We compared published wild type yeast mutations, with strains lacking RAD14 (rad14 deletion) and/or a mutator variant of Pol Zeta (rev3-L979F). The 2,579 collected mutations revealed distinct mutational patterns in each strain. Base substitution rates were strongly increased by rad14 deletion, independent of REV3 status. Complex mutation rates were highly elevated in all three mutant strains, with strong synergy between rev3-L979F and rad14 deletion. This suggests a critical interdependence between the translesion synthesis pathway mediated by Pol zeta and the nucleotide excision repair process mediated by NEF1. The mutation patterns were independent of replication timing, nucleosome occupancy, or coding status, and thus largely recapitulated the patterns found in reporter genes. Such consistency contrasts with mutation patterns in other processes, such as DNA replication or Mismatch Repair. This research describes the relationship between Pol Zeta and NEF1, offering mechanistic insights to advance genomic stability research. This study also bridges the gap between whole-genome and targeted mutagenesis research and sets the stage for future investigations into the complex mechanisms of mutational processes in eukaryotic organisms.

182. Krystal A Orlando, PhD

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Mentor: **Dr. Paul Wade**
Study Section: **Epigenetics**

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183. Sohal Puja, Ph.D.

GLIS3 plays a critical role in the regulation of inflammation and fibrosis in cystic kidney disease.
Mentor: **Dr. Anton Jetten**
Study Section: **Immunology – General**

The Krüppel-like zinc finger transcription factor GLIS3 plays a critical role in the regulation of normal renal functions and in the development of polycystic kidney disease (PKD). GLIS3-deficiency in humans and mice causes PKD, which is characterized by progressive growth of kidney cysts that leads to progressive loss of renal function. Development of interstitial inflammation and fibrosis are major pathological features of PKD. Masson's Trichrome staining showed that collagen deposition was significantly increased in Glis3-KO kidneys, while immunohistochemical analysis showed increased infiltration of lymphocytes and macrophages.
To obtain greater insights into the role of GLIS3 in the regulation of renal inflammation and fibrosis, RNA-Seq analysis was performed using kidneys from WT and Glis3-KO mice at different time points of PKD progression. This analysis showed that various inflammatory chemo/cytokine genes (Ccl2, Cxcl10, Ccl9, Cxcr3, Il1b), markers of immune cell types (Cd4, Cd163, Cd84, Adgre1), and fibrosis related (Ltbp2, Col1a1, Mmp9, Cd44, Tgfb) genes were expressed at significantly higher level in KO kidneys. ChIP-Seq analysis showed that several of these genes were directly regulated by GLIS3. These data are consistent with the histochemical analysis showing increased inflammation and fibrosis during the progression of PKD.
Analysis of different immune cell types by flowcytometry using antibodies for specific immune cell surface markers on single cells isolated from perfused kidneys from 1 and 2 months-old kidney-specific Glis3-PAX8Cre-KO mice demonstrated that the number of T cells, neutrophils, eosinophils, and macrophages were significantly increased in Glis3-PAX8Cre-KO kidneys as compared to WT. Both proinflammatory M1 and pro-fibrosis M2 macrophages were significantly enhanced with M2 being the predominant cell type. Accumulation of these cells in Glis3-PAX8Cre-KO kidneys steadily increased with age and the progression of the disease.
Our study identifies GLIS3 as a new critical regulator of renal inflammation and fibrosis by directly regulating the transcription of several chemo/cytokine and fibrosis-related genes mediated by increased infiltration of both proinflammatory M1 and pro-fibrosis M2 macrophages. Currently, we are analyzing immune cell (sub)populations, and their gene expression profiles by scRNA-Seq using CD45+ cells isolated from WT and KO kidneys.

184. Lenka Radonova, PhD.

The power network: mitochondria-endoplasmic reticulum interactions in oocyte and egg
Mentor: **Dr. Carmen Williams**
Study Section: **Reproductive Biology**

Embryo development starts at fertilization, when sperm trigger repetitive increases in cytoplasmic calcium levels (Ca^{2+} oscillations) in the egg. The egg acquires its ability to be fertilized during meiotic maturation. Meiotic maturation is a process in which an oocyte, the precursor of an egg, undergoes nuclear and cytoplasmic changes necessary for normal Ca^{2+} oscillations at fertilization. The cytoplasmic changes include increased endoplasmic reticulum (ER) Ca^{2+} stores, reorganization of the ER, and increased

mitochondrial activity. In somatic cells, the ER and mitochondria closely associate at mitochondria-ER-associated membranes (MAMs), which are essential for maintaining Ca2+ homeostasis and proper mitochondrial function. It is unknown if MAMs are present or have important functions in eggs, whose mitochondria have an unusual morphology. We hypothesize that MAMs are present in oocytes and undergo changes during meiotic maturation to support Ca2+ oscillations triggered in the egg at fertilization. We first tested for the presence of MAMs at the ultrastructural level in mouse eggs by performing focused ion beam-scanning electron microscopy and 3-D reconstruction. As expected, the mitochondria were spherical with small, immature cristae. They were often clustered in groups in the egg cortex, closely associated at specific surface locations with ER tubules, suggesting close interactions of both organelles. Next, we examined MAMs in live mouse oocytes and eggs using a fluorescence-based probe designed to detect ER-mitochondrial interactions at less than 10-15 nm. There were differences in MAM distribution in oocytes and eggs. In oocytes, the MAMs localized throughout the cytoplasm, with modest accumulation around the nucleus. In eggs, MAMs surrounded the meiotic spindle with a "dispersal pattern" localization from the spindle through the center of the cell. In both cases, MAM localization correlated with the mitochondria distribution. These results provide the basis for subsequent experiments to disrupt MAMs in eggs and study the impact on Ca2+ signaling during fertilization. Knowledge of the structural basis and core interactions of ER and mitochondria in female gametes will promote an understanding of the effects of health conditions, aging, and environmental factors on female reproduction.

185. Danielle R Stevens, PhD

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Mentor: **Dr. Kelly Ferguson**
Study Section: **Epidemiology/Biostatistics – General**

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186. Dimitrios Theofilatos, PhD

Exposure to high temperatures alters Glucocorticoid Receptor activity
Mentor: **Dr. Trevor K Archer**
Study Section: **Epigenetics**

A critical outcome of climate change is an increase in the frequency of prolonged periods of high or extreme high temperatures. Epidemiological evidence has highlighted the correlation between these heat events and an increase in heat-related deaths and chronic disease complications. The Glucocorticoid Receptor (GR) is a nuclear hormone receptor that binds to glucocorticoid hormones and enters the nucleus to modulate the expression of genes participating in biological processes critical for cellular physiology and organ homeostasis. Glucocorticoids are widely utilized to treat a broad spectrum of ailments and diseases, and understanding how extreme heat may disrupt or alter the cellular response to glucocorticoids is critical for human health. Here, we investigate how the prolonged exposure to heat influences the transcriptional activity of GR. For this purpose, we cultured cells for at least 72h at either 40°C or at 37°C (control condition). We used the T47D A1-2 breast cancer cell line in which we have extensively characterized the normal transcriptional response to glucocorticoids. RNAseq revealed that heat dramatically altered the transcriptional response observed after 4-hour treatment with Dexamethasone (Dex), a synthetic glucocorticoid. We observed over 100 known GR target genes that were overactivated by Dex upon heat exposure and identified over 1000 genes as heat-specific GR targets that only responded to Dex in cells at 40°C. Over a broader time-course of Dex treatment, we validated these results by qPCR and found that the over-activation was maintained across multiple time points. Focusing on the overactivated GR target genes, single cell RNAseq showed that these genes were not only induced at higher levels, but were also induced in more cells at 40°C. Moreover, western blots revealed that heat increased GR protein levels without affecting hormone-dependent nuclear translocation. Currently, we seek to determine the molecular mechanisms underlying the effect of heat on GR activity and characterize the heat-specific GR target genes. To better understand this phenomenon, we are also investigating how the duration and severity (eg temperatures higher than 40°C) of heat exposures differentially affect GR activity. Ultimately, we hope our findings will provide novel insights into how a hotter environment can shape molecular events in human cells and emphasize the necessity for considering how climate change will alter basic human biology.

187. Christina Wilkinson, Genetics, PhD

CD301b+ lung dendritic cells confer tolerance to inhaled allergens
Mentor: **Dr. Don Cook**
Study Section: **Immunology - Mucosal Immunity**

The incidence and severity of allergic asthma is controlled by a fine balance between allergen-specific T regulatory (Treg) and T helper (Th)2 cells. Thus, an improved understanding of how these two CD4+ T cell lineages develop is critical for designing novel strategies to treat asthma and other allergic diseases. Type 2 conventional dendritic cells (cDC2s) in the lung can promote the differentiation of both Tregs and Th2 cells, but it is unknown whether these two CD4+ cell lineages are promoted by different subsets of cDC2s. Accordingly, we purified lung cDC2s at various times after allergic sensitization and subjected them to single-cell RNA sequencing (scRNA-seq). At baseline, the majority of the cDC2s were marked by high display of the surface protein CD301b. However, following allergic sensitization through airway, several additional clusters of cDC2s became apparent. Purification and ex vivo analysis of these different

cDC2s revealed that CD301b+ lung resident cDC2s strongly promote Treg differentiation, even after allergic sensitization, whereas CD200+ cDC2s preferentially direct Th2 induction. Pseudotime analysis of the scRNA-seq data and adoptive transfer experiments revealed that during allergic sensitization some CD301b+ lung resident cDC2s transition to CD200+ cDC2s in a stepwise manner. The CD301b+ lung resident cDC2s express high amounts of GM-CSF receptor alpha, and mice overexpressing GM-CSF cytokine had increased numbers of CD301b+ cDC2s in the lung. Conversely, mice lacking the receptor Csf2rb in CD11c+ antigen presenting cells had fewer CD301b+ cDC2s and had a reduced capacity to induce Treg differentiation ex vivo. Adoptive transfer of allergen-loaded lung CD301b+ cDC2s to naïve mice reduced airway inflammation and type 2 cytokine production in a mouse model of asthma. CD301b+ cDC2s could also be expanded from bone marrow cells in the presence of GM-CSF and these cells displayed elevated Treg inducing activity, suggesting a potential use for them in immunotherapy. Mechanistically, Treg-inducing CD301b+ cDC2s have relatively low costimulatory molecule display and express higher levels of Tgfb1, as well as genes encoding the TGF-beta processing molecules, Furin and LRRC33. In agreement with this, an inhibitor of TGF-beta signaling effectively suppressed Treg induction by CD301b+ cDC2s. Together, these data provide new insights into cDC2-mediated development of Tregs in vivo and reveal novel potential approaches to immunotherapy.

188. Xiaoyue Wu, Ph.D.

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Mentor: **Dr. Xiaoling Li**
Study Section: **Cell Biology - Cell Cycle and Metabolism**

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National Institute of General Medical Sciences

189. Jong Park, Ph.D.

Studying lung vascular endothelial cells using zebrafish gills
Mentor: **Dr. Brant M Weinstein**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

The blood vasculature of the lung plays an essential role in gas exchange function. Recently, a novel population of lung capillary cells aptly named “Aerocytes” was discovered that is thought to play a gas-exchange role due to its extensive surface area in the lung alveolus. However, the specific functions of these novel cells are still largely unknown due to difficulties in observing and experimentally manipulating them in vivo in mammalian lungs. Although fish gills function underwater, they also facilitate efficient gas exchange and share many functionally equivalent cell types with lungs, including neuroepithelial cells that sense oxygen and gas-permeable fibroblasts that help facilitate selective oxygen uptake. However, unlike mammalian lungs, the externally situated gills are readily accessible for high-resolution optical imaging and experimental manipulation, making the fish a powerful comparative vertebrate model for studying the development and function of gas-exchange blood vasculature in an in vivo context. We performed single-cell RNA sequencing on dissected adult gills and identified various cell types with transcriptional profiles similar to those found in mammalian lungs, including a blood vessel cell population with a transcriptional profile that strongly resembles the recently discovered mammalian Aerocytes. In situ hybridization chain reaction (HCR) using conserved Aerocyte markers revealed that zebrafish Aerocytes localize to highly vascularized gill lamellae, equivalent to lung alveoli, where gas exchange takes place. Using conserved Aerocyte markers, we are generating zebrafish Aerocyte transgenic lines via Tol2 integration and CRISPR homology-directed repair to visualize and functionally manipulate these unique and essential cells in their normal in vivo context. Our findings are helping to establish a new experimentally accessible comparative vertebrate model for studying gas-exchange blood vasculature, specifically, the development, morphology, and function of the newly discovered Aerocyte.

National Institute of Mental Health

190. Miguel Arenivar, Neuroscience

The role of distinct somatostatin cell peptidergic transmission in regulating learning
Mentor: **Dr. Hugo Tejeda**
Study Section: **Neuroscience - General**

The prefrontal cortex (PFC) is integral to cognition, encompassing decision-making and adaptive learning. Within the PFC, somatostatin (SST)-expressing interneurons play a pivotal role in modulating neural circuits, crucial for mitigating cognitive functions. Disturbances in the inhibitory balance of the PFC, particularly involving SST-positive interneurons, have been linked to neuropsychiatric conditions such

as schizophrenia, and depression. While the general function of SST-positive interneurons in the cortex has been explored, the specific contributions of distinct subpopulations of these cells within the PFC to behavioral outcomes remain poorly understood. Previous work in our lab has shown that SST-peptidergic transmission is vital for learning cue and outcome associations. However, the role of subpopulations of SST interneurons in these behaviors is still unknown. This study aims to dissect the roles of different SST-expressing interneuron subpopulations in the PFC, using advanced genetic tools for cell-type-specific manipulation, in-vivo calcium imaging, and a suite of behavioral assays. Here, we unravel the nuanced contributions of SST interneuron subpopulations on cognitively-based behavior. First, we used high-plex RNA sequencing data, to reveal that SST co-expresses with a wide range of neuropeptides, specifically, dynorphin (DYN), enkephalin (ENK), and neuropeptide-y (NPY), in superficial, middle, and deep layers within the PFC, respectively. We then used a targeted SST knockdown approach using a CRE-dependent SST-CRISPR virus and transgenic mouse lines, to underscore the role of these subpopulations in fine-tuning behavior. We also utilized in-vivo fiber photometry to assess the activity of DYN, ENK, and NPY co-expressing SST subpopulations, during learning cue and outcome associations. Lastly, we establish the heterogeneity of SST interneuron neuronal activity within the PFC utilizing miniaturized endoscopes to obtain single-cell recordings of SST neurons during cue and outcome associations. In short, this study unveils the specificity of SST subpopulations within the cortex and how they regulate cognitively-based behaviors, as well as the anatomical differences between these subpopulations. By pinpointing the specific interneuron subpopulations and how they contribute to various aspects of behavior, this study opens new avenues for targeted interventions in neuropsychiatric disorders characterized by PFC dysfunction and SST dysregulation.

191. Karen Christopher Durairaj, Ph.D.

Cholinergic control of vocal expression in the developing marmoset (Callithrix jacchus)
Mentor: **Dr. Yogita Chudasama**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

Cholinergic neurons play an essential role in refining cognitive-emotional components of executive function by interacting with the activity of prefrontal-hippocampal networks. A major population of these neurons originate from the basal forebrain nuclei that lie as a continuum extending from the medial septum, vertical and horizontal diagonal bands of broca, through the nucleus basalis of Meynert to the globus pallidus. During development, acetylcholine modulates neuronal proliferation, differentiation, and synaptic plasticity but its contribution to the development and maturation of cognitive, social and emotional processing remains unknown. In this longitudinal study we show how early life degeneration of medial septal cholinergic projections in infant marmosets alters the normal development of vocal expression. We targeted the medial septal-basal forebrain nuclei by injecting ME20.4-IgG-saporin (saporin) in 14-day old neonatal marmosets (n=3). Saporin is an immunotoxin that selectively destroys the p75-positive cholinergic cells projecting to the hippocampus. Another group of infant marmosets received sham control surgery (n=3). We examined changes in isolation induced vocal calls before and after the saporin immunolesion. Vocalizations were recorded using a Sennheiser ME 64 microphone and acoustics were analyzed using Raven Pro software. Vocal calls such as phee, twitter, trill and tsik were preserved before and after the saporin immunolesion. Unlike the controls, two saporin injected infants stopped crying by postnatal week 5 and one did not cry at all. In addition, they made fewer social contact calls (mean: saporin: 23; controls: 117) which normally dominate the vocal repertoire as a means of connecting with distantly located family members. At the same time, they increased the number of non-social calls (mean: saporin: 53; controls: 13). These preliminary data indicate that the normal development of medial septal-basal forebrain cholinergic projections are integral to the postnatal development of vocal-emotional behavior. Further examination of the animals social and cognitive behavior will reveal the impact of the central cholinergic system to emotional development.

192. Paul M Dement, Chemistry

Establishing New Chemical Methods of Producing PET Imaging Agents that are Used for the Study of Neuropsychiatric Disorders
Mentor: **Dr. Victor W Pike**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

Establishing new imaging agents for the study of neuropsychiatric disorders (NPD) by, for example, positron emission tomography (PET), is desirable for biomedical research and drug development. PET imaging agents have value in the development of new treatments and therapies. One process which is shown to contribute to NPD is neuroinflammation (NI). Cyclooxygenases and cannabinoid type 2 receptors (CB2R) are potential biomarkers for NI. Improving the potency and selectivity of tracers for the PET imaging of these and other targets would be beneficial for early detection and monitoring of NI.
Positron-emitting carbon-11 (C-11; half-life 20 min) and fluorine-18 (F-18; half-life 110 min) are commonly used radionuclides for labeling tracers for PET imaging of brain. Producing carbon-11 or fluorine-18 labeled PET tracers is however exceptionally challenging due to their short half-lives. This limits the scope of chemical reactions that are useful for labeling to methods to those that can be completed in 20 minutes or less. For this reason and others, methods for labeling PET tracers with C-11 or F-18 are presently restricted to a few chemotypes. Expanding the range of chemotypes that is accessible for carbon-11 or fluorine-18 labeling could facilitate the development of new radioligands for the study of NI, or indeed other pathologies.
One entity that has potential for introducing either C-11 or F-18 into PET tracer of new chemotype is radiolabeled fluoroform (HCF3). Of the very few methods for synthesizing C-11 or F-18 containing groups, there has been none for directly coupling a radiolabeled trifluoromethyl group (CF3) to an alkyl (sp3-hybridized) carbon. Here in, we report the first method for coupling a radioactive CF3 group with an alkyl carbon. Conditions found to give high yields (e.g, 71%) in non-radioactive syntheses gave only very low yields (e.g., 3%) in

attempts to transfer conditions to radiochemistry. However, judicious changes and optimization of conditions now give high radiochemical yields (>50%). Reactions are complete within 10 minutes. This method works with a range of complex chemical structures. A large range of functional groups, including alcohols and phenols, and commonly encountered heterocycles, including indoles, are well-tolerated. A radioactive trifluoromethyl (CH_2CF_3) group can now be considered for the first time in the design of new PET tracers for PET imaging of NI.

193. Franco Giarrocco, PhD

Motor system-dependent effects of amygdala and ventral striatum lesions on explore-exploit behaviors
Mentor: **Dr. Bruno Averbeck**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

Living in a changing environment requires choosing between exploiting known options or exploring novel options. This decision is known as the explore-exploit dilemma and mediating it properly requires learning the values of choice options to determine when exploration is advantageous. Understanding how the brain mediates explore-exploit behaviors is crucial, as alteration of explorative behavior has been linked to many brain disorders.

Early studies linked exploration to cortical brain regions, suggesting a top-down control in shifting between exploitative and explorative behaviors. Recent studies have shown that subcortical motivational regions, including the amygdala and ventral striatum (VS), also play a major role in explore-exploit behaviors. However, in previous studies subjects mediated explore-exploit behaviors and learned the values of choice options through responses made with a single motor system (e.g., eye movements or arm movements). This approach reflects the common assumption that there exists a single value representation in motivation regions, independently of the motor system.

Here we evaluated the causal role of amygdala and VS in explore-exploit behaviors when monkeys learned the values of choice options with two different motor systems. We compared the behavior of control monkeys (n=12) against monkeys that received excitotoxic lesions of either the amygdala (n=6) or the VS (n=3) on two different tasks, in which choices were made with either saccades or arm movements. In both tasks we presented the monkeys with explore-exploit tradeoffs by repeatedly introducing novel choice options with unknown value. We found that monkeys showed higher explorative tendency with saccades but better value learning with arm movements. VS lesions increased exploration with arm movements compared to saccades and affected learning novel stimulus-value associations in both tasks. Further, VS and amygdala lesions reduced the monkeys' ability to choose better options only when choices were made with a saccade. These findings indicate that non-human primates manage explore-exploit behaviors in a motor system-depended way and offer new insights into the contribution of the amygdala and ventral striatum to reinforcement learning. Further, we challenge current theories and hypothesize that a different value representation might be driving learning in the oculomotor and skeletomotor systems.

194. Meijuan Jiang, PhD in Chemistry

Robust Radioligands for Quantifying Phosphodiesterase-4D in Monkey Brain with PET Imaging
Mentor: **Dr. Victor W Pike**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

Phosphodiesterase-4 (PDE4) enzymes hydrolyze the important secondary messenger, cyclic adenosine monophosphate. PDE4 enzymes exist as four subtypes (A–D). Notably, inhibitors selective for the PDE4D subtype are being developed as cognition enhancers and for the treatment of Fragile X Syndrome. A radioligand capable of quantifying human brain PDE4D with positron emission tomography (PET) would be valuable for drug development and biomedical research. An earlier developed radioligand, [11C]T1650, was unable to quantify PDE4D robustly in human brain, likely because of metabolism at a vulnerable nitro group that leads to radiometabolite contamination of enzyme-specific PET signal. Therefore, we pursued further structural elaboration of T1650 to meet the goal of producing an effective PDE4D PET radioligand. In our structure-activity study, we discovered a highly quantitative correlation ($R^2 = 0.988$) between PDE4D inhibitor structural fragments and their binding affinities. This relationship enabled efficient discovery of several new highly potent inhibitors ($IC_{50} < 10$ nM) as potential PET radioligand candidates. Considering major screening criteria for brain PET radioligands (e.g., high affinity, moderate lipophilicity), we then selected five high-affinity PDE4D-selective inhibitors (IC_{50} = 1.1–2.7 nM; 193–793-fold selectivity versus PDE4B) having no nitro group. They were successfully labeled with cyclotron-produced carbon-11 ($t_{1/2}$ = 20.4 min) in useful radiochemical yields. Each radioligand was then evaluated in monkeys with PET imaging of brain at baseline and after treatment with the pan-PDE4 inhibitor, rolipram or the PDE4D-selective inhibitor, BPN 14770. All five radioligands showed sizeable PDE4D-specific signal in monkey whole brain (baseline binding potential, 0.5–1.7). Among them, two radioligands showed time-stable specific signals from 90 to 120 min after injection. Both were found to be stable in vitro in fresh rat brain homogenates for 60 min, unlike [11C]T1650. Moreover, contamination of monkey brain with radiometabolites at 30 min after intravenous injection of either radioligand was found to be negligible (< 3%), as assessed by ex vivo analysis. Above all, these two radioligands were affirmed to robustly quantify PDE4D in monkey brain. The one with higher PDE4D baseline binding potential is now being taken forward for evaluation with PET in human subjects.

195. Alexis Kidder, BA Psychology

Evidence from mouth-specific visual distortions in prosopometamorphopsia for independent representations of face features
Mentor: **Dr. Chris Baker**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

Faces play a crucial role in our daily lives, and the human visual system excels at accurately extracting a range of information from a single glance at a face. How our visual system represents this face information remains an open question. Generally, it is thought that we process faces holistically, meaning that how we interpret one portion of the face impacts our perception of other areas of the face. Recent human neuroimaging data have suggested that different face processing regions prefer some facial features over others. While these studies suggest that face processing is not wholly holistic, it is unknown whether facial features are represented independently. We investigated whether there are mouth-specific representations in the human face processing system from a case study of a 39-year-old man who experiences prosopometamorphopsia (PMO). PMO is a visual disorder in which individuals see distortions on faces. The current patient's unique distortions occur exclusively to mouths, and include whole mouth duplications, illusory mouth motion, and changes to lip shape. We tested predictions of four alternative accounts of the process producing these distortions: (1) low-level visual processing, (2) general visual object processing, (3) the lower half of viewer-centered face representations, or (4) face-centered, mouth-specific representations. We ran a series of 43 behavioral tasks to characterize the patient's distortions and test which hypothesis best accounted for his experience. Of the evoked distortions, 94.8% (165 of 174) of the distortions were to faces, of which 94.5% were to single features (156 total), and 91.7% only affected mouths (143 total). To test accounts 1 and 2, the patient viewed 360 simple shapes and 586 common objects and scenes, and reported only two distortions. To evaluate accounts 3 and 4, faces were displayed at six picture-plane orientations. Mouth distortions occurred at each orientation and no distortions occurred on features in the lower half of the face other than on mouths. Other abnormal processing was ruled out with additional neuropsychological tests and neuroimaging. Taken together, these results indicate that the distortions experienced by this patient reflect disruptions to feature-specific, face-centered representations. These are the first data to show that mouths are encoded independently from other face features and suggest other facial features may also be represented independently.

196. Paul A Parcon, MD, PhD

Ketamine Rapidly Increases cAMP signaling in rat and monkey brain, as measured by PET neuroimaging of [11C](R)-rolipram
Mentor: **Dr. Robert B Inni**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

Background: Major Depressive Disorder is a heterogeneous disorder with relatively few options for treatment targets. One possible target for the development of novel treatments for major depressive disorder (MDD) is the cyclic adenosine monophosphate (cAMP) pathway. Phosphodiesterase-4 (PDE4) regulates cAMP via negative feedback, and can thus be an indirect marker of cAMP signaling. It was previously shown that [11C](R)-rolipram, a PET radioligand which binds to active PDE4, is decreased in individuals with MDD, implying decreased cAMP signaling compared to healthy controls. Further, 8 weeks of antidepressant treatment rescued this decrease in [11C](R)-rolipram binding. Unlike SSRIs, ketamine is a rapid-acting antidepressant, although its mechanism is not fully understood. We sought to determine if ketamine would rapidly increase cAMP signaling in rats and rhesus macaques. Methods: PET imaging was conducted using [11C](R)-rolipram. The rats (n=6) were imaged pre- and post-IV ketamine at 10mg/kg over 40 minutes, with post-ketamine scan performed within 1 hour of infusion. Data was compared as area under the curve of standard uptake values between 30 to 90 minutes (SUV AUC30-90), which is a measure of radioactivity in the brain. [11C](S)-rolipram, which has no specific binding to PDE4, was used as a control for the effects of ketamine on blood flow and radioligand delivery. Three monkeys were also scanned twice with [11C](R)-rolipram, before and after ketamine infusion (0.5mg/kg over 40 minutes). Data was analyzed with 2-tissue compartmental modeling with arterial blood sampling to determine distribution volume VT. Data analyzed by paired t-test. Results: Ketamine increased [11C](R)-rolipram binding to PDE4 in rats (mean SUV increase=24%±14%, range=3%-42%, p=0.004) and monkeys (mean distribution volume (VT) increase=14%±2%, range=12%-16%, p=0.003), demonstrating that ketamine increased cAMP signaling in animals. Ketamine had no consistent effect on [11C](S)-rolipram. Conclusions: Ketamine infusion increased [11C](R)-rolipram binding in rats and in monkeys compared to baseline scans within 1 hour of ketamine infusion. This implies that ketamine infusion can rapidly increase cAMP signaling and may be an underlying mechanism for ketamine's rapid antidepressant effect. This data supports a common pathway for cAMP and antidepressant action, and provides evidence for targeting cAMP signaling for Major Depressive Disorder.

197. Lina Teichmann, PhD

Reading out individual color experiences from the human brain
Mentor: **Dr. Chris Baker**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

We typically assume that others see the world in a similar way to us. However, even something as simple as color perception varies widely among individuals as shown by differences in color discrimination, color constancy, color appearance and color naming across languages. Despite such individual differences, the neuroscience literature is dominated by studies using central tendencies to

understand perceptual processes. In my work, I use color as a model to map subjective experiences in the human brain, as color is a biophysically well-defined and constrained space. Many attempts have been made to construct formal, uniform color spaces that aim to capture universally valid similarity relationships, but there are discrepancies between these models and individual perception.
My work aims to reconstruct individual color experiences from brain activation patterns, measured with magnetoencephalography (MEG), and relate those signals to sources of variability in color perception. To do this, we densely sampled neural responses to thousands of colors from six individuals across 10 hours of experimental sessions to reconstruct individual fine-grained color-space geometries from neural signals with millisecond accuracy. In addition, we collected psychophysical judgments for several thousand color comparisons and asked people to name hundreds of colors to capture individual color experiences behaviorally. Lastly, we also collected genetic data to extract potential differences in photoreceptor expression that can lead to different types of color blindness. Our approach integrates these multi-modal data to gain a comprehensive insight into how individual differences can arise at different levels (i.e., genetic, language, experience) affecting the neural signal and ultimately subjective color experience. We find that color information for all participants is present in the neural signal from approximately 80ms onwards but that neural color-space geometries unfold non-uniformly over time and vary across individuals. For example, we found that several broad color categories such as red, green, and blue, evoke distinctive neural signatures in all participants but that the timing and the relative strength of these activations differs across individuals. These findings highlight the gap between theoretical color spaces and color perception and represent a novel avenue to gain insights into the subjective nature of perception.

198. Runyi Tian, PhD

Psilocin Alters Mitochondrial Dynamics: Implication for the Hallucinogenic Mechanism of Psychedelics
Mentor: **Dr. Zheng Li**
Study Section: **Neuroscience - Cellular and Synaptic**

Mounting evidence suggests that the serotonergic psychedelics psilocybin and its active metabolite psilocin have promising therapeutic potentials for psychiatric disorders. However, the clinical application of psychedelics is hindered by their undesired hallucinogenic effects, which cause “bad trip” experiences, making the oversight of trained physicians during a psychedelic therapy necessary. This limits the feasibility and cost-effectiveness of psychedelic therapy. The hallucinogenic effect is believed to stem from activation of 5-HT2A receptors on cortical neurons, yet the precise molecular and cellular mechanisms remain largely elusive. To address these questions, we examined the effect of psilocin on primary cortical neurons. We used Instant Structured Illumination Microscopy (iSIM) to monitor mitochondria and cytosolic calcium before and after psilocin treatment. We found that psilocin increases fission and fusion of mitochondria in dendrites and mitochondrial movement in axons. These mitochondrial dynamic changes occurred transiently within 20 minutes after psilocin treatment. Additionally, spontaneous firing of neurons as reported by calcium transients increases within the same time frame. Prolonged treatment of psilocin for 24 hours causes a reduction of mitochondrial length, suggesting an alteration of the mitochondrial fission and fusion balance. Injection of psilocin into mice induces head twitching, a locomotor behavior commonly used as a proxy of human hallucination. Intriguingly, psilocin induces a higher incidence of head-twitching in Drp1S616A transgenic mice, characterized by deficient mitochondrial fission due to a phosphor-null mutation in the mitochondrial fission protein Drp1, compared to wild-type mice. These findings suggest that psilocin alters mitochondrial fission and fusion and that this is potentially involved in the psychoactive effect. We are currently investigating the mechanism by which psilocin regulates mitochondrial dynamics and if the mitochondrial effect of psilocin contributes to its hallucinogenic action.

National Institute of Neurological Disorders and Stroke

199. Maleeha Akram, Ph.D.

A BAIAP3 VARIANT IN MALES WITH PUBERTAL FAILURE: ROLE IN HPG AXIS
Mentor: **Dr. Susan Wray**
Study Section: **Neuroscience – Developmental**

A mutation in the BAIAP3 gene was identified in a male patient having failed puberty. BAIAP3 (brain-specific angiogenesis inhibitor I-associated protein 3) encodes a dense core vesicle (DCV) protein involved in recycling at the trans-Golgi network in neuroendocrine and endocrine cells and is associated with endosomes. Therefore, the present study was designed to determine the function of BAIAP3 in hypothalamo-pituitary-gonadal (HPG) axis. During embryonic development, cells expressing BAIAP3 mRNA were detected in nasal areas, coinciding with the migratory route taken by gonadotropin releasing hormone (GnRH) neurons. Microarray data obtained from migrating vs non-migrating GnRH neurons maintained in explants showed expression of BAIAP3 was higher in GnRH cells that had stopped migrating. Cessation of migration of GnRH cells in explants correlates with maturation of peptide processing and secretion. Expression of BAIAP3 protein was examined using double-label immunofluorescence. Results to date show that BAIAP3 protein is expressed in primary GnRH cells in explants and in adult mice, more often associated with the cell soma than process and in mature sperm in testis in mouse. BAIAP3 protein was also detected in GT1-7 GnRH cell line and the KissAR Kisspeptin cell line. Preliminary results suggest that BAIAP3 and previously established endosomal markers, Rab9 and Rab11 are co-expressed in GnRH cells. In addition, after stimulation of explants with KCl, the expression of BAIAP3 quickly increased in GnRH cells, indicating its possible role in

vesicle recycling. Future work will focus on delineating the role of BAIAP3 in DCV processing/recycling and in spermatogenesis using siRNA in testis cell lines and testis markers and to study the effect of mutated BAIAP3 on the function of GnRH neurons and/or spermatogenesis.

200. lujia Chen, ph.D.

Drifting activity fields in medial entorhinal cortex grid cells reveal experience dependent plasticity
Mentor: **Dr. Yi Gu**
Study Section: **Neuroscience – General**

The medial entorhinal cortex (MEC) plays a key role in spatial representation and episodic memory. Grid cells, which are abundant in the MEC, have one of the most mysterious activity patterns in the brain, i.e., their hexagonal firing fields covering the arena the animals are exploring. While theoretical models have proposed mechanisms for generating grid cell activity pattern per se, little is known about how the pattern is shaped during spatial learning. Some studies have suggested experience-dependent changes in grid cell activity patterns upon novel environment exposure and environmental modifications. However, these studies suffered from the limitation of electrophysiology, which can only track the activity of tens of MEC neurons over several days or tens to hundreds of neurons within the same day. To overcome the limitation, we utilized in vivo two-photon imaging to measure calcium dynamics of hundreds of grid cells over multiple days during learning in a one-dimensional (1D) virtual reality (VR) environment. The data collected in this study allowed us to investigate activity development of individual grid cells during spatial learning. Using a novel algorithm that iteratively connecting individual grid cell activity events in consecutive laps, we found that within the same day, a significant fraction of grid fields exhibited a lap-by-lap shift toward earlier track positions (backward drifting). The fraction and the degree of backward drifting grid fields decreased across days during spatial learning, indicating a gradual stabilization of grid cell activity. Notably, backward-drifting grid fields slowly approached visual cues, implying that after learning, grid cell activity tends to represent salient cues in the environmental. Furthermore, concatenating activity of the same grid cells across multiple days revealed a subset of grid fields that continuously backward-drifting across multiple days (cross-day drift), which could be related to continuous memory encoding. Lastly, Theoretical modeling result suggests that the drift was mediated by Hebbian plasticity at grid cell synapses that receive environment-specific inputs. Overall, our results reveal the experience-dependent plasticity that shapes grid cell activity during learning. Our study provides valuable information for improving the current theory of grid cell activity pattern formation and advance the field’s understanding of memory processing in the MEC.

201. Erin Fingleton, BS

Removed at request of author
Mentor: **Dr. Katherine W Roche**
Study Section: **Neuroscience - Cellular and Synaptic**

Removed at request of author

202. Nagela Ghabdan Zanluqui, PhD

Venous plexus-associated lymphoid hubs support meningeal humoral immunity
Mentor: **Dr. Dorian McGavern**
Study Section: **Immunology - General**

There is an increasing interest in how immune cells in the meninges, the membranes surrounding the brain and spinal cord, contribute to homeostasis and disease in the central nervous system. The outer layer of meninges, the dura mater, contains both innate and adaptive immune cells and functions as a site for B cell development. In this study, we identified previously undescribed lymphoid structures surrounding fenestrated venous plexus in the dura mater. We found the most elaborate immune organization, including lymphatic vessels, surrounding the rostral-rhinal confluence of sinuses, interfaced with the skull bone marrow and a comparable venous plexus at the skull base, that we named the rostral-rhinal venolymphatic hub. This hub emerged during development in mice at P8/9 before the formation of the superior sagittal sinus. Single cell RNA sequencing demonstrated that rostral rhinal hub hosts a diverse array of resident innate and adaptive immune cells during steady state and infections. Immune aggregates were present in this structure in homeostasis and expanded with age or following challenge with systemic or nasal antigens. Following intranasal VSV infection, the rostral-rhinal hub supported local germinal centre (GC) reactions consisting of T follicular helper cells as well as GC B cells that underwent proliferation, somatic hypermutation, class switch, and conversion into plasma cells locally. Blockage of cell trafficking at the time of infection and sustained for 10 days completely prevented B cell recruitment and GC B cells development in the hub. However, delayed blocking (from day 6 post infection) allows VSV-specific B cells to be recruited into the hub and undergo to local activation and differentiation, sustaining GC response independent of circulating immune cell input. These data demonstrate lymphoid architecture around vascular plexus in the dura mater that can sample antigens and rapidly elaborate local antigen specific immune responses and likely help to protect the meninges and underlying brain parenchyma from pathogens.

203. Ronald Kim, PhD

Ventral pallidal cholinergic input to the basolateral amygdala mediates valence encoding
Mentor: **Dr. David A Talmage**
Study Section: **Neuroscience - Neural Circuits**

Proper valence encoding is critical for survival in a changing environment. The ability to appropriately encode valence ensures apt behavioral responses toward rewarding (i.e., approach) and harmful (i.e., avoidance) stimuli. The basolateral amygdala (BLA) is a brain region involved in encoding valence. Numerous studies using distinct methodologies have demonstrated the BLA contains two non-overlapping populations of neurons that encode positive vs. negative valence. Cholinergic neurons in the ventral pallidum (VP) also play a role in encoding valence. Importantly, the primary output target of VP cholinergic neurons is the BLA. However, it is unknown if valence encoding VP cholinergic neurons mediate the activity of valence encoding BLA neurons, thereby influencing approach/avoidance behaviors. To investigate these questions, I utilized longitudinal, in-vivo, single-cell calcium imaging of BLA neurons, in conjunction with simultaneous optogenetic stimulation of VP cholinergic terminals in the BLA. Mice were first exposed to either a positive valence stimulus (appetitive odor) or a negative valence stimulus (aversive odor) concurrent with single-cell calcium imaging of BLA neurons. In a distinct imaging session, mice were then exposed to the opposite odor. Longitudinal registration across both imaging sessions was used to identify BLA neurons exclusively activated by each odor. A greater number of BLA neurons were identified as positive valence neurons (i.e., more BLA neurons were exclusively activated by the appetitive odor) than negative valence neurons. Pairing odor delivery with optogenetic excitation of VP cholinergic terminals significantly increased the number and calcium activity of negative valence BLA neurons, but not positive valence BLA neurons. This increased responsiveness of negative valence BLA neurons was also accompanied by corresponding behavioral changes. While mice typically exhibit approach behavior towards the appetitive odor, optogenetic excitation of cholinergic terminals in the BLA not only blocked approach but led to active avoidance behavior of the appetitive stimulus. Optogenetic stimulation did not affect avoidance behavior in response to the aversive odor. These results suggest a potential valence encoding microcircuit between VP cholinergic neurons and valence encoding BLA neurons, which mediates approach/avoidance behaviors.

204. Maxwell T Laws, Doctor of Medicine

Identification and Targeting of Tumor Driver Schwann(Chr22-) Cells in Sporadic and NF2-Related Vestibular Schwannomas
Mentor: **Dr. Prashant Chittiboina**
Study Section: **Oncology - Development and Metastasis**

Introduction:
Vestibular schwannomas (VS) arise from myelinating Schwann cells of the eighth cranial nerve and are associated with severe morbidity. VS are sporadic or associated with neurofibromatosis type 2, and their molecular underpinnings remain obscure. Our objective was to perform multiomic profiling of sporadic and NF2 related VS at single cell resolution and to investigate novel tumorigenic mechanisms.

Methods:
We annotated surgical samples from 15 patients undergoing surgery for VS (6 sporadic, 9 NF2), and performed single-cell or single-nucleus RNA sequencing (scRNAseq, n=4; snRNAseq, n=15), as well as transposase-accessible chromatin (snATAC-seq, n=9) and bulk DNA methylation assays (n=15). Specific transcription factor binding was assayed using bulk chromatin immunoprecipitation and sequencing (ChIPseq). We validated our key findings at the mRNA and protein levels using RNAscope (n=8) and multiplex immunocytochemistry (mIHC; n=8). Mechanistic studies were conducted using NF2^fl/fl;Periostin-Cre+ mice versus their Cre- control littermates treated with novel small molecule inhibitors of TEAD auto-palmitoylation (VTs).

Results:
We mapped the VS transcriptome at single cell resolution and identified markers for Schwann cells, macrophages, T cells and endothelial cells. We unexpectedly identified a subset of VS Schwann cells with parallel chromosome 22 loss and a high expression of VEFGA (Schwann(Chr22-)). We confirmed VEGFA expression in Schwann(Chr22-) cells with orthogonal RNAscope and mIHC, and found that these cells were central regulators of macrophage (M2 subtype, tissue resident) through IL-34 signaling. Schwann(Chr22-) cells were enriched for signaling via YAP1/TAZ-TEAD, laminin, NRXN and NEGR pathways. Schwann(Chr22-) cells showed increased TEAD1 expression as well as chromatin accessibility for TEAD family transcription factor motifs. We performed ChIPseq and confirmed increased TEAD1 binding at the promoters of (714) genes including VEGFA, EGFR, with strong enrichment for mitogenic pathways. NF2^fl/fl;Periostin-Cre+ mice showed increased VEGFA expression, and that was reversible by TEAD inhibition in-vivo with VTs.

Conclusions:
We performed detailed epigenetic and transcriptomic profiling of sporadic and NF2-associated VS and uncovered mechanistic TEAD1-mediated overexpression of VEGFA in VS. This pathway can be targeted with therapeutic agents. TEAD inhibition may constitute a novel therapeutic target for VS.

205. Uma R Mohan, PhD

Modeling and predicting neural responses to multisite direct electrical brain stimulation in humans
Mentor: **Dr. Kareem Zaghloul**
Study Section: **Neuroscience - Cognitive and Behavioral Neuroscience**

Direct electrical brain stimulation combined with intracranial electrophysiological recordings hold the potential to modulate and probe neural circuits in the awake human brain. While clinicians have used direct electrical brain stimulation for functional mapping and treatment of neurological and psychiatric disorders, the effects of stimulation on neural activity are poorly understood. Changes in neural activity from stimulation in local and remote areas are often highly complex and variable. Stimulation has most often been delivered at locations individually, however, simultaneous or patterned stimulation at multiple locations holds the potential to modulate distributed networks more precisely. To better understand and precisely control the responses to stimulation in individual patients, we took the approach of modeling the effects of stimulation on neural dynamics across the brain. We collected human electrocorticographic recordings from 9 neurosurgical epilepsy patients while systematically delivering cortical stimulation at different frequencies, amplitudes, durations, and locations while patients were at rest. Using a dynamic linear state-space model framework, we fit input-output models to timecourses of neural activity, represented by high frequency activity, while patients received stimulation. We first show that latent state space models can accurately predict dynamic responses in brainwide neural activity following stimulation at individual locations across brain regions. We further show patient-specific models can predict responses to novel stimulation locations. Lastly, we analyzed changes in large-scale neural activity in response to multisite stimulation and compare these responses to those predicted from patient-specific state-space models built while patients were stimulated at individual locations. We were able to reliably predict responses to stimulation delivered at novel combinations of multiple simultaneous locations. The ability to characterize and model neural responses to novel locations as well as patterns of multisite stimulation could allow clinicians and researchers to design stimulation protocols for precise modulation of neural activity. Stimulation parameters and locations may be selected to elicit specific changes to ongoing behaviorally relevant neural signals to modulate higher-order cognitive functions in humans and to more effectively probe functional brain networks and treat neurological disorders.

206. Brian R Roome, Ph.D

Ontogeny of the structure and neuronal diversity of the spinal cord dorsal horn
Mentor: **Dr. Ariel J Levine**
Study Section: **Neuroscience – Developmental**

The nervous system is arguably the most complex organ system in the body by the sheer diversity of molecularly distinct neuron types. The spinal cord's relative simplicity experimental tractability has placed it at the front of studies attempting to understand neuronal diversification, which occurs in the spinal cord by specification of progenitor domains within the neural tube (a consequence of opposing gradients of Wnt and Shh morphogens), and by progenitors varying the neurons they produce over time, resulting in the two grey matter structures: the dorsal and ventral horns. While this model adequately explains the ontogeny of the ventral horn, it has yet to explain dorsal horn ontogeny: rather than remaining throughout neurogenesis, the five dorsal-most progenitor domains transform into one large domain (dIL; dorsal interneuron-late) which produces almost the entirety of the dorsal horn, itself containing two-thirds of all spinal cord neurons.

To begin forming a theory of dorsal horn ontogeny, we comprehensively birthdated spinal neurons using EdU at 12-hour intervals throughout neurogenesis in separate cohorts of mouse embryos. This showed that the deepest excitatory neurons were born earliest and the most superficial excitatory neurons were born latest, while inhibitory neuron birth order followed a discontinuous pattern of laminar formation. We hypothesized therefore that the structure of the laminae is dependent on the orderly production of excitatory dIL neurons. Indeed, embryos absent of excitatory neurons (null for Gsx1 and Gsx2), but not inhibitory (null for Ptf1a) or sensory neurons (Pax3:Cre; Isl2:DTA), showed highly disorganized lamination. We next developed a single-cell RNA-sequencing database of 95000 embryonic spinal neurons, showing six progressively-born families of excitatory and inhibitory dIL neurons respectively. These families could be separated into neuron subtypes which varied in their expression of Zic family transcription factors. We hypothesized that Zic genes pattern dIL progenitors as they participate in Wnt and Shh morphogenesis, and indeed found graded Zic gene expression in progenitors, as well as that embryos null for Zic1/4 are deficient in Zic-high neuron variants and abundant in Zic-low variants. Comparing these data with adult single-cell RNA-seq atlases, we can reconcile all spinal neuronal diversity with progenitor mechanisms, producing a relatively complete theory of neuronal diversification in the spinal cord.

National Institute on Aging

207. Fulya Akcimen, PhD

Black and African American Connections to Parkinson's Disease Study (BLAAC PD)
Mentor: **Dr. Sara Bandres-Ciga**
Study Section: **Genetics – Diseases**

Our current insights into the genetics underlying PD etiology have been disproportionately based on European ancestry populations. This has led to a significant gap in our knowledge about the disease's genetics and clinical characteristics in underrepresented populations, particularly individuals of African and African-admixed ancestries.

BLAAC PD is a multi-center study recruiting Black and African American individuals with Parkinson's Disease (PD) and healthy controls. BLAAC PD aims to address disparities in genetics research by exploring the genetic architecture leading to PD in the Black and African American population and potentially investigate genotype-phenotype correlations. The goal is to create a foundational cohort to assess diverse aspects of PD in this historically excluded population and serve as a model for diversity and equity in research.

BLAAC PD collects samples from six sites across the United States. A total of 196 cases and 326 controls have been collected. Following DNA extraction, genotyping, and imputation, an ancestry assessment through a pre-trained machine-learning model was applied. Then, samples undergo whole-genome sequencing (WGS). A comprehensive assessment was conducted to investigate known and novel genetic contributors. SNVs, structural variants, and expanded repeats were examined. In addition, we utilized WGS data of 79 cases and 12,455 controls with African American ancestry from the All of Us biobank.

Our analyses showed consistent differences in variant frequencies, magnitude of effects, and risk alleles for causal mutations in PD-known genes, including SNCA, VPS35, LRRK2, PRKN, PINK1, DJ1, and GBA. Furthermore, we identified expanded repeat in ATXN3 in two PD cases (45 and 54 repeats), expanding the phenotypic spectrum of ATXN3.

These findings highlight the need for diverse representation in genetic research. Insufficiently diverse genetic data may exacerbate health disparities once translated to the clinic. BLAAC PD will help resolve the cross-ancestry applicability of drug targets, treatments, and preventative measures by elucidating the genetic architecture of disease in African-admixed ancestries. BLAAC PD aims to provide a platform for discovery and replication studies to explore the relevance of genetic findings reported in other populations. Looking ahead, BLAAC PD plans to continue recruiting and genotyping participants to serve as a foundational cohort for diverse genetic studies.

208. NIGUS Asefa, Ph.D.

Accelerated aging elevates the risk for mortality in persons with dementia: the Age, Gene/Environment Susceptibility- Reykjavik Study cohort
Mentor: **Dr. Lenore J Launer**
Study Section: **Epidemiology/Biostatistics - Prevention and Risk**

Introduction: Biological aging (BA) is the gradual deterioration of physiological functions over time, manifested at the cellular and molecular levels. The gap between chronological and BA, as determined by DNA methylation levels, is called Epigenetic Age Acceleration (EAA). Elevated EAA serves as a significant indicator for predicting neurodegenerative diseases such as dementia, as well as all-cause mortality. We investigated whether EAA moderates the association between dementia and mortality risk.

Methodology: Analyses are based on data from the Age, Gene/Environment Susceptibility- Reykjavik Study (AGES-RS) study (n=2602, 57.5% females, mean age =75.8 years, 30% demented). Data were collected from 2002 to 2006, with mortality data available until 2015. EAA was computed with the DunedinPACE algorithm and grouped as follows: EAA scores > 1 SD of the mean were labeled as 'fast', EAA < 1 SD as 'slow', and EAA scores within +/-1 SD as 'average'. Dementia was ascertained in a study exam or through follow-up of health records. Cox proportional hazard analysis was used to evaluate the hazard ratio (HR, 95%CI) of mortality and the interaction between EAA categories and dementia. Models were adjusted for sex, chronological age, education, smoking, diabetes and stroke.

Results: Fast agers had higher mortality HRs than average agers (HR = 1.62, 95% CI: 1.29-2.03, p = 3.86 x 10-5). Fast agers with dementia had a significantly increased risk of death (HR = 2.68, 95% CI: 2.11-3.38, interaction p =4.08 x 10-2) compared to non-demented average agers; this interaction was reduced after adjusting for total brain volume (p = 9.91 x 10-1). Conversely, slow aging was associated with reduced mortality risk (HR = 0.71, 95% CI: 0.52-0.98, p = 3.62 x 10-2); however slow agers with dementia had risk estimates similar to non-demented average agers (interaction p =1.14 x 10-1) but lower than demented fast agers. Controlling for total brain volume reduced the differences between slow and average agers with and without dementia.

Conclusion: Individuals aging at a faster rate than their chronological age were at a higher risk for death. The rate of BA had an effect on the association between dementia and mortality. The association was partially explained by brain atrophy, suggesting other factors related to dementia contributing to mortality risk in people with dementia.

209. Reema M Banarjee, Ph.D.

Unmasking the Cell Surfaceome to Identify and Target Senescent Cells In Vivo
Mentor: **Dr. Nathan Basisty**
Study Section: **Omics - Metabolomics/Proteomics**

Cellular senescence is a complex stress response to sub-lethal stressors characterized by permanent cell-cycle arrest and a pro-inflammatory secretory phenotype. Accumulation of senescent cells contributes to aging and age-related pathologies. Consequently, their selective elimination is a promising approach for treating such pathologies. However, translation of senescence-targeting therapies requires specific cellular markers to assess senescent cell burden in vivo. The cell surface proteome or 'Surfaceome' includes all plasma membrane proteins with exposed extracellular domains and is an unexplored repertoire of potential biomarkers. We applied biorthogonal chemistry in combination with quantitative proteomics to cellular models of senescence to identify robust and highly specific senescence-associated surface proteins that can be established as therapeutic targets and biomarkers for clinical translation of Senotherapeutics.
We established in vitro models of senescence in human IMR90 lung fibroblasts and THP1 monocytes, that were phenotypically validated using canonical senescence markers such as reduced cell proliferation, increased senescence-associated β-galactosidase activity, and increased expression of p21, p16 and IL6. Surface proteins from senescent and quiescent control cells were labelled using an optimized surface N-glycan biotinylation approach called Glyco-Cell Surface Capture, followed by data-independent LC-MS/MS analysis (DIA) on a Q-Exactive HF Orbitrap mass spectrometer. Proteomic data was analyzed using an R pipeline developed in-house for selective quantification of surface proteins based on the presence of N-glycosylation sites.
Our optimized mass spectrometry workflow identified 334 and 301 surface proteins from fibroblasts and monocytes, respectively, of which 68 and 78 proteins were over-expressed in the senescent cells, including the known senescence marker DPP4 and a novel marker CDCP1. Gene ontology analysis showed that the over-expressed markers are involved in inflammation, as well as cell adhesion and migration. Interestingly, many of these candidates are also over-expressed by cancer cell lines and can be potential biomarkers for cancer cells in vivo. The increased senescent cell surface abundance of CDCP1 and DPP4 was also validated via flow cytometry. Our study thus illustrates the potential of mass spectrometry-based proteomics to identify surface biomarkers that have the potential to target senescent cells in vivo.

210. Kimberley Billingsley, PHD

Decoding the genetics of Neurodegeneration in the global population with large-scale Long read sequencing
Mentor: **Dr. Andy Singleton**
Study Section: **Neuroscience – Molecular**

Neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, significantly impact millions of individuals worldwide. While our knowledge of the genetic underpinnings of these conditions has progressed, a substantial portion of the genetic variation contributing to disease risk remains unknown, particularly within historically underserved populations. To date, most existing genetic studies for these diseases have focused on one form of genetic variation, single nucleotide variants. While other important forms of genetic variation such as short-tandem repeats and structural variants are mostly ignored due to the complexity of detecting these variants with traditional sequencing methods. Yet these forms of genetic variation play crucial roles in gene expression and regulation in the human brain and are causative of numerous neurological disorders. Long-read sequencing significantly increases our power to detect short-tandem repeats and structural variants but has not been a feasible option for large-scale genomic projects because it was low throughput and too costly. Here we developed a scalable wet-lab protocol and computational pipeline (Napu). We applied this framework to neurologically normal control samples obtained from the North American Brain Expression Consortium cohort (n=222 of European ancestry), and the Human Brain Collection Core cohort (n=165 of African ancestry). Through this work, we present the first publicly available long-read sequencing data resource derived from hundreds of human brain samples. We discover over 200k structural variants. Leveraging expression datasets for these samples we apply quantitative trait locus (QTL) analyses and identify novel variants that drive gene expression in frontal cortex brain tissue. Further, we determine methylation rates of millions of CpGs and with this identify structural variants driving differential methylation in the brain. In summary, these results highlight that long-read sequencing at population scale can identify disease-relevant, population-specific regulatory loci that were inaccessible using conventional technologies. This new resource will be pivotal in comprehending the biological implications of genetic variation in the human brain. Finally, we are currently applying this framework to sequence thousands more case and control samples to build the first highly accurate catalog of structural variants in neurodegenerative diseases.

211. Yi-Han Hu, Ph.D.

Towards improved dementia prediction in the population: accounting for peripheral factors associated with levels of AD biomarkers
Mentor: **Dr. Lenore J Launer**
Study Section: **Epidemiology/Biostatistics – General**

Background: Identifying reliable, low-cost biomarkers for dementia risk in older populations with diverse health profiles is crucial for early intervention strategies. Blood-based biomarkers, glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL), are promising candidates for stratifying dementia risk. However, it is not known what health characteristics, other than dementia, influence levels of these biomarkers in a population-based setting.
Objectives: To investigate whether integrating information on health-related factors to a predictive model with plasma GFAP and NfL improves the prediction of incident dementia compared to a basic model including age, sex, education, APOE e4 status, and eGFR.
Method: Data are from 910 baseline non-demented participants (mean age 76.6 yo, 52% females, 6.7% developed dementia over 10 years) of the Age, Gene/Environment Susceptibility-Reykjavik Study (2002-2015). We examined 360 factors across sociodemographic,

clinical, sensory, cardiometabolic, musculoskeletal, medical history, and lifestyle domains, clustered into 34 groups. Predictor sets included: biomarker only; plus age and sex; plus basic factors (education, APOE e4, eGFR); and a full model with associated peripheral biomarker clusters. For each set, we used logistic regression models with 5000 bootstraps to assess the predictive power of GFAP and NfL and compared them using measures of precision, sensitivity, and the area under the precision-recall curve (AUPRC).
Results: Initial analysis showed significant overlap in biomarker levels between dementia cases and non-cases and overall poor performance to predict dementia (biomarker-only models' AUPRCs, GFAP: 0.17, NfL: 0.20). However, incorporation of peripheral clusters significantly enhanced predictive accuracy beyond basic models, especially for NfL. The final model, which included a set of associated peripheral clusters, showed a precision of 30.4% for GFAP and 61% for NfL, reflecting improved prediction accuracy. The GFAP's AUPRC improved to 0.339, accurately identifying 5 out of 100 incident dementia cases, while the NfL's AUPRC was 0.367, predicting 17% of cases.
Conclusion: In a population-based setting with a typically low presence of dementia, the standalone use of plasma GFAP and NfL biomarkers has limited predictive utility for dementia screening. Accounting for multi-morbidity in the community by adding selected health-related factors may improve their predictive performance.

212. Apostolos Manolopoulos, MD

Biomarkers from neuron-derived extracellular vesicles predict resilience to cognitive impairment in the presence of ApoE ε4
Mentor: **Dr. Dimitrios Kapogiannis**
Study Section: **Clinical and Translational Research - Clinical Trials**

The ApoE ε4 allele is the strongest genetic risk factor for late-onset Alzheimer's disease (AD) and is associated with earlier cerebral amyloid-beta (Aβ) deposition and age of onset. However, many carriers of one or even two ε4 alleles survive to old age without developing AD or experiencing cognitive decline. Using neuron-derived extracellular vesicles (NDEVs), we sought to identify biological factors that promote cognitive resilience and their interplay with ApoE genotype. We analyzed 1130 plasma samples from 676 women participating in the Women's Health Initiative (WHI)/ Long Life Study (LLS), with ApoE ε4 or ε3/ε3 genotype, who had provided blood samples at baseline and LLS visit (13-17 years later) and remained cognitively healthy or developed Mild Cognitive Impairment (MCI)/dementia. We isolated NDEVs using immunoaffinity capture for the neuronal marker L1CAM and quantified the core AD pathogenic proteins (Aβ42, p181-Tau, total tau), cellular mediators of the neuroinflammatory response (TNFR1 and pSer536-NFκB), and mitochondrial Complex V mediating energy generation. Neuronal origin of isolated EVs was confirmed by Flow Cytometry for neuronal markers (VAMP2, Tuj1, L1CAM). For statistical analysis, we fitted repeated measures mixed models, including covariates for EV yield, trailing age and years of education. Women ε4 carriers had higher Aβ42 levels compared to ε3/ε3 carrier women; however, no differences were seen between resilient and declining groups for Aβ42, p181-Tau, total tau. Women ε4 carriers who remained unimpaired had higher pSer536-NFκB and TNFR1 compared to ε4 carrier women who developed cognitive impairment and ε3/ε3 carrier women both at baseline and at the LLS visit. Additionally, at baseline, women ε4 carriers who remained unimpaired had higher Complex V compared to ε4 carrier women who became impaired. This large NDEV biomarker study provides insights into the mechanisms underlying cognitive resilience in the presence of ApoE ε4. Augmented TNFR1/NFκB pathway response to neuroinflammation and mitochondrial energy production characterize the so-called ε4 "escapees". These findings motivate novel therapeutic avenues for AD prevention.

213. Yeuran Oh, Ph.D

Modeling Mechanisms of Il12b Gene Regulation by NF-κB Dimers in Macrophages
Mentor: **Dr. Myong-Hee Sung**
Study Section: **Computational Biology/Systems Biology**

The interleukin 12 (IL-12) family of cytokines, particularly IL-12 and IL-23, are important proinflammatory cytokines that act as a bridge between the innate and adaptive immune responses. M1-like macrophages produce IL-12 and IL-23 that share a component called p40 subunit encoded by the Il12b gene. The Il12b gene is transcriptionally regulated by the transcription factor NF-kappaB (NF-κB). NF-κB consists of five family members: RelA, RelB, c-Rel, p50, and p52, which can form homo- and heterodimer complexes. Of the NF-κB family members, c-Rel and RelA draw particular interest in Il12b regulation. Early studies of Il12b gene regulation indicated a significant role for c-Rel, highlighting its importance over RelA in the induction of Il12b. However, the mechanisms governing the involvement and proportional impact of c-Rel in the regulation of Il12b remain unclear. Understanding the binding patterns of c-Rel- and RelA-containing dimers at various regulatory sites near the Il12b gene could provide essential clues to explaining the mechanism underlying the role of c-Rel in Il12b gene regulation. Considering the difficulties in conducting direct experiments, we propose a mathematical model to investigate Il12b gene regulation, which incorporates the actions of c-Rel- and RelA-containing dimers at proximal and distal regulatory sites near the Il12b gene. Mathematical modeling of Il12b makes it possible to test diverse knockout scenarios, including the knockout of c-Rel or RelA, as well as the knockout of specific target regulatory sites. The simulation results provide predictive insights into unexplored experimental domains and have the potential to propose innovative avenues for further investigation into the mechanisms of Il12b regulation. Furthermore, to test the mathematical model against experimental data, we use fluorescence live-cell imaging of macrophages derived from c-Rel and Il12b dual reporter mice. We analyze the live-cell imaging of nuclear c-Rel and Il12b gene expression and extract the quantitative features of their dynamics from the time-series data. Collectively, the integrated approach will

enhance our ability to uncover deeper biological insights into the complex mechanisms of Il12b regulation, which are crucial for innate and adaptive immune responses.

214. Hasitha U Premathilake, Ph.D.

Pig Taste Cell-Derived Organoids Synthesize Insulin
Mentor: **Dr. Josephine M Egan**
Study Section: **Clinical and Translational Research - Drug Discovery**

Studies in taste stem cell homeostasis and the preservation of the taste buds from which they are derived received renewed interest during the recent COVID-19 pandemic. Despite the sense of taste being essential to determine the quality of food and life, and the ease of access to taste buds and stem cells for investigation, we know very little about the nature of the taste cells in age and disease. To date, there are limited reports of studies on rodent taste organoids and none on higher-order animals. Recent clinical trials in which pig organs have been transplanted into humans have highlighted the similarity of pig organs to human ones. We have established the first porcine static and dynamic taste organoid cultures that can be expanded indefinitely and maintained long-term in culture. Gene expression analysis of these organoids revealed Lgr6-expressing stem cells but no Lgr5 expression was evident. Pig foliate organoids harvested at Day 14 and beyond exhibited the expression of Type I, II, III taste receptor cells (TRC) marked by the expression of ENTPD2, TRPM5 and OTOP1 (determined by qPCR and RNA-Seq), respectively. We formerly discovered biologically active insulin in a subset (Type II) of mammalian TRCs, thus warranting further investigation into the effect of insulin in maintaining the proper function of taste buds, and in our taste organoids. A minimum of 10nM insulin was necessary for proper growth and differentiation of taste organoids. Higher concentrations (50nM) of insulin resulted in a denser organoid culture with higher numbers of reticulate organoids and significant upregulation in both taste stem cell (LGR6/SOX2) and TRC markers (ENTPD2/GNAT3). Mature taste organoids expressed insulin (as determined by qPCR and immunostaining), as well as the insulin transcription factors PAX4 and MAFA but not PDX1 (essential for glucose-controlled insulin expression). Growth factor arrest and induction of quiescence increased insulin expression >1000-fold over normal growth media. Following on from the pig model, we also established human taste organoids (that express Type I, II, and III TRC) markers from fungiform papillae, and propagated them up to passage 3. This is an important milestone in the greater scheme of our work where we intend to develop these organoids into islet-like, insulin-producing structures (by inducing PDX1 expression) as a potential therapeutic for aging-related conditions where little or no insulin is being produced in islets.

215. Stormy E Ruiz, B.S. Biological Chem

Differences in transcriptional regulation in the Variable region between germinal center and ex vivo-stimulated B cells reflect differences in AID recruitment
Mentor: **Dr. Patricia Gearhart**
Study Section: **Immunology - Lymphocyte Development and Activation**

Activation-induced deaminase (AID) is a B cell-specific enzyme that initiates the processes of somatic hypermutation (SHM) and class switch recombination (CSR). While we understand how AID is targeted to the switch region to initiate CSR, relatively little is known about how AID can be accurately targeted to the variable (V) region to induce SHM. Transcription must occur to expose single-strand DNA, but transcription alone is insufficient for AID targeting. Previous research has shown that without retention of SPT5, a transcriptional regulator, into the V gene exon, AID is unable to be recruited, and SHM does not occur in ex vivo-stimulated cells. We propose that distinctive regulation of RNA Polymerase II activity at the V gene allows AID targeting, and thus SHM, in germinal center cells. To study this, we developed a knock-in mouse model called JH1, which utilizes a rearranged VDJ containing endogenous gene segments knocked-in directly in the locus, representing endogenous B cells generated from the bone marrow. Furthermore, the V2.2 gene segment is relatively easy to identify apart from the retained upstream Vs. Using this model, we have adapted CUT&RUN for probing transcriptional regulators in both sorted germinal center B cells and ex vivo stimulated B cells. Initial testing showed that this technique gives high-quality data comparable to ChIP-seq, with the benefit of needing far less input (</=100k nuclei). Additionally, we can easily map data to the V region of the JH1 mouse, highlighting the power of the model and technique combined. We have performed CUT&RUN for many major transcription elongation regulators, including negative elongation factor (NELF), SPT4, SPT5, and phosphorylation states so that we can begin to develop a picture of the transcriptional landscape at the V region in germinal center vs. ex vivo stimulated B cells. We have observed differences between these two conditions at the V region: germinal center B cells contain less RNA Pol II Ser2p (a sign of stable, long-range elongation) downstream of the promoter and more RNA Pol II Ser5p (a sign of early elongation) up to the Emu enhancer. The results suggest that transcription between these conditions is not the same, potentially leading to a partial explanation for AID's selectivity between germinal center and ex vivo-stimulated cells. Understanding how AID is targeted to the V region may be useful to fine-tune humoral responses via vaccines and develop further technologies.

216. Veronica Ryan, PhD

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Mentor: **Dr. Andy Singleton**
Study Section: **Neuroscience - Neurological and Neurodegenerative Disorders and Injury**

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217. Chang Hoon Shin, Ph.D.

RBMS1-mediated ANKRD1 increase in early senescence promotes the migration of recipient cells by suppressing production of EXT1
Mentor: **Dr. Myriam Gorospe**
Study Section: **RNA Biology**

Cellular senescence is a dynamic biological process triggered by sublethal cell damage and driven by specific changes in gene expression programs. In addition, although cellular senescence exacerbates aging-associated declines and diseases, senescent cells have beneficial roles, including wound healing. However, the wound healing phenotype of senescent cells is poorly understood. We recently identified ANKRD1 (ankyrin repeat domain 1) as being strongly elevated after triggering senescence in fibroblasts. Here, we set out to investigate the mechanisms driving the elevated production of ANKRD1 and its physiological roles in early stages of senescence.

We found that the rise in ANKRD1 levels during Etoposide (Eto)-induced senescence was the result of increased transcription, mRNA stabilization, and enhanced translation efficiency. A proteomic approach followed by antisense oligomer (ASO) pulldown revealed that the RNA-binding protein RBMS1 interacts with ANKRD1 mRNA. RBMS1 increased in the cytoplasm following Eto treatment, supporting the hypothesis that RBMS1 may participate in post-transcriptional regulation of ANKRD1 expression. In agreement with this possibility, silencing RBMS1 reduced, while overexpressing RBMS1 increased ANKRD1 mRNA stability. We propose that the increased expression of ANKRD1 during the early stages of senescence Is partly due to the stabilization of ANKRD1 mRNA by RBMS1.

We then studied the function of ANKRD1 on wound repair in a cell culture model of migration. After collecting conditioned media (CM) from fibroblasts expressing different levels of ANKRD1 and testing them on keratinocytes, we found that treatment of keratinocytes with CM from ANKRD1-depleted fibroblasts reduced the migration of keratinocytes, while CM from ANKRD1-overexpressing fibroblasts promoted the migration of keratinocytes. To identify the effectors of cell migration mediated by ANKRD1, mass spec analysis of CM revealed that the secreted protein EXT1 (exostosin glycosyltransferase 1) was a functional effector of ANKRD1 actions on keratinocyte migration, as blocking EXT1 production by fibroblasts robustly increased keratinocyte migration. In conclusion, an early rise in ANKRD1 production during senescence lowers the production and secretion of EXT1, in turn increasing the migration of neighboring cells.

218. Xin Wang, PhD

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Mentor: **Dr. Arya Biragyn**
Study Section: **Immunology – Immunotherapy**

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219. Xingliang Zhu, Doctor

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Mentor: **Dr. Weidong Wang**
Study Section: **Neuroscience - Neurological and Neurodegenerative Disorders and Injury**

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220. Wei Zhu, M.D.

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Mentor: **Dr. Yie Liu**
Study Section: **Clinical and Translational Research - Animal Models**

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National Institute on Alcohol Abuse and Alcoholism

221. Yohannes Getiye Estifanos, PhD

Acute electronic cigarette vaping induces profound pulmonary inflammation, emphysema phenotype and cardio-pulmonary dysfunctions in male but not in female mice

Mentor: **Dr. Pal Pacher**
Study Section: **Heart, Lung, and Vascular Disease and Biology**

Cigarette smoking is considered to be the single most preventable cause of cardiovascular and pulmonary diseases. Efforts to curb smoking have succeeded in the last decades, however a new type of nicotine delivery system called electronic cigarette (e-cigarette) is gaining tremendous popularity in recent years, in part because of the perceived “safety”. Through a process called vaping, these battery-based devices produce an aerosol by heating e-liquid containing nicotine and other constituents. Statistics shows 1 in 20 Americans vape; youth e-cigarette use increased by 1800% in the last few years. Despite such popularity, studies on the effects of vaping yield conflicting results. Owing to these variabilities, it is necessary to characterize the effects of acute vaping in a clearly defined animal model in both sexes. We employed state-of-the art inhalational exposure system, and exposed male and female mice for 10 days with puffing profile that mimic human use. Validation of our exposure protocol by measuring the level of blood cotinine, a nicotine marker, confirmed similarity with human values. Gene expression and protein levels of multiple inflammatory cytokines and chemokines in the lung were measured by qPCR and ELISA. Interestingly, male mice exhibited significantly more severe lung inflammation compared to their female counterparts, which was also substantiated by histopathological analysis. We next investigated whether the pulmonary inflammation coincided with lung dysfunction. Our analysis revealed notable functional changes in male but not female mice, pointing towards the emergence of emphysema characterized by abnormal dilation of the alveoli. We then performed detailed cardiac hemodynamic analysis using echocardiography and invasive pressure–volume conductance catheter technique, the gold standard method to assess myocardial contractile function independent from loading conditions and heart rate. Surprisingly, our data revealed a profound systolic and diastolic cardiac dysfunction in exposed male mice. Collectively, to the best of our knowledge, this study is the first to show acute vaping-induced pulmonary inflammation that is linked with cardiopulmonary dysfunction in sex-dependent manner. Using this model, we will further assess the gene profiling of the lung and heart to elucidate mechanistic pathways which can be targeted for treatment of vaping-induced abnormalities.

222. Tommy Gunawan, PhD

Comorbid alcohol use disorder with trauma disorder risk is conferred by parental history and genetic factors through negative emotionality factors.
Mentor: **Dr. Vijay Ramchandani**
Study Section: **Drug and Alcohol Abuse**

Alcohol use disorder (AUD) and trauma disorders (TD) are often comorbid and are characterized by dysregulated negative emotionality (NE). Genetics and family history (FH) contribute to the risk of both disorders. The present study utilized polygenic risk score (PRS) , an index of the genetic risk for problematic alcohol use (PAU), and parental FH of PAU to understand how these factors confer the risk of AUD with and without TD via negative emotionality.

N=300 individuals completed questionnaires and neurocognitive tasks that assessed negative emotionality constructs such as affect, resilience, anxiety, depression, personality, and aggression. Factor analyses were used to derive latent factors of negative emotionality. Parental FH (FH-Mother and FH-Father) was captured using the Family Tree Questionnaire. Participants were grouped as Healthy Controls (HC), AUD only or AUD with TD based on a structured clinical interview. PRS were computed using a genome-wide meta-analysis of PAU as our discovery dataset. Associations between parental FH, PRS, negative emotionality factors, and psychiatric diagnoses were modeled using path analysis with age, sex, and race as covariates.

Three factors underlie negative emotionality: internalizing, externalizing, and psychological strength. Individuals with AUD+TD exhibited greater PRS, internalizing and externalizing scores relative to HC and AUD only. Additionally, individuals with AUD were more likely to report a positive FH for PAU compared to HC. In the path analyses, FH-father, but not FH-mother, was associated with PRS. FH-mother was associated with greater internalizing, while FH-father was associated with greater externalizing and lower psychological strength. Greater internalizing scores increased risk of AUD, while greater externalizing and lower psychological strength scores increased risk of AUD+TD. Finally, FH-father conferred risk to AUD+TD via greater PRS, while FH-mother conferred risk to AUD+TD via greater internalizing scores.

These results highlight how parental history increased risk of psychiatric comorbidities through negative emotionality factors. Each parent contributed to the risk through different mechanisms, with paternal history conferring risk via both genetic and non-genetic paths, while maternal history conferring risk via non-genetic paths. Future research will elucidate the specific genetic loci and non-genetic mechanisms driving the risk of these psychiatric comorbidities.

223. Bryan Mackowiak, Ph.D.

Circulating albumin levels regulate inflammation in ethanol-induced liver injury
Mentor: **Dr. Bin Gao**
Study Section: **Drug and Alcohol Abuse**

Rising alcohol consumption during the pandemic has led to concurrent increases in alcohol-associated liver disease (ALD). There is an urgent need to identify novel mechanisms of early ALD progression that are potential therapeutic targets, as late-stage ALD has poor prognosis and limited treatment options. Albumin, a liver-derived circulating protein, plays key roles in oncotic pressure, molecular transport, and immune response. Circulating albumin is decreased in late-stage ALD and albumin supplementation is used therapeutically to address complications. However, when circulating albumin levels decrease during ALD development and whether albumin plays a role in ALD progression are unknown. Therefore, I analyzed clinical parameters from healthy controls, heavy drinkers with recent drinking, and treatment-seeking heavy drinkers who had been abstinent >10d. I found that heavy drinking is sufficient to reduce plasma albumin levels by ~25% and albumin levels are negatively correlated with average drinks per day. Since this decrease in circulating albumin likely occurs in people with alcohol use disorder prior to ALD initiation, I utilized the 10-day plus binge model of ethanol consumption in wild-type (WT), albumin knockout (ALB KO), and albumin heterozygous (ALB Het) mice to determine whether decreases in albumin affect ALD progression. I found that ALB Het mice exhibit ~25% decrease in circulating albumin and a trend of improvement in ethanol-induced liver injury compared to WT mice, which is abolished in ALB KO mice. RNA-seq analysis of liver samples found that both ALB Het and ALB KO mice exhibit decreased liver inflammation after ethanol consumption while ALB KO mice also exhibit vascular dysfunction that likely worsens liver injury. I then analyzed correlations between albumin and immune cell populations in heavy drinkers and severe alcohol-associated hepatitis (sAH) patients which revealed that albumin is positively correlated with lymphocyte counts. This matched the preclinical model, as immunohistochemical analysis of liver tissue found that both ALB Het and ALB KO mice exhibited decreased numbers of liver lymphocytes, largely driven by decreases in CD4+ T-cells. Overall, these studies demonstrate that decreases in circulating albumin happen early in ALD and point to a previously unrecognized role for albumin in ALD progression by modulating lymphocyte populations.

224. Lenny Pommerolle, PhD

Sexual dimorphism of the lung immunometabolism dysregulation induced by chronic alcohol consumption
Mentor: **Dr. Resat Cinar**
Study Section: **Drug and Alcohol Abuse**

In the US, according to the National Survey on Drug Use and Health, 78.5% of people aged 12 and older reported they drank alcohol at some point in their lifetime, and 10.5% of them developed an Alcohol Use Disorder (AUD). Chronic alcohol consumption impairs lung immunity which makes AUD patients more susceptible to develop inflammatory lung conditions, which can lead to acute respiratory distress syndrome and death. However, the reasons behind lung immune vulnerability in AUD remain unclear. We focused on investigating the effects of alcohol ingestion on lung immunity in male and female subjects using population-based human lung transcriptomics data from the Genotype-Tissue Expression (GTEx) project, encompassing 328 alcohol drinkers and 110 nondrinkers and an experimental mouse model by using a ten-day chronic alcohol drinking plus one binge of ethanol (EtOH-fed). We demonstrated males are more sensitive than females to the effect of chronic alcohol drinking on down-regulating lung immunity both in humans and mice. Cellular numbers of both innate (macrophages, eosinophils, NK cells) and adaptative immunity (T-CD8, Th17, B cells) were dramatically downregulated only in male mice lungs. We compared transcriptomics changes in lungs and liver from the same mice and human subjects. The lungs displayed approximately 60% of immune-related pathways downregulated whereas almost no significant changes in the liver. This underscores the lungs as one of the primary organs immunocompromised by alcohol misuse. Comparative analysis of lung transcriptomes using a system biology approach between mice and human subjects not only confirmed similar dysregulation on lung immunity but also provided evidence that immunometabolic changes are a central driver in altering the lung transcriptome. These changes involve the downregulation of immune pathways and the upregulation of metabolic pathways such as branch-chain amino acid (BCAA) degradation. Furthermore, we identified a decrease of mTOR signaling along with an increase of BCAAs (known as mTOR activators) in the lung of EtOH-fed mice. In addition, daily use of mTOR inhibitor considerably worsened the alcohol-induced lung immunodeficiency which highlights the BCAA/mTOR axis as a pivotal upstream regulator in lung immune dysregulation. Accordingly, restoring the balance between mTOR signaling and BCAA degradation might be essential for preventing and developing therapeutic strategies for alcohol-induced lung immunodeficiency.

225. Burhan Yokus, MD

Cannabinoid receptor 2 activation is a novel approach to reducing renal inflammation and fibrosis
Mentor: **Dr. Pal Pacher**
Study Section: **Clinical and Translational Research - Drug Discovery**

Chronic kidney disease represents a critical global health issue, culminating in renal fibrosis as its end-stage manifestation, for which effective treatments are scarce. Cannabinoid receptor 2 (CB2R) is a promising target for inflammatory disorders, and numerous selective agonists have been entering clinical trials for various indications. In this study, we investigated the localization of CB2R in the diseased kidney and its role in kidney injury, inflammation, and fibrosis. We used a combination of pathology, biochemistry, molecular biology, pharmacology (e.g., selective agonists and antagonists), and genetic (knockout mice) approach to explore the role of CB2R in kidney injury, inflammation, and fibrosis induced by ischemia/reperfusion (I/R) or unilateral ureteral obstruction (UUO) in mice. Because of the absence of selective CB2R antibodies, we also utilized advanced molecular and cell biology techniques (e.g., digital droplet PCR, RNA sequencing, 3D high-resolution RNAscope) to explore the localization and expression of CB2R in normal and diseased mouse and human kidneys and various isolated cell types. Kidney I/R and UUO models were characterized by parenchyma injury, oxidative

stress, inflammation, and/or fibrosis. Increased renal inflammation and fibrosis mirrored the rise in CB2R expression in damaged kidneys. The CB2R upregulation originated from infiltrating immune cells (macrophages and lymphocytes) and activated fibroblasts only, as revealed by ultra-high-resolution 3D RNAscope. Treatment with selective structurally distinct potent CB2R agonists (AM1241, HU910, RO6839828, and RO6871304) co-developed in our lab markedly ameliorated renal injury, inflammation, and fibrosis. In contrast, these pathological processes were exacerbated by CB2R antagonists (XL-001 and SR144528) or in CB2R knockout mice. In conclusion, CB2R is not expressed in normal kidneys. Kidney injury is associated with a marked increase in CB2R expression originating primarily from infiltrating immune cells but not kidney tubular cells. Furthermore, endocannabinoid/CB2R signaling represents an endogenous protective mechanism against kidney injury and inflammation. Targeting CB2R in inflammatory cells with selective agonists may emerge as a promising novel treatment for acute and chronic kidney disease.

National Institute on Deafness and Other Communication Disorders

226. Cathy Yea Won Sung, Ph.D.

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Mentor: **Dr. Lisa L Cunningham**
Study Section: **Neuroscience - Therapeutics and Translational Research**

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227. Kirupa Suthakar, BSc, BMedSci, PhD

Mechanisms Underlying Serotonergic Excitation of Medial Olivocochlear Efferent Neurons of the Auditory System
Mentor: **Dr. Catherine J Weisz**
Study Section: **Neuroscience – Sensory**

In the auditory system, the sensitivity of afferent neurons (i.e. from ear to brain) are directly modulated by efferent neurons (i.e. from brain to ear). Medial olivocochlear efferent neurons (MOCs), located in the auditory brainstem, decrease cochlear amplification by inhibiting outer hair cell electromotility via specialized nicotinic acetylcholine receptors. Given the proposed role of MOCs in context dependent tasks such as selective attention, signal extraction from noise and protection from acoustic trauma, we are interested in elucidating the full complement of non-auditory input to MOCs. Serotonin (5-HT) has been identified as a potential neuromodulator in central auditory circuits, and nucSeq data suggests MOCs express a diverse repertoire of 5-HT receptors. However, it is unclear if MOCs receive direct 5-HT synaptic input, and how such input may affect their excitability.

We use ChAT-IRES-Cre;tdTomato mice to identify cholinergic MOCs. Immunohistochemical data confirmed serotonergic terminals on both retrogradely-labeled and genetically identified MOCs in mouse. To demonstrate functional synapses, whole cell patch clamp recordings were made in current clamp from MOCs in brainstem slices. Neurons were held at -60mV and 100μM 5-HT was bath applied for ~10 minutes (N=15). 5-HT increased MOC excitability, but the magnitude of the effect varied. Compared to control, 5-HT significantly decreased action potential threshold (1.65+/-0.41mV; p=0.001), and significantly increased firing rate in response to 100pA of injected current (9.2+/-1.9Hz; p=0.0004). Elsewhere in the auditory system, 5-HT signaling is mechanistically linked to hyperpolarization activated cyclic nucleotide gated (HCN) channels. In contrast, preliminary pharmacology experiments suggest an alternative mechanism in MOCs because 50μM ZD7288 (a HCN channel antagonist) did not block the excitatory effects of 5-HT. Experiments investigating the contribution of multiple voltage gated potassium channels (important for modulating neuronal excitability) are ongoing.

Serotonin increases MOC excitability, which ultimately decreases cochlear sensitivity and could provide a means of fine-tuning cochlear gain in a context specific manner. Overall, investigating the role of 5-HT within the descending auditory system will aid in our understanding of central auditory processing and how factors such as mood and attention are involved in modulating MOC responses in complex listening situations.

National Institute on Drug Abuse

228. Elliot J Glotfelty, PhD

Excitotoxic glutamate levels cause the secretion of resident endoplasmic reticulum proteins
Mentor: **Dr. Brandon Harvey**
Study Section: **Neuroscience – Molecular**

Dysregulation of synaptic glutamate levels can lead to excitotoxicity such as that observed in stroke, traumatic brain injury, and epilepsy. The role of increased intracellular calcium (Ca2+) in the development of excitotoxicity is well established. However, less is known regarding the impact of glutamate on endoplasmic reticulum (ER)-Ca2+-mediated processes such as proteostasis. A subset of proteins are localized to the ER lumen via C-terminal amino acid sequences, ER retention/retrieval sequences (ERS). During cellular stress associated with ER Ca2+ depletion, we discovered these ERS-containing proteins are secreted as part of a process called exodosis. With this knowledge, we expressed a luciferase-based ERS reporter called GLuc-SERCaMP in primary cortical neurons to monitor exodosis. With this engineered reporter protein, we can measure GLuc activity in the media to determine the relative levels of exodosis. As a control, we expressed eYFP and performed a series of experiments with expression of GLuc without the ERS (GLuc-no tag). Activation of glutamatergic receptors (GluRs) led to an increase in GLuc-SERCaMP secretion, indicating ER resident protein secretion. GLuc-no tag was not increased in the media indicating that glutamate is not increasing overall protein secretion or the production of the reporter protein. Antagonism of ER Ca2+ channels attenuated the effects of glutamate and GluR agonists on GLuc-SERCaMP release. In addition, we demonstrated the secretion of an endogenous protein containing an ER retention/retrieval sequence (ERS) following GluR activation. This supports our finding that neuronal activation by glutamate promotes ER exodosis. Over-expression of KDEL receptors, which are known to maintain the ERS proteins in the ER, attenuated the secretion of ERS-containing proteins caused by GluR agonists. Taken together, our data indicate that excessive GluR activation causes disruption of neuronal proteostasis by triggering the secretion of ER resident proteins through ER Ca2+ depletion. This work has implications for a wide variety of conditions characterized by excitotoxicity.

229. Ido Maor, PhD

Representations of contradictory schemas in the orbitofrontal cortex during continuous learning
Mentor: **Dr. Geoffrey Schoenbaum**
Study Section: **Neuroscience - Neural Circuits**

Behavioral schemas are mental frameworks, developed through experience and learning, that serve as cognitive shortcuts to simplify decision-making in everyday situation. For example, when ordering food at a restaurant, we follow a schema that helps streamline the ordering process: we expect to be given a menu, to review it, and then to signal to the waiter when we're ready to order. However, when a schema is no longer beneficial (e.g. the restaurant has no waiters), we can implement a different schema, that is more appropriate to the current situation. Deploying the same behavioral schemas, even when they are no longer appropriate, is a primary phenotype of many compulsive-behavior disorders. The orbitofrontal cortex (ofc) has been identified as a key brain region involved in the execution of behavioral schemas. Yet, its exact role in the process of applying the proper schema when confronted with a new contradictory problem is still poorly understood, both in the normal and the dysfunctional brain.
To address this gap, rats were implanted with microelectrodes targeting their ofc and trained on a sequence of odor-based tasks that were governed by two contradictory rules, one in which reward was based on application of a 'non-match' rule and another in which it was based on 'cue identity'.
Rats have learned to implement two behavioral schemas based on the two contradictory rules. When transitioned to a new rule, they still showed signs of following the old irrelevant rule but gradually learned to ignore it as the new behavioral schema has been deployed.
Analysis of neural activity in the OFC during this learning process revealed intriguing insights into the underlying mechanisms. The neural activity in the ofc has converged, with learning, into a lower-dimensional space representing the appropriate behavioral schema. When deploying the contradictory schema, the neural representation shifted toward the new schema. However, even after complete behavioral adaptation to the new rule, traces of the prior schema persisted in the neural representation, observed through both low-dimensional representation space comparison and SVM decoder analysis, which revealed a significant decoding accuracy of the irrelevant rule.
This principle of multiple cognitive maps, multiplexed in the OFC population activity, would support efficient deployment of different behavioral schemas in different situations to facilitate continuous learning of multiple tasks.

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230. Qiao Jin, M.D.

Matching Patients to Clinical Trials with Large Language Models
Mentor: **Dr. Zhiyong Lu**
Study Section: **Artificial Intelligence - Machine Learning**

Background: Clinical trials play a vital role in drug development and evidence-based medicine. However, their successes are often hindered by the challenge of patient recruitment. Matching patients to clinical trials requires checking their eligibility with numerous inclusion and exclusion criteria, but manual efforts are typically labor-intensive and error-prone.

Objective: In this work, we aim to utilize the state-of-the-art language understanding and generation capabilities of large language models (LLMs) such as Generative Pre-trained Transformers version 4 (GPT-4) to facilitate the process of patient-to-trial matching.

Methods: We propose a first-of-its-kind model based on LLMs for patient recruitment. Given a patient note and a candidate clinical trial, the proposed model predicts the patient's eligibility on a criterion-by-criterion basis and generates natural language explanations. Then, the model consolidates these criterion-level predictions to assess the overall eligibility of the patient. Finally, our model returns a ranked list of potential clinical trials based on patient eligibility, accompanied by detailed explanations.

Results: Evaluations were conducted at both criterion-level and trial-level. We engaged three physicians to label over 1,000 patient-criterion pairs to assess the criterion-level prediction accuracy of the proposed model. We also evaluate the trial-level prediction performance of the model on three publicly available cohorts of 184 patients with over 18,000 trial annotations. Experimental results show that our model achieves a criterion-level accuracy of 87.3% with faithful explanations, close to the human expert performance (88.7%–90.0%). The aggregated model scores are highly correlated with human eligibility judgments, and they outperform the best-competing methods by 32.6% to 57.2% in ranking and excluding clinical trials. Furthermore, our user study reveals that the proposed model can significantly reduce the screening time by 42.6% in a real-life clinical trial matching task.

Conclusions: The proposed model demonstrates state-of-the-art performance in matching patients to clinical trials, significantly reducing the screening time. Because it can also be used by non-experts, our model has the potential to decrease the disparities in clinical trial enrollment. The integration of our model into clinical settings holds great promise of improving patient recruitment and ultimately accelerating clinical care.

231. Zhizheng Wang, Doctor

Empower Large Language Model with Self-verification for Gene Set Knowledge Discovery
Mentor: **Dr. Zhiyong Lu**
Study Section: **Bioinformatics - algorithms, packages and tools**

Gene set knowledge discovery is critical for human functional genomics, but it relies on manually curated databases of gene functions that are incomplete and unaware of biological context. Recent studies based on Large Language Models (LLMs) have achieved promising results by designing the effectiveness instructions and in-contexts. However, these methods have not harnessed the reasoning capability of LLMs in-depth to explore the most precise biological functions of gene sets and still suffered from the challenge of hallucinations. Therefore, we propose an advanced language agent built upon the GPT-4 to generate biological function names for gene sets in an interpretable and contextually coherent manner. Our method can autonomously interact with different domain-databases to supervise the process of inference, which can also offer standard assertions of gene functions to support the discovery of biological process. In this method, we develop a cascade structure to connect four steps, i.e., "generation, verification, modification, and summarization". Each step corresponds a well-designed prompt to boost the reliable of inference. Benchmarking on multiple gene sets in GO, NeST, and MsigDB, our approach achieves 16.1%, 5.2%, and 3.4% higher accuracies than the standard GPT-4. Notably, our method has exactly matched the golden terms for 165 (14.9%) gene sets. Additionally, enriched term test on MsigDB demonstrate that our method is more efficient in summarizing the significant terms that are consistence with the standard enrichment analysis. Further discussion on specific cases also proves that the proposed language agent can effectively alleviate the hallucination problem in GPT-4 and generate reliable analytical texts for gene functions.

NIH Clinical Center

232. Sayantan Bhadra, Ph.D.

Deep Learning using Weak Supervision for Improved Subcutaneous Edema Segmentation on Abdominal CT
Mentor: **Dr. Ronald M Summers**
Study Section: **Artificial Intelligence - Machine Learning**

Background: Anasarca is described as excessive accumulation of interstitial fluids within the subcutaneous adipose tissue, causing generalized edema. It mainly occurs due to organ dysfunction, such as heart, kidney or liver failure. Volumetric assessment of edema from abdominal CT scans can be useful for monitoring the progression of these diseases, particularly in ICU patients. However, due to the diffused nature of edema, manual annotation for supervised deep learning-based 3D segmentation is impractical. Recently, an unsupervised deep learning method was proposed for edema segmentation using intensity priors of edema and adipose tissue. However, using only intensity priors for edema segmentation leads to a high occurrence of false positives or under-segmentation errors. To improve the segmentation performance, we propose to use intensity prior-based edema segmentation as pseudo-labels, along with pseudo-labels of surrounding adipose tissue and muscle for additional context, in a weakly supervised framework.
Methods: We employed the state-of-the-art nnU-Net as the deep learning segmentation backbone in two stages: (Stage 1) to produce fat and muscle pseudo-labels and (Stage 2) train using combined pseudo-labels of fat, muscle and edema labels from the intensity prior

method. The training data for the Stage 1 nnU-Net consisted of 101 contrast-enhanced CT scans from patients without edema (52F, 49M, 66.6 +/- 5.1 years). The muscle and fat annotations for training the Stage 1 nnU-Net were obtained using a previously existing body composition analysis software. We trained the Stage 2 nnU-Net using 99 CT scans of patients without edema (45F, 54M, 48.1 +/- 17.7 years) and combined multi-class pseudo-labels.
Results: We evaluated the segmentation performance on 16 CT scans of patients with edema (10F, 6M, 52.4 +/- 8.7 years), with five slices randomly selected from each scan and manually annotated under the supervision of an experienced radiologist. Our method improved the average Dice Similarity Coefficient and relative volume difference of edema by 4-5 % compared to the intensity prior method. Qualitatively, we observed that weak supervision with multi-class pseudo-labels significantly mitigated the false positives and under-segmentation errors produced by the unsupervised intensity prior method.
Conclusion: Weakly supervised learning using multi-class pseudo-labels is an efficient way to improve direct quantification of edema for monitoring anasarca.

233. Verity Ford, MD

Sepsis Induced Cardiomyopathy Occurs Independently of Catecholamine Surges and Myocardial Microvascular Ischemia In a Canine Model of Septic Shock
Mentor: **Dr. Charles Natanson**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

During human septic shock and in our well-established large animal model, there are profound falls in left ventricular ejection fraction (LVEF) two days after the onset of hypotension that in survivors are reversible over 7-10 days. The mechanism of this cardiac dysfunction associated with septic shock is unknown. Clinically, Troponin I levels are commonly elevated in septic shock suggesting an ischemic-like injury. Septic shock is confounded by the use of catecholamines in the treatment of hypotension, which at high levels can cause ischemia and a reversible cardiomyopathy that is independent of sepsis. We examined if the presence of high dose catecholamines are necessary for the cardiomyopathy occurring during septic shock.

Sequential cardiac magnetic resonance (CMR) adenosine-stress perfusion imaging (ASPI, perfusion reserve) was utilized over 96h in a S.aureus canine model of pneumonia. Septic animals received either a 40h epinephrine infusion (n = 15, Epi) starting at 4h after challenge or a saline equivalent (n = 15, septic control). No other catecholamines were given. Sedation was titrated to suppress endogenous catecholamine release. Invasive hemodynamics, and laboratory data including plasma catecholamine and Troponin I levels were collected throughout.

Serial plasma catecholamine levels in septic controls were within or near the normal range throughout with no significant elevations from baseline. Despite this, these septic controls still had significant decreases in mean CMR derived LVEF at 48 and 66h after challenge which were alike to the LVEF depressions seen in septic Epi-animals. ASPI at 66h was significantly elevated in septic-controls, however ASPI was significantly blunted in septic Epi-animals. Plasma Troponin I levels were significantly elevated from baseline in Epi-animals and were not elevated in septic-controls.

We demonstrate here that sepsis-induced myocardial depression does not depend on high catecholamine levels nor cardiac microcirculatory perfusion inadequacies that give rise to tissue ischemia. Epi infusions during sepsis causes a form of cardiac injury that is tangential to the myocardial depression of sepsis. Specifically, Epi infusions depressed the microcirculatory perfusion reserve and increased Troponin I levels indicating a secondary prolonged mild myocardial ischemic effect. We show here that the mechanism underlying septic shock induced myocardial dysfunction is not primarily caused by catecholamines.

234. Charles Hesswani, MD

A Comparative Study of Novel Exclusively Transperineal Ultrasound Guided Prostate Biopsy versus Standard Transperineal Biopsy with Transrectal Ultrasound Technique
Mentor: **Dr. Peter Pinto**
Study Section: **Biophysics and Biomedical Engineering**

Introduction/Background

New biopsy techniques are emerging to enhance the detection rate of prostate cancer (PCa). For years, transrectal (TR) ultrasound (US)-guided biopsies remained the standard approach. More recently, a transition from TR biopsy to transperineal (TP) biopsy with a TR probe has gained popularity, primarily over concerns of infection risk. The use of a TR probe, however, remains a suboptimal option from a patient comfort and hygiene standpoint. In this study, we assess the performance of a totally TP (TTP) MRI fusion biopsy technique that utilizes a TP US probe instead of a traditional TR probe.

Methods/Materials

Between 2021 and 2023, patients were enrolled on a clinical trial undergoing TTP and TP MRI fusion targeted prostate biopsy. TP biopsies were performed using the UroNav MRI/US fusion biopsy platform with a TR side fire US probe, while TTP biopsies used a TP curved array US probe. A novel software platform was developed to allow TP US fusion in real time with prostate MRI utilizing electromagnetic tracking. Cancer detection rates (CDR) of TTP and TP biopsy were compared using McNemar's test on a per-patient and per-lesion basis. Baseline and clinical characteristics including grade group (GG), percentage of biopsy core involvement, size of tumor on biopsy, prostate volume and lesion size on MRI were assessed using two-sample t-tests and X2 tests.

Results

A total of 276 prostate lesions were biopsied from 143 patients who underwent prostate biopsy using both TTP and TP methods for each lesion in the same biopsy session. On a per-patient analysis, McNemar's test showed no significant difference in CDR using TTP vs TP methods for overall PCa (57%, p=1) and for csPCa (32% vs 36%, p=0.4). On a per-lesion analysis, McNemar's test showed no significant difference in CDR using TTP vs TP methods for overall PCa (46% vs 48%, p=0.6) and for csPCa (22% vs 26%, p=0.3).

Conclusions

Our novel TTP biopsy method has shown comparable CDR to the traditional TP biopsy in detecting prostate cancer, without the need for an US probe in the rectum. Further design development and technique refinement will aim to optimize this new platform's availability for commercialization.

235. Ravichandra Tagirasa, Ph.D

IKAROS negatively regulate memory T cell formation in humans.
Mentor: **Dr. Sergio D Rosenzweig**
Study Section: **Immunology - Lymphocyte Development and Activation**

IKAROS encoded by IKZF1, is a zinc finger (ZF) transcription factor of IkZF family, expressed throughout hematopoiesis. Patients with heterozygous germline IKZF1 mutations have progressive B cell deficiency and impaired immune functions, leading to recurrent infections, immune dysregulations, and an increased risk of malignancies. However, its role in T cell maturation and differentiation is not fully understood. In this study, we discovered that patients with mutations in IKAROS show T cell differentiation defects, particularly affecting the generation of naive (CD45RA+) and memory (CD45RO+) T cell subsets. Remarkably, loss-of-function (LOF) variants (N159K, H167R, Y503*) predominantly presented with increased memory T cells, skewed towards Th1 subset, and few naive T cells. However, the dominant negative variant (N159S another LOF variant) exhibits an accumulation of naive T cells, which can be attributed to its failure in T cell activation and proliferation. Unlike the LOF variants, the IKAROS gain-of-function (GOF) variant R183C primarily manifests with naive T cells. Additionally, to mimic the LOF mutations in vitro, we degraded the IKAROS through lenalidomide or knockdown by lentiviral shRNA, resulting in a significant increase in memory T cell formation from naive T cells. To further study the role of IKAROS on early T cell maturation and differentiation in thymus, we generated artificial thymic organoids from the patient's CD34+ cells, which clearly revealed that IKAROS LOF variants induce CD45RO+ T cells even at the early stage of T cell development in thymus. Mechanistically, we demonstrated that IKAROS alters the CD45RA+ and CD45RO+ T cell pools in vivo and in vitro by negatively regulating the heterogeneous nuclear ribonucleoprotein L-like protein (hnRNPLL), a master regulator of CD45 alternative splicing in T cells. Altogether, here we illustrate that IKAROS negatively regulates memory T cell generation by inhibiting the hnRNPLL expression. These insights further suggest that targeted degradation of IKAROS can be used to generate robust homogenic memory T cells, which is one of the challenges in CAR T cell generation.

236. Yan Zhuang, PhD

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Mentor: **Dr. Ronald M Summers**
Study Section: **Radiology/Imaging/PET and Neuroimaging**

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