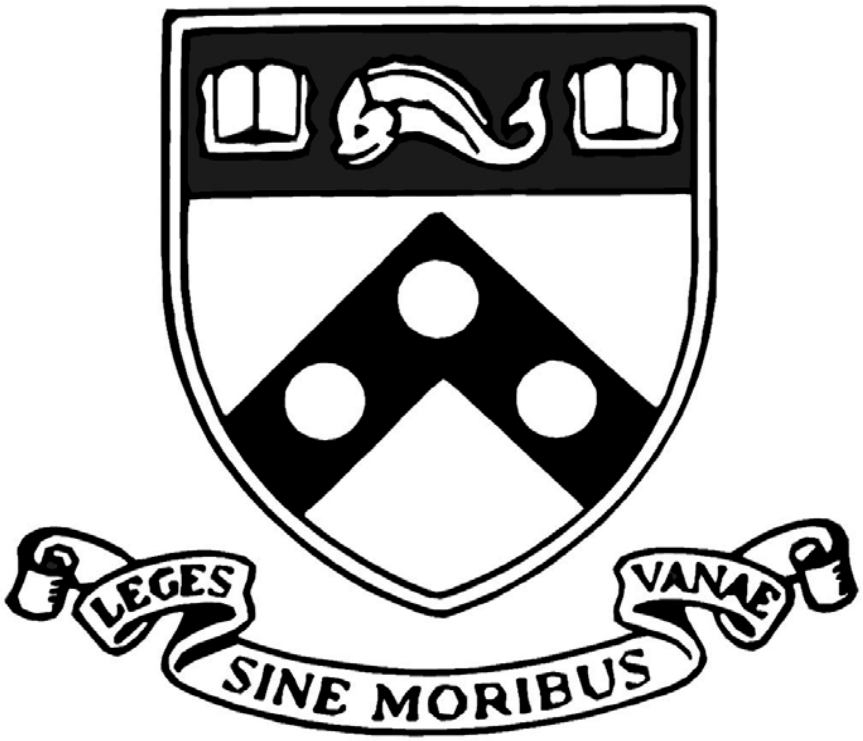
Perelman School of Medicine
at the University of Pennsylvania

Combined Degree Program Annual Retreat



August 2, 2024
Villanova University
Villanova, Pennsylvania

The Combined Degree and Physician Scholar Programs Administration

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David Bittner, MA	Coordinator, MD-PhD Program
Ameena Atif	Coordinator, VMD-PhD Program

Table of Contents

Welcome to the Retreat – from the MD-PhD Program Director

Welcome to the MSTP Retreat for 2024

For those here for the first time, the MSTP retreat is much more than a train ride to the suburbs, a few talks and a whole lot of posters. As a tradition of the very best sort, the retreat is a chance to unwind a bit and remind us that we are part of a community. Our community extends to both sides of Spruce Street, to the Vet school and back again, from the classrooms in JMEC and the Vet School, to the clinical spaces in HUP, VHUP and CHOP, and, perhaps most importantly, to hundreds of research settings. Scholarship and discovery require sustained focus, so it can be easy to forget all the others who are also moving ahead. Some of them started with you. Some started much earlier. Some much later. Today is a day for embracing old friendships, creating new ones, and, above all, learning from each other. Not so long ago COVID-19 kept us apart.

One of my not so secret pleasures is that I get to see all of you arrive at your first retreat as newbies and then progress year by year, becoming ever more confident and chimeric over time. This year 15 people graduated from the MD/PhD program. 31 are joining us, bringing the program to its greatest size ever. The 850-plus alumni of the combined MD/PhD and VMD/PhD programs have spread across the country, making important contributions in academia, research institutes, biotech and pharma. Wherever they land, they carry our community with them.

Some of our alums have remained at or returned to Penn, becoming faculty colleagues as well as friends, taking on new roles while remaining members of our community. Whether at Penn or elsewhere, they become role models, thesis advisors, program advisors, and preceptors in TiMM and CSTR. As members of admissions committees, they help to select the next generation of clinicians who can discover and translate. Whether you are an incoming first year member of our community, a veteran who joined a while ago, a faculty member who enjoys the community or an alum who cares, Rahul, Maggie and I are glad to have you here. Welcome to the retreat and welcome to our community. Many thanks to the members of this year's organizing committee. Enjoy the day and the official start of a new year.

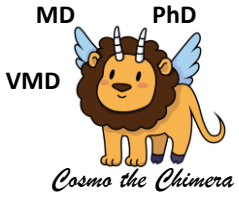
All the best,

Skip

Skip Brass, MD PhD

MSTP Director

7/24/24



P.S. Special thanks to Jasmine Larrick for Cosmo's facelift.

Welcome to the Retreat – from the VMD-PhD Program Director

Welcome to the Penn Combined Degree Retreat 2024!

Hi folks. It has been a good year for the VMD-PhD program. The year included numerous meetings to mesh the VMD-PhD program with the new curriculum that rolled out at the School of Veterinary Medicine over the past two years. This new curriculum, in many ways, is advantageous for VMD-PhD students and is likely to augment their pathways through the program. In the process of implementing the new curriculum transition, and perhaps synergizing with pandemic interruptions, we have many students near the end of the program who either have graduated or will graduate shortly.

Defenses and Graduations

The past 12 months have been busy with 5 thesis defenses including Ashley Vanderbeck, Suna Li Cranfill, Sabina Hlavaty, Nathaniel Sutoyu, and Megan Clark. In Spring 2024 five students graduated including Brinkley Raynor, Jaclyn Carlson, Philip Hicks, Monica Jimenez, and Elisabeth Lemmon, and two more are scheduled to graduate in December 2024 (Suna Li Cranfill and Ariel Shepley-McTaggart).

Entering Students

Three very accomplished students are entering the Program this Fall (Caroline Davis from the University of Virginia, Michael Lyons from the University of Michigan, and Anne Yauch from Northeastern University).

We wish our graduating students much success and look forward to working with those now entering the program. I hope you have a great MSTP retreat day and enjoy the advantages of the large critical mass and diversities that our program enjoys.

Welcome again to all current and incoming students.

Sincerely,

Michael Atchison, PhD

Many, Many Thanks To the Retreat Planning Committee

We asked the third and fourth year MD-PhD and equivalent VMD-PhD students to take responsibility for planning this event. They did a fabulous job, and we'd especially like to thank the students who were most active in attending the meetings and organizing.

Sam Barnett Dubensky
Andres Fernandez del Castillo
William Gao
Sanam Kavari
Sam Kim
Mattia Mah'moud
Andrew Nelson
Carson Poltorack
Alexander Post
Pav Ravindran
Han-Seul Ryu
Henry Utset
Caroline Wechsler
Daniel Yen
Jack You

2024 Incoming Class

MD/PhD

Sydney Alderfer	Bioengineering	Colorado State / Buck Inst.
Seth Anderson	Immunology	Yale / Broad
Kessh Bhasiin	Neuroscience	Mount Holyoke / Wash U
Ether Dharmesh	Bioengineering	St. Louis U
Jonathan Gaither	Cell and Molecular Biology	Howard / CHOP
Akshay Govindan	Genomics & Computational Biology	Wash U / Northwestern
Sagar Gupta	Biochemistry, Biophysics, Chemical Biology	Penn
Zahraa Hotait	Cell and Molecular Biology	Georgetown / Rockefeller
Kate Jones	Immunology	Amherst / Penn
Ashwin Kammula	Cell and Molecular Biology	U Maryland / Broad
Sajeev Kohli	Cell and Molecular Biology	Harvard / NIH
Suma Kotha	Neuroscience	Johns Hopkins
Jenny Lu	Epidemiology and Biostatistics	Harvard / City of Hope
Claire Millett	Neuroscience	Harvard / Kallyope
David Moon	Cell and Molecular Biology	Duke
Rachana Mudipalli	Cell and Molecular Biology	Stanford
Anika Nerella	Health Care Management & Economics	Northwestern / West Point
Samuel Neuman	Bioengineering	U Wisconsin / NIH
Rachel Palmer	Chemistry	Georgetown / NIH
Danealle Parchment	Immunology	Wash U / NIH
Vish Rao	Bioengineering	Columbia / Tsinghua U
Elizabeth Rubin	Cell and Molecular Biology	Pitt
Jake Shapira	Cell and Molecular Biology	Pitt
Rishi Shridharan	Neuroscience	Rice / KU Leuven

Nick Simpson	Anthropology	SMU / Gartner, Inc
Miranda Song	Genomics & Computational Biology	UCSD / NIH
Jose Velarde	Immunology	Harvard / MIT
Eric Wang	Immunology	USC / Weill Cornell
Nathan Wang	Bioengineering	Johns Hopkins
Rachel Xiang	Cell and Molecular Biology	Tufts / NIH
Daniel Yong	Immunology	U Chicago / NIH

VMD/PhD

Caroline Davis	Bioengineering	U Virginia
Michael Lyons	Epidemiology and Biostatistics	U Michigan
Anne Yauch	Cell and Molecular Biology	Northeastern

Graduating Students and Thesis Information

MD/PhDs

Omar Abdoun
Design, Modeling, and Optimization of a Tape Spring Steerable Needle
Thesis Advisor: Dr. Mark Yim

Ryan Boe
Lost in transcriptional noise: how allelic correlation marks a trade-off between noise transmission and signal responsiveness
Thesis Advisor: Dr. Arjun Raj

Marc Bornstein
A fluxomics approach toward quantifying the metabolic response to cold stress
Thesis Advisor: Dr. Zoltan Arany

China Byrns
Glia: friend and foe in traumatic brain injury and (aging)
Thesis Advisor: Dr. Nancy Bonini

Chuan Hao (Alex) Chen
Safecraft: Race, Space, and the Building of American Biosecurity Against Emerging Diseases
Thesis Advisors: Drs. Adriana Petryna, Deborah Thomas, Ramah McKay & Andrew Carruthers

Daniel Connolly
Reading and writing DNA methylation in the mammalian brain
Thesis Advisor: Dr. Zhaolan (Joe) Zhou

Charles Danan
RNA Methylation Sustains Intestinal Homeostasis and Regeneration
Thesis Advisor: Dr. Kathryn Hamilton

Rajiv Deshpande
MRI-Based Quantification of Renal Oxygen Utilization
Thesis Advisor: Dr. Felix W. Wehrli

Dan Dou
Axonal trafficking deficits caused by Parkinson's-associated mutations in LRRK2
Thesis Advisor: Dr. Erika Holzbaur

Diego Espinoza
Cross-compartment immunology of pediatric neuroinflammatory disease
Thesis Advisor: Dr. Amit Bar-Or

Jordan Harris
Cutaneous immunity at the interface of sebaceous biology and the microbiota: uncovering the phenomenon of host-microbe inheritance
Thesis Advisors: Drs. Taku Kambayashi & Elizabeth Grice

Naveen Jain
Beyond Luck of The Draw: Uncovering Cell Intrinsic Determinants in Reprogramming Somatic Cells to Pluripotency
Thesis Advisor: Dr. Arjun Raj

Elle Lett
Centering the multiply marginalized: Using intersectionality to characterize health inequities faced by ethno-racial minoritized subpopulations within the transgender community
Thesis Advisor: Dr. Nadia Dowshen

Andrew Lin
Development of Magnetic Nanopore-based Extracellular Vesicle Subpopulation Sorting for the Multimodal Diagnosis and Prognosis of Neurological Disease and Cancer
Thesis Advisor: Dr. David Issadore

Joyce Liu
Divergent Roles for ACLY and Bempedoic Acid in Hepatic Lipid
Thesis Advisor: Dr. Kathryn Wellen

Graham Lobel
Glutamine availability regulates tissue specific cDC subsets
Thesis Advisors: Drs. Malay Haldar & Celeste Simon

Kelly Martins
Investigating Recombinant and Wild-Type Adeno-Associated Virus Integrations in the Macaque and Human Genomes Following In Vivo Exposure
Thesis Advisor: Dr. Jim Wilson

Sarshan Pather
Reading, writing, and perturbing human neural cell biology
Thesis Advisor: Dr. Ophir Shalem

Henry Sanchez
Pooled tagging of endogenous proteins for exploration of the human proteome driven by prime editing
Thesis Advisor: Dr. Ophir Shalem

Ben Sieff
Leveraging clinical material: an ethnography of buprenorphine-based treatment in greater Pittsburgh
Thesis Advisor: Dr. Adriana Petryna

Folasade Sofela
The role of metabolism in a Drosophila model of Neurofibromatosis 1.
Thesis Advisor: Dr. Amita Sehgal

Teddy Steinbock
The RNA biology of DNA viruses: Insights from Human Adenovirus
Thesis Advisor: Dr. Matthew D. Weitzman

Iulia Tapescu
Wanted DEAD or alive: Antiviral roles for DEAD-box helicases
Thesis Advisor: Dr. Sara Cherry

Stephanie Teeple
Missingness and Equity of Clinical Model Predictive Performance: Considering the Social Construction of EHR Data
Thesis Advisor: Dr. Elizabeth Grice

Monica Wei
Molecular Investigation of Competitive Microbial Interactions in the Porcine Skin Microbiome
Thesis Advisor: Dr. Sara Cherry

Ellen White
An understudied bacterium of the wound microbiome promotes diabetic healing
Thesis Advisor: Dr. Elizabeth Grice

Daniel Xu
Investigating the Effects of H3K27ac Reduction in an iPSC Neuron Model of Familial
Thesis Advisor: Dr. Shelley Berger

Jason Xu
Identification and targeting of treatment resistant progenitor populations in T-cell Acute Lymphoblastic Leukemia
Thesis Advisor: Dr. Kai Tan

Kevin Yang
Machine learning optimized targeted detection of alternative splicing
Thesis Advisors: Drs. Yoseph Barash & Peter Choi

Alexandra Zezulin
RUNX1 is required in granulocyte-monocyte progenitors to attenuate inflammatory cytokine production by neutrophils
Thesis Advisor: Dr. Nancy Speck

Jenna Zhang
Investigating cellular innate immune responses to intestinal Yersinia infection
Thesis Advisors: Drs. Sunny Shin & Igor Brodsky

VMD/PhDs

Jaclyn Carlson
Fibrillar Collagen Modulation of Extracellular Matrix Structure and Organization Following Tendon Injury
Thesis Advisor: Dr. Louis Soslowsky

Megan Clark
Mitochondrial NDUFA4 is a functional switch controlling tumor-associated macrophages and tumor immunity
Thesis Advisor: Dr. Jorge Henao-Mejia

Suna Li Cranfill
Determining the role of the lateral habenula in pain and itch processing
Thesis Advisor: Dr. Wenqin Luo

Sabina Hlavaty
ACSS1-dependent acetate utilization rewires mitochondrial metabolism to support tumor growth and metastasis
Thesis Advisor: Dr. Zachary Schug

Ariel Shepley-McTaggart
Investigating host regulation of the Filovirus lifecycle
Thesis Advisor: Dr. Ron Harty

Nathaniel Sotuyo
GABAergic interneuron transplants as a potential therapy for Dravet Syndrome
Thesis Advisors: Drs. Ethan Goldberg & Stewart Anderson

Ashley Vanderbeck
Notch in the niche: How stromal notch ligands regulate T cell development and differentiation in secondary lymphoid tissue
Thesis Advisor: Dr. Ivan Maillard

Agenda

Please note – all the rooms listed below are located in Villanova's Connelly Center

Student Arrival, Poster Set-Up & Continental Breakfast
Villanova Room
8:00 – 9:00am

Opening Remarks
Dr. Skip Brass, MD, PhD
Dr. Mike Atchison, VMD, PhD
Cinema Room
9:00 – 9:15am

Keynote Talk
Cinema Room
9:15 – 10:15am

Dr. Todd Golub
Director at Broad Institute of MIT and Harvard
Perspectives on Cancer Precision Medicine

Lightning Talks/Poster Pitches
Cinema Room
10:15 – 10:30am

Group & Incoming Class Photos
10:35 – 11:00am

Student Poster Session A
Villanova Room
Presenters will stand by posters during their session
11:05 – 11:50am

Lunch with Faculty Lab Pitches
Villanova Room
11:50 – 12:50pm

Student Poster Session B
Villanova Room
Presenters will stand by posters during their session
12:50 – 1:35pm

Student Talks
Cinema Room
1:40 – 2:25pm

Claudia Lovell	*Xist Deletion in B Cells Results in Systemic Lupus Erythematosus Phenotypes*
Ben Kahn	*The lymph node is an intrinsically tolerant destination for metastasizing tumor cells*
Brian Goldspiel	*Eating The Seeds of Your Own Destruction; Understanding the Metabolic Determinants of Inflammatory Cell Death Pathways*

Snack Break
Please enjoy at assigned social activity table
2:30 – 2:40pm

Student Group Activity*
Villanova Room
2:40 – 3:20pm

Closing Remarks
Villanova Room
3:20 – 3:30pm

Departure from Villanova

Post Retreat Happy Hour

The Post at Cira Centre South
129 S 30th St, Philadelphia, PA
5:00 – 7:00pm

Student Talks

Claudia Lovell

Xist Deletion in B Cells Results in Systemic Lupus Erythematosus Phenotypes
Advisor: Dr. Montserrat Anguera

Ben Kahn

The lymph node is an intrinsically tolerant destination for metastasizing tumor cells
Advisor: Dr. Ben Stanger

Brian Goldspiel

Eating The Seeds of Your Own Destruction; Understanding the Metabolic Determinants of Inflammatory Cell Death Pathways
Advisor: Dr. Will Bailis

Xist Deletion in B Cells Results in Systemic Lupus Erythematosus Phenotypes

Claudia D. Lovell, Nikhil Jiwrajka, Hayley K. Amerman, Michael P. Cancro, and Montserrat C. Anguera

Submitted by: Claudia Lovell, CAMB - Genetics and Epigenetics
Email: Claudia.Lovell@pennmedicine.upenn.edu
Advisor: Dr. Montserrat Anguera

Systemic lupus erythematosus (SLE) is an autoimmune disease preferentially observed in females. X-linked gene expression in XX females is normalized to that of XY males by X-Chromosome Inactivation (XCI). However, B cells from female SLE patients and mouse models of SLE exhibit mislocalization of Xist RNA, a critical regulator of XCI, and aberrant expression of X-linked genes, suggesting that impairment of XCI may contribute to disease. Here, we find that a subset of female mice harboring a conditional deletion of Xist in B cells ("Xist cKO") spontaneously develop SLE phenotypes, including expanded activated B cell subsets, disease-specific autoantibodies, and glomerulonephritis. Moreover, pristane-induced SLE-like disease is more severe in Xist cKO mice. Activated B cells from Xist cKO mice with SLE phenotypes have increased expression of proinflammatory X-linked genes implicated in SLE. Together, this work indicates that impaired XCI maintenance in B cells directly contributes to the female-bias of SLE.

The lymph node is an intrinsically tolerant destination for metastasizing tumor cells

Benjamin Kahn, Il-Kyu Kim, Cody Eskandarian, Alfredo Lucas and Ben Stanger

Submitted by: Benjamin Kahn, CAMB – Cancer Biology
Email: Benjamin.kahn@pennmedicine.upenn.edu
Advisor: Dr. Ben Stanger

Lymph nodes (LNs) are the first site of metastasis for most solid cancers. As LNs are also the staging grounds for anti-tumor immunity, their high susceptibility to metastatic colonization is a paradox. Previous studies have suggested that extrinsic tumor-derived factors precondition the draining LN to enable tumor cell survival by promoting a state of immune-suppression. Here, we investigate whether intrinsic qualities of the LN may also impede its immune response to metastasizing tumor cells. Using multiple transplant models, we show that LNs possess intrinsic features independent of preconditioning which make them an advantageous site for tumor cells to evade T cell killing. Regulatory T cells locally suppressed the cytolytic capacity of tumor-specific CD8 T cells in the LN to facilitate survival of antigenic tumors. These findings identify the regulatory T cell population within lymph nodes as an intrinsic mechanism that explains the lymph node's paradoxical susceptibility to metastatic colonization.

Eating The Seeds of Your Own Destruction; Understanding the Metabolic Determinants of Inflammatory Cell Death Pathways

Brian Goldspiel*, Mikel Haggadone*, Ashley Yang, Aaron Wu, Nora Kiledijan, Jimmy Xu, Clementina Mesaros, Crystal Conn, Sunny Shin, Will Bailis

Submitted by: Brian Goldspiel, CAMB – Microbiology, Virology, and Parasitology
Email: Brian.Goldspiel@pennmedicine.upenn.edu
Advisor: Dr. Will Bailis

There is no doubt that the amount and content of calories we consume bears tremendous impacts on our overall health. Decades of research focused on the western diet, for instance, have demonstrated how these diets increase rates of a variety of pathological insults, ranging from cardiovascular to infectious diseases. Yet, despite endless misinformation and countless social media videos of influencers touting specific diets, there is still little mechanistic insight into how and why diets can predispose patients to inflammatory disease. Thus, a more thorough understanding of the diet-disease axis will not only inform novel patient treatments and risk factors but may offer a broader understand of why some diets, but not all, may be useful in preventing severe disease.

A central driver of inflammation is a form of inflammatory cell death called pyroptosis, which leads to the production of pro-inflammatory cytokines like IL-1ß and subsequently terminal cell lysis. Here, we demonstrate that a class of amino acids – the branched chain amino acids (BCAAs) – are central to pyroptotic-driven inflammation, and that the BCAAs may be fundamental regulators of cell death decisions. We find that the BCAAs control the specific cytokines that are produced and the rate of terminal cell death before and during pyroptosis. We show that the central regulator of this process is the mTORC1 pathway, the master anabolic regulator of cellular metabolism. We find that pyroptosis is regulated by environmental amino acids through a translational mechanism. Indeed, we find that the NLRP3 inflammasome, which orchestrates pyroptosis, is regulated through the induction of specific transcripts in a BCAA-dependent manner. Through polysome profiling, we show that NLRP3 inflammasome proteins shift their localization in translation to occupy less higher order polysomes, suggesting translational suppression of NLRP3 proteins as it pertains to metabolic environment. This shift leads to a reduction in inflammasome complex formation, reduced terminal cell death, and an inhibition of pyroptotic driven cell death. Proving its relevance *in vivo*, we demonstrate that mice on BCAA reduced diets are less susceptible to LPS mediated sepsis. We propose that environmental amino acid sensing serves as a nexus for pyroptotic cell death by controlling the rates and location of translation of inflammasome proteins.

This work presents a novel means of understanding how our diet can modify inflammation. Indeed, it provides credence to the idea that diet not only dictates how our cells choose to live, but also how they may die. Our studies illustrate how dietary interventions may reduce both the severity of inflammatory diseases as well as downstream functional consequences of inflammatory disease. Moreover, it provides unique mechanistic insight into how our diets converge on cellular metabolism to orchestrate inflammation.

Poster Pitches

Kevin Sun

Transdiagnostic Polygenic Risk Scores Underlying Overall Psychopathology and Personalized Functional Networks in Early Adolescence
Advisors: Drs. Aaron Alexander-Bloch & Theodore D. Satterthwaite

Henry Utset

CAR-T cell therapy for idiopathic pulmonary fibrosis
Advisor: Dr. Ellen Puré

Randall Burson

Neglect Protocol: Cultural and Ethical Negotiations During a Case of Suspected Child Neglect
Advisor: Dr. Adriana Petryna

Faculty Lab Blurbs

Dr. Hajera Amatullah
hajera.amatullah@pennmedicine.upenn.edu

Dr. Amatullah is an Assistant Professor in the Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania. The Amatullah Lab's overarching research interest is in understanding the chromatin mechanisms regulating our innate immune system and how loss of this regulation leads to disease, particularly in the context of an ever-changing environment. Our current research efforts focus on understanding mechanisms of how genetic variants in chromatin regulators, L3MBTL3 and KDM4C, lead to chronic immune diseases like Asthma, Inflammatory Bowel Disease and Systemic lupus erythematosus. To pursue our research questions, the Amatullah lab employs a range of molecular biology, immunology and epigenetic tools. Interested trainees are invited to reach out to Dr. Amatullah for rotation projects!

Dr. Caroline Bartman
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The Bartman lab's goal is to target the metabolism of immune and tumor cells to improve cancer therapy or reduce inflammation. Both cancer and immune cells have extreme metabolism. This makes metabolism a good target to alter the survival or function of these cells. We aim to identify metabolic fluxes altered in immune or tumor cells, then use genetic and pharmacologic approaches to target these pathways to improve tumor clearance or reduce inflammation. A key approach we use is infusing heavy non-radioactive metabolic tracers, and then performing mass spectrometry to track tissue and cell type metabolism.

Dr. Michael Hogan
mihogan@upenn.edu

The overarching goals of the Hogan Lab (hoganlab.org) are to understand how protective immune responses are generated against viral diseases and to translate these lessons into new and improved vaccines. We have particular focuses on: 1) unconventional T cell responses (e.g. non-classical MHC restriction and unconventional epitopes), 2) elucidating the cooperativity between CD4 T cells, CD8 T cells, and antibody responses in fighting respiratory infections, 3) mechanisms of mRNA vaccine immunogenicity, and 4) evaluation of mRNA vaccine technologies in different animal species.

Dr. Guilherme Nader
naderg@chop.edu

Dr. Nader seeks to reconstitute the complex interface between mechanical and chemical signaling during cell migration in dense microenvironments and tissue crowding. His lab employs microfluidics, microfabrication and organ-on-a-chip devices to apply controlled and precise confinement to cells and mechanical stress to the nuclei. He combines these tools with diverse fluorescence microscopy techniques and live cell imaging to investigate how the tissue microenvironment regulates cell function through its impact on nuclear deformation and integrity. Ultimately, our goal is to identify signaling pathways associated with nuclear mechano-sensing in cells that experience confinement/mechanical stress. This will allow us to establish a link between different degrees of nuclear deformation and different cellular behaviors, from orchestrated signaling cascades to cellular perturbations and damage.

Dr. Manolis Roulis
manolis.roulis@pennmedicine.upenn.edu

The mesenchymal microenvironment and its primary constituents, the extracellular matrix and the millions of fibroblasts that build it, is one of the most enigmatic areas of tissue biology today. By integrating single cell studies in human tissues and in mice, with functional analyses in mouse models, human organoids and organotypic systems, the Roulis laboratory aims to uncover specific cellular and molecular mechanisms through which fibroblasts control intestinal inflammation, fibrosis and tumorigenesis. Using single-cell technologies, we found that "stromal cells" are in fact a family of different cell types, each with a distinct transcriptional program and localization within the tissue. With the tools that we have available, we are mapping the full extent of cellular diversity in the mesenchyme and its spatial organization. Based on this knowledge, we aim to uncover the functional specialization of each fibroblast type in homeostasis, and also understand its implications for disease.

Dr. Juan Alvarez
juan.alvarez@pennmedicine.upenn.edu

Our laboratory uses human stem cell-derived pancreatic islet organoids and mice as model systems to study islet development, physiology, and pathology, and to develop replacement therapies for insulin-dependent diabetes. We focus on the interplay between circadian rhythms, metabolism, and islet cell maturation. Our work addresses three fundamental questions:

1. How do cells and tissues become functionally specialized?

2. How does organ-level physiology entrain to circadian rhythms?

3. How do circadian disturbances lead to organ and systemic metabolic dysfunction?

To answer these questions, we employ single-cell transcriptomic, epigenomic, proteomic methods and genetic, nanoelectronics-based approaches to study and control islet cell fate. We are especially interested in advancing replacement therapies for insulin-dependent diabetes, and harnessing control over the function and applications of any human organoid. Interested students are invited to reach out to Dr. Alvarez about rotation projects!

Dr. Joel Babdor
joel.babdor@pennmedicine.upenn.edu

Dr. Joel Babdor is an assistant professor in the department of Systems Pharmacology and Translational Therapeutics at the University of Pennsylvania. He leads the Precision Immunology and Microbiome Medicine laboratory that aims at understanding immune health, through a multi-systems approach that explores the dialogue between the human immune system and the microbial exposome. The lab uses high-throughput, high-dimensional profiling approaches and multimodal computational methods to study clinical and animal model biology. The lab focuses on diseases that can be treated with immunomodulators, which encompasses autoimmunity, cancer, transplantation and vaccine. Through this broad scope, the lab dissects the impact of the microbiome on disease development, severity and treatment response. One of the long-term goal of the lab is to define immune health and contribute to the development of precision microbiome-medicine therapies to improve immune interventions for patients.

Dr. Evan Weber
weberew@chop.edu

Engineered T cell therapies (e.g. CAR T cell therapy) have revolutionized the cancer immunotherapy landscape by mediating remarkable clinical responses. However, T cell exhaustion and poor persistence limit efficacy in patients and are major barriers to progress for the treatment of solid tumors. The Weber Lab at CHOP and UPenn seeks to overcome these barriers by developing engineering-based approaches to endow human CAR T cells with exhaustion resistance and improved durability. We are identifying and modulating transcriptional and epigenetic pathways that redirect human T cells towards more therapeutic cell states. Multiomics analyses on experimental and patient CAR T cells enable us to link biological pathways to cell phenotype, function, and patient outcomes, thereby informing our T cell engineering efforts. Collectively, our work will uncover molecular programs that drive human CAR T cell dysfunction, identify targets for therapeutic intervention, and inform universal strategies that improve CAR T cell efficacy in cancer patients.

Dr. Glennis Logsdon
glogsdon@pennmedicine.upenn.edu

Dr. Glennis Logsdon is an Assistant Professor in the Department of Genetics and a Core Member of the Epigenetics Institute at the University of Pennsylvania Perelman School of Medicine, where she studies centromere variation, evolution, and function. Her lab uses a combination of long-read sequencing technologies, innovative computational methods, and synthetic biology approaches to investigate how centromeres vary among humans and throughout evolution. Her lab also designs and engineers centromeres from scratch on human artificial chromosomes to better understand the human genome. More information about the Logsdon Lab can be found on their lab website: logsdonlab.com.

Dr. Julia Warren
warrenjt@chop.edu

The Warren lab studies normal and malignant hematopoiesis using primary human hematopoietic stem cell cultures, cell lines, CRISPR screens, and mouse models. We are motivated by the devastating consequences for patients who have an inability to form blood cells, and by a desire to understanding the basic biology of hematopoiesis. Hopefully we can one day translate these findings back to patients with marrow failure or leukemia. We are especially interested in three main areas: 1) using mouse models to understand mechanisms of marrow failure in rare genetic diseases; 2) using cell-based models to understand the mitochondrial determinants of myeloid precursor-to-granulocyte cell fate choices; and 3) applying knowledge from rare germline disorders of granulocyte production to develop new leukemia therapeutics. The PI strongly values open communication and independent thinking, and as an early-career investigator has time for more hands-on training of graduate students. Our group participates in several joint lab activities including quarterly neutrophil journal club, monthly joint group meeting with a colleague in biochemistry studying our favorite gene (CLPB), and weekly blood club (work-in-progress alternating with journal club together with the Speck, Tong, Klein, Bowman, Pear, Paralkar, and other leading hematopoiesis labs). When we aren't thinking about science, you can find lab members out to dinner, visiting the PI's chickens, or taking a daytime break to go turtle-viewing at the biopond.

Poster Session A

Anthropology

Poster 1A
Neglect Protocol: Cultural and Ethical Negotiations During a Case of Suspected Child Neglect
Presenter: Randall Burson | Advisor: Dr. Adriana Petryna

Biochemistry, Biophysics, Chemical Biology

Poster 2A
Genomic context shapes DNA methylation & hydroxymethylation landscapes: A high-throughput enzymology study of TET dioxygenase activity
Presenter: Noa Erlitzki | Advisor: Dr. Rahul Kohli

Poster 3A
Winning Designs for Winter 2024 Rosetta TEV protease Design Games
Presenter: Samuel Garfinkle | Advisor: Dr. Dan Kulp

Poster 4A
Intrinsically disordered regions of TOX regulate chromatin binding dynamics to coordinate protein function during CD8 T cell exhaustion
Presenter: Matthew Sullivan | Advisor: Dr. John Wherry

Bioengineering

Poster 5A
Subject-level segmentation accuracy weights for volumetric studies involving label fusion
Presenter: Christina Chen | Advisor: Dr. Taki Shinohara

Poster 6A
CMR Imaging Traits Associated with Right Ventricular Remodeling in Repaired Tetralogy of Fallot
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Poster 1A | Anthropology

Neglect Protocol: Cultural and Ethical Negotiations During a Case of Suspected Child Neglect

Randall Burson

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A young Mapuche Indigenous couple brings their newborn for an ultrasound study at a community-run "intercultural" clinic in Southern Chile. During the visit, the nurse notices soot on the baby's cheeks and hands, and probes for signs of child neglect. The visit turns tense, and the family breaks ties with the clinic, leaving the clinical team divided about what to do next. Rather than focus on this Mapuche newborn and her family as the subjects of this case, I turn my focus towards the clinical team—an interdisciplinary group of biomedical professionals, both Indigenous and wingka (non-Mapuche). I follow the team as they try to re-engage the family and begin to draft a clinical protocol to standardize the clinical response to suspected child neglect. Through these efforts, the team must navigate its liminal position between Mapuche families and state institutions like child protective services, which have historically torn apart Indigenous communities. Ultimately, this case examines how clinicians navigate their ethical and legal obligations to care and intervene when those obligations might expose communities to further systemic harm. Finally, I reflect on how I use my anthropological insights and position within the clinical team to advocate for a clinical protocol that prioritizes community resources, such as Mapuche ancestral leaders, to help buffer vulnerable patients and families from state interventions.

Poster 2A | Biochemistry, Biophysics, Chemical Biology

Genomic context shapes DNA methylation & hydroxymethylation landscapes: A high-throughput enzymology study of TET dioxygenase activity

Noa Erlitzki and Rahul Kohli

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DNA demethylation in the mammalian genome reverses the gene silencing program imposed by 5-methylcytosine (5mC) and is facilitated by Ten-Eleven Translocation (TET) enzymes, which catalyze the stepwise oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC). Oxidation of 5hmC to 5fC commits the modified cytosine to a pathway for active demethylation. By contrast, persistence as 5hmC allows, but does not necessitate, passive demethylation through an alternate pathway. 5hmC is thought to play key roles in various developmental pathways including neuronal development, while its dysregulation is prominently associated with oncogenesis. Interestingly, it has been observed not only as a transient intermediate but also as a stable modification that likely has independent epigenetic functions. The *in vitro* kinetics of TET-mediated 5mC oxidation have been previously described and were found to be strongly influenced by DNA sequence context, with potential implications for the determinants of demethylation dynamics *in vivo*. However, prior methods have been unable to parse the generation and decay of 5hmC, a key fulcrum in progression through distinct demethylation pathways. This experimental "blind spot" has precluded a comprehensive understanding of iterative TET activity. We took a high-throughput enzymology approach utilizing dual-pipeline deep sequencing to observe, for the first time and at base resolution, the dynamic generation and depletion of 5hmC *in vitro*. Analysis of TET dynamics at single CpGs and across clusters of CpGs reveals distinct sequence context preferences and provides rationale for exploring the role of local sequence context in the genome in governing the process of gene reactivation in the setting of normal development or in disease.

Poster 3A | Biochemistry, Biophysics, Chemical Biology

Winning Designs for Winter 2024 Rosetta TEV protease Design Games

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Here, we present the results of the 2024 Protein Design Games, sponsored by Rosetta Commons and Liberum Bio. The contest involved designing catalytically improved variants of the tobacco etch virus (TEV) protease, a commonly used laboratory reagent. With two weeks to submit designs, the rules of the competition allowed the use of any mutations from the literature, or any sequence less than 267 residues in length including *de novo* computational designs. Five teams submitted 24 total designed sequences, which were expressed in Liberum's proprietary cell-free expression system and screened for activity using a fluorophore/quencher based assay. Our team generated the top 3 overall designs, earning a $5000 prize and the chance to present our findings at RosettaCon.

Poster 4A | Biochemistry, Biophysics, Chemical Biology

Intrinsically disordered regions of TOX regulate chromatin binding dynamics to coordinate protein function during CD8 T cell exhaustion

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Immune checkpoint blockade and chimeric antigen receptor T cell therapies have collectively demonstrated substantial therapeutic benefit for many cancers, including advanced refractory, recurrent, or metastatic solid malignancies. The breadth, efficacy, and durability of these immunotherapies remain limited, however, by the sustained cellular dysfunction and restricted epigenetic plasticity of exhausted CD8 T cells ("Tex"). Exhaustion encompasses a highly regulated differentiation response by CD8 T cells to chronic stimulation by persistent antigen. Development of next-generation immunotherapies requires novel strategies to selectively enhance Tex function or durably "reprogram," at least partially, Tex lineage-defining epigenetics. The transcription factor TOX is essential for the initiation of Tex development, coordinating via unknown mechanisms the acquisition of Tex-specific transcription and chromatin accessibility while inhibiting alternate differentiation trajectories. Although reduced or absent TOX activity can limit the magnitude of achievable exhaustion phenotypes, this benefit is offset by significant time- and differentiation-dependent defects in cell proliferation and/or survival. To improve mechanistic understanding of how Tex identity is established and how TOX activity may be more selectively modulated, we sought to identify the biochemical features of TOX responsible for its function and test how these features may be used to alter TOX function.

Because the TOX HMG-box DNA-binding domain is predicted to lack strong sequence specificity and the other large regions of the protein were previously completely uncharacterized, we focused on these N- and C-terminal "domains" ("NTD," "CTD") and found that in the absence of DNA, NTD and CTD are highly disordered without stable secondary structure. Using the murine lymphocytic choriomeningitis virus model of chronic infection and other *in vitro* assays of TOX function in primary CD8 T cells, we show that these regions are required for intact TOX activity and for maintaining the native spatial distribution of TOX in the nucleus. Using live-cell single molecule tracking microscopy in mouse fibroblasts, we show that NTD and CTD both tune the chromatin-binding behavior of the HMG-box via opposing kinetic effects, with apparent NTD-driven control of off-rate and CTD-driven control of on-rate. Within the NTD, we identify negatively charged and asparagine or glutamine, but not serine, residues as being required for TOX-dependent PD-1 expression. We further show that the negative charge and serine content of the NTD programs its kinetic effects on the overall protein, and are testing whether charge and serine-focused perturbations can modify DNA target selection by TOX. Collectively, we establish NTD and CTD as intrinsically disordered regions required for TOX function, at least in part

because of their roles in regulating chromatin binding dynamics, that may be readily modified to tune the functional strength or genomic positions of TOX activity.

Poster 5A | Bioengineering - Imaging

Subject-level segmentation accuracy weights for volumetric studies involving label fusion

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In neuroimaging research, volumetric data contribute valuable information for understanding brain changes during both healthy aging and pathological processes. Extracting these measures from images requires segmenting the regions of interest (ROIs), and many popular methods accomplish this by fusing labels from multiple expert-segmented images called atlases. However, post segmentation, current practices typically treat each subject's measurement equally without incorporating any information about variation in their segmentation precision. This naïve approach hinders comparing ROI volumes between different samples to identify associations between tissue volume and disease or phenotype. We propose a novel method that estimates the variance of the measured ROI volume for each subject due to the multi-atlas segmentation procedure. We demonstrate in real data that weighting by these estimates markedly improves the power to detect a mean difference in hippocampal volume between controls and subjects with mild cognitive impairment or Alzheimer's disease.

Poster 6A | Bioengineering – Imaging

CMR Imaging Traits Associated with Right Ventricular Remodeling in Repaired Tetralogy of Fallot

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Background
Deterioration of right ventricular (RV) function and best timing for pulmonic valve replacement (PVR) in repaired tetralogy of Fallot (rToF) are poorly understood. Cardiovascular magnetic resonance (CMR) is used for monitoring, but its analysis is user-dependent and time-consuming.
Methods
A longitudinal cohort of rToF patients underwent CMR at the Children's Hospital of Philadelphia. The nnU-Net method was used to train a machine learning model to segment the left ventricular (LV) blood pool, LV myocardium, and RV blood pool from 2D short-axis CMR images. Conventional and novel measures were calculated and studied in association with PVR and remodeling rate using Cox proportional hazards, Kaplan-Meier curves, and multivariable linear regression. Remodeling rate was calculated as (($RVEDVi_{scan2}$ – $RVEDVi_{scan1}$)/years between scans).
Results
The cohort was comprised of 758 patients, of whom 210 underwent PVR. In univariable Cox modeling, traditional right and left-sided, as well as novel variables RV end-diastolic volume (EDV)/LVEDV (Hazard ratio [HR] per 10% increase=1.23, 95% confidence interval [CI] 1.18-1.28, p<0.001), peak systolic LV dV/dt (HR=1.04, 95% CI 1.02-1.06, p<0.001), and peak systolic RV dV/dt (HR=1.01, 95% CI 1.01-1.01, p<0.001) were associated with PVR. In multivariable modeling adjusting for RVEDV index (i)>150 mL/m2 and RVEF<45%, peak systolic RV dV/dt remained predictive of PVR (HR=1.05, 95% CI 1.00-1.10, p=0.032). In patients with 2 CMRs, RV remodeling rate was associated with LVEDVi (odds ratio [OR]=0.93, 95% CI 0.88-0.99, p=0.026), LV end-systolic volume index (OR=0.89, 95% CI 0.80-1.00, p=0.043), and LV mass index (OR=0.87, 95% CI 0.80-0.96, p=0.006), while LV stroke volume and LV dV/dt approached significance.
Conclusions
Peak systolic RV dV/dt predicted PVR even when controlling for metrics used to guide PVR referral, and several left-sided variables were associated with RV remodeling rate.

Poster 7A | Bioengineering

Single-cell determinants of self-organization in the gastruloid

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A characteristic of developmental systems is their ability to organize into a spatially-structured assembly of distinct cell types. While much research into self-organization, particularly in the context of embryogenesis, focuses on the ability of systems to form highly-reproducible structures, their morphologies nevertheless remain heterogeneous. Our work focuses on the gastruloid model of pre-implantation development, which spontaneously breaks symmetry to form the three germ layers and an anteroposterior body axis. Gastruloids display spontaneous emergence of variation in several morphological characteristics even under controlled conditions. The extent to which cell-extrinsic (e.g. morphogen gradients and relative cell position) or intrinsic (e.g. gene expression or lineage) factors dictate the spatial arrangements of cell types in the developing gastruloid therefore remains unclear. Here, we build a latent space of gastruloid morphologies to provide a quantitative description of their heterogeneity. Using fluorescent reporters for gene expression and cell lineage, we track cells through gastruloid development to connect individual cells' behaviors to a gastruloid's body plan. We identify distinct propensities for clonal mESC lineages to differentiate and occupy particular tissue structures of the gastruloid. Ultimately, this work hopes to establish a framework for quantitatively mapping the characteristics of single cells to their differentiation trajectories in the context of a developing tissue.

Poster 8A | Bioengineering

Non-muscle myosin II knockdown disrupts tenocyte morphology and contractility

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Introduction: Mechanical loading is essential to the normal development, homeostasis, and repair of tendon. It is well established that resident tenocytes are mechanoresponsive, but the mechanisms by which these cells sense, transmit, and respond to mechanical stimuli are still unclear. The interaction between actin fibers and non-muscle myosin II (NM-II) is essential for force generation within cells. Non-muscle myosin IIA and IIB, encoded by the genes *Myh9* and *Myh10*, are known to drive morphological changes in epithelial force-generating tissues. The purpose of this study was to clarify the role of non-muscle myosin in tendon cell morphology and contractility using cells isolated from *Myh9*/*Myh10* double-floxed mice. The toxicity and variability of adenoviral vectors are a common pitfall of *in vitro* knockdown models. To address this challenge, we utilized a novel method of *in vitro* recombination, employing recombinant Cre protein modified to include a TAT cell-penetrating peptide in addition to a nuclear localization sequence.

Methods: Animal work was IACUC approved. Tail tendons were dissected from Ai9 Rosa-tdTomato Cre reporter mice (n=3) or *Myh9*$^{f/f}$*;Myh10*$^{f/f}$ mice (n=4) and digested in 2% collagenase IV/1.5% dispase II. Cells were treated with TAT-Cre at 0.5μM or 3μM for 5 hrs in basal media without FBS. Cells were transduced with Ad5-CMV-Cre at an MOI of 50 or 300 for 24 hrs. Twenty-four hrs after treatment, tdTomato signal was assessed in live Ai9+ cells. *Myh9*$^{f/f}$*;Myh10*$^{f/f}$ cells were seeded on glass coverslips. Two days after treatment cells were stained with anti-paxillin and phalloidin. Cell area and solidity were quantified using CellProfiler. Tendon fascicles were isolated from *Myh9*$^{f/f}$*;Myh10*$^{f/f}$ mice (n=4) and cut to 15mm. Free floating explants were cultured in growth media in 12-well plates. Explants were treated with TAT-Cre, blebbistatin, or nothing. The TAT-Cre group was incubated with 3μM TAT-Cre for 5 hrs on days 1, 4, and 7. The blebbistatin group received fresh media with 10μM blebbistatin every 3 days. After 16 days, explants were incubated with 2μM calcein AM and 4μM EthD-1 for 30 minutes and imaged. Treatment groups for monolayer experiments and the live/dead assay were compared using a one-way ANOVA with Tukey post-hoc tests (α=0.05). Explant groups were compared using a repeated measures two-way ANOVA with Tukey post-hoc tests (α=0.05).

Results: Incubation with 3μM TAT-Cre resulted in significantly higher Ai9 recombination than control cells ($p=0.02$). The average recombination rate of cells treated with 3μM TAT-Cre was 26.32% (SD=11.03%) compared to 7.79% (SD=6.72%) among control cells. Neither concentration of Adeno-Cre resulted in effective recombination, even with previous concentrations used successfully by our group. Based on the recombination rates seen in Ai9 cells, only the higher doses of Adeno-Cre and TAT-Cre were used for NM-II knockdown. *Myh9*$^{f/f}$*;Myh10*$^{f/f}$ cells treated with TAT-Cre had disrupted morphology compared to control cells. NM-II knockdown resulted in decreased cell area ($p<0.0001$) and cell solidity ($p<0.0001$). Given the lack of response to Adeno-Cre, functional outcomes were tested in an explant model using only TAT-Cre and the NM-II inhibitor, blebbistatin. Similar to blebbistatin, treatment with TAT-Cre disrupted the ability of tendon cells to contract the free-floating fascicle compared to control tendons ($p=0.05$). There was no significant difference in cell death between groups.

Discussion: This study demonstrates that TAT-Cre is an effective tool for inducing *in vitro* and *in situ* recombination of tendon cells. A concentration of 3μM was sufficient to induce Ai9 recombination in over 25% of cells without noticeable effects on cell morphology or viability. After confirming its efficacy, we used TAT-Cre to illustrate the vital role of NM-II in directing tendon cell morphology through stress fiber and focal adhesion formation. Within 48 hours of knockdown, cells showed altered morphology with decreased cell spreading and solidity. Furthermore, these data establish the necessity of *Myh9* and *Myh10* in tendon contractility as TAT-Cre-mediated gene excision in our explant model was equal to blebbistatin-mediated NM-II inhibition.

Poster 9A | Bioengineering

Ionizable lipid nanoparticles for *in utero* prime editing of Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD), the most prevalent and lethal genetic muscular disorder, affects ~1 in 5000 male births. It is typified by progressive muscle fiber necrosis leading to respiratory insufficiency, cardiac failure, and eventually death**.** Unlike FDA-approved antisense oligonucleotide (AON) therapies and steroidal agents, CRISPR-Cas9 gene editing strategies can permanently restore gene function. Prime editing (PE) represents the most promising improvement on traditional CRISPR-Cas9 by enabling insertions, deletions, and substitutions of nearly any sequence up to ~100 bp. *In utero* gene editing using adeno-associated viruses has enabled partial restoration of dystrophin prior to irreversible muscle pathology while leveraging the small size, naïve immune system, and increased vascular permeability of the fetus. However, viral-based therapies carry significant genotoxicity and risk of insertional mutagenesis. Lipid nanoparticles (LNPs) have emerged as a durable, potent, and biocompatible non-viral delivery platform for nucleic acids in both preclinical and clinical models. LNP-mediated prime editing efficacy *in vivo* remains low (~2%) in part due to the lack of rationally designed formulations for delivering large RNA payloads. Moreover, the pronounced size discrepancy between prime editor RNA (~6.3kb) and guide RNAs (~100-200bp) suggests that engineering LNPs for separate encapsulation of these components may enhance PE efficacy, diverging from the co-delivery approach employed in most gene editing applications. In particular, the impact of ionizable and helper lipid choice and relative ratios of lipid components on the encapsulation and endosomal release of PE RNA components in fetal myocytes is poorly understood. It is hypothesized that the specific lipid components and their relative ratios that will enhance encapsulation and delivery of editor versus guide RNAs to fetal myocytes will differ; and rationally designed PE LNPs will demonstrate greater therapeutic amelioration of DMD with similar cytotoxicity compared to an LNP clinically validated for general RNA delivery. First, this study will assess the efficacy of conjugating antibodies targeting receptors specific to fetal myocytes to LNPs for muscle-specific delivery. Further screening will evaluate the efficacy of novel ionizable and helper lipids containing functional groups that destabilize LNPs preferentially within the endosomal compartment. Finally, formulations engineered with these novel lipids and their relative ratios to other lipid components will be evaluated *in vivo* for their therapeutic efficacy in a mouse model of DMD.

Poster 10A | Biology

Spatial Association of Dog Mobility and Development of a Spill-Over Model of Hydatid Disease in an Echinococcosis-affected area in Junín, Peru

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Cystic Echinococcosis (CE) is a zoonotic parasitic disease that is caused by *Echinococcus granulosus* and generates a significant burden in humans. While dogs serve as the definitive host for *E. granulosus*, eggs shed in dog feces hatch upon consumption by an intermediate host, such as sheep or other herbivores. In the intermediate host, parasite larva contribute to slow-growing hepatic and pulmonary cystic lesions. Humans that consume *E. granulosus* eggs can become infected and develop such lesions, which often go undetected for years until cysts grow large enough to contribute to life-threatening disease that warrants surgical intervention. While the prevalence of CE in the Central highlands of Peru has previously been estimated around 5-7%, disease experts agree that the true prevalence is likely much higher given the protracted period of asymptomatic disease, reduced medical access of at-risk populations and poor understanding of local disease epidemiology.

This study sought to quantify the epidemiological behavior of CE in Pachacayo, Peru, with the goal of developing and measuring the efficacy of future intervention programs. To address this goal, this study implemented three distinct strategies: 1) placement of GPS-collars to tract dog mobility to understand the movement of the definitive host of *E. granulosus* on the landscape, 2) detection of *E. granulosus* positive dogs using copro-ELISA, and 3) engagement with local community leaders and healthcare providers to better understand the impact of disease in the study area and surrounding communities.

Preliminary results suggest that dogs are visiting local slaughterhouses, which may provide access to infected viscera from slaughtered sheep, allowing the persistence of the *E. granulosus* life cycle. Results from the copro-ELISA indicate that approximately 75% (15/20) of the dogs tested in our study were likely positive for *E. granulosus*. Moreover, through conversations with community leaders and healthcare providers, we were informed that there are 410 people who are either suspected or confirmed to be infected with CE within the last 3 years. This number represents 52% of the human population in the study area, suggesting a very high infection rate. If accurate, this estimate of human disease corroborates our copro-ELISA findings and is likely correlated with the high parasite prevalence in dogs. These preliminary results are being used to inform our understanding of the local disease ecology and the development of future intervention strategies targeted to reduce human cases.

Poster 11A | CAMB - Cancer Biology

Identification of regulators of CAR T persistence in solid tumors

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Chimeric Antigen Receptor (CAR) T cell therapy has had success in certain hematological malignancies. However, this therapy has faced challenges to translating to solid tumors, including inadequate *in vivo* persistence of CAR T. Recently, several novel regulators of CAR T function have been identified through CRISPR screening. However, these approaches primarily utilized in vitro assays, which often fail recapture the full complexities of an in vivo microenvironment, including CAR T tumor-honing and infiltration, as well as persistence in a metabolically challenging and immunosuppressive environment. As such, successful performance of an *in vivo* screen may better identify directly translatable perturbations. We perform a genome-scale *in vivo* genome scale CRISPR knockout (KO) screen in tumor-infiltrating human CAR T cells (CAR-TIL), using an osteosarcoma NSG model targeted by HER2.BBz CAR T cells. As we utilize a compact screening approach through Cas12a, an alternative Cas nuclease, our genome-scale screen only requires 30 mice. Results from this screen faithfully recapitulate known regulators of CAR T persistence, such as MED12, CCNC, and FLI1, while also identifying novel regulators that have yet to be described.

Poster 12A | CAMB - CAMB – Cancer Biology

Effects of KRAS inhibition on anti-tumor immunity in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is estimated to become the second-leading cause of cancer-related deaths by 2030, highlighting the need for alternative targeted therapies. In over 90% of patients, PDAC is driven by activating mutations of the small GTPase KRAS. Mutations render KRAS active in its GTP-bound state, leading to constitutive downstream pathway activation and promotion of cancer cell proliferation, invasion, and survival. In addition to its cell-autonomous role, mutant KRAS signaling drives the establishment of an immunosuppressive tumor microenvironment (TME) characteristic of PDAC tumors, including through the inhibition of cytotoxic CD8+ T cells and recruitment of myeloid-derived suppressor cells (MDSCs). The "cold" PDAC TME can explain the lack of response to immunotherapies that relies on robust adaptive T cell immunity. Therefore, targeting oncogenic KRAS is an attractive therapeutic strategy to combat both cell-autonomous and non-cell-autonomous phenomena that make PDAC highly unresponsive to existing therapies.
Recently, our lab and others showed that pharmacological inhibition of $KRAS^{G12D}$, the predominant mutant form of KRAS in PDAC (40%), has dramatic anti-tumor effects in multiple PDAC models. Notably, $KRAS^{G12D}$ inhibition also promotes the infiltration of T cells into cold PDAC tumors, suggesting that KRAS inhibition can augment anti-tumor immunity. However, recent data from clinical trials have shown that resistance to KRAS inhibitors that target single allele mutations can arise quickly, including through upstream pathway reactivation of wild-type RAS signaling. To circumvent resistance to allele-specific inhibitors, an emerging therapeutic strategy is broadly targeting multiple RAS mutations and RAS isoforms using "RAS^{multi}" inhibitors. Importantly, the effects of broad-spectrum RAS inhibition on immune cells, which utilize RAS signaling for their function, remains unknown. Thus, in this study, we investigated the effects of RAS^{multi} inhibition on the PDAC TME. To recapitulate human disease, we utilized the "KPC/Y" autochthonous mouse model in which mutant $KRAS^{G12D}$ and mutant $Trp53^{R172H}$ are expressed in pancreatic epithelial cells that can be identified with a YFP lineage label. Clonal cell line derivatives from KPC/Y mice give rise to tumors with varying amounts of T cell infiltration when re-implanted into immunocompetent mice, allowing us to model a "hot" or "cold" PDAC TME. We find that RAS^{multi} inhibition dynamically alters the TME of cold tumors by promoting the infiltration of anti-tumor T cells and macrophages while reducing the infiltration of MDSCs. Importantly, T cell depletion studies show that T cells can contribute to the anti-tumor response of RAS^{multi} inhibition *in vivo*. Moreover, RAS^{multi} inhibition has minimal effect on CD8+ T cell activation and proliferation at relevant doses *in vitro* and can potentiate antigen-specific CD8+ T cell expansion *in vivo* at an early timepoint. Furthermore, RAS^{multi} inhibition in combination with immunotherapy effectively and tolerably improves the depth and durability of tumor regressions in multiple PDAC models. Taken together, we conclude that broad-spectrum RAS inhibition can successfully relieve oncogenic KRAS-driven immunosuppression in the PDAC TME while sparing anti-tumor T cell function. Ultimately, RAS^{multi} inhibition can effectively combine with immunotherapy regimens and can sensitize cold PDAC tumors to immunotherapy.

Poster 13A | CAMB - Developmental, Stem Cell, and Regenerative Biology

Illuminating the Molecular Mechanisms of Replication and Transcription Coordination

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The goal of this proposal is to understand how replication and transcription are spatially and temporally coordinated at molecular scales during embryonic development. A long-standing assertion is that RNA Pol II (Pol II) is completely evicted from DNA during replication to avoid steric conflict between transcription and replication machinery. This eviction hypothesis rests largely on in vitro or population and time-averaged genomics or biochemical experiments. These either may not recapitulate endogenous contexts or are unable to capture the interactions kinetics of Pol II's association with chromatin with adequate temporal resolution (of seconds to minutes). In fact, recent in vitro experiments suggest that Pol II is not completely evicted from chromatin but remains in proximity (~40nm) to promoters and gene bodies during replication. This relationship is facilitated by Pol II interacting with PCNA, a sliding clamp protein associated with actively replicating domains in the nucleus that grow and disappear as replication completes. These contradictory findings call into question the molecular mechanisms that govern the coordination of transcription and replication machinery. I propose to use advanced live microscopy and single molecule tracking to directly measure the interaction kinetics of transcription and replication proteins in real time in developing *Drosophila* embryos. This work will provide molecular scale insights on the coordination of replication and transcription in an in vivo context.

Our lab has established high resolution light-sheet microscopy which enables tracking of single protein molecules within the nuclei of live *Drosophila* embryos. Single molecule tracking reveals how individual proteins move within the nucleus and the kinetics of protein-protein and protein-chromatin interactions. Using these approaches along with perturbations to replication and transcription, I will investigate the chromatin binding kinetics of Pol II and PCNA as transcription is activated post-replication. To further understand how transcription is reactivated post-replication I will similarly investigate the distributions and kinetics of the pioneer transcription factor Zelda that is a ubiquitous activator in *Drosophila* embryos. Preliminary data from our lab shows that Zelda is excluded from domains of active replication marked by PCNA yet is detected near these sites. I hypothesize that i) Pol II is retained within actively replicating domains to avoid complete eviction from chromatin to enable rapid re-engagement and that ii) Zelda swiftly transitions from its excluded state post-replication to engage at these sites to facilitate transcriptional activation.

Poster 14A | CAMB - Developmental, Stem Cell, and Regenerative Biology

Investigating the Formation and Remodeling of Uterine Spiral Arteries during Pregnancy

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Proper uterine vascularization is critical for a healthy pregnancy. Abnormalities in uterine vascular density are associated with infertility. Moreover, incomplete remodeling of the spiral arteries (SAs), vessels which transport maternal blood into the placenta for maternal-fetal nutrient exchange, is associated with the hypertensive disorder preeclampsia. Although uterine blood vessels, especially SAs, play an essential role in supporting pregnancy, little is known about how these vessels develop and remodel. The objectives of this proposal are therefore to 1) characterize the formation of SAs on a cellular and transcriptional level, and 2) define the molecular mechanisms underlying SA remodeling. While it is known that SAs are present in the uterus by mid-gestation, a mechanism for their formation has not been previously proposed. Our preliminary data indicate that SAs do not form via vasculogenesis or arterial sprouting, two common mechanisms by which new arteries develop. Thus, we hypothesize that SAs form via an alternative mechanism: angiogenesis from uterine veins. In Aim 1, we will rigorously test this hypothesis using light sheet imaging, lineage tracing, and single nucleus RNA sequencing. Additionally, we have detected high expression of the vascular remodeling protein Angiopoietin 2 (ANG-2) in the uterus during pregnancy. ANG-2 destabilizes blood vessels in a number of developmental and pathologic contexts by promoting the dissociation of smooth muscle cells from endothelial cells. In the uterus, the loss of smooth muscle cells from SAs appears to initiate SA remodeling. We hypothesize that ANG-2 is required for this process. In Aim 2, we will use pharmacologic and genetic methods to investigate how loss of ANG-2 signaling affects SA remodeling and pregnancy outcomes. Together, these studies will bring novel insights into the mechanisms by which blood vessels form and remodel in the uterus, which could have important implications for our understanding of infertility, preeclampsia, and other vascular complications of pregnancy.

Poster 15A | CAMB - Gene Therapy and Vaccines

Development of therapeutic myeloid cells to deliver IL-12 specifically within the glioblastoma tumor microenvironment

Orlando Arevalo and Saar Gill

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Glioblastoma (GBM) is the most common primary brain tumor with a poor overall survival that has not significantly improved after decades of research. Recent advances in tumor immunotherapy have highlighted the potential for leveraging the patient's immune system to control GBM tumors but have failed to demonstrate significant efficacy, potentially due to the immunosuppressive tumor microenvironment (TME). Therefore, recent work has aimed at shifting the GBM TME to enhance immune cell activity by delivering proinflammatory cytokines, such as interleukin-12 (IL-12), to the TME. While administration of IL-12 has demonstrated efficacy in controlling GBM tumors, systemic exposure to IL-12 leads to dose-limiting toxicity. I herein propose a strategy to achieve spatiotemporal control of IL-12 delivery within the GBM TME by introducing synthetic genetic circuits, such as synthetic Notch (synNotch) receptor circuits, into myeloid cells capable of trafficking to the GBM TME. In cells engineered with these circuits, the desired transgene, within a synNotch responsive cassette, is only expressed when the synNotch receptor binds its ligand and is cleaved to release a synthetic transcription factor. In my first aim, I will generate and characterize an optimal synNotch circuit for transgene induction specifically within the human GBM TME. I will generate circuits targeting different GBM-associated antigens, introduce them into human monocytes, and assess the basal and induced transgene expression. Optimal circuits will be subsequently tested in human GBM xenograft mouse models to assess engineered cell trafficking to the GBM tumor as well as intratumoral and systemic IL-12 production. In Aim 2, I will assess the use of circuit-engineered hematopoietic stem and progenitor cells (HSPCs) for perpetual production of therapeutic myeloid cells as a novel platform for GBM immunotherapy. Engineered macrophages are capable of trafficking to the GBM TME and exerting antitumor efficacy, but their therapeutic potential is limited due to lack of *in-vivo* proliferation and persistence. Therefore, I will engineer murine HSPCs to express a synNotch receptor circuit, currently used in GBM clinical trials, only within myeloid cells and transplant these cells into an immune-competent murine GBM model. I will then determine the tumor control capability, systemic proinflammatory toxicity, and persistence of this novel cell therapy. Overall, I hypothesize that inserting a GBM-specific synNotch receptor circuit within a myeloid specific locus within HSPCs will lead to the perpetual production of myeloid cells with the ability to produce IL-12 specifically in the GBM TME, leading to continuous tumor control with limited systemic toxicity. The studies proposed here will establish a novel autologous cellular therapeutic platform with the potential to control spatiotemporal expression of potent anti-tumor therapies within the GBM TME that would not otherwise be tolerated using systemic administration. Additionally, by simply changing the antigen of the synthetic receptor or the cytotoxic cargo, this platform can be used to target any myriad of solid tumors, highlighting the modularity and clinical translatability of this potential therapy.

Poster 16A | CAMB - Genetics and Epigenetics

Role of Sympathetic Innervation in the Development of Brown Adipose Tissue

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The heat-generating activity of brown adipose tissue (BAT) increases resting energy expenditure and is protective against obesity in mice. In response to cold temperatures, BAT undergoes tissue remodeling and increased heat production (thermogenesis). In adult mice, BAT remodeling and thermogenesis are completely dependent on sympathetic signaling. However, BAT initially acquires its thermogenic phenotype during embryonic development, and the role of sympathetic innervation in this process is unknown. Here, we show that brown adipocytes are able to induce the thermogenic gene expression program in the absence of sympathetic innervation. In contrast, as soon as 2 days after birth, BAT requires sympathetic innervation for cold-induced gene expression changes. These results indicate that alternative mechanisms underly the molecular development of brown adipocytes during embryogenesis. Further work will determine whether downstream components of sympathetic signaling, such as adrenergic receptors and second-messenger factors, remain engaged in and necessary for BAT development in the absence of sympathetic nerves.

Poster 17A | CAMB - Genetics and Epigenetics

Thrombin signaling in placental development

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Coagulation is initiated by exposure of tissue factor (TF) to clotting factors in the blood. This results in a cascade of proteolytic cleavage steps ending with the conversion of prothrombin to mature thrombin. Thrombin is the most potent activator of human platelets, and this response is mediated by the GPCR PAR1. Approximately 50% of *F2* (encoding prothrombin) and *F2r* (encoding PAR1) knockouts (KOs) die around E10.5. The cause of this incompletely penetrant lethality has not been adequately explained. Data from our lab shows that endothelial cell (EC) or placental PAR1 deficiency recapitulates the partially penetrant lethality seen in the global *F2r* KO. Together, these data suggest that thrombin-PAR1 signaling is specifically required in placental ECs during midgestation. I hypothesize that thrombin activity promotes placental angiogenesis through PAR1. I propose to characterize the placental vascular requirement for PAR1 through lightsheet imaging of mutant placentas, and observation of EC PAR1 activation through the use of a PAR1-Tango reporter. I will investigate the role of PAR1-mediated adherens junction remodeling in angiogenesis by assessing for genetic interaction between *F2r* and *Cdh5* (encoding VE-cadherin). Lastly, I will evaluate the requirement for trophoblast TF in activating EC PAR1 through conditional ablation of *F3* (encoding TF) in epiblast and trophoblast and assessing PAR1-Tango activity and lethality.

Poster 18A | CAMB - Genetics and Epigenetics

Mapping of the neural connectome

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In the human brain, billions of neurons are interconnected via trillions of synapses, transmitting electrical signals that underlie brain function including perception, learning, and memory. Proper synaptic connections are crucial for healthy brain function; dysregulated connectivity contributes to various neurological disorders such as autism, psychological disorders, and Alzheimer's disease. Current methods for mapping neural connectomes are limited by low throughput and specialized technological requirements, limiting them to a select few labs globally. These constraints hinder progress in understanding brain diseases and developing effective therapies.

To overcome these challenges, I am developing SynGram-seq, a novel molecular technology utilizing cell barcoding and in situ sequencing. SynGram-seq aims to facilitate rapid and reproducible mapping of neural connectomes. Using a model of rat hippocampal neurons, we have established localization of cell barcodes throughout the neuron and developed an improved method of in situ sequencing to readout barcodes in both the soma and distal neurites. We are currently optimizing in situ sequencing to increase efficiency of signal in the neurites and have validated a system of neural activation in preparation for testing engineered proteins to label activated synapses.

Poster 19A | CAMB - Genetics and Epigenetics

A novel mendelian neurodevelopmental disorder caused by germline variants in MAP2K4

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Our group has recently built a cohort of individuals with novel variants in Mitogen-Activated Protein Kinase Kinase 4 (MAP2K4). MAP2K4 is an enzyme that modulates cytoskeletal proteins and nuclear substrates, including transcription factors, by activating c-Jun N-terminal kinase (JNK) through phosphorylation. Based on gnomAD constraint metrics, *MAP2K4* is predicted to be highly intolerant to both loss-of-function (LOF, pLI=1) and missense variants (z=3.22). Our cohort of affected individuals harbor germline variants in *MAP2K4* that are heterozygous, predominantly *de novo*, a combination of missense and nonsense variants, and all cluster in the kinase domain. While germline mutations in *MAP2K4* have not been previously described, somatic mutations are implicated in various cancer types with three recurrent mutations seen in the Catalogue of Somatic Mutations in Cancer (COSMIC) — Ser184Leu, Arg134Trp, Arg281*. Notably, the Arg281* nonsense substitution is also seen in our cohort.

In this cohort of 9 unrelated individuals, clinician reported phenotypes include developmental delay (71%, 5/7), intellectual disability (75%, 3/4), limb anomalies (71% 5/7), craniofacial differences (62.5%, 5/8) and other variable systemic differences. Guided by our patients' predicted LOF variants and predominantly neurological phenotypes, we leveraged an existing systemic knockout *Map2k4* mouse model to investigate global perturbations in CNS signaling using paired proteomics and phospho-proteomics. Our proteomics data showed dysregulation of pathways involved in dendrite formation, axonogenesis, and intracellular trafficking, which is in line with neurodevelopmental phenotypes in our cohort. On-going functional work includes interrogating kinase activity of missense variants *in vitro*; structural modeling *in silico*; growth and developmental assessments *in vivo;* and micro-CT evaluation of craniofacial phenotypes *in vivo* with the objective of further elucidating the pathogenic mechanism of this novel disorder.

Poster 20A | CAMB - Genetics and Epigenetics

Investigating the mechanisms of polycomb group protein mediated fetal hemoglobin silencing

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The fetal to adult hemoglobin switch is a developmental event in erythroid cells that reconfigures the beta globin locus from a transcriptional environment permissive to transcription of fetal type globin (HbF) to an environment that favors transcription of adult beta globin. We recently demonstrated that polycomb group proteins (PcG) participate in fetal globin silencing not by directly repressing the fetal globin genes but instead repressing the fetal cell fate drivers LIN28B and IGF2BP1/3 (Qin et al, Blood 2023). PcG proteins are a highly diverse group of epigenetic modifiers, and their collective activity is critical for modulating gene expression in a wide range of tissues. Moreover, PcG proteins are involved in silencing important developmental and cell cycle regulatory genes, and can be aberrantly expressed or mutated in a variety of malignancies. Given this multitude of PcG functions, there is an incentive to develop PcG inhibitors that are more selective for HbF activation. Due to the inherent complexity of PcG proteins, specific PcG compositions may exist in erythroid cells that preferentially affect genes involved in HbF silencing such as LIN28B or IGF2BP1/3. By defining the specific PcG proteins involved in HbF silencing, it may be possible to selectively perturb HbF silencing without inducing potential pleiotropic effects on erythroid cell function. To identify novel PcG proteins and specific regions of PcG proteins involved in HbF silencing, we performed a high density CRISPR-Cas9 screen targeting nearly all PAM sites in the polycomb repressive complex 2 (PRC2) as well as most domain encoding regions of polycomb repressive complex 1 (PRC1). This identified a novel domain in EZH2 (part of PRC2) and a novel subunit of PRC1 as HbF regulators. In validation studies, we used CRISPR-Cas9 to introduce targeted indels in these key candidate regions and were able to markedly decouple HbF silencing from defects in erythroid development. These findings suggest that perturbing specific PcG proteins or specific regions of PRC2 subunits may allow for significant de-repression of fetal hemoglobin without causing dramatic effects on erythroid cell viability or maturation. We will present these findings and discuss ongoing studies on deciphering the molecular mechanisms of PcG target selectivity.

Poster 21A | CAMB - Microbiology, Virology, & Parasitology

Antiviral Role of Interferon Epsilon in Human Neurons

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There are several human antiviral cytokines with distinct roles in physiology and pathology. Notably, type I interferon (IFN) has many subtypes that signal through the same receptor. Interestingly, different type I IFN subtypes exert varying downstream responses depending on cell type. Fascinatingly, I have uncovered that IFNε, a type I IFN, is constitutively expressed in the human CNS, independent of acute virus infection. Moreover, IFNε expression in the human CNS increases with age. Despite this identified expression pattern, the role of IFNε constitutive expression in the human CNS remains unknown.

To examine the regulation and antiviral role of IFNε in human neurons, I employed human iPSC-derived neurons in the form of 2-D i3Neurons and 3-D forebrain organoids. Stimulation of neurons with IFNε induces upregulation of interferon-stimulated genes (ISGs), such as IFIT1 and OAS2, and resulted in restriction of neurotropic virus infection. To further explore alternative roles of IFNε in neurons, I compared ISG expression signatures in IFNε-treated i3Neurons and a control cell line. Additionally, I demonstrate that IFNε expression increases significantly during neuronal maturation from iPSCs, paralleling human age-related expression data. Promoter analysis of IFNε suggests regulation by NF-κB and progesterone. Using pharmacological agents, I show that NF-κB limits IFNε mRNA expression, while progesterone exerted no significant effect on IFNε expression, which differs from the regulation seen in female reproductive tract. Lastly, I plan to knockout IFNε in both model systems to assess impact of constitutive IFNε expression on viral susceptibility and overall neuron health. Exploring the significance of constitutive IFNε signaling in neurons can uncover novel underlying mechanisms and therapeutic targets to combat neurotropic virus infections.

Poster 22A | Chemistry

Complementary Chemical and Cell Autonomous Strategies to Control Engineered Cell Therapy

Angela Gong and Mark Sellmyer

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Given CAR T cells' inherent self-regulating behaviors as "living drugs," the ability to control CAR expression, and thus CAR-T cell signaling and activity, could help mitigate toxicities and/or improve therapeutic efficacy. In this proposal, I will explore and compare two different strategies for controlling therapeutic proteins, such as CARs. In Aim 1, I will test methods to systematically improve PROTACs, which can control CARs that have been appended with a genetic tag. Our lab has previously investigated the ability of trimethoprim (TMP)-based proteolysis-targeting chimeras (PROTACs) to regulate *E. coli* dihydrofolate reductase (eDHFR)-tagged proteins, including CARs. *E. coli* dihydrofolate reductase (eDHFR) and trimethoprim (TMP) is a highly specific protein tag-ligand pair that has been engineered for many experimental and biomedical applications. While our lab has previously optimized *in vitro* efficacy, the *in vivo* pharmacokinetics of TMP-PROTACs need to be further optimized. Aim 1 seeks to improve TMP-PROTACs via the development of data-driven workflows to inform exploration of PROTAC linker space and eventual compound optimization for biological activity and pharmacokinetic properties. However, it may be difficult to optimize the perfect time to administer existing small molecules for CAR regulation in the setting of acute toxicity. Genetically encoded, *ex vivo* engineered technologies could address this challenge. Aim 2 investigates the incorporation of CAR-targeting bioPROTACs into synthetic circuits for cell autonomous modulation of CAR-T cells. Together, these aims will explore both chemical and synthetic biology strategies to regulate CAR-T therapy.

Poster 23A | Epidemiology & Biostatistics – Biostatistics

Performance drift in a national mortality risk prediction model

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Risk prediction algorithms are used to guide clinical decision making, to inform risk adjusted payment plans, and to generate hospital quality metrics. Algorithms built on patient electronic health records are prone to performance changes after deployment. One main reason is that clinical practice patterns and documentation change over time, leading to a deviation between the distributions of the data used to train an algorithm and those of the data generated in real time. This algorithmic drift can lead to misinformed clinical decision-making and have consequential impacts on patients and health care systems. We study the extent, data shift mechanisms, and impact of model performance drift in a nationally deployed mortality risk prediction model. We find that between 2016 and 2021, the model declines in positive predictive rates and true positive values, leading to a longitudinal under-identification of patients who are at high risk for the mortality outcome. We also identify covariates related lab and hospital utilization as well as demographic variables that significantly shift in distribution. Finally, we see a decline in the reliability of a national quality metric generated using algorithmic classifications.

Poster 24A | Genomics and Computational Biology

Decoding human cardiac aging with single-nucleus RNA sequencing

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Cardiovascular disease is the leading cause of death worldwide. Among the greatest risk factors for cardiovascular disease is age – the risk of heart disease doubles every decade after age 65. To identify changes in gene expression across human cardiac aging, we generated a single-nucleus RNA sequencing (snRNA-seq) dataset of left ventricle tissue from 17 human donors with no documented cardiovascular disease. We combined our cohort with 9 other undiseased human left ventricle snRNA-seq datasets, obtaining a combined dataset of 805,428 high-quality nuclei from 104 donors. Additionally, we generated a single-nucleus Assay for Transposase Accessible Chromatin sequencing (ATAC-seq) of 6 non-diseased donors which we complemented with 8 snATAC-seq samples from 2 external datasets. Although we identify genes with sex-specific gene expression and chromatin accessibility differences, we recover few genes that are differentially expressed with age. However, we do observe a decrease in the proportion of cardiac neuronal cells with increased age. We also identify a loss of cell-cell interactions with aging. Our study combines a new dataset with existing datasets to provide a comprehensive analysis of changes in the cardiac transcriptome across the human lifespan.

Poster 25A | Health Care Management and Economics

Healthcare Heroes' Shield of Armor: Causes and Consequences of Callousness in Healthcare Workers

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Research Objective: Callousness—the display of impersonal, insensitive, and cynical behavior towards others—is increasing among healthcare professionals, yet there has been little theoretical or empirical investigation of it. Some literature suggests that callousness is a protective mechanism to prevent internalizing work-related trauma, but callous behavior in healthcare, where compassionate bedside manner and effective teamwork are paramount, is likely to have significant negative consequences. This study aims to: (1) distinguish callousness from related interpersonal workplace phenomena, (2) identify organizationally related predictors of callousness and its incidence by work context, and (3) identify consequences of callousness for healthcare workers and quality of care. Addressing these aims allows for development of recommendations of strategies that healthcare leaders can use to mitigate increasing callousness in the healthcare workforce.
Study Design: We conducted a two-time point study of healthcare workers using surveys administered six months apart, and linked workers' survey data to their units' patient mortality indices and other characteristics obtained from administrative records. The survey assessed self-reported callousness, psychological safety, work-life balance, intent to stay in one's job, and proactive behavior. We calculated descriptive statistics and used ordinary least squares regression and multi-level mixed effect models to assess associations between work-life balance and psychological safety as predictors of callousness, and intent to stay in job, proactive behavior, and patient mortality as consequences of callousness.
Population Studied: We analyzed data from clinical staff (attending physicians, fellows, residents, physician assistants, nurse practitioners, and registered nurses) affiliated with 20 intensive care units (ICU) across 7 hospitals in one health system (N = 557 and 153 at Time 1 and Time 2, respectively).
Principal Findings: Work-life balance and psychological safety had significant, negative relationships with callousness (b = -0.39, $p < 0.01$ and b = -0.53, $p < 0.01$, respectively). Type of ICU (specialized versus mixed care) significantly moderated the negative relationship for work-life balance (b = -0.2, $p < 0.01$) and marginally moderated for psychological safety (b = -0.41, $p < 0.1$) such that that the mitigating effects of these factors against callousness were stronger for mixed compared to specialized units, and more significant for work-life balance than for psychological safety. Individuals' own callousness was negatively associated with intent to stay across time points (Time 1: b = -0.23, $p < 0.01$; Time 2: b = -0.21 , $p < 0.01$), and negatively associated with proactive behavior at Time 1 (b = -0.08, $p < 0.01$). We found no effect of aggregate unit-level callousness on unit-level patient mortality.
Conclusion: Callousness at work has significant individual and organizational consequences that merit continued study. It is associated with lower intent to stay and decreased proactive behavior which have been linked to increased workforce turnover, decreased clinical quality improvement efforts, and decreased organizational learning. Work-life balance and psychological safety are two contextual predictors of callousness that managers can address to protect against its development, and the protective effect of these predictors varies by practice setting.
Practice implications: Healthcare organizations should consider interventions to improve work-life balance and psychological safety to mitigate the development of callousness in the workforce.

Poster 26A | Health Care Management and Economics

Identifying Low Acuity Emergency Department Visits with a Machine Learning Approach: The Low Acuity Visit Algorithms (LAVA)

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This study aimed to enhance the identification of low acuity Emergency Department (ED) visits by using machine learning methods to improve upon existing ICD code rule-based algorithms. Using National Hospital Ambulatory Medical Survey data from 2016-2020, baseline performance metrics were established with seven published rule-based algorithms. Logistic regression, random forest, and gradient boosting models were trained on five covariate sets of demographic and clinical data, and their performance was compared using a validation dataset. The primary metric was the positive predictive value (PPV). Training data included 53,074 observations, and validation data included 9,542 observations. Model-based algorithms consistently outperformed existing ones, with the most effective model, XGBoost trained on all variables, improving PPV by 83% (to 0.64 (95% CI [0.62, 0.66]). These models also demonstrated higher PPV across all demographic subgroups. The findings suggest that machine learning models significantly outperform existing ICD code-based algorithms in predicting low acuity ED visits. However, variations in performance highlight the need for further research to ensure equitable applicability across diverse populations.

Poster 27A | Health Care Management and Economics

Allocation of Medicare Reimbursement Based on Social Disadvantage: Empirical Assessment Using Area-Level Indices

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Background: The Centers for Medicare and Medicaid Services (CMS) adjust reimbursements in population-based payment models to account for baseline clinical risk of attributed patients. New payment models, including ACO REACH, seek to also account for the impact of health-related social needs on patient outcomes. These models allocate additional resources to providers who care for socially disadvantaged populations, to advance health equity goals including increasing safety-net participation in payment models, preventing selection against disadvantaged patients, and expanding access. However, it is unclear whether social risk contributes to variation in health outcomes independent of clinical risk. The objective of this study was to determine the association of an area-level index of social disadvantage, the Area Deprivation Index (ADI), with mortality and hospitalizations among Medicare beneficiaries after adjustment for clinical risk using Hierarchical Condition Categories (HCCs).
Methods: We conducted a retrospective, cross-sectional study using administrative claims data for Medicare fee-for-service (FFS) beneficiaries. We used patient-level multivariable generalized linear models to estimate the association between national ADI percentile in 2018, as determined by patient 9-digit ZIP code, and mortality and hospitalization outcomes in 2019, adjusting for covariates including age, sex, dual-eligibility for Medicare and Medicaid, disability status, and 86 distinct HCCs. We used a random sample of 5 million Medicare FFS beneficiaries. We excluded those with end-stage renal disease, Medicare Advantage enrollment, or a non-Medicare primary payer. In our final sample of 3,408,730 beneficiaries, mean age was 72.4 (SD 11.4), 55.0% were female, 18.4% were dual-eligible, 8.0% were of Black race, and 5.4% were of Hispanic ethnicity.
Results: In 2019, 147,230 (4.3%) beneficiaries died and 536,194 (15.6%) were hospitalized. After adjusting for patient characteristics, ADI percentile was significantly associated with mortality. For each percentile increase in ADI, mortality increased by 0.01 percentage points (pp, 95%CI 0.01 to 0.01). Hospitalization also increased by 0.03pp (95%CI 0.02 to 0.03) for each percentile increase in ADI. Patients in the highest (most disadvantaged) ADI decile had 1.4pp greater mortality and 2.6pp greater incidence of hospitalization than those in the lowest decile.
Conclusions and Implications: An area-level index of social disadvantage was associated with mortality and hospitalizations for Medicare beneficiaries even after risk adjustment for chronic illness burden and other patient factors. This study provides empirical support for allocating resources to providers on the basis of social risk of their attributed patients, in addition to clinical risk, as in CMS's ACO REACH and other models. Recent literature shows that adding area-level social indices directly into existing models that predict healthcare spending would *decrease* resource allocation to socially disadvantaged populations. However, our findings demonstrate that patients living in areas with greater social disadvantage experience higher rates of mortality and hospitalization independent of clinical risk, highlighting an opportunity for additional investment to prevent adverse outcomes and improve health equity. Future studies should investigate implications of different approaches to measuring and adjusting for social risk.

Poster 28A | Immunology

Hepatic CD9 regulates adipose tissue inflammation and metabolic dysfunction during obesity

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Obesity is a major cause of morbidity and mortality as it increases the risk of type 2 diabetes and cardiovascular disease, among other conditions. In response to excess caloric intake, adipose tissue (AT) undergoes extensive remodeling leading to expansion, inflammation, and ultimately fibrosis. Extracellular vesicles (EVs) are nanometer-sized lipid particles that facilitate intercellular and interorgan communication via the transport of cargo molecules. Recently, it has been suggested that EV-mediated communication between the liver and adipose tissue is critical for proper AT remodeling, however, the mechanisms of this communication network are not well known. The tetraspanin CD9 is a membrane protein necessary for the biogenesis, content selection, release, and uptake of a subset of EVs. We hypothesized that CD9 regulates an EV-mediated liver-AT axis during obesity. Using a novel hepatic-cell-specific CD9 knock-out mouse (CD9 HKO), we found that deletion of CD9 leads to decreased production of EVs by primary hepatocytes. Next we subjected CD9 HKO and control mice to a model of diet-induced obesity. We found that obese CD9 HKO mice have increased AT inflammation, including a 4-fold increase in adipose tissue macrophages. Furthermore, CD9 HKO mice are more susceptible to obesity-associated sequelae, including glucose intolerance, ectopic lipid deposition, and hyperlipidemia, despite similar weight gain compared to controls. Overall, our results suggest that hepatic CD9 dampens AT inflammation, ultimately protecting against the development of obesity-associated sequelae. Our work has identified a novel axis by which liver-derived CD9 regulates AT inflammation and metabolic dysfunction during obesity. Future studies will aim to understand the mechanisms by which CD9 influences hepatic EV contents and functions in adipose tissue.

Poster 29A | Immunology

Optimizing a mouse model to study the longevity of vaccine-induced humoral responses

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Nucleoside-modified messenger RNA (mRNA) vaccines encapsulated in lipid nanoparticles (LNPs) can elicit potent germinal center reactions, which drive the generation of affinity-matured plasma cells and protective antibodies (Abs). Published studies highlighted a discordance between Ab kinetics in mice and humans after mRNA vaccination, calling into question whether mice are suitable models for predicting the durability of humoral responses in humans. Indeed, Balb/c and C57BL/6 mouse models of mRNA-LNP vaccination have demonstrated virtually no Ab decay up to a year post-immunization. By contrast, human studies have suggested that Ab titers decline rapidly in the first months after mRNA-LNP vaccination (one paper suggesting $t_{1/2}$ = 66 days in SARS-CoV-2-naïve individuals; another suggesting a 5-fold decay in the first three months), followed by a stabilization phase observed up to a year. Since murine Ab kinetics do not mimic this early-phase decay seen in humans, we aimed to optimize a mouse model of mRNA-LNP vaccination that would more accurately predict the durability of humoral responses in humans.

We pursued two hypotheses: 1) mRNA-LNP vaccine doses used in mouse studies are exceedingly high compared to human dosages. Most published studies of mRNA-LNP vaccination in mice use 75-150x the standard human mg/kg dosage. By comparing vaccine doses between 1 ug and 30 ug mRNA per mouse, we found that the magnitude of binding Ab titers followed a dose-response curve, but waning was not apparent even at our lowest doses. 2) Adult laboratory mice have more naïve immune systems than adult humans because of a lack of pathogen exposure in specific-pathogen-free housing facilities. Published work has suggested that mice with a more experienced immune system might mount less durable humoral responses to vaccination. We developed a sequential immunization/infection model to expose mice to a multitude of antigenic stimuli. We then immunized these mice (and control mice) with low-dose mRNA-LNP vaccination. Our preliminary data suggest that sequentially immunized/infected mice do not differ from mock immunized/infected mice in the magnitude of Ab titer elicited, and ongoing efforts are aimed to assess the longevity of humoral response. Together, with these two complementary strategies we seek to develop an optimized mouse model that better recapitulates human Ab kinetics post-vaccination.

Poster 30A | Immunology

Can microbial molecular mimics protect from type 1 diabetes?

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The early-life gut microbiota protects from type 1 diabetes (T1D), but the mechanisms of this protection remain poorly understood. Interestingly, colonization by a gut commensal that expresses an insulin-peptide molecular mimic promotes diabetes in adult non-obese diabetic (NOD) mice. This finding provokes the hypothesis that molecular mimicry protects from T1D when instead encountered during known early-life tolerogenic windows. Early-life colonization of NOD mice with PedsCom, a novel defined consortium of nine early-life commensals, significantly reduces diabetes incidence compared to both germ-free (GF) NOD mice and NOD mice colonized with PedsCom as adults. Bioinformatics analysis of PedsCom members identifies several potential insulin-peptide mimics. In preliminary experiments, we have observed *in vitro* recognition of one predicted mimic by an insulin-recognizing T cell hybridoma. Our ongoing experiments aim to determine which PedsCom microbes induce specific T cell responses, and to identify potential cross-reactive T cell receptors (TCRs) from PedsCom-colonized NOD mice using single-cell RNA sequencing (scRNA-seq) and TCR sequencing (scTCR-seq) of T cells from the intestinal lamina propria and pancreatic islets.

Poster 31A | Immunology

Elucidating the role lamina propria macrophages on the pathogenesis of Hirschsprung Disease-Associated Enterocolitis (HAEC)

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Hirschsprung disease (HSCR) is a congenital defect in which the enteric nervous system (ENS) – the intrinsic innervation of the bowel responsible for motility – is absent in the distal gut. Children with HSCR often develop a life-threatening inflammation called HSCR-associated enterocolitis (HAEC). Lamina propria macrophages play a crucial role in both pathogen defense as well as epithelial barrier integrity, and while microbiome, diet, pathogens, hypoxia has been shown to modulate macrophage behavior, the role of the ENS in macrophage biology remains ambiguous. Here, using a spontaneous *Ednrb* mutation as a model for HSCR, we assess the macrophage landscape using flow cytometry and whole-mount imaging to discern the immunological consequences of an absent ENS. Preliminary results suggest an accumulation of CD11b+F4/80+ macrophages as well as a bias towards anti-inflammatory M2 macrophage phenotype in the aganglionic regions of the gut. Our findings point toward a possible role of neurotransmitter signaling in control of macrophages critical to gut homeostasis. Understanding the mechanisms underpinning the ENS's control of macrophages open the doors for novel immunotherapies for both HSCR and other immune-related disorders.

Poster 32A | Immunology

A genetically attenuated *Cryptosporidium* strain protects mice from reinfection

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The parasite *Cryptosporidium* is an important enteric pathogen of livestock and a leading cause of diarrhea and death in immunocompromised people and malnourished children. One strategy to reduce the public health impact of *Cryptosporidium* would be the development of a vaccine to mitigate clinical disease and reduce transmission. The ability to develop a rational strategy for vaccination requires an appreciation of the parasite lifecycle, its interactions with the intestinal epithelial cell, and the immune mechanisms that mediate resistance to this organism. With our rapidly growing knowledge of the parasite genetic program over the course of its life cycle and improved tools for molecular engineering, we have opportunities to develop genetically attenuated parasites. Here we introduce an inducible knockout strain of *Cryptosporidium* that can answer questions about the requirements of the protective memory immune response to *Cryptosporidium.*

Poster 33A | Immunology

Role of T cells in alteration of egg sncRNA profile

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Classically, immune cells are defined by their roles in infection and inflammation. However, a growing body of literature suggests that immune cells have critical roles in the support of diverse tissue functions at homeostasis. Of note, germ-free (GF) mice, which are raised in the absence of microbes, have many documented defects in T cell function and corresponding downstream defects in the function of non-immune organs at steady-state. Studies in this area have focused primarily on somatic tissues. However, little is known about whether perturbations in immune function could affect gametes, and through this, inform progeny phenotypes.

Preliminary data from our lab suggest significant changes in the small non-coding RNA (sncRNA) of eggs derived from GF mice when compared to eggs from conventionally-raised (CR) mice. We have also uncovered alterations in downstream embryonic gene expression in zygotes formed from these eggs. Given the T cell defects which are observed in GF mice, we hypothesize that the absence of appropriate T cell function could underlie the perturbations we observe in the sncRNA of eggs from GF mice. To determine to what extent homeostatic T cell function determines the sncRNA profile of eggs, we propose the following studies. First, we will test whether mice lacking T cells (TCRbKO) bear differences in the sncRNA profile of eggs when compared with their wildtype, T-cell sufficient counterparts. Secondly, we will test whether acute depletion or adoptive transfer of T cells can determine the sncRNA profile of eggs. Finally, we propose to test whether challenge with transient immune stimuli can acutely alter the sncRNA profile of eggs. To complete these studies, we have developed methods to reliably profile sncRNA from the limiting quantity of eggs released following super-ovulation (10-20 eggs). We have also developed methods to release the T cell compartment from ovaries for analysis by flow cytometry. Taken together these studies will reveal whether, and to what extent, T cells may inform egg sncRNA and subsequent embryonic gene expression. Data from these studies could (1) suggest a novel homeostatic role for T cells beyond somatic tissue function, in gamete biology and (2) form the mechanistic basis for further study in how immune cells might transmit critical environmental cues to progeny.

Poster 34A | Immunology

Mechanisms of T Cell Dysfunction in Activated PI3Kδ Syndrome

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Activated Phosphoinositide 3-Kinase δ Syndrome (APDS) is a rare immunodeficiency characterized by hyperactive PI3K-Akt signaling, resulting in severe immune dysregulation and heightened susceptibility to infections. APDS manifests with several dysfunctions in T cell physiology, including skewed differentiation towards effector phenotypes, enhanced metabolic activity, altered cytokine production, and increased apoptosis. This study aims to elucidate how two potential downstream targets of PI3K signaling, namely suppression of FoxO1 and autophagy, contribute to T cell dysfunction within the context of PI3K hyperactivity. Leveraging a cohort of APDS patients with paired PBMCs collected pre- and post- disease-specific treatment, the study will investigate the extent of FoxO1 suppression and autophagy impairment. Furthermore, an in-vitro model using healthy CD8 T cells expanded in the presence of 740Y-P, a PI3Kδ agonist, will simulate APDS conditions to assess whether enhancing FoxO1 signaling and autophagy can restore T cell function. By integrating these approaches, this research aims to advance our understanding of immune dysregulation in APDS and provide significant insights into the mechanisms through which hyperactive PI3K-Akt signaling contributes to T cell dysfunction.

Poster 35A | Immunology

Investigating the effects of TSLP on regulatory T cells in a mouse model of atopic dermatitis

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The skin maintains barrier integrity through the coordinated efforts of keratinocytes and resident immune cells. Barrier disruption results in the release of alarmins that alert the immune system. One such alarmin is thymic stromal lymphopoietin (TSLP) which is traditionally recognized for its pro-inflammatory role through its pleiotropic effects on immune cells such as dendritic cells (DCs) and T helper 2 (Th2) cells. However, similar to other barrier cytokines, TSLP may also possess anti-inflammatory properties. Yet, it remains unclear whether TSLP can act on classically anti-inflammatory cell types such as regulatory T cells (Tregs) to modulate inflammation. To investigate this, we employed the MC903-induced atopic dermatitis (AD) mouse model, where mice receive topical treatment with MC903 (a vitamin D analog) for several days, resulting in a dry, scaly skin characteristic of atopic disease. Using this model, preliminary evidence suggests that mice with conditional deletion of the TSLP receptor on Tregs ($Foxp3^{Cre}TSLPR^{fl/fl}$) exhibit worsened ear inflammation. This was accompanied by a decrease in both the percentage and number of Tregs in ear skin compared to control mice. These findings imply that TSLP at the skin can also play an anti-inflammatory role by engaging skin Tregs within this specialized microenvironment. Studying the role of TSLP on skin Tregs will provide insights into novel local tissue regulations and site-specific adaptations.

Poster 36A | Neuroscience

Galactosylceramide triggers cell death in *Galc^{twi}* macrophages

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Brain macrophages, important contributors to debris clearance and the resolution of inflammation, become dysfunctional in Krabbe disease (KD) – a neurodegenerative condition caused by mutations in the lysosomal hydrolase, *GALC*. These cells develop rounded lipid-laden morphology and adopt pathologic transcription states. Notably, conditional knockout studies have strongly suggested that these lipid-laden cells worsen disease. A prevailing hypothesis is that the accumulation of lipids, due to defective galactosylceramide (galcer) catabolism by GALC, triggers macrophage dysfunction.

To test this hypothesis, we optimized an in vitro model for galcer macrophage challenge, and compared responses between GALC deficient macrophages from the Krabbe model (twitcher, *Galc^{twi}*) and macrophages from control mice (*Galc^{wt}*). We found that galcer induced rounded morphology in both *Galc^{wt}* and *Galc^{twi}* macrophages, but that *Galc^{twi}* cells were far more sensitive. Notably, these rounded macrophages were viable, did not activate apoptotic caspases, and exhibited signs of lysosomal stress. Bulk RNA sequencing showed that galcer dramatically reprogrammed *Galc^{twi}* macrophages while vehicle-treated cells resembled *Galc^{wt}* macrophages. Importantly, galcer-treated *Galc^{twi}* macrophages showed enrichment of pathologic macrophage signatures also seen in vivo by *Galc^{twi}* microglia.

To gain further insight into the consequences of lipid accumulation, we performed pathway analyses and found increased gene expression in pathways related to oxidative stress, lipid metabolism, and cell death, suggesting a pre-death state. Indeed, longer galcer treatment resulted in pronounced cell death in *Galc^{twi}* but not *Galc^{wt}* macrophages. However, the precise mechanism of galcer-induced death remains elusive, as pharmacologic inhibition of ferroptosis, apoptotic caspases, necroptotic executioners, and cathepsins, did not prevent lipid-induced macrophage death.

Together, these studies show that 1) galcer treatment can be used to study pathologic macrophages seen in twitcher mice 2) defective GALC may not cause macrophage dysfunction in the absence of lipid challenge 3) galcer challenge precipitates macrophage dysfunction by inducing metabolic stress and atypical cell death. Ongoing studies continue to probe the mechanisms of lipid-induced cell death and its contribution to KD pathogenesis.

Poster 37A | Neuroscience

An analysis pipeline to investigate neuronal entrainment to visually-evoked traveling waves after the presentation of different types of visual stimuli

Claudia Heymach and Alex Proekt

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Traveling waves of neural activity have been implicated in multiple processes in the visual system, including modulating perceptual sensitivity and predicting visual inputs. In awake mice, visual stimuli evoke a 3-6 Hz wave that travels across the cortex from a higher order association area, the posterior parietal area (PPA), to the primary visual cortex (V1). The phase of this wave modulates neuronal firing, a process referred to as "entrainment," in a subset of neurons in both V1 and PPA. Thus far, it is unknown whether the specific neurons that become entrained to the 3-6 Hz wave — and the phases these neurons become entrained to — differ depending on the type of visual stimulus. To address this knowledge gap, I first constructed simulations of neurons responding to different visual stimuli. I used these simulations to develop an analysis pipeline to distinguish between two possibilities: 1) that entrainment is agnostic to which stimulus was shown, and 2) that entrainment is stimulus-specific. Ultimately, the goal of developing this pipeline is to apply it to real world data and provide insight into how visually-evoked traveling waves orchestrate neuronal firing.

Poster 38A | Neuroscience

Exploring the Functional Impact of Neuron-Glioblastoma Synapses on Brainstem Circuits

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Glioblastoma (GBM), the most common primary brain tumor in adults, carries a devastating prognosis, with a median survival time of only 14.6 months. The emerging field of cancer neuroscience reveals that neuron-glioma interactions play a pivotal role in brain tumor progression, with neuronal activity promoting glioma growth and gliomas reciprocally promoting neuronal hyperexcitability. Strikingly, neurons can form direct synapses onto tumor cells, and signaling through these synapses promote tumor proliferation and invasion. While much of the cancer neuroscience field is dedicated to understanding how these synapses can benefit tumor progression, the consequences of these interactions on the nervous system are poorly understood. As each neuron within the brain can have many postsynaptic targets, hyperexcitability induced by neuron-tumor synapses could alter the dynamics of other circuits. As GBM typically occurs in cortical areas, cortical neuron-tumor synapses may be sufficient to cause dysfunction in distant regions. Through monosynaptic rabies tracing, we have found that neuromodulatory regions of the ascending reticular activating system (ARAS), brainstem areas that are critical for arousal, frequently innervate cortically implanted GBM. Specifically, the serotonergic dorsal raphe nucleus most frequently innervates GBM cells regardless of tumor transplantation location. Preliminary scRNAseq data and patch-clamp data suggest that neurons synapsing with tumor cells are indeed more hyperexcitable. As serotonergic neurons in the dorsal raphe are implicated with arousal and wakefulness, we hypothesize that hyperexcitability of these neurons would result in activation of wake-promoting areas during sleep, leading to sleep fragmentation. Preliminary EEG/EMG data demonstrate shorter and more frequent bouts of sleep in mice with cortically implanted GBM compared to sham controls. We plan to perform fiber photometry to examine the activity of labeled neurons during sleep-wake cycles and will also seek to rescue the phenotype by chemogenetically inhibiting labeled serotonergic neurons in the dorsal raphe. Ultimately, our findings may suggest that tumor cells may not have to physically migrate to a particular brain region to cause dysfunction there.

Poster 39A | Neuroscience

Exploring the Glutamatergic Underpinnings of Within-Network Functional Connectivity and Motor Performance in a Transdiagnostic Cohort

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Background
Alterations in glutamate (Glu) concentration and functional connectivity (FC) within the somatomotor network (SomMot) have independently been linked to psychosis spectrum (PS) symptoms. Yet, the relationship between regional Glu and FC patterns has been largely understudied due to methodological limitations. This study leverages a transdiagnostic, multimodal dataset to explore links between Glu, FC, and motor performance.

Methods:
51 adults (23 healthy control [HC], 28 PS) underwent 7T Glu Chemical Exchange Saturation Transfer (GluCEST) imaging, 3T resting-state functional MRI, and completed a standardized tapping task. Neuroimaging data were processed using in-house and field-standard pipelines (e.g., fMRIPrep, XCP-D). SomMot was defined using the Schaefer100 atlas. Statistical analyses were performed in Python.

Results:
PS patients showed significantly lower GluCEST in SomMot ($p=0.03$) than HCs. There was a significant positive correlation between SomMot GluCEST and within-network functional connectivity ($R^2=0.14$, $p=0.01$) and a trend-level association between SomMot GluCEST and tapping performance. SomMot FC was negatively associated with tapping performance ($R^2=0.26$, $p=0.03$) in the PS group, but not HCs.

Conclusions:
Reduced SomMot GluCEST aligns with reports of cortical glutamatergic deficits in PS cohorts. The positive correlation between SomMot GluCEST and FC suggests that the robustness of local excitatory glutamatergic signaling may correlate with macroscopic connectivity dynamics. Glu or FC dysfunction may underlie PS-associated somatomotor issues. However, the divergent relationship between SomMot FC and tapping performance implies nuanced behavioral implications of Glu-FC coupling in this population, urging further investigation.

Poster 40A | Neuroscience

Monosynaptic tracing defines brain-wide circuit connectivity of human glioblastoma

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Glioblastoma (GBM), a deadly brain cancer, infiltrates the brain and can be synaptically innervated by neurons. Synaptic inputs onto GBM cells identified so far are largely short-range and glutamatergic in nature. The extent of integration of GBM cells into the brain-wide neuronal circuitry is therefore not well understood. We report the application of transsynaptic viral tracing approaches to study the neuronal connectome of GBM. We applied rabies virus-mediated retrograde tracing and herpes simplex virus (HSV)-mediated anterograde tracing approaches to characterize presynaptic partners *in vivo* in the adult mouse brain and *ex vivo* with iPSC-derived neurons, cortical organoid-GBM assembloids, and human surgical specimens. After transplantation into adult mice, GBM cells derived from multiple patients rapidly integrated into brain-wide neuronal circuits. GBM exhibited increased connectivity rates compared to transplanted neural progenitor cells, highlighting functional connectivity as a hallmark of malignant cells. Beyond glutamatergic inputs, we identified neuromodulatory inputs across the brain, including cholinergic inputs from the basal forebrain. We validated these long-range cholinergic neuron-to-glioma projections signaling through the metabotropic CHRM3 receptor by high-resolution microscopy, anterograde monosynaptic HSV tracing, calcium imaging, and patch-clamp electrophysiology. To interrogate the functional effects of acetylcholine (ACh) on GBM, we performed calcium imaging and RNA sequencing analyses, which showed that ACh stimulation induced sustained calcium oscillations and long-lasting transcriptional reprogramming of GBM cells into a more invasive state via CHRM3. Importantly, CHRM3 activation promoted GBM cell invasion, whereas CHRM3 downregulation suppressed GBM cell invasion *in vitro* and *in vivo*, suggesting CHRM3 as a potential therapeutic target for this disease. Together, these results reveal the capacity of human GBM cells to robustly integrate into anatomically and molecularly diverse neuronal circuitry in the adult brain. They also support a model wherein rapid connectivity with GBM cells may promote to a long-lasting increase in tumor cell fitness.

Poster Session B

Biochemistry, Biophysics, Chemical Biology

Poster 1B
Using heterobifunctional ligands to induce the selective arginylation of Alpha Synuclein fibrils in a Parkinson's model system
Presenter: Andres Fernandez del Castillo | Advisor: Dr. Mark Sellmyer

Poster 2B
Deep sequence design using ProteinMPNN as a refinement module for protein design
Presenter: Joseph (Andrew) Nelson | Advisor: Dr. Dan Kulp

Bioengineering

Poster 3B
Generative Adversarial Bayesian Optimization for Surrogate Objectives
Presenter: Michael Yao | Advisors: Drs. Jim Gee & Osbert Bastani

Poster 4B
Microfabricating a multimodal neural interface integrating microLEDs and transparent Ti3C2Tx MXene electrodes for colocalized neural recording, imaging, and light-based stimulation
Presenter: Royce Dong | Advisors: Drs. Brian Litt & Flavia Vitale

Poster 5B
Cell type-specific relationship between higher-order chromatin folding and short tandem repeat instability in Huntington's disease
Presenter: Han-Seul Ryu | Advisor: Dr. Jenn Cremins

Poster 6B
Extracellular vesicle liquid biopsy to improve breast cancer screening accuracy
Presenter: Griffin Spychalski | Advisor: Dr. Dave Issadore

Cell and Molecular Biology

Cancer Biology

Poster 7B
Spatial and single cell transcriptomics uncovers metabolic reprogramming and NETosis of neutrophils in human pancreatic cancer
Presenter: Carson Poltorack | Advisors: Drs. Sydney Shaffer & Celeste Simon

Poster 8B
CellSlime: Visualizing T-Cell Trajectories to Unravel Migration- Exhaustion Interplay in Tumors
Presenter: Pavithran Ravindran | Advisor: Dr. Arjun Raj

Poster 9B
Investigating the Role of Diet and Genetics on Colorectal Cancer Metabolism and Host Physiology
Presenter: Prateek Sharma | Advisors: Drs. Christoph Thaiss & Kathryn Wellen

Genetics and Epigenetics

Poster 10B
Ferroptosis Modulators in Breast Cancer Dormancy and Recurrence
Presenter: Emily Shea | Advisor: Dr. Lewis Chodosh

Cell Biology, Physiology, and Metabolism

Poster 11B
Investigating How a Small Molecule Activator of VCP Reduces TDP-43 Aggregates
Presenter: Katherine Xia | Advisor: Dr. Eddie Lee

Developmental, Stem Cell, and Regenerative Biology

Poster 12B
Leveraging the murine allantois to redefine the roles of VEGFR1 and VEGFR2
Presenter: Erica Nguyen | Advisor: Dr. Mark Kahn

Poster 13B
Runx1's Regulation of Granulocyte Monocyte Progenitors in RUNX1 Familial Platelet Disorder
Presenter: Daniel Yen | Advisor: Dr. Nancy Speck

Poster 14B
Determining the Role of the Epidermis in Regulating Hair Follicle Phenotype
Presenter: Jack You | Advisor: Dr. George Cotsarelis

Gene Therapy and Vaccines

Poster 15B
Reducing Kidney Fibrosis by FAP-CAR-T Cells in Mouse Models of Kidney Disease
Presenter: Blake Jardin | Advisor: Dr. Jon Epstein

Genetics and Epigenetics

Poster 16B
Genome Folding and Speckle Association Cooperate to Orchestrate Fibroblast Activation
Presenter: Zachary (Zach) Gardner | Advisor: Dr. Raj Jain

Poster 17B
Pathological characterization, genome-wide methylation, and in vitro modeling of trophoblast overgrowth in Beckwith-Wiedemann Syndrome
Presenter: Sanam Kavari | Advisors: Drs. Jenn Kalish & Marisa Bartolomei

Poster 18B
Lsd1 is a critical mediator of epidermal development, homeostasis, and oncogenesis
Presenter: Napasorn (Nina) Kuprasertkul | Advisors: Drs. Brian Capell & Kathryn Wellen

Poster 19B
Hemodynamics and KLF2/4 regulate myxomatous valve formation
Presenter: Jesse Pace | Advisor: Dr. Mark Kahn

Poster 20B
Critical assessment of CGG short tandem repeat length, FMR1 DNA promoter methylation, heterochromatin, and transcriptional repression of neural, synaptic genes in fragile X syndrome
Presenter: Kenneth Pham | Advisor: Dr. Jenn Cremins

Poster 21B
Deciphering the role of cytoskeletal-nuclear interactions in peripheral chromatin organization
Presenter: Kaitlyn Shen | Advisor: Dr. Raj Jain

Microbiology, Virology, and Parasitology

Poster 22B
Effect of Mutations in the Glycoprotein of Dabie Bandavirus (SFTSV)
Presenter: Raegan Petch | Advisor: Dr. Paul Bates

Poster 23B
Mitochondrial oxidative phosphorylation restricts SARS-CoV-2 replication via metabolic remodeling
Presenter: Yentli Soto Albrecht | Advisor: Dr. Doug Wallace

Chemistry

Poster 24B
Synthesis and design of macrocyclic collagen mimetic peptides for targeting the cancer-implicated DDR2 kinase
Presenter: Diane Rafizadeh | Advisor: Dr. Dave Chenoweth

Epidemiology & Biostatistics

Poster 25B
Racial Differences in Background Parenchymal Enhancement, a Novel Marker of Breast Cancer
Presenter: Mattia Mah'moud | Advisor: Dr. Anne Marie McCarthy

Genomics & Computational Biology

Poster 26B
A Longitudinal Single-Cell and Spatial Multiomic Atlas of Pediatric High-Grade Glioma
Presenter: Jonathan Sussman | Advisor: Dr. Kai Tan

Poster 27B
Towards a universal metric for comparing cellular neighborhoods
Presenter: Barbara Xiong | Advisor: Dr. Kai Tan

Poster 28B
The phenotypic basis of CT-derived kidney traits and their utility in predicting estimated glomerular filtration rate
Presenters: David Zhang & Rachit Kumar | Advisors: Drs. Daniel Rader & Marylyn Ritchie

Health Care Management & Economics

Poster 29B
Hospital Responses to Financial Risk
Presenter: Michael Sielski | Advisor: Dr. Alexander Olssen

Poster 30B
Physician consults in the hospital
Presenter: Zachary Templeton | Advisor: Dr. Guy David

History & Sociology of Science

Poster 31B
Making Fun, Making Doctors: "Spoof" shows and professionalization in US Medical Education, 1950-2000
Presenter: Caroline Wechsler | Advisor: Dr. Robby Aronowitz

Immunology

Poster 32B
Tonsillar versus circulating CD4 T Follicular Helper polarization states delineated by trimodal single-cell sequencing and spectral flow cytometry in a healthy pediatric cohort
Presenter: Sam Barnett Dubensky | Advisors: Drs. Derek Oldridge & Laura Vella

Poster 33B
Inherited C-terminal TREX1 variants disrupt homology-directed repair to cause senescence and DNA damage phenotypes in Drosophila, mice, and humans
Presenter: Samuel Chauvin | Advisor: Dr. Jonathan Miner

Poster 34B
Spatial transcriptomic map of CAR-T cells in situ in the human tumor microenvironment
Presenter: Samuel Kim | Advisor: Dr. Carl June

Poster 35B
Dietary long chain fatty acids shape innate immune cell tone and modulate inflammatory responses in the lung
Presenter: Samuel McCright | Advisor: Dr. David Hill

Poster 36B
Excess IL-18 suppresses EAE by augmentation of CD8Tregs to inhibit autoreactive T-cell activity
Presenter: Jeremy Morrissette | Advisor: Dr. Scott Canna

Poster 37B
CAR-T cell therapy for idiopathic pulmonary fibrosis
Presenter: Henry Utset | Advisor: Dr. Ellen Puré

Neuroscience

Poster 38B
Investigating the role of GABAergic interneurons in the dorsomedial striatum in value-based decision-making
Presenter: Evan Iliakis | Advisor: Dr. Marc Fuccillo

Poster 39B
Ndnf interneuron excitability is spared in a mouse model of Dravet syndrome
Presenter: Sophie Liebergall | Advisor: Dr. Ethan Goldberg

Poster 40B
Transdiagnostic Polygenic Risk Scores Underlying Overall Psychopathology and Personalized Functional Networks in Early Adolescence
Presenter: Kevin Sun | Advisors: Drs. Aaron Block-Alexander & Ted Satterthwaite

Pharmacology

Poster 41B
Engineering cellular systems for biomedical imaging & diagnostics
Presenter: Jonathan Pham | Advisor: Dr. Mark Sellmyer

Poster 1B | Biochemistry, Biophysics, Chemical Biology

Using heterobifunctional ligands to induce the selective arginylation of Alpha Synuclein fibrils in a Parkinson's model system

Andres Fernandez del Castillo, Robert H. Mach, E. James Petersson and Mark A. Sellmyer

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Alpha synuclein (α-syn) is a small (15kDa) intrinsically disordered protein believed to play roles in vesicular trafficking and mediating mitochondrial oxidative stress, and its dysregulation or overexpression leads to proteotoxic stress and cell death. α-syn has the propensity to aggregate into oligomers or fibrils. α-syn has histological and genetic associations with neurodegenerative disorders, including Parkinson's disease. There are no currently approved medications to slow or treat the underlying pathologies of α-syn-associated neurodegenerative diseases. For this reason, α-syn has been the focus of intense study to elucidate its contributions to the progression of neurodegeneration. There are many known post-translational modifications (PTMs) of alpha synuclein, some of which are considered neuropathological hallmarks of Parkinson's disease. A growing body of work has shown that some of these PTMs are directly neurotoxic, while others are potentially neuroprotective. Mid-chain arginylation of alpha synuclein has recently been shown to negatively correlate with pathologic PTMs, and knockout of ATE1 (the enzyme shown to be an inducer of α-syn arginylation) in mice leads to symptoms of neurodegeneration and increases the fraction of insoluble α-syn fractions in brain lysates. However, we lack tools to directly induce arginylation of α-syn fibrils, which limits our ability to draw causal inferences on the role of arginine PTMs in preventing neurodegeneration. In this project, I intend to use chemical biology tools to induce the colocalization of ATE1 with α-syn fibrils and produce selective and controllable arginylation of α-syn in living cells. Since there are no known ligands of ATE1 I will create fusions of ATE1 and e. coli Dihydofolate Reductase (eDHFR). Using a small molecule binder of α-syn fibril developed by the Petersson lab, I have designed and synthesized novel heterobifunctional binders of α-syn fibrils and eDHFR. Using analytical ultracentrifugation, I have shown that the molecules are capable of colocalizing α-syn fibrils and eDHFR. Using these chemical tools I will study the effect of α-syn arginylation on the cellular processing, abundance, and cytotoxicity of α-syn aggregates in cellular models of Parkinson's disease. I will study the extent and distribution of arginylation using immunocytochemistry, western blot, and mass spectroscopy. I hypothesize that mid-chain α-syn arginylation will reduce the prevalence and cytotoxicity of α-syn aggregates. These novel chemical-biological tools will allow us to explore the interplay of α-syn arginylation, α-syn aggregation and disaggregation, cellular dysfunction, and cytotoxicity. Additionally the demonstration that arginylation is capable of reducing the cytotoxicity of α-syn fibrils would point to novel future directions for Parkinson's treatment.

Poster 2B | Biochemistry, Biophysics, Chemical Biology

Deep sequence design using ProteinMPNN as a refinement module for protein design

Andrew Nelson and Daniel Kulp, PhD

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ProteinMPNN is a deep learning-based sequence design tool, that, when given a protein backbone, generates one or more sequences capable of folding into a prescribed backbone. Here, I investigate how to use ProteinMPNN as a refinement module for protein design. Specifically, I interrogate the optimal number of sequences to be output by ProteinMPNN and how the quality of the starting protein backbone seed affects performance. In addition, I show that ProteinMPNN performs best in a blinded validation when used iteratively. Lastly, I show that ProteinMPNN score does not correlate with performance and that blinded validation with a deep learning-based structure prediction tool is necessary for model validation.

Poster 3B | Bioengineering - Imaging

Generative Adversarial Bayesian Optimization for Surrogate Objectives

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Offline model-based policy optimization seeks to optimize a learned surrogate objective function without querying the true oracle objective during optimization. However, inaccurate surrogate model predictions are frequently encountered along the optimization trajectory. To address this limitation, we propose **generative adversarial Bayesian optimization (GABO)** using *adaptive source critic regularization*, a task-agnostic framework for Bayesian optimization that employs a Lipschitz-bounded source critic model to constrain the optimization trajectory to regions where the surrogate function is reliable. We show that under certain assumptions for the continuous input space prior, our algorithm dynamically adjusts the strength of the source critic regularization. GABO outperforms existing baselines on a number of different offline optimization tasks across a variety of scientific domains.

Poster 4B | Bioengineering

Microfabricating a multimodal neural interface integrating microLEDs and transparent Ti3C2Tx MXene electrodes for colocalized neural recording, imaging, and light-based stimulation

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Light-based stimulation and control of neural activity is a powerful tool for neuroscience. However, studying complex brain activity with light-based stimulation in large animal models such as non-human primates is currently limited by the lack of scalable neurotechnologies integrating light sources and neural recording electrodes. For the few existing multimodal neural devices - mostly based on graphene - manufacturability, throughout, and material processing are still unaddressed issues. This work describes the microfabrication and characterization of a multimodal neural interface consisting of microLEDs and transparent Ti3C2Tx MXene microelectrode arrays, allowing for colocalized optical stimulation and electrical recording with high spatiotemporal resolution. We report scalable MXene microelectrode arrays with a transmittance of 60% at the reference wavelength of 550 nm and impedance of 587±152 kOhms at the reference frequency of 1 kHz. Importantly, we show that Ti3C2Tx MXene interacts minimally with microLED light output at 460 nm and 630 nm and does not give rise to photoelectric artifacts that are commonly observed in metal-based multimodal microelectrodes.

Poster 5B | Bioengineering

Cell type-specific relationship between higher-order chromatin folding and short tandem repeat instability in Huntington's disease

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One of the most common of short tandem repeat (STR) expansion disorders is Huntington's disease (HD), a neurodegenerative condition caused by more than 36 CAG triplets in exon 1 of the *HTT* gene. Recent studies have shown that age of HD onset correlates with increased CAG length from somatic instability. A central mystery in HD pathogenesis is how CAG STR instability arises and leads to selective degeneration of striatal and cortical projection neurons, despite ubiquitous *HTT* expression. This study aims to understand the mechanism of cell-type specific degeneration in HD by elucidating the relationship between higher-order chromatin folding and *HTT* CAG instability across multiple cell types. Chromatin is folded into megabase (Mb)-sized topologically associating domains (TAD) and subTADs, and their boundaries are often occupied by architectural proteins such as CTCF and cohesin. Based on many studies demonstrating the close link between domain boundaries and STR instability, we hypothesize that vulnerable cell types in HD display somatic instability of the CAG STR due to its localization to domain boundaries enriched with CTCF/cohesin. To test this hypothesis, we have differentiated isogenic human embryonic stem cell lines with various knock-in CAG lengths to different cell types. We plan to measure somatic instability of the CAG STR with targeted long-read Nanopore sequencing and map chromatin folding with single nucleus methyl 3C seq (sn-m3c-seq). This study begins to shed light on why certain cell types are more susceptible to the same inherited *HTT* CAG tract than others through the study of higher-order chromatin folding and STR instability in a cell type-specific manner.

Poster 6B | Bioengineering

Extracellular vesicle liquid biopsy to improve breast cancer screening accuracy

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Breast cancer screening mammograms commonly identify BI-RADS category 4 lesions, indeterminate lesions with a broad likelihood of malignancy. We seek to determine whether a multiplexed extracellular vesicle liquid biopsy can improve the classification of patients with BI-RADS category 4 lesions for early-stage breast cancer detection. Here, we analyzed plasma from 114 prospectively enrolled subjects with BI-RADS category 4 breast lesions, including 87 with benign lesions and 27 with malignant lesions (12 with stage I invasive carcinoma and 14 with ductal carcinoma *in situ*). From each plasma sample, we used track etched magnetic nanopore (TENPO) technology to separately isolate HER2 and CD24 expressing extracellular vesicles and measured their miRNA cargo using next-generation sequencing. We evaluated the performance of EV miRNA biomarkers for classifying malignancy and compared the miRNA cargo of HER2+ and CD24+ extracellular vesicles, and then applied LASSO regression to identify a panel of five complementary EV miRNA that were validated by qPCR. We found that individual miRNAs accurately classified patients with breast cancer, including an area under the receiver-operator characteristic curve (AUC) of 0.87 for miR-340-5p from HER2+ EVs. The differentially enriched EV miRNA were found to be weakly correlated with a mean absolute Kendall tau correlation coefficient of 0.20, suggesting that these biomarkers are well suited for algorithmic combination into a panel. Following LASSO regression, we report a panel of five miRNA selected from both HER2+ and CD24+ EVs, indicating that these EV subpopulations contain unique, complementary information for classifying breast cancer. Together, we report a biomarker panel comprised of miRNA from breast cancer-associated EVs for the noninvasive classification of malignancy in patients with BI-RADS category 4 breast lesions. Future work will evaluate this assay in a larger cohort with EVs from multiple cell types.

Poster 7B | CAMB - Cancer Biology

Spatial and single cell transcriptomics uncovers metabolic reprogramming and NETosis of neutrophils in human pancreatic cancer

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Pancreatic ductal adenocarcinoma is the most hypovascular solid tumor, imposing harsh constraints on tumor metabolism. Cancer cells cope with reduced access to vascular-derived nutrients and O2 by cooperating with abundant stromal cells. Specifically, critical nutrients including alanine, proline, glycosaminoglycans, branched chain ketoacids, lysophosphatidic acid, and unsaturated fatty acids can be provided by fibroblasts *in vitro* and *in vivo*. However, the extent of metabolic support provided by other cell types in the PDAC TME is relatively unexplored. We hypothesized that cell types that are cooperating metabolically with cancer cells will be spatially positioned near cancer cells in these tumors. We performed Xenium spatial transcriptomics on 8 treatment-naive PDAC resections and generated a spatial atlas of nearly 700,000 cells across a range of expected cell types. We noted striking neutrophil aggregates, nucleating in the glands of classical-like malignant cells and elaborating extraglandularly. Cellular neighborhood analysis demonstrates neutrophil aggregation is strongly correlated with malignant cell proliferation and low endothelial content, suggesting neutrophils may be an important source of pro-tumoral, non-vascular nutrients in the PDAC TME. Reanalysis of published scRNAseq data revealed 3 neutrophil metabolic states in human PDAC tumors. Metabolic cluster 7 neutrophils were found only in PDAC patient samples but not healthy controls, indicative of PDAC-driven peripheral reprogramming of neutrophil metabolic gene expression. Cl7 neutrophils were enriched for Type I interferon response, the pentose phosphate pathway and iron uptake. Metabolic cluster 2 neutrophils were strikingly depleted from PDAC tumor samples compared to blood, suggesting decreased infiltration or increased cell death. Cl2 neutrophils were enriched for several catabolic pathways including glycogenolysis, autophagy, and proteasome, and selectively express the *PADI4* which controls induction of NETosis, a form of nonapoptotic neutrophil cell death. Multiplexed immunofluorescence demonstrated that neutrophil aggregates in PDAC are citH3+/MPO+ large neutrophil extracellular traps (NETs). We reasoned pancreatic cancer cells could proliferate under tumor-like metabolic stress by repurposing amino acids liberated by autophagy, glucose liberated by glycogenolysis, or simply consuming bulk neutrophil dead cell debris via macropinocytosis. Using tumor interstitial fluid media (TIFM) and 0.5% O2 to mimic tumor-like metabolic stress, we cocultured pancreatic cancer cells with human primary neutrophils and HL60-derived neutrophil-like cells. However, under tumor-like metabolic conditions, coculture with human primary neutrophils failed to substantially increase cancer cell proliferation and NETotic neutrophils were cytotoxic. In contrast, we noticed enhanced neutrophil viability in tumor-like metabolic conditions, suggesting metabolic regulation of neutrophil cell death. Together, these data shed light on the metabolic phenotypes and interactions with cancer cells of neutrophils in the PDAC tumor microenvironment.

Poster 8B | CAMB – Cancer Biology

CellSlime: Visualizing T-Cell Trajectories to Unravel Migration- Exhaustion Interplay in Tumors

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Adoptive cell therapy, such as tumor-infiltrating lymphocyte therapy and chimeric antigen receptor T-cell therapy, holds promise in treating difficult-to-treat cancers with durable remissions. However, many patients, especially those with solid tumors, find that T-cell therapies do not work. Typically, this inactivity is due to T-cell exhaustion, a process by which T-cells no longer can kill tumor cells nor release cytokines for proper activation, and lack of tumor infiltration. While these processes have been studied in key detail separately, few experiments link them together. Specifically, how does a T-cell become exhausted as it traverses a heterogeneous tumor? Furthermore, research has shown that higher T-cell migration speed increases tumor infiltration and clearance. Do less exhausted T-cells have higher migration speeds? Prior work has failed to specifically answer such questions because either one can track where T-cells are using intravital imaging or molecularly characterize the cells using single-cell techniques like FISH, single-cell transcriptomics, or spatial transcriptomics. Connecting past locations with current cell phenotype in a fixed image is currently not possible. Our proposal aims to use a combination of in vitro and in vivo experiments to uncover the connections between migration path and speed to T-cell exhaustion. The key innovation that enables this is the development of CellSlime. As an infiltrating cell, in this case, T-cells, pass through recipient tissue (tumor), a trail of GFP and BFP is left behind. In this way, a fixed image that can be taken to a spatial transcriptomics platform for molecular characterization also contains the past locations of the infiltrating cells. In Aim 1, we will first use in vitro mixing experiments of T-cells with PDAC cell lines that either develop a highly infiltrated tumor or a lowly infiltrated one when implanted into mice. This will provide us a foothold into how migration path may affect T-cell exhaustion. Next, we will use CellSlime in vivo with the same PDAC tumor lines to connect the migration path in vivo with T-cell exhaustion. In Aim 2, we will isolate T-cells that have high in vitro migration speed and profile them for exhaustion markers. Next, we will improve our CellSlime technology to include a starting time component such that path length can also be viewed as cell speed. In this manner, we will characterize T-cells with high in vivo speed for exhaustion. Overall, the results of this work will define the connection between T-cell exhaustion, migration path, and migration speed which may help rationally engineer T-cells to infiltrate and eliminate tumors better.

Poster 9B | CAMB – Cancer Biology

Investigating the Role of Diet and Genetics on Colorectal Cancer Metabolism and Host Physiology

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Colorectal cancer (CRC) is the second leading cause of cancer-related mortality in the United States. Identifying new factors that promote or inhibit CRC is critical to mitigating cancer risk and improving patient outcomes. Alongside genetic factors, diet influences CRC by altering normal gut physiology, microbial composition, and available metabolites. These metabolites include fructose and short-chain fatty acids (SCFA) comprised of acetate, propionate, and butyrate. We recently asked whether these divergent diet-derived metabolic signals integrate to exert a net effect and showed that, in mice, a high-fiber diet leads to greater glucose tolerance compared to a low-fiber diet while supplementation of high-fructose corn syrup to a high-fiber diet mitigates this protective benefit. Metabolomics analysis revealed sets of metabolites in cecal contents that cluster with each nutritional condition. These results suggest that nutrients with opposing metabolic functions integrate to exert a net effect. However, whether central metabolites at the intersection of fructose and fiber metabolism are primarily mediating this phenotype, and how host enzymes that process these nutrients are regulated in this context is poorly understood. One major class of metabolites impacted by these nutrients is SCFAs. The enzyme acyl-Coenzyme A (CoA) synthetase short chain family member 2 (ACSS2) is responsible for metabolizing nuclear and cytosolic acetate, the most abundant SCFA, to acetyl-CoA, a central metabolite that is used in histone modifications and biosynthetic pathways. To assess correlations of ACSS2 with CRC biology, we analyzed publicly available Cancer Genome Atlas (TCGA) gene expression datasets and found that ACSS2 is underexpressed in CRC compared to normal colon tissue. Moreover, ACSS2 expression is inversely correlated with survival. We thus asked if loss of ACSS2 expression had a functional impact on CRC. TCGA analysis showed that ACSS2-low CRC patients had increased epithelial-mesenchymal transition (EMT) and inflammation gene expression signatures. Collectively, our data suggest that loss of ACSS2 expression, through currently undefined mechanisms, is correlated with de-differentiation and EMT in CRC and that dysregulation of ACSS2 may play an important role in colorectal cancer in part through the processing of dietary nutrients. Through ongoing work, we are testing the role of ACSS2 in diet-dependent CRC tumorigenesis.

Poster 10B | CAMB – Cancer Biology

Ferroptosis Modulators in Breast Cancer Dormancy and Recurrence

Emily Shea and Lewis Chodosh

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Lipids are involved in cellular signaling, are critical to the formation and maintenance of cellular membranes, and are a major energy source. When damaged by reactive oxygen species, lipid peroxides promote cell death via ferroptosis. We conducted a targeted CRISPR knockout screen using a library of 331 guides to find functional targets in the ferroptosis pathway regulating breast cancer dormancy and recurrence. Three of the top hits by clonal enrichment were *GOT1*, *GPX4*, and *PNPLA2*. *PNPLA2* codes for adipose triglyceride lipase, the rate-limiting step of triglyceride lipolysis. In addition to providing free fatty acids for biosynthesis and oxidation, *PNPLA2* promotes expression of PPARα and its target genes, thus also serving as a key node for cellular signaling. *GOT1* encodes glutamate-oxaloacetate transaminase 1, a critical node in many cellular metabolic processes, including the citric acid cycle and urea cycle. *GPX4* encodes glutathione peroxidase 4, which is the key last line of defense against ferroptotic cell death by reducing toxic lipid peroxides into non-toxic alcohols. Intriguingly, all three genes are predicted to have roles in inhibiting ferroptosis. Counterintuitively, guides knocking out each of the genes were selected for in recurrent tumors in the CRISPR screen. I have further validated in a recurrence-free survival assay that knockout of each gene accelerates recurrence. In addition to studying these three genes, I have modulated ferroptosis in our model system by treating our *Her2*-inducible cells with erastin, a canonical ferroptosis inducer. As expected, proliferative cells die with increasing doses of erastin. However, when I induce dormancy in these *Her2*-inducible cells first, the cells not only survive erastin treatment but actually increase in cell number. I thus have concordant, counterintuitive results in response to genetic and pharmacological ferroptotic stimuli in dormancy and recurrence. I hypothesize that dormant cells respond differently to these stimuli to survive and proliferate instead of dying. I have further conducted untargeted lipidomics in our dormancy model. Analysis is ongoing but ferroptosis is the top metabolite set identified in dormant timepoints compared to proliferative by metabolite set enrichment analysis. This is likely due to a drastic enrichment of polyunsaturated fatty acids, including arachidonic acid, in dormancy which further supports my model of an alternative response to ferroptotic stimuli. Ongoing studies include identifying the mechanism of cell number increase in response to erastin by assaying for mitosis, proliferation, and lipid peroxidation. I am further studying the mechanism of *GPX4*, *GOT1*, and *PNPLA2* accelerating recurrence via measuring reactive oxygen species, multiple types of cell death, and metabolite use and production. Together, these studies may reveal a counterintuitive cellular response to ferroptotic stimuli in dormancy and implicate these stimuli in promoting recurrence.

Poster 11B | CAMB - Cell Biology, Physiology, and Metabolism

Investigating How a Small Molecule Activator of VCP Reduces TDP-43 Aggregates

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Neurodegenerative diseases are progressive and fatal diseases that currently have no cure. There is, however, a promising target for therapeutics – protein aggregates, a hallmark of neurodegenerative diseases and a contributor to disease progression and severity. TDP-43 aggregates are one major class of protein aggregates found in multiple neurodegenerative diseases. These aggregates are predominantly found in neuronal nuclei in multisystem proteinopathy (MSP) and in neuronal cytoplasm in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In these diseases, TDP-43 aggregates can sequester and inactivate normal functioning TDP-43, which play important roles in RNA regulation. Lost TDP-43 function can thus lead to neuron dysfunction, and eventually, death. While TDP-43 aggregates are implicated in multiple neurodegenerative diseases, no treatment exists to clear them.

Our lab proposes a novel approach to clear TDP-43 aggregates with small molecule activators of valosin containing protein (VCP). VCP is a AAA+ ATPase that unfolds polyubiquitinated structured proteins for efficient proteasomal degradation. Recently, we showed that VCP colocalized to nuclear TDP-43 aggregates in cell models of MSP and that VCP-specific inhibitors also inhibited clearance of these aggregates. We then showed that VCP ATPase activity could be enhanced with the small molecule UP109, specifically at the domains that generate the force to unfold proteins. Importantly, when UP109 was added to cell models of MSP, the cells had reduced amounts of nuclear TDP-43 aggregates compared to untreated cells. These initial results lead us to believe that UP109 could enhance VCP to facilitate the efficient clearance of TDP-43 aggregates through the proteasome. Now, we aim to clarify the exact mechanisms by which UP109 reduces TDP-43 aggregates, as well as the impact UP109 has upon cytoplasmic TDP-43 aggregates. To do so, we aim to identify intracellular protein clearance pathways that can be enhanced by UP109. We also aim to determine whether UP109 enhances VCP to reduce cytoplasmic TDP-43 aggregates and whether this function could potentially restore normal TDP-43 functions in cells. Ultimately, we will work to elucidate a mechanism for how small molecule activators of VCP could clear pathologic aggregates and potentially offer a new therapeutic approach for TDP-43-mediated neurodegenerative diseases.

Poster 12B | CAMB - Developmental, Stem Cell, and Regenerative Biology

Leveraging the murine allantois to redefine the roles of VEGFR1 and VEGFR2

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The allantois is an extraembryonic structure that serves as the precursor to the umbilical cord and placental vasculature. Before somitogenesis, the allantois begins as a small mesodermal bud arising from the primitive streak. Over the course of 24 hours, the allantois elongates towards the chorion and becomes increasingly vascularized. Proper vessel growth in this tissue is essential for the survival of the embryo as it increases its dependence on the umbilical cord and placenta for gas, nutrient, and waste exchange with the maternal bloodstream. It is currently accepted that vessel growth is driven by pro-vascular signal from vascular endothelial growth factor receptor 2 (VEGFR2) and anti-vascular signal from VEGFR1. This project utilizes genetic mouse models to show a novel role for VEGFR1. Our lab has developed a Hoxa13-Cre mouse line that deletes genes in the allantois. Our preliminary data suggests that VEGFR1, previously considered an antagonist of vessel growth, can act as an agonist when VEGFR2 is absent in the allantois. Here, immunohistochemistry and wholemount confocal imaging of the allantois will function as a vascular readout for how vessel growth responds to the absence of VEGFR1, VEGFR2, or both in the allantois specifically. Overall, this project not only studies the development of a tissue essential for human health but utilizes this same tissue to learn broadly about vessel development as it may relate to disease.

Poster 13B | CAMB - Developmental, Stem Cell, and Regenerative Biology

Runx1's Regulation of Granulocyte Monocyte Progenitors in RUNX1 Familial Platelet Disorder

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RUNX1 Familial Platelet Disorder (RUNX1-FPD) is an autosomal dominant disorder caused by germline mutations in the RUNX1 transcription factor. RUNX1-FPD patients have a high risk of developing hematopoietic malignancies. Approximately 44% of RUNX1-FPD patients develop a hematopoietic malignancy, with an average age of onset of 33. RUNX1-FPD patients also exhibit a high prevalence of inflammatory disorders, such as excema, psoriasis, and systemic lupus erythematosus, and have markedly elevated levels of many inflammatory cytokines in the bone marrow. It is thought that chronically elevated inflammation acts as a driver for cancer in RUNX1-FPD. Previous work from our lab has demonstrated that RUNX1 plays a significant role in epigenetically constraining inflammatory signaling in the myeloid lineage. Complete loss of RUNX1 in granulocyte-monocyte progenitors (GMPs) results in the production of neutrophils that oversecrete inflammatory cytokines in response to Toll-Like-Receptor 4 (TLR4) stimulation. We determined that in wildtype GMPs, RUNX1 binds to members of the TLR4 and Type 1 Interferon pathways. Genetic deletion of RUNX1 in GMPs using *Cebpa-Cre* resulted in increased chromatin accessibility at, and increased expression of RUNX1-bound genes in both of these pathways. Neutrophils inherit these pro-inflammatory epigenetic alterations and become primed to oversecrete inflammatory cytokines. Thus, RUNX1 may play a direct role in epigenetically restraining these and other inflammatory pathways in GMPs. To confirm the relevance of our findings to RUNX1-FPD, we have profiled chromatin accessibility in neutrophils from 5 RUNX1-FPD patients and 5 controls, and found that RUNX1-FPD patients preliminarily appear to exhibit increased chromatin accessibility in inflammatory pathways.

The Ait-Oufella group demonstrated that mice fed a high fat diet for 4 weeks, returned to normal chow for 8 weeks, and then re-challenged with an additional 4 weeks of high fat diet greatly increase inflammatory neutrophil production during the second high fat diet feeding challenge. Thus, initial exposure to a high fat diet primes the myeloid system for an inflammatory and neutrophil-specific response to future periods of high fat diet feeding. RUNX1 expression in GMPs decreases after the initial high fat diet feeding, and remains low during the second high fat diet period. To test whether decreased levels of RUNX1 in GMPs are responsible for the inflammatory neutrophil phenotype, *Runx1$^{fl/fl}$; Cebpa-Cre* (RUNX1$^{\Delta GMP}$) mice were fed high fat diet for 4 weeks and neutrophil production was tracked. RUNX1$^{\Delta GMP}$ mice exhibit enhanced granulopoiesis and increased bone marrow neutrophil IL-1beta production in response to an initial high fat diet feeding period, as compared to control animals. These data support a model in which decreased RUNX1 expression in GMPs controls the inflammatory potential of neutrophils produced by the bone marrow.

Poster 14B | CAMB - Developmental, Stem Cell, and Regenerative Biology

Determining the Role of the Epidermis in Regulating Hair Follicle Phenotype

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Reciprocal interactions between the epidermis and dermis are necessary for proper organogenesis and patterning of hair. Classical tissue recombination between reptiles, avians, and mice have led to the current dogma that the dermis influences to the overall phenotype of the hair follicle including size, morphology, and cycling pattern. Using interspecies hair reconstitution assays whereby trichogenic epidermal and dermal cells from different species are grafted onto nude mice to form de novo hair, our laboratory has preliminary evidence that the epidermis wields a greater influence in determining the overall phenotype of the hair follicle that forms. We thus hypothesize that the epidermis, as opposed to the dermis, encodes the phenotype of the hair follicle. Once these results are thoroughly confirmed, we hope to use this system to further investigate the dynamics that underly formation of the hair follicle. In doing so, we hope to better understand how the epidermis is capable of restricting the developing follicle to produce a specific phenotype of hair.

Poster 15B | Poster 15B | CAMB - Gene Therapy and Vaccines

Reducing Kidney Fibrosis by FAP-CAR-T Cells in Mouse Models of Kidney Disease

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Chronic kidney disease (CKD) is characterized by the progressive decline in kidney function. Histologically, this disease is marked by extensive tubular destruction, fibrosis, and inflammation, contributing to global nephron loss. Over time, this loss of function necessitates renal replacement therapy. Importantly, no specific therapy yet exists for targeting fibrosis, the major contributor to the underlying pathology of CKD. Chimeric antigen receptor T (CAR-T) cells could be a potential therapy by specifically targeting the pathologic fibroblasts responsible for fibrosis. Notably, pathologic fibroblasts specifically express the cell surface marker fibroblast activation protein (FAP). We have previously shown CAR-T cells targeting FAP (FAP-CAR-T cells) can eliminate cardiac fibrosis in models of hypertensive heart disease. Given these data, we hope to translate FAP-CAR-T cell treatment to a model of CKD in proof-of-concept studies. Here, we demonstrate that FAP is specifically expressed in both human disease samples and a mouse model of CKD, correlating with histologic fibrosis. Pilot studies have shown that FAP-CAR-T cells can infiltrate the injured kidney and are functional, as demonstrated by trogocytosis or chewing up of FAP from fibroblasts. However, current data do not show an obvious reduction in either fibrosis or FAP in mice treated with FAP-CAR-T cells compared to saline treated controls. Future studies are devoted to optimizing treatment and determining if this paradigm has efficacy in this model. These studies include titrating the amount of injury the mice experience, changing the dosage and timing of FAP-CAR-T cell administration, and armoring FAP-CAR-T cells to express soluble factors to neutralize other components of the injured niche that exacerbate fibrosis and hinder tissue recovery.

Poster 16B | CAMB – Genetics and Epigenetics

Genome Folding and Speckle Association Cooperate to Orchestrate Fibroblast Activation

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Cell-state changes serve a crucial rheostatic function, allowing the cell to adapt to shifting environmental cues. Changes in cell-state require coordinated changes in gene expression. A crucial regulator of gene expression is the multileveled organization of the genome within the nucleus. At one level of organization, the ring-shaped protein complex cohesin folds chromosomes into regions of increased self-interaction. At another level of organization, genomic regions lose or gain association with subnuclear structures. Yet the interplay of these organizational units and their role in cell-state changes remains unclear. Here, we report that proper genome folding and spatial positioning are required for fibroblast activation, a model cell-state change. Fibroblasts activate in response to inflammatory or injurious stimuli. Activated fibroblasts contract and secrete extracellular matrix proteins. Using DNA fluorescence in situ hybridization (FISH) and confocal microscopy we found that cell-state specific genes undergo changes in genome folding during fibroblast activation, correlating with changes in gene expression. Moreover, knock-down of positive or negative regulators of cohesin toggled not only the folding of model loci, but also the expression of the genes they contained. However, changes in genome folding alone did not adequately account for the observed changes in gene expression. Using immunofluorescence and intronic-RNA FISH we found that fibroblast activation specific genes were preferentially transcribed at nuclear speckles, subnuclear bodies that boost gene expression. Speckle knock-down impeded activation associated gene expression changes, but did not alter local genome folding. Furthermore, we found that enhanced cohesin function compensated for suppressed speckle function and rescued gene expression defects, consistent with synergistic crosstalk between these two levels of genome organization. Our study provides insight into how genome folding and spatial positioning cooperate during cell-state changes.

Poster 17B | CAMB - Genetics and Epigenetics

Pathological characterization, genome-wide methylation, and *in vitro* modeling of trophoblast overgrowth in Beckwith-Wiedemann Syndrome

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The pediatric overgrowth disorder Beckwith-Wiedemann Syndrome (BWS) is characterized by distinct pathological changes in the placenta, including increased number and size of the extravillous trophoblasts (EVTs) known as EVT hyperplasia and cytomegaly. The EVTs are the invasive cell population of the placenta and defects in their differentiation from the germinative cytotrophoblasts are associated with preeclampsia and intrauterine growth restriction. Therefore, understanding the etiology of EVT abnormalities in BWS has implications for this rare syndrome and more common adverse pregnancy outcomes. BWS is caused by genetic and epigenetic alterations on chromosome 11p15, most commonly loss of methylation at the imprinting control region 2 (IC2 LOM) leading to loss of expression of the cyclin-dependent kinase inhibitor 1C (*CDKN1C*). Previous studies have reported an association between loss of *CDKN1C* expression and EVT hyperplasia and cytomegaly in BWS placentas; however, these reports have been limited in their sample size and have not characterized the mechanism by which *CDKN1C* may influence EVT growth or differentiation. In this study, we performed pathological characterization of a cohort of BWS placentas with a focus on patients with IC2 LOM and *CDKN1C* gene mutations. We have found an association between loss of *CDKN1C* expression in the trophoblasts and the presence of EVT hyperplasia and cytomegaly. We also identified an association between EVT hyperplasia and prematurity. To better understand the role of *CDKN1C* in these EVT phenotypes, we established an *in vitro* model of BWS trophoblast differentiation. In collaboration with the Penn iPSC core, our lab has established an iPSC model of BWS by allele-specific deletion of the IC2 region (BWS-iPSCs). We performed a two-step differentiation protocol of BWS-iPSCs and control iPSCs toward cytotrophoblast-like cells followed by terminally differentiated trophoblasts, including EVT-like cells. We found that BWS-iPSCs had reduced expression of *CDKN1C* and increased proliferation index at the cytotrophoblast stage but demonstrated no difference in efficiency of the first step of differentiation. The finding of increased proliferation of BWS-iPSC derived cytotrophoblasts is supported by cell-type deconvolution analysis of genome-wide methylation array data of BWS placentas, which shows an increase in the proportion of cytotrophoblast cells and a decrease in stromal cells in BWS placentas compared to control. We also found that BWS-iPSCs demonstrated increased expression of an EVT marker during the second step of differentiation, indicating more efficient differentiation toward the EVT lineage. These findings provide preliminary evidence that loss of *CDKN1C* expression promotes trophoblast proliferation and EVT differentiation in BWS. To follow up this finding, we aim to knockdown *CDKN1C* expression in human trophoblast stem cells (hTSCs), then assess the differentiation efficiency of these cells toward the EVT lineage *in vitro*. We will then determine if

the impact of *CDKN1C* on trophoblast differentiation is dependent on its canonical function as a cell cycle regulator or on other noncanonical functions of this protein.

Poster 18B | CAMB - Genetics and Epigenetics

Lsd1 is a critical mediator of epidermal development, homeostasis, and oncogenesis

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Alterations in epigenetic modifiers have been recognized as key determinants in differentiation and oncogenic signaling. Specifically, histone lysine-specific demethylase 1 (LSD1) represses expression of crucial differentiation genes and preserves progenitor-like cell fates. As such, LSD1 is frequently overexpressed in human malignancies, leading to interest in LSD1 inhibitors for cancer therapy. Despite this, the role of LSD1 in skin homeostasis and oncogenesis is virtually unknown. Previously, our lab discovered that LSD1 inhibition *in vitro* unleashes epidermal differentiation to prevent cSCC (Egolf & Aubert, *Cell Reports*, 2019). Here, we provide new evidence that LSD1 is a vital epigenetic regulator in the skin *in vivo*. Mice with constitutive epidermal deletion of *Lsd1* are embryonic lethal stemming from profound barrier dysfunction and lack of epidermal stratification. In contrast, inducible knockout of Lsd1 in adult mice is tolerated and results in epidermal thickening, increased immune cell recruitment to the skin, and dramatic activation of retinoic acid signaling. Next, we were able to successfully recapitulate the results of our genetic knockout through our development of a topical catalytic inhibitor of Lsd1. Strikingly, we find that concomitant treatment with a RAR antagonist rescued the gross histologic effects and reversed the epidermal thickening and immune cell recruitment caused by the Lsd1 inhibitor. Lastly, we demonstrate that this topical Lsd1 inhibitor can reduce skin tumorigenesis *in vivo*, potentially through an immune-mediated effect. Taken together, our data supports a pivotal role for LSD1 in establishing the skin barrier and maintaining epidermal homeostasis through the regulation of retinoic acid signaling and immune cell function. The data also provide preliminary rationale for LSD1 inhibition for cutaneous diseases such as keratinocyte cancers.

Poster 19B | CAMB - Genetics and Epigenetics

Hemodynamics and KLF2/4 regulate myxomatous valve formation

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Valvular heart disease affects up to 2% of people worldwide and often requires surgical intervention since effective medical therapies do not exist. Myxomatous valve disease (MVD) is among the most common forms of valvular heart disease and is characterized by pathologic thickening and degeneration of valve leaflets, leading to regurgitation and valve prolapse. MVD can be syndromic, occurring due to single gene mutations such as in Marfan's Syndrome, but often presents in the absence of known mutations suggesting that environmental factors influence MVD formation. We have previously shown that shear-responsive transcription factors KLF2 and KLF4 (KLF2/4) are required for cardiac valve formation by transducing hemodynamic forces. However, whether hemodynamic forces and KLF2/4 play a role in adult cardiac valves remains unknown. Here we demonstrate that hemodynamic forces are required for valve homeostasis and endothelial cell quiescence in the adult cardiac valve. Loss of blood flow across the mouse mitral valve in transplanted hearts leads to pathologic myxomatous valve formation, characterized by proliferation and decreased expression of KLF2/4. Using a genetic inducible system, loss of KLF2/4 from the adult valve endothelium is sufficient to drive rapid and severe myxomatous valve pathology. Mechanistically, loss of KLF2/4 from valve endothelial cells leads to increased TGFb/SMAD signaling in both valve endothelial and valve interstitial cells. Co-deletion of *Tgfbr1* partially rescues myxomatous valve formation following loss of KLF2/4. These data support a model in which hemodynamic forces acting through KLF2/4 maintain valve homeostasis, in part, through suppression of pathologic TGFb/SMAD signaling.

Poster 20B | CAMB - Genetics and Epigenetics

Critical assessment of CGG short tandem repeat length, *FMR1* DNA promoter methylation, heterochromatin, and transcriptional repression of neural, synaptic genes in fragile X syndrome

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Diseases can vary in their presentations across individuals with the same diagnosis. In fragile X syndrome (FXS), the expanded CGG short tandem repeat (STR) in *FMR1* leads to promoter DNA methylation and transcriptional silencing of *FMR1*. Recently, ectopic Megabase-scale domains of heterochromatin with higher-order genome folding ablation were found in FXS epigenomes. Whether variation in heterochromatinization and higher-order genome folding exists and correlates with transcriptional repression in FXS is unknown. Here, by profiling multiple FXS patient derived induced pluripotent stem cells differentiated to neural progenitor cells (iPSC-NPCs), we demonstrate that variation in heterochromatin, loss of chromatin folding and transcriptional repression, are independent of known correlates of FXS severity: mosaicism of STR length and or *FMR1* promoter DNA methylation. We find that *SLITRK4* and *SLITRK2*, genes implicated synaptic processes and associated with neurodevelopmental disorders, correlate with H3K9me3 deposition in FXS patient iPSC-NPCs. When we derive subclonal lines from a singular FXS patient line, we find that variation in H3K9me3 deposition correlates with repression of *SLITRK4* and *SLITRK2*. Our results identify heterochromatin as a facet of epigenomic variation in FXS genomes that correlates with transcriptional silencing of genes associated with neurodevelopmental disorders.

Poster 21B | CAMB - Genetics and Epigenetics

Deciphering the role of cytoskeletal-nuclear interactions in peripheral chromatin organization

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The mammalian genome is organized into various regions at different scales as one mechanism to regulate gene expression and mediate cellular identity. One type of region is the lamina-associated domain (LAD), which contains chromatin regions that directly interact with the nuclear lamina (NL) at the nuclear periphery. Found across all chromosomes, LADs dynamically interact with the NL to release or attach genes and regulatory elements in accordance with cell-type and differentiation state-specific gene expression programs. Patients with mutations in *LMNA*, encoding the A and C type lamins in the NL, develop a heterogenous group of diseases, known as laminopathies. We previously used induced pluripotent stem cells (iPSC) to determine the impact of a patient *LMNA* mutation (T10I) on peripheral heterochromatin organization. T10I iPSC-derived cardiomyocytes (iPSC-CMs) exhibited gross nuclear abnormalities and demonstrated loss of lamina-bound chromatin enriched in genes and lower lamin B1 contact frequency. We then modeled *LMNA* haploinsufficiency in iPSC-CMs using siRNAs against LMNA/C. *LMNA* knockdown also resulted in abnormal nuclear morphology, and relocalization of LAD loci away from the nuclear periphery. Mapping LADs using CUT&RUN demonstrated that LAD regions sensitive to *LMNA* loss were enriched at the edges of weaker LADs. Evidence from mouse models and human genetic studies have suggested a potential role for the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex in mediating genome organization. When we disrupted the LINC complex, we found that a subset of *LMNA*-sensitive LADs were prevented from losing laminB1 enrichment, and a subset of genes were no longer dysregulated. We plan to continue to use a combination of population-based genomics analyses and single-cell microscopy to test the hypothesis that cytoskeletal-lamina interactions destabilize LADs in lamin mutant cells. These studies will provide mechanistic insights into how the nuclear lamina and LINC complex are involved in LAD organization in cardiomyocytes, which will begin to provide novel understanding of the molecular basis of laminopathy phenotypes.

Poster 22B | CAMB - Microbiology, Virology, and Parasitology

Effect of Mutations in the Glycoprotein of *Dabie Bandavirus* (SFTSV)

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Dabie Bandavirus is an emerging tick-borne virus found predominantly in Southeast Asia. It is also referred to as Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) due to the clinical symptoms associated with infection. Some infected patients only exhibit mild flu-like symptoms, but approximately 12-30% of patients develop severe hemorrhagic disease and succumb to infection. Currently, there are no FDA-approved therapeutics or vaccines for the treatment or prevention of SFTSV. Due to this and the high mortality rate of infected patients, the World Health Organization has designated SFTSV a priority pathogen, and there is an urgent need for a safe and effective vaccine.

Recent research has focused on developing a recombinant VSV vaccine that expresses the SFTSV glycoprotein (rVSV-SFTSV). VSV can readily incorporate foreign glycoproteins onto its surface and induce a protective immune response. Moreover, VSV is minimally pathogenic and has a very low seroprevalence in humans. An rVSV vaccine for Ebola was the first FDA-approved viral vectored vaccine, highlighting the safety and efficacy of this platform. To date, the rVSV-SFTSV vaccines that have been developed are very attenuated in cell culture, making it difficult to produce the large quantities of virus necessary for vaccination. Recent studies by our lab and others have identified mutations in the SFTSV glycoprotein that increase the titer of rVSV-SFTSV in cell culture, but these mutations have never before been combined into one vaccine candidate.

We sought to determine the effect of three different mutations on incorporation of the SFTSV glycoprotein onto VSV pseudoviruses. We discovered that each mutation individually increases the viral titer, and the mutations have a synergistic effect in combination. The combination of mutations significantly increased the titer of pseudoviruses by approximately 95 times. We have shown that the increased titer is not a result of altered steady state glycoprotein expression, and the titer cannot be completely explained by altered surface expression. Future work will elucidate the mechanism(s) by which these mutations increase the titer of pseudoviruses. We will also launch replication competent rVSV-SFTSV viruses carrying these mutations to study replication kinetics and the efficacy of each mutant as a vaccine candidate.

Poster 23B | CAMB - Microbiology, Virology, and Parasitology

Mitochondrial oxidative phosphorylation restricts SARS-CoV-2 replication via metabolic remodeling

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SARS-CoV-2 rewires host metabolism, and this is thought to optimize virus production. While glycolysis is necessary for virus production, whether mitochondrial oxidative phosphorylation (OXPHOS) is required for SARS-CoV-2 replication is unknown. Mitochondrial DNA (mtDNA) codes for 13 critical oxidative phosphorylation (OXPHOS) polypeptides of the electron transport chain (ETC) as well as the mitochondrial translation machinery necessary for their production. I discovered ~5 to 100-fold greater SARS-CoV-2 virus production in infected human ACE2-expressing A549 cells where OXPHOS was inhibited by mtDNA depletion (ρ^0 cells). A similar infection enhancement is observed by blocking mitochondrial translation and chemically inhibiting ETC complexes. Cells with inhibited OXPHOS demonstrate increased size and distribution of viral replication centers and promote infectious particle production and release two hours earlier than WT cells following infection. Notably, mitochondria-associated inflammation remained intact, and enhanced glycolysis underpins the replication advantage. Reintroduction of mtDNA into ρ^0 cells reinstates OXPHOS, impairs SARS-CoV-2 viral replication compared to parental r^0 cells, and reverses pro-viral correlates. In summary, my findings support that metabolic balance can regulate SARS-CoV-2 replication. Specifically, OXPHOS exerts a metabolically-regulated antiviral effect on SARS-CoV-2 infection, which may offer mechanistic insight into the antiviral effect of metformin in acute and long COVID-19 clinical trials.

Poster 24B | Chemistry

Synthesis and design of macrocyclic collagen mimetic peptides for targeting the cancer-implicated DDR2 kinase

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Collagen lays the foundation of bodily tissues, serving to strengthen, connect, and signal from the micro to the macro scale. The importance of collagen in cancer biology ranges from regulation of the tumor microenvironment to dysregulation of cancer cell signaling. Key to these effects is its interaction with DDR2, a receptor tyrosine kinase implicated in multiple cancers. This work seeks to develop synthetic collagen mimetic peptides (CMPs) for interrogation of the collagen-DDR2 interaction. As a triple helical polymer involved in biochemical signaling, collagen has potential as a tool for modulating protein-protein interactions. These applications have heretofore been limited by its tripartite nature, which restricts its thermal and entropic stability. Synthetic linkage and cyclization of the three collagen strands may overcome these limitations. Herein, we present the design and synthesis of miniaturized, cyclic and linear CMPs with desirable thermal stability and capacity to interact with DDR2. Cyclization of strands as well as incorporation of aza-glycine residues strongly improve thermal stability of DDR2-targeted CMPs, allowing for development of 25% smaller peptides with up to 23 ºC higher melting temperature. This work develops chemical tools for modulation of the cancer-implicated interaction between collagen and DDR2 via an innovative chemical biology approach, with potential for applications in drug discovery.

Poster 25B | Epidemiology and Biostatistics – Epidemiology

Racial Differences in Background Parenchymal Enhancement, a Novel Marker of Breast Cancer

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Background: Breast density is a risk factor for breast cancer that can guide eligibility for supplemental screening, for which Black women are less likely to qualify for due to having less dense breasts on average despite higher rates of breast cancer-associated mortality. Background parenchymal enhancement (BPE), the enhancement of fibroglandular breast tissue on contrast enhanced MRI, is a novel biomarker that has been shown to be a superior breast cancer risk marker to breast density across racial groups. Women with high BPE levels have up to a four fold increased risk of breast cancer, even after adjusting for density, however, no studies have examined BPE exclusively among Black women. We sought to examine racial differences in BPE.
Methods: We identified Black and White women ages 40-74 who had a mammogram and subsequent MRI as part of a screening or diagnostic work-up from 2016-2022, with no history of breast cancer. BPE was qualitatively defined according to BI-RADS (Breast Imaging Reporting and Data System), with four ordinal levels of increasing enhancement — minimal, mild, moderate, and marked. We employed a cross-sectional study design to perform a logistic regression with race as the exposure and BPE as the outcome, using minimal and mild BPE as the reference group, adjusted for age and BI-RADS density.
Result: We included 1027 Black women and 4834 White women in our analysis. Few Black women have extremely dense breasts compared to White women (6% vs 13%, p= 0.04), however there is a larger proportion of high BPE (moderate or marked) in Black women compared to White women (32% vs 28%, p = 0.03) (Table 1). There was an inverse association between age and BPE levels (p = 0.03), and a positive association between density and BPE levels regardless of race (p< 0.01) (not shown). Black women are 39% more likely to have high BPE levels (95% CI: 1.39 [1.19, 1.63]), adjusted for age and density.
Conclusion: Black women have lower density but higher BPE than White women, suggesting that BPE may improve identification of breast cancer risk for Black women. Methods to assess BPE levels among Black women are urgently needed to identify "high risk" Black women that may benefit from supplemental screening with MRI.

Poster 26B | Genomics and Computational Biology

A Longitudinal Single-Cell and Spatial Multiomic Atlas of Pediatric High-Grade Glioma

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Pediatric high-grade glioma (pHGG) is an incurable central nervous system malignancy that is a leading cause of pediatric cancer death. While pHGG shares many similarities to adult glioma, it is increasingly recognized as a molecularly distinct, yet highly heterogeneous disease. In this study, we longitudinally profiled a molecularly diverse cohort of 16 pHGG patients before and after standard therapy through single-nucleus RNA and ATAC sequencing, whole-genome sequencing, and CODEX spatial proteomics to capture the evolution of the tumor microenvironment during progression following treatment. We found that the canonical neoplastic cell phenotypes of adult glioblastoma are insufficient to capture the range of tumor cell states in a pediatric cohort and observed differential tumor-myeloid interactions between malignant cell states. We identified key transcriptional regulators of pHGG cell states and did not observe the marked proneural to mesenchymal shift characteristic of adult glioblastoma. We showed that essential neuromodulators and the interferon response are upregulated post-therapy along with an increase in non-neoplastic oligodendrocytes. Through in vitro pharmacological perturbation, we demonstrated novel malignant cell-intrinsic targets. This multiomic atlas of longitudinal pHGG captures the key features of therapy response that support distinction from its adult counterpart and suggests therapeutic strategies which are targeted to pediatric gliomas.

Poster 27B | Genomics and Computational Biology

Towards a universal metric for comparing cellular neighborhoods

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Spatial organization of a tissue microenvironment provides key insights into functional biological mechanisms. In organ development, the precise spatial arrangement of cells is crucial for proper tissue formation and function. In cancer, spatial heterogeneity of the tumor microenvironment plays a major role in the development of resistance to treatment. Advances in single-cell resolution spatial omics technologies have facilitated the exploration of tissue microenvironments and their role in normal function and disease. Recent developments in quantitative methods for tissue cellular neighborhood (TCN) detection have provided further insights into the organization of spatial heterogeneity within individual tissue samples. However, methods to compare neighborhoods between samples of varying time points and clinical features are limited. We introduce a distance metric that enables quantitative comparison of TCNs across conditions, built upon a novel *N-Orbit* formalism of a neighborhood. We benchmark our N-Orbit-based neighborhood distance metric on spatial omics datasets with manually annotated ground truth anatomical regions and on patient datasets with corresponding clinical variables. We demonstrate that our approach has the potential to augment findings from existing neighborhood detection methods in the spatial analysis of tissue microenvironments broadly.

Poster 28B | Genomics and Computational Biology

The phenotypic basis of CT-derived kidney traits and their utility in predicting estimated glomerular filtration rate

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The growing size of patient biobanks have given researchers unprecedented access to large-scale datasets of linked genomic and phenotypic data, including patient radiology scans. However, there is a particular need to study the biological basis of the radiomic signatures to validate their clinical utility. We hypothesize that kidney imaging-derived phenotypes (IDPs) are both biologically and clinically informative and can be used to predict estimated glomerular filtration rate (eGFR). We extracted CT scans of the thorax, abdomen, and/or pelvis for 20,289 unique individuals in the Penn Medicine Biobank and segmented the kidneys using TotalSegmentator. For scans where the complete kidney is captured, we derived quantitative imaging traits for genome and phenome association studies. All associations were adjusted for sex, age, age^2, BMI, and genetic principal components 1-10. A simple feed-forward neural network was also trained on a regression task to predict eGFR using the imaging-derived kidney traits as well as age and sex. The dataset was split into 70%/15%/15% training/validation/testing splits. We performed phenome-wide association studies (PheWAS) against multiple quantitative kidney IDPs. For kidney volume, we observed strong negative associations with end-stage renal disease as well as related circulatory conditions such as hypertension and congestive heart failure. Similar trends were also identified for kidney surface area and mean attenuation. Our neural network model for predicting eGFR from kidney traits was trained on eGFR values documented within 7 days of a CT scan. The model exhibited robust predictive ability using only kidney IDPs and had a mean squared error of 413.93 on the testing dataset. Our association studies not only demonstrate the strong correlations between CT imaging-derived kidney traits and kidney-related conditions, but also begin to elucidate the granularity in how different kidney diseases affect specific kidney traits and not others. We also plan to perform genome-wide association studies to study the genetic architecture of our kidney IDPs. Furthermore, the quantitative IDPs showed predictive potential for approximating eGFR, demonstrating the utility of such a tool that could be integrated into a clinical workflow and used to indicate further testing in relevant patients.

Poster 29B | Health Care Management & Economics

Hospital Responses to Financial Risk

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There has been an increasing interest in reimbursing health care providers through methods other than the traditional fee-for-service paradigm, such as through value-based payments (VBPs). VBPs typically involve shifting financial risk from health care payers (e.g., insurers) to providers (e.g., hospitals). The goal of this project is to explore how hospitals manage bearing financial risk, to gain a better understanding of the potential consequences of this shift towards VBPs and how to design policy to minimize such consequences. Of particular interest to policymakers is if hospital risk-bearing results in potential changes of the operating status of hospitals (e.g., closures) or if hospitals seek to spread that risk across a larger pool by engaging in consolidation activity (e.g., mergers). To achieve this, I consider the introduction of the Inpatient Prospective Payment System (IPPS) by Medicare in 1983 as an exogenous shock to the amount of risk borne by hospitals. I find that the IPPS substantially increases the volatility in profits faced by hospitals, with the impact being the greatest among smaller hospitals. I intend to utilize hospital data from the state of California to analyze the strategic responses of hospitals to this significant increase in risk.

Poster 30B | Health Care Management & Economics

Physician consults in the hospital

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Physician consultations are an important yet understudied facet of hospital care delivery. Consults occur when the primary attending physician formally requests the help of another physician in the diagnosis or treatment of a patient. Because most non-surgical, hospitalized patients are treated by hospitalists or other general medicine physicians, consults are the main mechanism by which specialty care is delivered in the inpatient setting. Previous work finds that more than half of all hospitalizations among traditional Medicare beneficiaries involved at least one consultation, and more than 20% involved consultations of two or more specialties. Consults could improve quality of care if they increase diagnostic accuracy, change patient management, or provide access to treatments and services not otherwise available. On the other hand, consults could represent "flat-of-the-curve" medicine if they do not improve care, result in unnecessary tests and procedures, and increase patients' length of stay in the hospital.

Despite their importance and frequency, there is little high-quality evidence on the effects of consults on health and utilization outcomes. Because consults are ostensibly requested when the primary team has a question or needs help, they are highly correlated with severity of illness and clinical information not commonly observed in administrative datasets. To address the endogeneity of consults, I conduct an instrumental variable analysis using detailed electronic medical record data from a large health system in Pennsylvania over 2017-2022. Specifically, I exploit variation in demand for infectious disease (ID) consults during the first 24 hours of admission to the general medicine service. I show that patients are less likely to receive a consult when consultants are busier due to consult requests made by other physicians. Estimation of the effect of an ID consult on 30-day readmission via OLS gives a 2 percentage point (pp) increase, which reflects omitted variable bias. In contrast, estimation via 2SLS gives a 35 pp *decrease* in the probability of readmission. In addition, I find that ID consults greatly increase length of stay and charges of the index hospitalization and moderately increase 30-day episode, inpatient charges. Although the effect is imprecise, patients who receive an ID consult are 23 pp more likely to use post-acute care upon discharge, mostly in the form of home health. Physician consults may significantly affect the type and intensity of inpatient care, as well as the receipt of outpatient services after discharge. Given the prevalence of consults and limited specialist capacity, improved understanding of consults' tradeoffs in the inpatient setting, as well as their downstream consequences for outpatient care, is critical for improving care delivery.

Poster 31B | History & Sociology of Science

Making Fun, Making Doctors: "Spoof" shows and professionalization in US Medical Education, 1950-2000

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Comedy variety shows ("spoof" shows) are a common and consistent feature of medical student life across U.S. medical schools, then and now. Composed of parody songs and skits written by students for their peers, these shows are a window into medical student culture at a very early stage in their medical training and professionalization. I explore the medical school "spoof" shows from a range of U.S. medical schools from the 1950s to the 2000s. While student shows have been acknowledged in the historical literature on medical education and student rituals (i.e. cadaver work), they have yet to be broadly surveyed and critically examined. Using a rich body of recordings, images, scripts, and records from a variety of schools including University of Michigan Medical School, Harvard Medical School, and Women's Medical College of Pennsylvania, I showcase remarkable continuities in medical culture across geographic, temporal, and gender-based boundaries, as well as the strikingly local characteristics of these shows and the progressive changes in both style and content over the second half of the 20th century. I attend particularly to how shows discuss gender, prestige, and relationships with faculty. Ultimately, I argue that these shows represent an opportunity for students to be vulnerable in a controlled and professionally sanctioned manner: a safe space to make plain the unwritten and not-for-public-consumption realities about medical training. These shows offer a rare and raw glimpse of medical students' fears, anxieties, and frustrations and confusions with the process of professionalization, offering insight into why spoof shows are still part of medical education today.

Poster 32B | Tonsillar versus circulating CD4 T Follicular Helper polarization states delineated by trimodal single-cell sequencing and spectral flow cytometry in a healthy pediatric cohort

Sam Barnett Dubensky, Molly Gallagher, Nina De Luna, Kingsley Kumashie, Amy Baxter, Tianyu Lu, Yi Qi, Jonathan Tedesco, Neil Romberg, Sarah Henrickson, Derek Oldridge, and Laura Vella

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CD4 T Follicular Helper (Tfh) cells are an integral component of the adaptive immune system that coordinate antibody responses and establish long-term antigenic memory. Tfh cells can tailor humoral immunity to the specific pathogen or other challenges by differentiating into "polarized" subsets including Tfh1, Tfh2, and Tfh17. To provide rapid as well as long-term protection, Tfh are thought to further differentiate into effector memory (EM) and long-lived central memory (CM) subsets. However, it remains unknown how these memory fates are coupled with helper-polarity during Tfh differentiation. Delineating the gene regulatory mechanisms underlying this continuum of differentiation states may enable the rational design of immunotherapies to modulate Tfh activity in vaccination, cancer, autoimmunity, or other vital contexts.

However, measuring features of both Tfh polarity and memory fate has been limited by assays that cannot simultaneously capture dynamic gene regulation from the epigenetic to transcriptional and proteomic layers. Recently, TEA-seq (measuring mRNA Transcripts, surface protein Epitopes, and genome-wide chromatin Accessibility) has enabled deep multimodal molecular analysis at single-cell resolution. Using TEA-seq, our group identified substantial discordance between subset definitions of Naive, CM, and EM CD4 T cells based on gold-standard proteomic features versus RNA or ATAC reference atlas predictions. Consistent with recent literature, our findings underscore the need to integrate multimodal data to accurately discern specific Tfh states within the greater heterogeneity of the CD4 T cell compartment.

To capture baseline Tfh heterogeneity during childhood, we assembled a pediatric cohort of healthy peripheral blood donors and patients undergoing tonsillectomy for sleep-related indications, balanced across sex and age. From this cohort, we profiled peripheral blood and tonsillar lymphocytes by TEA-seq using 10x Multiome scRNA/ATAC-seq and a 155-parameter cocktail of TotalSeq antibody-oligos. To complement our TEA-seq analysis, we performed 30-color spectral flow cytometry on the same samples, providing an orthogonal approach to discern polarized memory Tfh states in healthy children.

By dimensionality reduction of these multimodal data, Tfh17 were distinguished from other polarized subset by CM features, including increased expression of IL-7 receptor rather than TIGIT across expression modalities. Targeting the balance of IL-7 versus TIGIT signaling may

enable therapeutic control over long-lived Tfh17 differentiation. Further analysis of our Tfh epigenomic and transcriptional data will detail genetic programs that underly CM Tfh17 fate.

Poster 33B | Immunology

Inherited C-terminal TREX1 variants disrupt homology-directed repair to cause senescence and DNA damage phenotypes in Drosophila, mice, and humans

Samuel D. Chauvin, Shoichiro Ando, Joe A. Holley, Atsushi Sugie, Fang R. Zhao, Subhajit Poddar, Rei Kato, Cathrine A. Miner, Yohei Nitta, Siddharth R. Krishnamurthy, Rie Saito, Yue Ning, Yuya Hatano, Sho Kitahara, Shin Koide, W. Alexander Stinson, Jiayuan Fu, Nehalee Surve, Lindsay Kumble, Wei Qian, Oleksiy Polishchuk, Prabhakar S. Andhey, Cindy Chiang, Guanqun Liu, Ludovic Colombeau, Raphaël Rodriguez, Nicolas Manel, Akiyoshi Kakita, Maxim N. Artyomov, David C. Schultz, P. Toby Coates, Elisha D. O. Roberson, Yasmine Belkaid, Roger A. Greenberg, Sara Cherry, Michaela U. Gack, Tristan Hardy, Osamu Onodera, Taisuke Kato & Jonathan J. Miner

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Age-related microangiopathy, also known as small vessel disease (SVD), causes damage to the brain, retina, liver, and kidney. Based on the DNA damage theory of aging, we reasoned that genomic instability may underlie an SVD caused by dominant C-terminal variants in TREX1, the most abundant 3′−5′ DNA exonuclease in mammals. C-terminal TREX1 variants cause an adult-onset SVD known as retinal vasculopathy with cerebral leukoencephalopathy (RVCL or RVCL-S). In RVCL, an aberrant, C-terminally truncated TREX1 mislocalizes to the nucleus due to deletion of its ER-anchoring domain. Since RVCL pathology mimics that of radiation injury, we reasoned that nuclear TREX1 would cause DNA damage. Here, we show that RVCL-associated TREX1 variants trigger DNA damage in humans, mice, and *Drosophila*, and that cells expressing RVCL mutant TREX1 are more vulnerable to DNA damage induced by chemotherapy and cytokines that up-regulate TREX1, leading to depletion of TREX1-high cells in RVCL mice. RVCL-associated TREX1 mutants inhibit homology-directed repair (HDR), causing DNA deletions and vulnerability to PARP inhibitors. In women with RVCL, we observe early-onset breast cancer, similar to patients with BRCA1/2 variants. Our results provide a mechanistic basis linking aberrant TREX1 activity to the DNA damage theory of aging, premature senescence, and microvascular disease.

Poster 34B | Immunology

Spatial transcriptomic map of CAR-T cells *in situ* in the human tumor microenvironment

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Chimeric antigen receptor (CAR) T cells have had tremendous efficacy in certain relapsed/refractory hematologic malignancies, yet have had minimal success against solid tumors, which comprise 90% of all cancers. The immunosuppressive tumor microenvironment (TME) is a barrier to successful adoptive immunotherapy and comprises cell types that are potentially targetable to enhance CAR-T therapy in solid tumors. Mouse models to study CAR-T dynamics within the TME are poorly predictive for a number of reasons, including immunodeficiency and/or experimental and biological differences in tumor progression between mice and humans. To resolve this gap, we will leverage high-plex spatial single-cell transcriptomics of CAR-T infiltrated human tumors from patients with metastatic castration-resistant prostate cancer (mCRPC). In preliminary data, we see that CAR-T cells are spatially enriched in proximity to macrophages and monocytes. Furthermore, TME macrophages after CAR-T therapy have increased transcripts of proteins associated with poor clinical outcome across multiple cancers, including CXCL8 (IL-8) and SPP-1 (osteopontin). Expanded analysis of all available CAR-T-infiltrated human prostate tumors will enable us to make correlations between TME features and clinical outcome, paving the way for experimental validation to enhance CAR-T cells in metastatic prostate cancer.

Poster 35B | Immunology

Dietary long chain fatty acids shape innate immune cell tone and modulate inflammatory responses in the lung

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Obesity is a risk factor for asthma, and obesity-associated asthma (OAA) is more severe and more difficult to treat than allergic asthma. Macrophages regulate the lung innate immune response to allergic stimuli, and obesity influences macrophage functional states outside the lung, implicating obesity as an immune pressure that may modify macrophage-dependent lung inflammation. However, the mechanisms by which obesity alters lung macrophage function, and the consequences of these effects on lung inflammation, are not well understood. Improving our understanding of these processes will facilitate the development of targeted therapies for OAA and other obesity-associated inflammatory lung conditions.

We first compared the cellular phenotype of lung macrophages from lean and obese mice and found that obesity expands lung macrophage populations with features of obesity-associated activation including surface CD9 and increased intracellular lipid. Additionally, obesity increases IRE1a endonuclease activity in lung macrophages, and increases production of IL-1b, a cytokine implicated in the development of OAA. Lipidomics analysis revealed increased stearate (SA), a saturated long chain fatty acid, in obese lung macrophages. In vitro, SA induces IRE1a-dependent priming of the macrophage NLRP3 inflammasome. In vivo, SA causes expansion of IL-1b producing, CD9+ lung macrophages and monocytes, and increases the severity of inflammation in a model of neutrophil-predominant asthma. Crucially, increased severity of neutrophilic inflammation after SA diet feeding was dependent on IRE1a endonuclease activity, myeloid cell caspase-1 expression, and IL-1b signaling. Finally, we identified a population of obesity- and asthma-associated CD9+ lipid-laden lung monocytes in humans, suggesting that obesity-associated activation of lung macrophages may be conserved across species.

Our work has identified an axis by which high fat diet-associated fatty acids prime lung macrophages for exaggerated, inflammasome-mediated inflammation in response to an allergic stimulus, with broad implications for understanding the etiology and refractory nature of OAA. Ongoing studies will investigate the molecular and metabolic mechanisms by which stearate alters macrophage activation and will inform future treatment efforts through dietary modification or targeted therapeutics.

Poster 36B | Immunology

Excess IL-18 suppresses EAE by augmentation of CD8Tregs to inhibit autoreactive T-cell activity

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Interleukin 18 (IL-18) is an IL-1 family cytokine that canonically induces IFNγ to amplify Th1 responses. Despite its likely pathogenic role in autoinflammation, clinical observations suggest an immunoregulatory role in CD4T-mediated autoimmunity. We recently uncovered a protective effect of excess IL-18 in murine experimental autoimmune encephalomyelitis (EAE), a model of CD4T cell CNS autoimmunity. We hypothesize that IL-18 is augmenting suppressive CD8T cells to enhance clearance of autoreactive CD4T cells and suppress CNS damage.

Il18bp$^{-/-}$, Il18tg, 2D2, various cre x Il18r1$^{fl/fl}$ and control mice underwent MOG$^{35-55}$ immunization to induce EAE. IL-18bp$^{-/-}$ mice lack IL-18's circulating antagonist while Il18tg mice express an IL-18 transgene, both modeling excess IL-18. 2D2 mice express an MHC-II restricted TCR transgene specific for MOG$^{35-55}$. Requirements for IL-18 signaling on T cells, CD4Tregs, and CD8 T cells were tested using CD4-Cre, FoxP3-Cre, and E8icre respectively.

Il18bp$^{-/-}$ and Il18tg mice demonstrated profound protection from disease which was abrogated with T cell-specific loss of IL-18 receptor (CD4-cre, Il18r1$^{fl/fl}$, Il18tg). Analysis of CD4 T cell cytokine production demonstrated decreased MOG-specific responses with excess IL-18. Spinal cords from mice with excess IL-18 showed decreased CD4 T cells with increased CD8Ts (relative to CD4Ts). These data suggested suppression of autoreactive CD4Ts by a population of regulatory T cells (CD4 Tregs or CD8Tregs). Protection did not require IL-18 signaling on CD4 Tregs (FoxP3-cre, Il18r1$^{fl/fl}$, Il18tg) but required signaling on CD8 Ts (E8i-cre, Il18r1$^{fl/fl}$, Il18tg). Further, IL-18-mediated protection was partially lost with CD8 T cell depletion. Taken together, these data implicated CD8 Tregs. The suppressive capacity of adoptively transferred CD8 Ts was enhanced by culture in IL-18 resulting in delayed EAE onset. Neutralization of IFNg eliminated all protective effects while knock-out of Perforin abrogated late protection. This suggests a role of IFNg-mediated suppression and Perforin-mediated cytotoxicity by CD8 Tregs targeting autoreactive CD4 T cells. Preliminary results suggest an increase of HELIOS-expressing CD8 T cells within mice with excess IL-18.

These data suggest that excess IL-18 augments a population of CD8Tregs cells to mediate protection from autoreactive CD4s in EAE. Work is ongoing to characterize the phenotype and

function of IL-18 amplified CD8 Tregs. This may inform novel strategies to amplify endogenous suppressive T cells or cellular therapeutics to treat autoimmunity.

Poster 37B | Immunology

CAR-T cell therapy for idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a fatal disease of the lung characterized by loss of gas exchange capacity and excessive deposition of dense extracellular matrix. In my work, I explore the effects of fibroblast-targeted CAR-T cells on patient-derived, ex vivo-cultured lung tissue, which has shown early promise in depleting fibrotic matrix and pathogenic fibroblasts.

Poster 38B | Neuroscience

Investigating the role of GABAergic interneurons in the dorsomedial striatum in value-based decision-making

Evan Iliakis, Alexandra Ramirez, Carlos Ivan Linares-Garcia, Luigim Vargas, Kyuhyun Choi, Sarah Ferrigno, Edgar Diaz-Hernandez, Elizabeth Holly, David Margolis, Marc Fuccillo

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Value-based decision-making aims to maximize rewards in changing environments. Abnormalities in value-based decision-making are a feature of a range of neuropsychiatric diseases and are functionally impairing. The dorsomedial striatum (DMS) is critical for the execution of value-based decision-making. Within the DMS, the role of sparse GABAergic interneurons in behavior is of increasing interest. Tyrosine hydroxylase-positive (TH+) interneurons (THINs) are necessary for maintaining goal-directed strategies, while somatostatin-positive (SST+) low-threshold spiking interneurons (LTSIs) exhibit novel reward-related activity that decays throughout operant learning. However, the role of DMS THINs and LTSIs in value-based decision-making is not known. To study their role in value-based decision making, we have developed a head-fixed joystick-based two-alternative forced choice behavioral paradigm in mice that assays the effect of varying relative reward values, reward probabilities, and net reward environment on value-based goal-directed choice and motor vigor. Fiber photometry recordings of these interneuron subtypes suggest key roles of their phasic activity in execution of value-based choice and modulation of motor vigor. Pilot bidirectional optogenetic manipulations suggest that LTSIs and THINs impact win-stay and lose-switch probabilities. These findings contribute to a growing evidence base on the role of striatal microcircuitry in striatal function and goal-directed behavior, with potential translational relevance suggested by evidence implicating striatal interneurons in a range of neuropsychiatric disease presentations.

Poster 39B | Neuroscience

Ndnf interneuron excitability is spared in a mouse model of Dravet syndrome

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Dravet syndrome (DS) is a neurodevelopmental disorder characterized by epilepsy, developmental delay/intellectual disability, and features of autism spectrum disorder, caused by heterozygous loss-of-function variants in *SCN1A* encoding the voltage-gated sodium channel α subunit Nav1.1. The dominant model of DS pathogenesis is the "interneuron hypothesis," whereby GABAergic interneurons (INs) express and preferentially rely on Nav1.1-containing sodium channels for action potential (AP) generation. This has been shown for three of the major subclasses of cerebral cortex GABAergic INs: those expressing parvalbumin (PV), somatostatin, and vasoactive intestinal peptide. Here, we define the function of a fourth major subclass of INs expressing neuron-derived neurotrophic factor (Ndnf) in male and female DS (*Scn1a*+/−) mice. Patch-clamp electrophysiological recordings of Ndnf-INs in brain slices from *Scn1a*+/- mice and WT controls reveal normal intrinsic membrane properties, properties of AP generation and repetitive firing, and synaptic transmission across development. Immunohistochemistry shows that Nav1.1 is strongly expressed at the axon initial segment (AIS) of PV-expressing INs but is absent at the Ndnf-IN AIS. In vivo two-photon calcium imaging demonstrates that Ndnf-INs in *Scn1a*+/- mice are recruited similarly to WT controls during arousal. These results suggest that Ndnf-INs are the only major IN subclass that does not prominently rely on Nav1.1 for AP generation and thus retain their excitability in DS. The discovery of a major IN subclass with preserved function in the *Scn1a*+/- mouse model adds further complexity to the "interneuron hypothesis" and highlights the importance of considering cell-type heterogeneity when investigating mechanisms underlying neurodevelopmental disorders.

Poster 40B | Neuroscience

Transdiagnostic Polygenic Risk Scores Underlying Overall Psychopathology and Personalized Functional Networks in Early Adolescence

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A critical question in developmental neuroscience is how genetic risk influences functional brain networks and psychopathology in early adolescence. A recent multivariate genome-wide association study found two genetic factors, F1 and F2, that capture the majority of genetic variability associated with transdiagnostic psychopathology in adulthood. F1 was found to be associated with more common psychiatric symptoms and F2 with rarer, more severe disease. Additionally, emerging evidence has suggested the biological importance of a latent overall factor, or p-factor, that quantifies an individual's generalized vulnerability to psychiatric symptoms. However, it is unclear how F1 and F2 are related to p-factor during adolescence, and how these variables are reflected in functional brain networks. We used personalized functional networks (PFNs)—which capture individual variation in functional network topography that is otherwise ignored by standard analyses based on group atlases—to elucidate the how these genetic factors relate to overall psychopathology and functional brain networks in the Adolescent Brain Cognitive Development (ABCD) cohort (N=7,459, ages 9-10). P-factor was found to be heritable based on twin analyses, and the polygenic risk score of F1 (PRS-F1) was found to be significantly correlated to p-factor, although the polygenic risk score of F2 (PRS-F2) was not. Furthermore, PFN topography was found to be heritable and robustly related to interindividual differences in p-factor, PRS-F1, and PRS-F2. The cortical regions and network topography driving these multivariate PFN associations converge between p-factor and PRS-F1, yet diverge between p-factor and PRS-F2. Our results expand upon prior literature, showing that personalized functional networks are both heritable and related to overall psychopathology in early adolescence. Specifically, we provide novel evidence that two psychiatric polygenic risk scores, one that manifests clinically during early adolescence (PRS-F1) and one that has yet to (PRS-F2), are both reflected in personalized functional topography during this stage of development.

Poster 41B | Pharmacology

Engineering cellular systems for biomedical imaging & diagnostics

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Despite advances in the sensitivity of biomedical imaging, the detection of micrometastatic disease remains a significant challenge. Because current imaging methods (e.g. FDG-PET, CT, MRI) rely on intrinsic tumor features (e.g. metabolic activity, tissue density, etc.) as a source of signal, the ability of these methods to identify micrometastases is fundamentally limited. Here, we aim to engineer cells which amplify a detectable signal upon sensing cancer, improving our ability to diagnose and monitor micrometastatic disease. The sensing component of the system will be derived from a synthetic intramembrane proteolysis receptor (SNIPR) with an scFv extracellular domain and a TEV protease as the intracellular domain. Upon antigen binding, proteolysis will liberate the intracellular protease domain. The reporter will consist of the fluorescent protein mCherry fused to the murine ornithine decarboxylase degron, which will target the protein for degradation by the proteasome. In this system, scFv-antigen binding will result in liberation of the TEV protease, which will cleave the degron from mCherry and increase the abundance of the reporter protein. We aim to demonstrate the generalizability and translational potential of this approach using luciferase, E. coli dihydrofolate reductase, and secreted embryonic alkaline phosphatase for detection by bioluminescence imaging, PET imaging, and serum ELISA in vitro and in a murine model of hematogenous metastasis. If successful, this work will provide the foundation for the development of cell-based diagnostic agents which enable the diagnosis and monitoring of micrometastatic disease.

Poster Session List
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Algur, Eda	25A	Mentor: Dr. Ingrid Nembhard *Healthcare Heroes' Shield of Armor: Causes and Consequences of Callousness in Healthcare Workers*
Arevalo, Orlando	15A	Mentor: Dr. Saar Gill *Development of therapeutic myeloid cells to deliver IL-12 specifically within the glioblastoma tumor microenvironment*
Ayyappan, Vinay	7A	Mentor: Dr. Arjun Raj *Single-cell determinants of self-organization in the gastruloid*
Bannerman, Carl	21A	Mentor: Dr. Kellie Jurado *Antiviral Role of Interferon Epsilon in Human Neurons*
Barnett Dubensky, Thomas (Sam)	32B	Mentors: Drs. Derek Oldridge & Laura Vella *Tonsillar versus circulating CD4 T Follicular Helper polarization states delineated by trimodal single-cell sequencing and spectral flow cytometry in a healthy pediatric cohort*
Bernstein, Elizabeth (Ellie)	8A	Mentor: Dr. Robert Mauck *Non-muscle myosin II knockdown disrupts tenocyte morphology and contractility*
Burson, Randy	1A	Mentor: Dr. Adriana Petryna *Neglect Protocol: Cultural and Ethical Negotiations During a Case of Suspected Child Neglect*
Chaluvadi, Venkata (Sai)	36A	Mentor: Dr. F. Chris Bennett *Galactosylceramide triggers cell death in Galctwi macrophages*
Chauvin, Samuel (Sam)	33B	Mentor: Dr. Jonathan Miner *Inherited C-terminal TREX1 variants disrupt homology-directed repair to cause senescence and DNA damage phenotypes in Drosophila, mice, and humans*
Chen, Angela	26A	Mentor: Dr. Guy David *Identifying Low Acuity Emergency Department Visits with a Machine Learning Approach: The Low Acuity Visit Algorithms (LAVA)*
Chen, Christina	5A	Mentor: Dr. Russell (Taki) Shinohara *Subject-level segmentation accuracy weights for volumetric studies involving label fusion*
Chini, Julia	28A	Mentor: Dr. David Hill

		Hepatic CD9 regulates adipose tissue inflammation and metabolic dysfunction during obesity
Chiu, Joy	29A	Mentor: Dr. Michela Locci *Optimizing a mouse model to study the longevity of vaccine-induced humoral responses*
Crowley, Aidan	27A	Mentor: Dr. Amol Navathe *Allocation of Medicare Reimbursement Based on Social Disadvantage: Empirical Assessment Using Area-Level Indices*
Deschaine, John	30A	Mentor: Dr. Michael Silverman *Can microbial molecular mimics protect from type 1 diabetes?*
Dong, Royce	4B	Mentors: Drs. Flavia Vitale and Brian Litt *Microfabricating a multimodal neural interface integrating microLEDs and transparent Ti3C2Tx MXene electrodes for colocalized neural recording, imaging, and light-based stimulation*
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Fein, Ethan	16A	Mentor: Dr. Patrick Seale *Role of Sympathetic Innervation in the Development of Brown Adipose Tissue*
Fernandez del Castillo, Andres	1B	Mentor: Dr. Mark A. Sellmyer *Using heterobifunctional ligands to induce the selective arginylation of Alpha Synuclein fibrils in a Parkinson's model system*
Frankfurter, Maxwell (Max)	17A	Mentor: Dr. Mark Kahn *Thrombin signaling in placental development*
Franklin, Brian	18A	Mentor: Dr. Jennifer E. Phillips-Cremins *Mapping of the neural connectome*
Gao, William	24A	Mentor: Dr. Hao Wu *Decoding human cardiac aging with single-nucleus RNA sequencing*
Gardner, Zachary (Zach)	16B	Mentor: Dr. Raj Jain *Genome Folding and Speckle Association Cooperate to Orchestrate Fibroblast Activation*
Garfinkle, Samuel (Sam)	3A	Mentor: Dr. Daniel Kulp *Winning Designs for Winter 2024 Rosetta TEV protease Design Games*
Gong, Angela	22A	Mentor: Dr. Mark Sellmyer *Complementary Chemical and Cell Autonomous Strategies to Control Engineered Cell Therapy*
Gonzalez, Elizabeth (Eli)	19A	Mentors: Drs. Elizabeth Bhoj & Becca Ahrens-Nicklas *A novel mendelian neurodevelopmental disorder caused by germline variants in MAP2K4*

Hapke, Robert (Rob)	11A	Mentors: Drs. Evan Weber & Andy Minn *Identification of regulators of CAR T persistence in solid tumors*
Heymach, Claudia	37A	Mentor: Dr. Alex Proekt *An analysis pipeline to investigate neuronal entrainment to visually-evoked traveling waves after the presentation of different types of visual stimuli*
Iliakis, Evan	38B	Mentor: Dr. Marc Fuccillo *Investigating the role of GABAergic interneurons in the dorsomedial striatum in value-based decision-making*
Jardin, Blake	15B	Mentor: Dr. Jonathan A. Epstein *Reducing Kidney Fibrosis by FAP-CAR-T Cells in Mouse Models of Kidney Disease*
Kaminski, Paul	20A	Mentor: Dr. Gerd Blobel *Investigating the mechanisms of polycomb group protein mediated fetal hemoglobin silencing*
Kavari, Sanam	17B	Mentors: Drs. Jennifer Kalish & Marisa Bartolomei *Pathological characterization, genome-wide methylation, and in vitro modeling of trophoblast overgrowth in Beckwith-Wiedemann Syndrome*
Kim, Samuel (Sam)	34B	Mentors: Drs. Carl June, Regina Young & Andrew Rech *Spatial transcriptomic map of CAR-T cells in situ in the human tumor microenvironment*
Kolla, Likhitha	23A	Mentor: Dr. Ravi B. Parikh *Performance drift in a national mortality risk prediction model*
Kumar, Rachit (& Zhang, D.)	29B	Mentors: Drs. Daniel Rader & Marylyn Ritchie *The phenotypic basis of CT-derived kidney traits and their utility in predicting estimated glomerular filtration rate*
Kuprasertkul, Napasorn (Nina)	18B	Mentors: Dr. Brian Capell & Kathryn Wellen *Lsd1 is a critical mediator of epidermal development, homeostasis, and oncogenesis*
Liebergall, Sophie	39B	Mentor: Dr. Ethan Goldberg *Ndnf interneuron excitability is spared in a mouse model of Dravet syndrome*
Lou, Meng	31A	Mentor: Dr. Robert Heuckeroth *Elucidating the role lamina propria macrophages on the pathogenesis of Hirschsprung Disease-Associated Enterocolitis (HAEC)*
Maganti, Rohin	9A	Mentors: Drs. Bill Peranteau & Mohamed-Gabriel Alameh *Ionizable lipid nanoparticles for in utero prime editing of Duchenne Muscular Dystrophy*
Mah'moud, Mattia	25B	Mentor: Dr. Anne Marie McCarthy *Racial Differences in Background Parenchymal Enhancement, a Novel Marker of Breast Cancer*
McCright, Samuel (Sam)	35B	Mentor: Dr. David Hill

		Dietary long chain fatty acids shape innate immune cell tone and modulate inflammatory responses in the lung
Merolle, Maria	32A	Mentor: Dr. Christopher Hunter *A genetically attenuated Cryptosporidium strain protects mice from reinfection*
Morrissette, Jeremy	36B	Mentor: Dr. Scott Canna *Excess IL-18 suppresses EAE by augmentation of CD8Tregs to inhibit autoreactive T-cell activity*
Morucci, Katherine	10A	Mentors: Drs. Ricardo Castillo-Neyra & Dustin Brisson *Spatial Association of Dog Mobility and Development of a Spill-Over Model of Hydatid Disease in an Echinococcosis-affected area in Junín, Peru*
Narayan, Sweta	14A	Mentor: Dr. Mark Kahn *Investigating the Formation and Remodeling of Uterine Spiral Arteries during Pregnancy*
Nelson, Joseph (Andrew)	2B	Mentor: Dr. Dan Kulp *Deep sequence design using ProteinMPNN as a refinement module for protein design*
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Pecsok, Margaret (Maggie)	39A	Mentor: Dr. David Roalf *Exploring the Glutamatergic Underpinnings of Within-Network Functional Connectivity and Motor Performance in a Transdiagnostic Cohort*
Petch, Raegan	22B	Mentor: Dr. Paul Bates *Effect of Mutations in the Glycoprotein of Dabie Bandavirus (SFTSV)*
Pham, Jonathan	41B	Mentor: Dr. Mark Sellmyer *Engineering cellular systems for biomedical imaging & diagnostics*
Pham, Kenneth	20B	Mentor: Dr. Jennifer Phillips-Cremins *Critical assessment of CGG short tandem repeat length, FMR1 DNA promoter methylation, heterochromatin,*

		and transcriptional repression of neural, synaptic genes in fragile X syndrome
Poltorack, Carson	7B	Mentors: Drs. Sydney Shaffer & Celeste Simon *Spatial and single cell transcriptomics uncovers metabolic reprogramming and NETosis of neutrophils in human pancreatic cancer*
Rafizadeh, Diane	24B	Mentor: Dr. Dave Chenoweth *Synthesis and design of macrocyclic collagen mimetic peptides for targeting the cancer-implicated DDR2 kinase*
Ravindran, Pavithran (Pav)	8B	Mentor: Dr. Arjun Raj *CellSlime: Visualizing T-Cell Trajectories to Unravel Migration- Exhaustion Interplay in Tumors*
Ryu, Han-Seul	5B	Mentor: Dr. Jennifer Phillips-Cremins *Cell type-specific relationship between higher-order chromatin folding and short tandem repeat instability in Huntington's disease*
Sharma, Prateek	9B	Mentors: Drs. Kathryn E. Wellen & Christoph A. Thaiss *Investigating the Role of Diet and Genetics on Colorectal Cancer Metabolism and Host Physiology*
Shea, Emily	10B	Mentor: Dr. Lewis Chodosh *Ferroptosis Modulators in Breast Cancer Dormancy and Recurrence*
Shen, Kaitlyn	21B	Mentor: Dr. Rajan Jain *Deciphering the role of cytoskeletal-nuclear interactions in peripheral chromatin organization*
Sielski, Michael	29B	Mentor: Dr. Alexander Olssen *Hospital Responses to Financial Risk*
Soto Albrecht, Yentli	23B	Mentor: Dr. Douglas C. Wallace *Mitochondrial oxidative phosphorylation restricts SARS-CoV-2 replication via metabolic remodeling*
Spychalski, Griffin	6B	Mentor: Dr. David Issadore *Extracellular vesicle liquid biopsy to improve breast cancer screening accuracy*
Sullivan, Matthew	4A	Mentor: Dr. E John Wherry *Intrinsically disordered regions of TOX regulate chromatin binding dynamics to coordinate protein function during CD8 T cell exhaustion*
Sun, Kevin	40B	Mentors: Drs. Aaron Alexander-Bloch & Theodore Satterthwaite *Transdiagnostic Polygenic Risk Scores Underlying Overall Psychopathology and Personalized Functional Networks in Early Adolescence*
Sun, Lillian (Lily)	33A	Mentors: Drs. Taku Kambayashi and Yasmine Belkaid *Role of T cells in alteration of egg sncRNA profile*

Sun, Yusha	40A	Mentor: Dr. Hongjun Song *Monosynaptic tracing defines brain-wide circuit connectivity of human glioblastoma*
Sussman, Jonathan	26B	Mentor: Dr. Kai Tan *A Longitudinal Single-Cell and Spatial Multiomic Atlas of Pediatric High-Grade Glioma*
Svirydava, Maryia (Mary)	34A	Mentor: Dr. Sarah Henrickson *Mechanisms of T Cell Dysfunction in Activated PI3Kδ Syndrome*
Tegegne, Saba	35A	Mentors: Drs. Taku Kambayashi & Elizabeth Grice *Investigating the effects of TSLP on regulatory T cells in a mouse model of atopic dermatitis*
Templeton, Zachary (Zach)	30B	Mentor: Dr. Guy David *Physician consults in the hospital*
Thompson, Elizabeth (Beth)	6A	Mentor: Dr. Walter Witschey *CMR Imaging Traits Associated with Right Ventricular Remodeling in Repaired Tetralogy of Fallot*
Utset, Henry	37B	Mentor: Dr. Ellen Puré *CAR-T cell therapy for idiopathic pulmonary fibrosis*
Wechsler, Caroline	31B	Mentor: Dr. Robert Aronowitz *Making Fun, Making Doctors: "Spoof" shows and professionalization in US Medical Education, 1950-2000*
Xia, Katherine (Kat)	11B	Mentor: Dr. Edward Lee *Investigating How a Small Molecule Activator of VCP Reduces TDP-43 Aggregates*
Xiong, Barbara	27B	Mentors: Dr. Kai Tan *Towards a universal metric for comparing cellular neighborhoods*
Yao, Michael	3B	Mentors: Drs. James C. Gee & Osbert Bastani *Generative Adversarial Bayesian Optimization for Surrogate Objectives*
Yen, Daniel	13B	Mentor: Dr. Nancy Speck *Runx1's Regulation of Granulocyte Monocyte Progenitors in RUNX1 Familial Platelet Disorder*
You, Jack	14B	Mentor: Dr. George Cotsarelis *Determining the Role of the Epidermis in Regulating Hair Follicle Phenotype*
Zhang, David (& Kumar, R.)	28B	Mentors: Drs. Daniel Rader & Marylyn Ritchie *The phenotypic basis of CT-derived kidney traits and their utility in predicting estimated glomerular filtration rate*