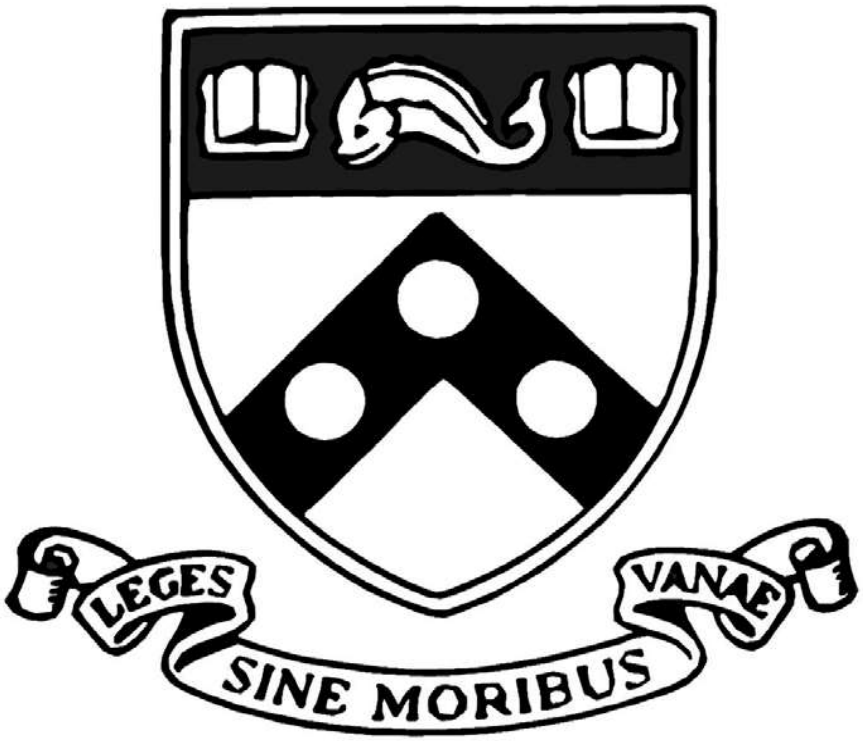
Perelman School of Medicine
at the University of Pennsylvania

Combined Degree Program Annual Retreat



August 5, 2022
Villanova University
Villanova, Pennsylvania

The Combined Degree and Physician Scholar Programs Administration

Skip Brass, MD, PhD	Associate Dean and Director
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Nicola Mason, B Vet Med, PhD, DACVIM	Steering Cmt Member, VMD-PhD program
Michael May, PhD	Steering Cmt Member, VMD-PhD program
Jennifer Punt, VMD, PhD	Steering Cmt Member, VMD-PhD program
Susan Volk, VMD, PhD	Steering Cmt Member, VMD-PhD program

Maggie Krall	Director of Administration
Jill Baxter	Director of Financial Operations
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Hope Charney, MA	Administrative Coordinator
David Bittner, MA	Coordinator, MD-PhD Program
Anastasia Brown	Coordinator, VMD-PhD Program

Table of Contents

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

Welcome everyone to the first in person Penn MSTP retreat since 2019.

Some of you will remember past retreats but some of you have never been to an in person MSTP retreat. If you fall into that latter camp, look upon today as an opportunity to become part of the larger MSTP community which includes current students, alumni, prospective students and faculty. We've told you that it existed, but Zoom faces on computer monitors don't have the same impact as actually being together. Please enjoy being here today. The program developed by the organizing committee has been built with community building as a priority. It is a mix of talks, posters, gathering time, photo sessions and opportunities to meet new people.

In the coming year there will be a record-setting 222 MD/PhD and 24 VMD/PhD students on campus. Many of them will be here at the retreat. Joining us are students in 2 of our pipeline programs (PennPREP and PASS) as well as faculty looking for thesis students. Say hello to as many people as you can, especially to people who are not in your usual orbit. If you are a seasoned member of Penn MSTP, take time to welcome the newer folks, including the 28 MD/PhD and 4 VMD/PhD candidates who are officially joining the program today. Recall how out of place you felt at your first retreat and make them feel welcome. Everyone should seek new friends and make new connections.

Hard to write this essay without acknowledging at least some of the many things that happened since our retreat in August 2021. Unfortunately, not all of them have been great. The less than wonderful parts include the continuing effects of the SARS-CoV2 pandemic, the events of January 6th, the unequal status of too many Americans and migrants to America, the invasion of Ukraine, and recent decisions by the United States Supreme Court.

There were, however, lots of positive things to be grateful for during the past year. A great group of MD/PhD students graduated two months ago and are off conquering the galaxy as residents, postdocs and entrepreneurs. Multiple MSTP babies have been born. The admissions season that just finished was one of our most successful ever. Penn MSTP community member, Yentli Soto Albrecht, has started her year of service as President of APSA, binding us even closer to that important organization. Although two of our most valued staffers, Amy Nothelfer and Maura Tucker, have moved on to other positions, our newest staff associate director, Carina Myers, has arrived to take Maura's place overseeing admissions. Carina has been a Penn person at Wharton, but she is new to PSOM and the world of physician-scientists. It can be a bit overwhelming. Please welcome her when you see her.

Last, but not least, the 1200 page magnum opus proposal for another 5 years of NIH funding for the MSTP was delivered to the NIH in January after a year-long team effort by Maggie K. (of course), Amy, Francia Portacio, Maura and Hope Charney with absolutely essential input from Mike Atchison, Aimee Payne and Rahul Kohli. The outstanding reception (and score) that the proposal received from the NIH study section reflects decades of student and alumni success along with a vision for the training and support of people who can blend the practice of medicine with the discovery and translation of new science (that's all of you). I look forward to telling you more about it in the coming months.

For now, my advice to all of us is to enjoy the day together today - then go be a chimera!

Skip

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

Welcome to the Penn Combined Degree Retreat!

This has been a historic year for the VMD-PhD program with celebration of its 50th anniversary by a 1.5 day symposium. We had 132 attendees including 34% of program alumni. Last March we held the Vet Student Research Day with talks by VMD-PhD and VMD students, and numerous posters. This July will be the third official meeting of the newly emerging veterinary combined degree association bringing together students and program directors of veterinary combined degree programs from around the nation. We are very proud of our current students and alumni, and congratulate them on their multiple successes, publications, and awards

Three new students are entering the Program this year (Kay Foos, Sabina Hlavaty, and Martha Stone), and one student (Jeffrey Carey) will be graduating in August. We welcome those entering the program and congratulate those graduating now, or in the near future.

I hope you enjoy this day and take time to enjoy the colleagueship of your fellow students and faculty. This is the largest MSTP program in the nation, and the only one that includes veterinary combined degree students. I hope you benefit from and appreciate the advantages of the large critical mass and diversity that our program enjoys.

Again, I welcome all current and incoming students. I hope you enjoy the day.

Sincerely,

Michael Atchison

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.

Noa Erlitzki
Ethan Fein
Sabina Hlavaty
Feng Hu
Emerson Hunter
Raymond Ng
Maggie Tamburro
Brian Goldspiel
Lev Litichevskiy
Matthew Sullivan

2022 Incoming Class

MD/PhD

Aleksia Barka	Biochemistry & Molecular Biophysics	Penn
Vasia Barka	Cell and Molecular Biology	Penn
Natalie Brown	Cell and Molecular Biology	Cornell / Harvard
Sabrina Da Silva	Neuroscience	Penn
Walla (Miguel) Disbennett	Cell and Molecular Biology	Ohio State / U Michigan
Islam Elsaid	Cell and Molecular Biology	Cornell
Sarah Finstuen-Magro	Cell and Molecular Biology	Carleton / Dana Farber
Giang Hoang	Bioengineering	Hopkins
Evan Jiang	Neuroscience	Penn
Maria (Gina) Kovalik	Immunology	Duke / Weill Cornell
Jasmine Larrick	Immunology	Berkeley / UCSF
Jialiu (Annie) Liang	Genomics & Computational Biology	Hopkins / NIH
Briana Macedo	Genomics & Computational Biology	Princeton
Nicolas (Nick) Marzolini	Bioengineering	U of Florida
Joseph (Joe) McGaunn	Cell and Molecular Biology	UMASS Amherst / Broad Inst
Pedro Mendez Fernandez	Cell and Molecular Biology	U of Puerto Rico / Penn
Casey Mogilevsky	Cell and Molecular Biology	UC Berkeley / U Cambridge
Charlotte Monsour	Chemistry	Cal State-LA / UCSF
Taylor Phillips-Jones	Neuroscience	Howard / U Maryland

2022 Incoming Class

Ryan Rahman	Neuroscience	Texas A & M
Diana Renteria	Cell and Molecular Biology	MIT
Daniel Shapiro	Neuroscience	UVA
Niharika Shukla	Biochemistry & Molecular Biophysics	Vassar / NIH
Montita Sowapark	Anthropology	Harvard / Health Adv
Amelia Taylor	Pharmacology	Vanderbilt
Chloe Winston	Neuroscience	U of Washington
David Wu	Genomics & Computational Biology	Stanford / Broad Inst
Vincent Xia	Bioengineering	Stanford

VMD/PhD

Theresa Astmann	Cell and Molecular Biology	U of Vermont
Breezy Brock	Cell and Molecular Biology	Northern Arizona U
Katherine Morucci	Epidemiology and Biostatistics	Penn
Daana Roach	Cell and Molecular Biology	U of Oklahoma

Graduating Students and Thesis Information

MD/PhDs

Adeeti Aggarwal
The characterization of visual evoked feedforward-feedback travelling waves in mice during waking and anesthetized states
Thesis Advisors: Drs. Max Kelz and Alex Proekt

Kate Beattie
TRPC3 Antagonizes Pruritus in a Mouse Contact Dermatitis Model
Thesis Advisor: Dr. Wenqin Luo

John Bernabei
Quantitative Methods for Guiding Epilepsy Surgery from Intracranial EEG
Thesis Advisor: Dr. Brian Litt

Gregory Chen
Leveraging Systems Immunology to Understand the Molecular Underpinnings of Chimeric Antigen Receptor T-cell Therapy
Thesis Advisor: Dr. Kai Tan

Maria Diaz Ortiz
From '-omics' to biomarkers and mechanisms in Parkinson's disease: Growth hormone receptor and GPNMB
Thesis Advisor: Dr. Alice S. Chen-Plotkin

Kevin Goff
VIP interneuron cell and circuit distinction underlying Dravet Syndrome
Thesis Advisor: Dr. Ethan M Goldberg

Naihua (Natalie) Gong
Molecular factors governing sleep circuit maturation and neurodevelopmental disorders
Thesis Advisor: Dr. Matthew Kayser

Bridget Gosis
The role of folliculin in hepatic lipid metabolism and the pathogenesis of NAFLD and NASH
Thesis Advisor: Dr. Zoltan Arany

Yoonhee Ha
Studies in kidney transplantation
Thesis Advisor: Dr. Peter Reese

Connie Jiang
Tracing cell type determination in directed differentiation of human induced pluripotent stem cells
Thesis Advisor: Dr. Arjun Raj

Aimee Juan
Tissue-specific Grb10/Ddc insulator drives allelic architecture for cardiac development
Thesis Advisor: Dr. Marisa Bartolomei

David Kersen
A computational study of the influence of cortical processes on the olfactory bulb
Thesis Advisors: Drs. Vijay Balasubramanian and Minghong Ma

Ivan Kuznetsov
Biophysical Dynamics of RGS-LOV Proteins as Systems For Light-Induced Membrane Recruitment
Thesis Advisor: Dr. Brian Chow

David S.M. Lee
Insights into functional noncoding RNA elements through the analyses of human genetic variation
Thesis Advisors: Drs. Yoseph Barash and Louis Ghanem

Emma Lewis
Immune Modulation at the Maternal-Fetal Interface Regulates Perinatal Outcomes
Thesis Advisor: Dr. Michal Elovitz

Ricardo Linares Saldana
Brd4 orchestrates genome folding to promote neural crest progenitor differentiation
Thesis Advisors: Drs. Rajan Jain and Jonathan A. Epstein

Jing Luan
Roles of CTCF in nascent transcription and chromatin architecture
Thesis Advisor: Dr. Gerd A Blobel

Danielle Murashige
Comprehensive fuel metabolism of the failing and nonfailing human heart
Thesis Advisor: Dr. Zolt Arany

Joseph Park
A genome-first approach to investigating the biological and clinical relevance of exome-wide rare coding variation using electronic health record phenotypes
Thesis Advisors: Drs. Dan Rader and Marylyn Ritchie

Aileen Ren
Investigating the Role of PI3K Signaling in Cerebral Cavernous Malformation Pathogenesis
Thesis Advisor: Dr. Mark Kahn

Jacob Sterling
Innate Immunity in Degenerative Diseases of the Retina and Brain
Thesis Advisors: Drs. Joshua L. Dunaief and Qi N. Cui

Tong Wang
Discovery and Application of Cytosine Carboxymethylation
Thesis Advisor: Dr. Rahul Kohli

Aaron Williams
Neuronal codes and circuits underlying audiovisual integration
Thesis Advisor: Dr. Maria Geffen

Daniel Zhang
Development and application of patient-derived glioblastoma organoids for studying tumor heterogeneity and the immune-related microenvironment
Thesis Advisor: Dr. Hongjun Song

VMD/PhDs

Ian Penkala
Interrogating alveolar epithelial type 1 cell plasticity across the lifespan
Thesis Advisor: Dr. Edward Morrisey

Sherrie Xie
Enhancing electronic health record data for population health studies
Thesis Advisor: Dr. Blanca Himes

Agenda

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

Student Arrival and Poster Set Up
Villanova Room
7:45 – 9:00am

Opening Remarks
Dr. Skip Brass, MD, PhD
Dr. Nicola Mason, BVetMed, PhD
Cinema Room
9:00 – 9:15am

Faculty Talks
Cinema Room
9:15 – 10:15am

Elizabeth Bhoj, MD, PhD
Assistant Professor, Division of Human Genetics, University of Pennsylvania, Perelman School of Medicine
Attending Physician, Children's Hospital of Philadelphia

My Journey as a Physician-Scientist: From Snake Tongues to Human Brains

Warren S. Pear, MD, PhD
Gaylord P. and Mary Louise Harnwell Professor, University of Pennsylvania, Perelman School of Medicine
Director, Experimental Pathology and Immunobiology Division

An accidental scientific journey

Elizabeth Lennon, DVM, PhD
Pamela Cole Assistant Professor of Internal Medicine, University of Pennsylvania, School of Veterinary Medicine
Diplomate, American College of Veterinary Internal Medicine (SAIM)

Tails from the (Intestinal) Crypt: Life as a Veterinary Clinician Scientist

Student Group Activity*
Villanova Room
10:20 – 11:05am

Group & Incoming Class Photos ('20, '21, '22)
11:10 – 11:45am

* *group # is on your nametag*

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

Student Talks
Cinema Room
12:55 – 1:40pm

Derek Sung	VE-cadherin enables trophoblast endovascular invasion and spiral artery remodeling during placental development
Fola Sofela	Effects of Metabolism on Behavioral Phenotypes in Neurofibromatosis 1
Philip Hicks	Abolishment of putative COPI binding motif in the Gc glycoprotein of bandaviruses enhances recovery of infectious VSV pseudoyptes

Lightning Talks/Poster Pitches
Cinema Room
1:40 – 1:55pm

Brian Goldspiel	Unraveling metabolic determinants of innate immune function in Maple Syrup Urine Disease
Yentli Soto Albrecht	Non-pathogenic variation in mitochondrial DNA modulates murine SARS-CoV-2 pathogenesis
Shreya Parchure**	Entorhinal Amygdala Network-based Biomarker for Closed-loop Deep Brain Stimulation in Anxiety Disorders: Results of First-in-human Invasive Electrophysiology
Ariel Shepley-McTaggart	Host Regulation of Ebola Virus Entry and Egress: Role of Cytoskeletal Filamin Proteins
Clayton Otter	Infection of primary nasal epithelial cells differentiates SARS-CoV-2, MERS-CoV, and HCoV-NL63
Emily Lubin	De Novo Mutations in Replication-Independent Histone Genes Elude Diagnosis by Exome/Genome Sequencing
Audrey Luo	Refinement of Functional Connectivity in Development Aligns with the Sensorimotor to Association Axis

** *recorded/virtual presentation*

Student Poster Sessions (with snacks & adult beverages!)
Villanova Room

Poster Session A
2:00 – 2:30pm

Poster Session B
2:30 – 3:00pm

Poster Session C
3:00 – 3:30pm

Wellness Talks
Villanova Room
3:30 – 4:15pm

Raymond Ng	*Dancing the Skies – Lessons I've learned as a Pilot*
Brian Goldspiel	*Board Games and You: In Philadelphia and Beyond (but Especially in Philly)*
Frederic Bushman, PhD	*Parking Your Brain in a Good Place: the Pleasures of Chess*

Retreat Happy Hour
Belle Air Terrace
4:30 – 6:00pm

Departure from Villanova

Student Talks

Derek Sung

VE-cadherin enables trophoblast endovascular invasion and spiral artery remodeling during placental development
Advisor: Dr. Mark Kahn

Fola Sofela

Effects of Metabolism on Behavioral Phenotypes in Neurofibromatosis 1
Advisor: Dr. Amita Sehgal

Phil Hicks

Abolishment of putative COPI binding motif in the Gc glycoprotein of bandaviruses enhances recovery of infectious VSV pseudoyptes
Advisor: Dr. Paul Bates

VE-cadherin enables trophoblast endovascular invasion and spiral artery remodeling during placental development

Derek C. Sung, Xiaowen Chen, Mei Chen, Jisheng Yang, Susan Schultz, Apoorva Babu, Yitian Xu, Siqi Gao, TC Stevenson Keller IV, Patricia Mericko-Ishizuka, Michelle Lee, Ying Yang, Joshua P. Scallan, Mark L. Kahn

Submitted by: Derek Sung, CAMB – Cell Biology, Physiology, and Metabolism
Email: derek.sung@pennmedicine.upenn.edu
Advisor: Dr. Mark Kahn

During formation of the mammalian placenta, trophoblasts invade the maternal decidua and remodel spiral arteries to bring maternal blood into the placenta. This process, known as endovascular invasion, is thought to involve the adoption of functional characteristics of vascular endothelial cells (ECs) by trophoblasts. The genetic and molecular basis of endovascular invasion remains poorly defined, however, and whether trophoblasts utilize specialized endothelial proteins in an analogous manner to create vascular channels remains untested. Vascular endothelial (VE-)cadherin is a homotypic adhesion protein that is expressed selectively by ECs in which it enables formation of tight vessels and regulation of EC junctions. VE-cadherin is also expressed in invasive trophoblasts and is a prime candidate for a molecular mechanism of endovascular invasion by those cells. Here, we show that VE-cadherin is required for trophoblast migration and endovascular invasion into the maternal decidua in the mouse. VE-cadherin deficiency results in loss of spiral artery remodeling that leads to decreased flow of maternal blood into the placenta, fetal growth restriction, and death. These studies identify a non-endothelial role for VE-cadherin in trophoblasts during placental development and suggest that endothelial proteins may play functionally unique roles in trophoblasts that do not simply mimic those in ECs.

Effects of Metabolism on Behavioral Phenotypes in Neurofibromatosis 1.

Folasade Sofela, Tom Jongens, and Amita Sehgal

Submitted by: Folasade Sofela, CAMB – Cell Physiology and Metabolism
Email: folasade.sofela@pennmedicine.upenn.edu
Advisor: Dr. Amita Sehgal

Background: NF1 is an autosomal dominant disorder characterized by the propensity to develop benign and malignant nervous system tumors. The disease is also associated with an increased prevalence of sleep disorders and ADHD. A Drosophila model for NF1 recapitulates many aspects of the human disease. Specifically, these animals exhibit reduced and fragmented sleep and marked locomotor hyperactivity. Clinical studies in humans with NF1 suggest that NF1 is associated with an altered state of metabolism.

Objectives: It is unclear if these metabolic changes arise and whether they contribute to disease phenotypes. We hypothesized that Nf1-KO Drosophila will have altered metabolism, and that the metabolic disruption contributes to abnormal sleep and activity phenotypes.

Results: Metabolomic analysis revealed reduced levels of glycolytic intermediates and elevated levels of several tricarboxylic acid cycle metabolites in Nf1-KO Drosophila as compared to age-matched controls, potentially indicating a shift toward glycolytic metabolism (Fig. 1). Whole body triglycerides are also reduced in Nf1-KO Drosophila (Fig. 2). Mitochondria in the indirect flight muscle of Nf1-KO animals are severely deformed (Fig. 3), and isolated mitochondria from Nf1-KO mutants exhibit abnormally elevated mitochondrial membrane potentials (Fig. 4). Nf1-KO mutants recover significantly more quickly from cold coma, suggesting excess heat production and inefficient use of metabolic fuel (Fig. 5). Taken together, these data suggest that Nf1-KO animals may be in a starvation-like state secondary to widespread metabolic dysfunction. In Drosophila, starvation activates a neuroendocrine signaling cascade that results in loss of sleep and hyperactivity. In support of this notion, a diet consisting of 5 times the typical amount of sucrose rescues hyperactivity and loss of sleep in Nf1-KO animals (Fig 6).

Conclusions: Metabolism, though frequently overlooked in the study of NF1, may play a crucial role in sleep symptomatology in this disease. Future experiments will elucidate the mechanism by which loss of Nf1 causes severe metabolic disruption. Further investigation of the interplay between the metabolic state induced by loss of Nf1 and other disease phenotypes could provide new and accessible paradigms for the treatment of disease.

Unraveling metabolic determinants of innate immune function in Maple Syrup Urine Disease

Philip Hicks, Tomaz Manzoni, Kendall Lundgreen, Alexander Jochmans, and Paul Bates. Department of Microbiology, Perelman School of Medicine, University of Pennsylvania.

Submitted by: Phil Hicks, CAMB – Microbiology, Virology, and Parasitology
Email: hicksph@vet.upenn.edu
Advisor: Dr. Paul Bates

Severe fever with thrombocytopenia syndrome virus (SFTSV) and heartland bandavirus (HRTV) are two recently emerged bunyaviruses with high case fatality ratios in humans. Additionally, the geographic range of a tick vector capable of transmitting both viruses is expanding. Despite the risk these viruses pose to public health, there are currently no approved vaccines or therapeutics available to prevent severe disease.

We recently demonstrated that rVSV-SFTSV, a recombinant vesicular stomatitis virus (rVSV) vectored vaccine encoding the glycoproteins Gn and Gc of SFTSV, protected interferon-incompetent mice from lethal challenge with SFTSV and HRTV. Like many rVSVs encoding bunyavirus glycoproteins, rVSV-SFTSV was challenging to launch from a plasmid recovery system and grew inefficiently in cell culture relative to wild type VSV. These phenotypes may be due to a mismatch between the plasma membrane assembly site of VSV virions and the Golgi localization of SFTSV Gn/Gc complexes. The mechanisms underlying bunyavirus Gn/Gc retention within the secretory system remain unknown. A putative COPI binding site was identified in the cytoplasmic tail of Gc by sequence similarity to known COPI targets. Fluorescent chimeric proteins containing the transmembrane domain and cytoplasmic tail of SFTSV Gc were retained in the endoplasmic reticulum of transfected A549 cells. Abolishment of the putative COPI binding site redistributed these fluorescent chimeras to the cell surface. Additionally, this point mutation redistributed Gn/Gc complexes to the cell surface. Finally, recovery of infectious VSV pseudotyped with SFTSV and HRTV Gn/Gc was improved by mutation of the putative COPI binding motif. Deletion of COPI binding sites from the cytoplasmic tail of Gc may be a strategy for improving the launch efficiency and immunogenicity of rVSVs encoding bunyavirus glycoproteins.

Poster Pitches/Micro Talks

Brian Goldspiel

Unraveling metabolic determinants of innate immune function in Maple Syrup Urine Disease
Advisor: Dr. Will Bailis

Yentli Soto Albrecht

Non-pathogenic variation in mitochondrial DNA modulates murine SARS-CoV-2 pathogenesis
Advisor: Dr. Douglas C. Wallace

Shreya Parchure
(pre-recorded presentation)

Entorhinal Amygdala Network-based Biomarker for Closed-loop Deep Brain Stimulation in Anxiety Disorders: Results of First-in-human Invasive Electrophysiology
Advisor: Dr. Casey Halpern

Ariel Shepley-McTaggart

Host Regulation of Ebola Virus Entry and Egress: Role of Cytoskeletal Filamin Proteins
Advisor: Dr. Ronald N. Harty

Clayton Otter

Infection of primary nasal epithelial cells differentiates SARS-CoV-2, MERS-CoV, and HCoV-NL63
Advisor: Dr. Susan Weiss

Emily Lubin

De Novo Mutations in Replication-Independent Histone Genes Elude Diagnosis by Exome/Genome Sequencing
Advisor: Dr. Elizabeth Bhoj

Audrey Luo

Refinement of Functional Connectivity in Development Aligns with the Sensorimotor to Association Axis
Advisor: Dr. Theodore Satterthwaite

Poster Session A

Anthropology

Poster 1A
Temporal Tolerance: The Un/Building of COVID-19 Laboratory Testing Infrastructures
Presenter: Alex Chen | Advisor: Dr. Adriana Petryna

Poster 2A
Leveraging clinical material: an ethnography of buprenorphine-based treatment in greater Pittsburgh
Presenter: Ben Sieff | Advisor: Dr. Adriana Petryna

Biochemistry & Molecular Biophysics

Poster 3A
Small Molecule Activation of Valosin-containing protein (VCP)
Presenter: Ben Creekmore | Advisor: Drs. Edward B. Lee and Yi-Wei Chang

Poster 4A
Controllable epigenome editing in CAR T cells
Presenter: Noa Erlitzki | Advisor: Dr. Rahul Kohli

Bioengineering

Poster 5A
LaxKAT: a more powerful method to test for association and localize signal in high-dimensional data
Presenter: Christina Chen | Advisor: Dr. Russell (Taki) Shinohara

Poster 6A
Quantification of bilateral renal oxygen consumption: a preliminary study
Presenter: Rajiv Deshpande | Advisor: Dr. Felix Wehrli

Poster 7A
Feasibility of machine learning for cardiovascular function analysis in patients with repaired tetralogy of Fallot
Presenter: Beth Thompson | Advisor: Dr. Walter Witschey

Poster 8A
Extracellular vesicle liquid biopsy to improve breast cancer screening accuracy
Presenter: Griffin Spychalski | Advisor: Dr. David Issadore

Cell and Molecular Biology

Cancer Biology

Poster 9A
Loss of the MGAT5 glycosyltransferase sensitizes pancreatic tumor cells to immune clearance
Presenter: Erin Hollander | Advisor: Dr. Ben Stanger

Poster 10A
Route-specific immune surveillance in Pancreatic Adenocarcinoma metastasis
Presenter: Ben Kahn | Advisor: Dr. Ben Stanger

Developmental, Stem Cell and Regenerative Biology

Poster 11A
Optical pooled CRISPR screens in human iPSC-derived neurons
Presenter: Sarshan Pather | Advisor: Dr. Ophir Shalem

Genetics & Epigenetics

Poster 12A
Single-Cell Transcriptomics and Multiplexed Imaging Resolves the Spatial and Cellular Heterogeneity of the Human Bone Marrow Microenvironment
Presenter: Shovik Bandyopadhyay | Advisor: Dr. Kai Tan

Poster 13A
Systematic characterization and manipulation of the trade-off between proliferation and invasion in melanoma
Presenter: Ryan Boe | Advisor: Dr. Arjun Raj

Poster 14A
Quantifying the Metabolic Response to Acute Cold Exposure in Mice
Presenter: Marc Bornstein | Advisor: Dr. Zoltan Arany

Poster 15A
Investigating ferroptosis in epidermal differentiation and tumorigenesis
Presenter: Nina Kuprasertkul | Advisor: Drs. Brian C. Capell and Kathryn E. Wellen

Poster 16A
Characterizing non-genetic mechanisms of adaptive resistance in metastatic melanoma
Presenter: Jessica Li | Advisor: Dr. Arjun Raj

Microbiology, Virology, & Parasitology

Poster 17A
Structural and functional determinants of caspase-11 inflammasome assembly in innate immune defense
Presenter: Daniel Akuma | Advisor: Dr. Igor Brodsky

Poster 18A
Distinct roles for cDC1s and cDC2s in CD4+ T cell responses against the intracellular parasite Cryptosporidium
Presenter: Ian Cohn | Advisor: Drs. Christopher Hunter and Boris Striepen

Poster 19A
Unraveling metabolic determinants of innate immune function in Maple Syrup Urine Disease
Presenter: Brian Goldspiel | Advisor: Dr. Will Bailis

Epidemiology & Biostatistics

Poster 20A
Voxel-wise intermodal coupling analysis of two or more modalities using local covariance decomposition
Presenter: Feng Hu | Advisor: Dr. Russell T. Shinohara

Poster 21A
Time of clinic appointment and advance care planning discussions in oncology
Presenter: Likhitha Kolla | Advisor: Dr. Jinbo Chen

Poster 22A
Racism and EHR data: examining missingness as a potential driver of inequities in predictive performance of a clinical decision support tool
Presenter: Stephanie Teeple | Advisor: Dr. Scott Halpern

Genomics & Computational Biology

Poster 23A
LSV-Seq: A Novel Targeted Sequencing Method to Measure Alternative Splicing Across Human Tissues
Presenter: Kevin Yang | Advisor: Drs. Yoseph Barash and Peter S. Choi

Immunology

Poster 24A
Hepatic CD9 regulates adipose tissue inflammation and metabolic dysfunction during obesity
Presenter: Julia Chini | Advisor: Dr. David Hill

Poster 25A
Are commensal-induced Tregs protective in a gnotobiotic model of type 1 diabetes?
Presenter: John Deschaine | Advisor: Dr. Michael Silverman

Poster 26A
Fate induction in chimeric antigen receptor T cells through asymmetric cell division
Presenter: Casey Lee | Advisor: Drs. Christoph T. Ellebrecht and Aimee S. Payne

Poster 27A
Immunometabolic reprogramming of pulmonary macrophages in obesity
Presenter: Sam McCright | Advisor: Dr. David Hill

Neuroscience

Poster 28A
Ndnf-IN dysfunction in a mouse model of Dravet Syndrome
Presenter: Sophie Liebergall | Advisor: Dr. Ethan Goldberg

Poster 29A
Refinement of Functional Connectivity in Development Aligns with the Sensorimotor to Association Axis
Presenter: Audrey Luo | Advisor: Dr. Theodore Satterthwaite

Poster 1A | Anthropology

Temporal Tolerance: The Un/Building of COVID-19 Laboratory Testing Infrastructures

Alex Chen

Submitted by: Alex Chen, Anthropology
Email: achenchc@sas.upenn.edu
Advisor: Dr. Adriana Petryna

The dearth of testing, essential supplies, and even building materials during the COVID-19 pandemic revealed not only the fragility of global supply chains, but also the complete dependence of contemporary biomedical care on such infrastructures. While anthropologists have long anticipated the limits of "preparedness" discourse among public health experts (Lakoff 2008), less attention have been paid to the material investments in "staff, stuff, space, and systems" (Farmer 2014), especially in places like the United States that have long been presumed to be "developed" (Benton 2014). What underlies the shiny veneer of techno-medical advancement (Good 2001)? In this poster, I follow a group of hospital leaders, laboratory technicians, and architects in their efforts to refurbish an existing space to increase their COVID-19 testing capacity during the pandemic. Focusing on discussions over how to "fit" several testing machines into the room, I experiment with "tolerance" – "the allowable deviation from a standard" (Merriam-Webster 2022) – as a concept for parsing scale, temporality, and the limits of pandemic preparedness and response along extractive capitalist logics. First, I highlight the importance of "viral speed" (Ngyuen 2017) in propelling the rushing affective response of my interlocutors. Second, I explore with COVID-19 diagnostic machines as "interscalar vehicles" (Hecht 2018) at the crux of pandemic time, hospital time, production time, and design time. Finally, in juxtaposing the racialized plantation politics of COVID-19 frontline testing (Oyarzun 2020) with the anticlimactic termination of the project, when the vendor notified the hospital that they could not deliver on the diagnostic machines that they promised, I advance time as a crucial analytic in the parsing the intersection of race, care, and material infrastructure. Such examination of the material details of design practice expands methodological and theoretical possibilities for an anthropology of pandemics.

Poster 2A | Anthropology

Leveraging clinical material: an ethnography of buprenorphine-based treatment in greater Pittsburgh

Ben Sieff

Submitted by: Ben Sieff, Anthropology
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Advisor: Dr. Adriana Petryna

Studies have found that buprenorphine-based treatment reduces morbidity and mortality among Opioid Use Disorder (OUD) patients (Wakeman et al. 2020), and a growing consensus about the efficacy of this medication has led to substantial public and private investments in increasing its availability across the U.S (Urban Institute 2019). However, as Markovitz et al. write, "Estimates suggest that up to 40–50% of patients will discontinue treatment prematurely… most within the first month following induction." How might we explain this considerable drop-out rate? Based on eighteen months of ethnographic research in rehabilitation centers and outpatient addiction clinics in the greater Pittsburgh area, this dissertation project considers the lived experiences of Medicaid-sponsored buprenorphine patients. What might their firsthand accounts of this modality of treatment reveal about an expanding political economy of addiction care in western Pennsylvania, a rustbelt region where productive industries like steelmaking and coal mining have given way to a local healthcare sector that, over the last several decades, has become the region's largest employer (Winant 2021; Simpson 2019)?

In the course of hundreds of conversations with patients and staff in the addiction treatment industry, I have found that a large contingent of current and former buprenorphine patients use the language of captivity (e.g., "chains," "leashes," "handcuffs," "slavery") to describe their experiences with this medication. They emphasize that, though far safer and less likely to cause overdose than heroin or fentanyl, buprenorphine is nevertheless a habit-forming opioid. Such expressions of discontent are, for the most part, less concerned with the intrinsic, pharmacological properties of buprenorphine than with the extractive and coercive relations of power that prolonged exposure to this partial opioid agonist can facilitate in specific circumstances and treatment programs. I suggest that these patient frustrations are inextricable from profound social transformations underway in western Pennsylvania, including processes of deindustrialization and carceral expansion, that have exposed many low-income patients to pronounced structural vulnerability (Bourgois et al. 2017).

Drawing on buprenorphine patients' embodied critiques and my own observations in outpatient clinics, I argue that the buprenorphine-based treatment enterprise is predicated on a widely shared entrepreneurial approach to patients as "clinical material." I use this phrase "clinical material" to designate the unspoken but widely accepted ways in which certain bodies, which have been rendered surplus to more formal modes of economic production in western Pennsylvania (such as coal mining, steel production, or more recently carework), are made useful and profitable by both carceral and medical entrepreneurs. These bodies become sources of value, not as wage laborers (Marx 1867) or patient-consumers (Tomes 2016), but as material to be collected, monitored, and leveraged in enterprising and rewarding ways by those charged with their care. In this sense, the enrollment and retention of Opioid Use Disorder patients—and the management of legal liability in a high stakes regulatory environment—has become the primary focus of many entrepreneurs and clinicians in the addiction treatment industry. I argue that this approach to patients as clinical material is not an exceptional but a structural feature of a commercially oriented, fragmented, and rapidly evolving American healthcare system.

Poster 3A | Biochemistry and Molecular Biophysics

Small Molecule Activation of Valosin-containing protein (VCP)

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Valosin-containing protein (VCP) is a AAA+ ATPase that plays a crucial role in protein quality control and membrane trafficking/fusion. Mutations in *VCP* have been associated with frontotemporal dementia clinically. Multisystem Proteinopathy (MSP) mutations in *VCP* lead to TDP-43 aggregates and increased ATPase activity, while a Vacuolar Tauopathy (VT) leads to tau aggregates and decreased ATPase activity. Despite MSP mutations increasing ATPase activity, the unifying hypothesis is that VCP loses function in crucial proteostasis pathways, leading to neurodegenerative disease. As such, there is potential therapeutic value to compounds that increase VCP activity.

We identified compounds that potentially increased VCP activity, validated with two orthogonal *in vitro* ATPase assays. We determined dose-dependence of all active compounds against WT VCP with and without *in vivo* relevant cofactors Ufd1 and Nploc4. We also determined effect of ATP concentration on activation and specificity to VCP by using AAA+ ATPase NSF. Walker B mutations of the D1 and D2 ATPase domains were used to determine which ATPase domain is most effected.

We identified novel activators of VCP that have variable potency and maximum activation. Addition of Ufd1 and Nploc4 has variable effect on the potency of compounds. All compounds exhibit specificity for VCP over NSF. All compounds increase D2 ATPase activity with a D1 Walker B mutation. Interestingly, some compounds significantly decrease D1 ATPase activity with a D2 Walker B mutation.

Our data characterizes novel activators of VCP that have variable potency, maximum activity, and effect on ATPase domains.

Poster 4A | Biochemistry & Molecular Biophysics

Controllable epigenome editing in CAR T cells

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Epigenetic regulation of gene expression is a dynamic program responsive to environmental stimuli that influences diverse biologic processes and can be permuted for the study and treatment of cancer. One aspect of such programming is the introduction of DNA modifications, such as the methylation of cytosine to generate 5-methylcytosine (5mC). 5mC is associated with gene silencing, particularly when it occurs at CpG islands (CGIs) of promoters. Active reversal of this epigenetic gene silencing program is initiated by TET family enzymes, which oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC). These oxidized methylcytosines (ox-mCs) are intermediates along demethylation pathways that result in replacement of 5mC with unmodified cytosine and subsequent relief of transcriptional repression. Oncogenic mutations that inactivate TET result in loss of ox-mCs and hypermethylation and are a hallmark of various cancers. Interestingly, TET2 knockout (KO) increases the cancer-fighting capacity of chimeric antigen receptor (CAR) T cells, lymphocytes that have been modified to express surface receptors against tumor antigens. A limitation of this immunotherapy is CAR T cell exhaustion, a state of dysfunction resulting from repeated stimulation by antigen. Critically, CAR T cells harboring TET2-KO *resist* exhaustion even with continued antigen stimulation. These persistent CAR T cells demonstrate long-lived anti-tumor activity and offer a window of opportunity for the development of exhaustion-resistant CAR T cells. While theoretical concerns about the oncogenic potential of TET2-KO CAR T cells preclude their clinical use, identification of targets downstream of TET2 could guide the design of persistent CAR T cells with favorable safety profiles. Current knowledge suggests TET2 activates genes that promote exhaustion, as demonstrated by the known gene activating effects of TET2 and the fact that TET2-KO prevents exhaustion. Such genes are potential candidates for inhibition to induce CAR T persistence. Inducible CRISPR-guided epigenome editors offer a unique genome screening approach for the identification of such targets by enabling controllable activation of genomic loci of interest. I hypothesize that the effects of TET2-KO in CAR T cells can be traced to sustained silencing of specific genes, and that reactivation of these genes by an inducible split-engineered TET-based epigenome editor (TETseEE) will identify them as targets for inhibition.

Poster 5A | BioengineeringImaging

LaxKAT: a more powerful method to test for association and localize signal in high-dimensional data

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In neuroimaging, researchers are often interested in testing for the association between a single outcome and imaging features across the brain. There are many methods that test for global association, including the sequence kernel association test (SKAT). However, the omnibus nature of this test makes interpretation difficult. We propose a new method called LaxKAT (linear maximum kernel association test) that enables testing for both global and local association in high-dimensional data in a way that controls the family-wise error rate (FWER) while also improving power over current methods.

Poster 6A | BioengineeringImaging

Quantification of bilateral renal oxygen consumption: a preliminary study

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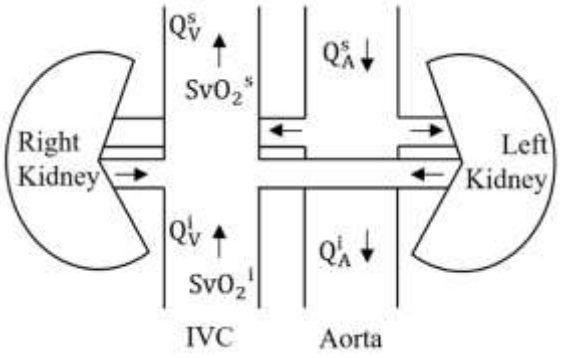
Type 2 diabetes mellitus is the leading cause of chronic kidney disease. Clinical measures of kidney function often detect dysfunction after irreversible damage has occurred. This highlights an unmet need for a biomarker that can enable earlier detection. Quantification of renal metabolic rate of oxygen (MRO_2) is a promising metric because oxygen utilization increases by 40-65% during the early stages of diabetic kidney disease. Here, we propose a "difference method" to quantify oxygen consumption of both kidneys (bilateral renal MRO_2) by imaging above and below the renal vessels and exploiting conservation of mass.

To quantify bilateral renal MRO_2, consider treating the kidneys as one system as illustrated to the right. Blood flow rates (Q, in units of volume per time, $L/\Delta t$) and venous oxygen saturations (SvO_2 expressed as percentages) are defined in the aorta (A) and inferior vena cava (V) at suprarenal (s) and infrarenal (i) locations.



Bilateral renal MRO_2 is then computed with the following expression:

$$r_{b.l.}MRO_2 = k \cdot \left(Q_A^s - Q_A^i\right) \cdot \left(SaO_2 - \left(\frac{\left(Q_V^s \cdot SvO_2^s\right) - \left(Q_V^i \cdot SvO_2^i\right)}{Q_V^s - Q_V^i}\right)\right)$$

The parameters required for quantification of bilateral renal MRO_2 were measured using MRI methods. Five healthy subjects (avg. 25 y/o, 3M) underwent imaging at 3T (Siemens Prisma) under a breath-hold after informed consent.

Poster 7A | BioengineeringImaging

Feasibility of machine learning for cardiovascular function analysis in patients with repaired tetralogy of Fallot

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Introduction: Tetralogy of Fallot (ToF) is the most common cyanotic congenital heart disease (CHD). Although early surgery can repair the defects associated with ToF (rTOF), there is a lack of understanding of individual risk for future adverse events. We propose to train a machine learning model to segment cardiac volumes quickly and accurately from cardiovascular magnetic resonance (CMR) to identify the structural and functional parameters of rToF that are associated with right ventricular (RV) remodeling.

Methods: We randomly selected 8 patients from The Single Center Outcomes Study in ToF (SCOUT-ToF) between 2016-2020 who all received CMR imaging at the Children's Hospital of Philadelphia (CHOP). CMR Imaging (1.5T) included steady-state free-precession cine CMR acquisitions in contiguous short-axis cine imaging from the atrioventricular junction through the cardiac apex. The left ventricular (LV) blood pool, LV myocardium, and RV blood pool were contoured from contiguous short-axis images at end-systole and end-diastole by trained experts at CHOP using cvi42. A U-net architecture was trained to segment the LV blood pool, LV myocardium, and RV blood pool from 2D CMR images using a Dice score loss function. 75% of scans were randomly selected as part of the training group. 2D segmentations were combined with voxel sizes to calculate end-systolic volume (ESV), end-diastolic volume (EDV), and ejection fraction (EF; defined as 100%*(EDV-ESV)/EDV). ESV, EDV, and EF for each ventricle were compared between manual and model.

Results: The model achieved median Dice scores of 0.91, 0.76, and 0.73 for the LV blood pool, LV myocardium, and RV blood pool, respectively. ESV, EDV, and EF medians and correlations for ground truth versus model-based segmentations were calculated. Spearman's rho correlations ranged from 0.59-0.97. Additionally, four patients had CMR performed at two post-repair timepoints, with a median of 2.94 years between scans.

Conclusion: The preliminary outcomes of this study demonstrate the potential of machine learning to accurately segment the LV and RV from CMR images. The results of these efforts will enable large-scale longitudinal studies of RV remodeling in rToF.

Poster 8A | Bioengineering

Extracellular vesicle liquid biopsy to improve breast cancer screening accuracy

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Breast cancer mortality has decreased by approximately 33% since 1990 due to advances in screening mammography and early detection of cancer. However, breast cancer screening often identifies BI-RADS category 4 lesions, indeterminate lesions with a broad likelihood of malignancy. Since each of these lesions are biopsied under current guidelines, many patients with BI-RADS category 4 lesions undergo unnecessary biopsies of benign lesions. We seek to create a non-invasive liquid biopsy to improve the accuracy of assessing likelihood of malignancy for patients with BI-RADS category 4 lesions. In this study, we will apply an immunomagnetic nanofluidic chip to isolate breast cancer-derived extracellular vesicles from plasma; we will sequence the miRNA contents of captured vesicles to identify transcriptional signatures that can classify breast cancer patients. Here, we quantified surface proteins on vesicles isolated from BT-474 breast cancer cell conditioned media using an ELISA, which identified EpCAM, HER2, and CD24 as candidate surface proteins for immunomagnetically labeling breast cancer-derived vesicles. We validated the immunomagnetic capture of breast cancer-derived vesicles with anti-EpCAM, anti-HER2, and anti-CD24 to isolate vesicles from BT-474 conditioned media spiked into healthy plasma. Next, we will isolate vesicles in plasma from patients with either malignant or benign lesions classified as BI-RADS category 4 using anti-EpCAM, anti-HER2, and anti-CD24 pulldowns; we will sequence the miRNA contents of the captured vesicles and apply machine learning to generate a panel of miRNAs that differentiate malignant samples. Finally, we will validate our assay by determining the accuracy of classifying malignant vs benign samples from a blinded test set. Together, the successful outcome of this study will provide a noninvasive extracellular vesicle-based liquid biopsy to inform clinical decision making for patients with indeterminate breast lesions.

Poster 9A | Cell and Molecular Biology - Cancer Biology

Loss of the MGAT5 glycosyltransferase sensitizes pancreatic tumor cells to immune clearance

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Pancreatic ductal adenocarcinoma (PDAC) is currently the third leading cause of cancer-related death in the United States. The five-year survival rate of less than nine percent is attributed mainly to a difficulty in early detection and a lack of effective treatments. Tumor-associated glycans represent a potential anti-tumor target for two reasons: (i) protein glycosylation is known to play a role in tumor progression, and (ii) alternatively glycosylated proteins may function as tumor neoantigens.

The glycosyltransferase MGAT5 catalyzes the formation of β1,6-N-acetylglucosamine branched glycans, and overexpression has been implicated in tumor growth and metastasis in multiple cancers. Using a panel of clonal cell lines that recapitulate the immune heterogeneity of PDAC, we found that knockout of MGAT5 in some clones ("T cell-inflamed" tumors) allows for complete clearance of tumors while in other clones ("non-T cell-inflamed") MGAT5 deficiency led to a marked decrease in tumor growth. This phenotype was confirmed in orthotopic injection as well as subcutaneous injection into syngeneic mice. By contrast, MGAT5 loss had no impact on tumor cell growth *in vitro*.

To probe immune system involvement in this robust rejection of tumor growth, the MGAT5 KO cells were injected into Nod/Scid mice, resulting in full rescue of the phenotype. Tumor eradication or growth inhibition *in vivo* was found to be dependent specifically on the presence of CD4/CD8 T cells and dendritic cells. Tumor challenge experiments, in which mice were immunized with MGAT5 KO cells and challenged with wild-type tumors four weeks later, revealed that tumor rejection was associated with a durable immunologic memory. Finally, therapeutic vaccination with the knockout line concomitantly with wild-type tumor cell injection led to decreased tumor growth.

These results are consistent with a model in which MGAT5 loss results in the formation of new tumor antigens and/or enhances the immunogenicity of pre-existing antigens. Ongoing work seeks to determine the identity of the responsible antigens and further investigate the relationship between altered glycosylation and anti-tumor immunity.

Poster 10A | Cell and Molecular Biology - Cancer Biology

Route-specific immune surveillance in Pancreatic Adenocarcinoma metastasis

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Pancreatic ductal adenocarcinoma (PDAC) has the worst 5-year survival rate of any major cancer type due to its high rate of metastasis. Cancer cells metastasize to distant organs through the blood and lymphatic vascular systems. Regional lymph node (LN) metastases are present in the majority of PDAC patients and are among the strongest predictors of poor prognosis, yet their contribution to cancer mortality has long been debated. The recent discoveries that LN metastases promote systemic metastasis both by seeding cells directly into the blood and by tolerizing the immune system suggest that lymphatic dissemination is a key driver of metastatic burden. Once metastasizing tumor cells enter blood or lymphatic vessels, they leave the immunosuppressive microenvironment of the primary tumor and become vulnerable to killing by circulating immune cells. Circulation may be the most vulnerable step of the metastatic cascade; therefore, understanding the immune vulnerabilities of cancer cells traveling in blood and in lymph is of critical importance. Blood and lymph have very different compositions of immune cells; however, the impact that these differences have on anti-tumor immune surveillance in each compartment has not been investigated. Using flow cytometry we find that effector immune cells in tumor-draining lymph nodes display suppressed cytotoxic response to cancer compared to those circulating in blood. We find cytolytic activation of peripheral blood NK cells in tumor-bearing mice however NK cell activation is inhibited in tumor draining LNs. Using the ovalbumin tumor antigen system we find that tumor-specific CD8 T cells are more mature and proliferative in circulating blood than in tumor-draining LNs, and tumor-specific regulatory T cells are more frequent in the draining LN than in blood. These preliminary results suggest that lymphatic dissemination may be an immune-privileged route of metastasis. To compare differences in immune surveillance of the hematogenous versus lymphatic routes of metastasis we will use cellular barcoding to quantify metastatic efficiency of cells injected directly into blood versus lymph in mice with and without an immune system. This experiment will be repeated following selective depletion of various immune cell populations. This study will identify immune-privileged routes of metastasis and identify route-specific immune vulnerabilities.

Poster 11A | CAMB - Developmental, Stem Cell, and Regenerative Biology

Optical pooled CRISPR screens in human iPSC-derived neurons

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Pooled CRISPR gene perturbation screening in human cells is a scalable platform to identify genetic architectures underlying phenotypes. Readily accessible phenotypes such as survival and proliferation and molecular phenotypes such as gene expression quantified via RNA and protein levels have been widely investigated in transformed cell lines. Such functional genomics approaches are limited in their ability to capture physiologically relevant cell type-specific phenotypes and limited in their scale to capture spatially and temporally dynamic phenotypes accessible via high-throughput imaging. Here, we report a platform combining induced pluripotent stem cell (iPSC) technology, pooled genome-wide CRISPR interference (CRISPRi) screening with anti-CRISPR-mediated inducibility, and optical phenotyping combined with targeted sgRNA in situ sequencing (ISS) to demultiplex a library of CRISPRi perturbations following imaging at single-cell resolution. As a proof-of-concept, we conduct a pooled CRISPRi screen for neuronal morphology in human iPSC-derived neurons using a novel spectral barcoding approach with inducible Brainbow technology to assist with neurite tracing and segmentation. Optical pooled screens in human iPSC-derived neurons presents an opportunity to uncover novel regulators of complex phenotypes accessible via high-throughput imaging.

Poster 12A | CAMB - Genetics and Epigenetics

Single-Cell Transcriptomics and Multiplexed Imaging Resolves the Spatial and Cellular Heterogeneity of the Human Bone Marrow Microenvironment

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The bone marrow is the critical organ for blood production. Acute myeloid leukemia (AML), a hematologic malignancy with abysmal prognosis, arises from the bone marrow. Despite apparent complete remission of many AML patients, residual leukemia cells may persist within the bone marrow microenvironment, which is associated with a much greater chance of AML relapse regardless of leukemia risk subtype. Murine and xenograft studies have suggested that the leukemic cells hijack chemoprotective niches in the bone marrow microenvironment which exist in normal physiology to preserve hematopoietic stem cells. However, the *in vivo* bone marrow microenvironment in humans remains poorly characterized. We therefore optimized and employed joint single-cell RNA sequencing and 44-parameter multiplexed imaging of femoral heads from total hip arthroplasty surgeries to deeply dissect the cellular heterogeneity of the human bone marrow niche. We found tremendous mesenchymal heterogeneity not captured by existing studies relying on bone marrow aspirate samples. Next, we employed the same techniques to study residual leukemia cells. We found that AML cells treated with venetoclax and hypomethylating agents persisted in spatial clusters within para-trabecular regions of the marrow, in a manner most similar to early myeloid precursors, but not CD34+ hematopoietic stem and progenitor cells, in healthy steady-state marrow. Overall, our work contributes the first spatially resolved, comprehensive atlas of the healthy human bone marrow niche. We demonstrate the power of this framework to delineate the spatial relationships of AML MRD to the bone marrow niche and suggest that the endosteal niche may be a key contributor to AML MRD persistence, which warrants further functional investigation.

Poster 13A | CAMB - Genetics and Epigenetics

Systematic characterization and manipulation of the trade-off between proliferation and invasion in melanoma

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Genotype-based targeted therapy for melanoma has given rise to great success stories often followed by resistance and relapse. Why do some cells survive treatment with targeted therapy, and how do these surviving cells change to cause relapsing disease? A predominant model is that melanoma cells exist in two distinct cellular states: the proliferative, drug-sensitive melanocytic state and invasive, drug-resistant mesenchymal state. However, despite detailed molecular profiling, it is currently unknown how to effectively target both populations in initial treatment and prevent switching between the phenotypes during treatment. We propose an alternative framework to understand phenotype switching which measures the task performance of individual cells. Repeated observations seem to indicate that in individual melanoma cells are subject to a **trade-off** between proliferation and invasion, i.e., for a cell to improve at one task, it must necessarily become worse at the other. We combine the Pareto Task Inference (ParTI) algorithm to find task optimization from gene expression data with our method of single cell barcoding and RNA-sequencing. We confirm that a clonal population of BRAF V600E WM989 mutant melanoma cells is optimized to perform both invasion and proliferation both before and after treatment with BRAF inhibitor. However, we find that the distribution of the enrichment of task performance shifts dramatically from proliferation to invasion following treatment. We also find that different cells in the same resistant colony perform different tasks and that some rare colonies are overall better overall at both proliferation and invasion. Finally, we find that expression of invasion-related genes is restricted to expression in G0/G1 of the cell cycle in resistant cells but not in drug-naive cells. Overall, our model is that extended time in G0/G1 is required for expression of invasion-related genes and transition into the invasive state, with cell cycle length naturally providing a trade-off between these two tasks.

Poster 14A | CAMB - Genetics and Epigenetics

Quantifying the Metabolic Response to Acute Cold Exposure in Mice

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Obesity is a growing healthcare challenge in the United States and around the world. Obesity stems from an imbalance between nutrient intake and energy expenditure, suggesting that finding ways to increase the latter may help treat obesity. Humans and other mammals can increase energy expenditure to maintain body temperature in response to cold exposure, a process known as thermogenesis. During cold exposure, brown adipose tissue (BAT) generates heat via UCP1-mediated uncoupling and other mechanisms. Cold exposure also promotes metabolic changes across many other organs including skeletal muscle, where shivering also contributes to heat generation. However, the effect of cold exposure on metabolic flux of nutrients, both systemically and in specific organs, remains poorly defined. To address this, we implemented a minimally-perturbative stable isotope-tracing infusion approach to quantify nutrient utilization, systemically and in specific organs, in awake and freely-moving mice at room temperature or acutely exposed to 4°C. Our results demonstrate that in response to cold exposure, circulating flux (plasma turnover) of both carbohydrates and fatty acids increase, with fatty acid flux accounting for a higher proportion of overall flux. Cold exposure also increases the rate of appearance of essential amino acids, including notably branched chain amino acids (BCAAs), indicating increased systemic proteolysis. At the tissue level, BAT consumes proportionally more circulating fatty acids at 4°C than at RT, though oxidation of circulating carbohydrates is largely maintained, suggesting there may be a decreased reliance on internal fuel stores. Substantial changes in nutrient preference also occur in other organs at 4°C. For example, cardiac oxidation of carbohydrates decreases by ~75%, whereas skeletal muscle increases its preference for BCAAs and ketone bodies and decreases its preference for glutamine. Together, these results provide a comprehensive, quantitative map of fuel use in response to cold exposure and offer new insights into the metabolic changes driving increased energy expenditure during thermogenesis.

Poster 15A | CAMB - Genetics and Epigenetics

Investigating ferroptosis in epidermal differentiation and tumorigenesis

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Ferroptosis is a recently described form of regulated cell death gaining significant interest for its involvement in a variety of disease states. It is broadly defined by iron-dependent, lethal accumulation of lipid peroxides and dysregulation of cellular redox homeostasis. However, both a physiologic role for ferroptosis and how it functions in skin homeostasis remains elusive. Recent evidence from our lab identified a critical link between ferroptosis, the epigenetic tumor suppressor MLL4, and epidermal differentiation (Egolf et al., *Science Advances*, 2021). We demonstrated that loss of Mll4 in mouse epidermis impairs epidermal differentiation and promotes hyperplasia tied to lost expression of key lipoxygenase enzymes (*Alox12*, *Alox12b*, *Aloxe3)* that mediate ferroptosis. This provoked the hypothesis that ferroptosis may play a broader role in terminal differentiation and tumor suppression in the skin. Staining for ferroptotic markers in human skin and inhibiting ferroptosis in 3D human organotypic skin models reinforced this idea. Here, we present further evidence for a functional link between ferroptosis and epidermal differentiation using global transcriptomic analyses. We show that triggering ferroptosis in keratinocytes upregulates endoplasmic reticulum (ER) stress signaling, and terminal differentiation genes. This suggests that perhaps ferroptosis-mediated ER membrane lipid peroxidation results in upregulation of ER stress and subsequent terminal differentiation. Taken together, our data supports the idea that ferroptosis alters key transcriptional pathways in the skin to orchestrate epidermal differentiation. Overall, this potentially opens exciting therapeutic avenues for regulating ferroptosis in skin ichthyoses and cancers, as well as other skin diseases characterized by altered differentiation dynamics.

Poster 16A | CAMB - Genetics and Epigenetics

Characterizing non-genetic mechanisms of adaptive resistance in metastatic melanoma

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A major long-term objective in the melanoma field is to develop new therapeutic strategies that can overcome relapse for the many patients suffering from melanomas that are not susceptible to immunotherapy. Although there have been various efforts to identify the molecular mechanisms driving melanoma resistance, most efforts had been focused on genetic mutations that resistance. Previous work from our lab and others have shown that non-genetic changes involving transient fluctuations of the activity of various signaling pathways within a genetically homogenous population can enable certain rare populations of cells to survive treatment with targeted therapies. Yet, **a key conceptual question remains**: are these pre-existing cells already fully resistant (a priori) to therapy and are thus selected for by the initiation of therapy? Or, upon exposure to therapy, are they able to adapt from a pre-resistant state towards a new, fully resistant state? We **hypothesize that adaptation is the result of a process of cellular reprogramming in which cells not initially resistant to therapy can become resistant.** We will test this hypothesis in **Aim 1** using live imaging, DNA barcode lineage tracking, and RNA and ATAC sequencing and validate in patient-derived xenograft (PDX) mouse models and in patient biopsy samples. In **Aim 2**, we will use a combination of pharmacological inhibition, fluorescent reporters, and CRISPR-Cas9 directed gene perturbations to test the hypothesis that AP-1 is a key regulatory factor driving targeted therapy resistance. Together these aims will address the critical gap in knowledge of how cancer cells can undergo adaptation to develop and maintain a resistant state in the presence of targeted therapies. Characterizing mechanisms of adaptation could provide new therapeutic options to prevent or treat therapy-resistant metastatic melanoma.

Poster 17A | CAMB - Microbiology, Virology and Parasitology

Structural and functional determinants of caspase-11 inflammasome assembly in innate immune defense

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Sepsis is caused by a dysregulated systemic hyper-inflammatory response to bacterial and viral infections. Globally, 27 million people die of sepsis each year. Despite decades of research, a definitive clinical treatment for sepsis remains to be found. Gram-negative sepsis is caused by the pathological immune response to lipopolysaccharide (LPS). In innate immune cells cytosolic LPS is sensed by the cysteine protease, caspase-11 (Casp11). LPS drives Casp11 assembly into a highly organized supramolecular complex known as the inflammasome. This Casp11 inflammasome (Casp4/5 in human cells) triggers inflammatory cell death that significantly contributes to sepsis in murine models. Yet, the structural and functional determinants of Casp11 inflammasome assembly remain poorly defined. Using a robust single-cell based fluorescent reporter, we have found that Casp11 catalytic activity and auto-processing are required for Casp11 inflammasome assembly. We show that the Casp11 catalytic site cys-254 drives the scaffolding and maintenance of a perinuclear Casp11 inflammasome, through intra- and inter-molecular auto-processing, as well as disulfide bridges. These findings have the potential to fundamentally change our understanding of Casp11 signaling in immune cells and provide novel targets for drug development against gram negative sepsis.

Poster 18A | CAMB - Microbiology, Virology and Parasitology

Distinct roles for cDC1s and cDC2s in CD4+ T cell responses against the intracellular parasite *Cryptosporidium*

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Cryptosporidium is a protozoan parasite that is a major cause of diarrhea and death in immunocompromised individuals and malnourished children. There is no vaccine, and the single FDA-approved therapy is largely ineffective. Protective immunity to *Cryptosporidium* requires CD4+ T cells and interferon gamma (IFN-γ). Few studies have investigated antigen-specific T cell responses to *Cryptosporidium.* Transgenic *C. parvum* were engineered to express the the LCMV-$gp_{61\text{-}80}$ (gp61) MHCII-restricted epitope attached to the parasite protein MEDLE2, which is exported into the host cell cytoplasm. Mice infected with these parasites showed expansion of adoptively transferred SMARTA CD4+ T cells (TCR transgenic specific for gp61). The majority of these cells were Th1, producing IFN-γ and expressing T-bet. This CD4+ T cell response required type 1 conventional dendritic cells (cDC1s), as mice lacking these cells (ΔcDC1, IRF8+32-/-) were unable to generate parasite-specific responses. Characterization of early events during T cell priming revealed that cDC1s were required for CD4+ T cell proliferation, up-regulation of the gut-homing receptor LPAM-1 (α4β7 integrin), and induction of the chemokine receptor CXCR3. cDC1s were not required for antigen presentation, however, consistent with a distinct role for cDC2s in priming naive CD4+ T cells. In line with this, CD4+ T cell responses were impaired in mice deficient in cDC2s ($IRF4^{\Delta CD11c}$). This transgenic system where both host and parasite are tractable will reveal additional insights into mechanisms of CD4+ T cell priming and activity during *Cryptosporidium* infection.

Poster 19A | CAMB - Microbiology, Virology and Parasitology

Unraveling metabolic determinants of innate immune function in Maple Syrup Urine Disease

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Human genetic diseases related to metabolism, like Inborn Errors of Metabolism (IEMs) represent unique opportunities to study metabolic determinants of immune function. One such IEM is Maple Syrup Urine Disease (MSUD), in which patients have genetic variants that disrupt branched chain amino acid (BCAA) catabolism. These patients have apparent immune dysfunction which persists despite dietary intervention, including increased rates and reduced clearance of bacterial infections. In addition, BCAA levels are known to be disrupted in a variety of other disease states, including in diabetes and cardiovascular disease. Therefore, there is a significant need to understand how BCAAs and their metabolites impact the putative immune systems in these patients. Here, we demonstrate that the branched chain amino acids (BCAAs) regulate cytokine secretion and inflammasome activity in macrophages in response to LPS. Our data suggests that the three BCAAs – leucine, valine, and isoleucine – have independent roles in regulating the activity of macrophages. By characterizing the signaling networks that are altered by BCAA availability, we are beginning to understand the metabolic mechanisms that underlie innate immune function. Future work will expand upon these findings by modeling MSUD through both mouse models as well as mutagenesis of healthy human monocytes via CRISPR-Cas9. Therefore, we will unravel the mechanistic and pathologic mechanisms that lead to innate immune deficits in patients with deficiencies in BCAA catabolism.

Poster 20A | Epidemiology and Biostatistics - Biostatistics

Voxel-wise intermodal coupling analysis of two or more modalities using local covariance decomposition

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When individual subjects are imaged with multiple modalities, biological information is present not only within each modality, but also between modalities - that is, in how modalities covary at the voxel level. Previous studies have shown that local covariance structures between modalities, or intermodal coupling (IMCo), can be summarized for two modalities, and that two-modality IMCo reveals otherwise undiscovered patterns in neurodevelopment and certain diseases. However, previous IMCo methods are based on the slopes of local weighted linear regression lines, which are inherently asymmetric and limited to the two-modality setting. Here, we present a generalization of IMCo estimation which uses local covariance decompositions to define a symmetric, voxel-wise coupling coefficient that is valid for two or more modalities. We use this method to study coupling between cerebral blood flow, amplitude of low frequency fluctuations, and local connectivity in 803 subjects ages 8 through 22. We demonstrate that coupling is spatially heterogeneous, varies with respect to age and sex in neurodevelopment, and reveals patterns that are not present in individual modalities. As availability of multi-modal data continues to increase, principal-component-based IMCo (pIMCo) offers a powerful approach for summarizing relationships between multiple aspects of brain structure and function. An R package for estimating pIMCo is available at: https://github.com/hufengling/pIMCo.

Poster 21A | Epidemiology and Biostatistics - Biostatistics

Time of clinic appointment and advance care planning discussions in oncology

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Early advance care planning (ACP) in oncology increases goal-concordant care. However, time pressures during a busy clinic day may prevent clinicians from engaging in necessary conversations. Given prior evidence of suboptimal clinician decision-making in non-oncology settings in latter parts of a clinic day, we investigated the association between appointment time and likelihood of ACP conversations. We used electronic health record data to identify medical oncology encounters. We ascertained the presence of ACP from either (1) a specific ACP note type in the EHR, or (2) an ACP smart phrase in clinical progress notes. Oncology clinicians usually practiced in a morning (8am to 11am) or afternoon (12pm to 4pm) session. We used generalized estimating equations, clustering by clinician, to estimate the probability of ACP documentation by session hour. We found that oncology clinicians' likelihood of having advance care planning conversations decreases as a clinic session progresses. Decision fatigue and falling behind schedule could be contributing reasons for this effect. Lower rates of discussions about goals of care later in a session could result in more aggressive end-of-life treatments. Proactive scheduling of high-risk patients earlier in a clinic session or scheduling separate visits for advance care planning could facilitate necessary conversations and should be further studied.

Poster 22A | Epidemiology and Biostatistics - Epidemiology

Racism and EHR data: examining missingness as a potential driver of inequities in predictive performance of a clinical decision support tool

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Background. Understanding how electronic health record (EHR) clinical prediction models can exacerbate health inequities is of critical importance. Previously, differences in predictive performance across racialized patient groups has been attributed to intrinsic differences between patients, an understanding shaped by medical racism. In this study, we instead examine how socially patterned error in common predictors affects predictions from a random forest model for emergency department (ED) triage. Specifically, we examine missingness in the problem list that may arise from structural (e.g., Black patients receiving more fragmented care across institutions) and interpersonal racism (e.g., underdiagnosis of conditions among Black patients).

Methods. We compare model performance for patients coded as Black vs patients coded as white in different scenarios where we manipulate the level of missingness in the problem list data. We use a nonparametric bootstrapping approach and both conventional (e.g., c statistic) and health equity relevant metrics (e.g., false negative rate (FNR)). The data include all visits to an urban academic ED between 2016 –2017 with and excluded patients with missing chief complaint(s), any missing vital signs, and psych patients. To manipulate the missingness, we 'look forward' to the end of the retrospective encounter and use this more complete problem list data to generate counterfactual predictions (e.g., if we knew all patients' true disease status at the point of triage).

Results. There were few between-race differences between Black and white patients that impacted triage predictions. For both Black and white patients there were significant within group differences in both conventional and health equity metrics: e.g., difference in c statistic [Black patients 0.056, 95% CI (0.048, 0.064); white patients 0.054, 95% CI (0.047, 0.061)] and difference in FNR [Black patients -0.115, 95% CI (-0.165, -0.065); white patients -0.106 95% CI (-0.167, -0.045)].

Conclusion. Socially patterned missingness in common EHR predictors impacts prediction for both Black and white patients. While missingness did not vary significantly between selected patient race groups in this sample, it is likely to vary in other contexts where health care is unequal, underscoring the importance of evaluating social drivers of predictive disparities.

Poster 23A | Genomics and Computational Biology

LSV-Seq: A Novel Targeted Sequencing Method to Measure Alternative Splicing Across Human Tissues

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Alternative splicing is a key regulatory process that allows multiple transcripts to be produced from a single gene. Splicing has been primarily studied on a high-throughput scale via RNA sequencing (RNA-Seq). However, most of the reads in standard RNA-Seq are not the junction-spanning reads required for detecting splicing changes and splice isoforms. To address this, we developed a cost-effective, targeted RNA-Seq method to quantify splicing variations across human tissues. Building on the previous MPE-Seq method (Xu et al 2019) for detecting splicing in yeast, our LSV-Seq method uses thousands of reverse transcription primers anchored near 3' splice sites. We created a new pipeline to identify targetable regions from previous RNA-Seq data using the MAJIQ algorithm and predict high yield primers. The library preparation protocol uses highly specific reverse transcription conditions to prevent off-target amplification. LSV-Seq achieves an overall median enrichment of ~500-fold compared to standard RNA-Seq and a median enrichment of ~800-fold for lowly expressed genes. Furthermore, LSV-Seq quantifications correlate well with RNA-Seq and detect numerous *de novo* junctions not found with RNA-Seq. We envision that LSV-Seq will be used to quantify splicing in large patient cohorts, detect splicing variation in lowly expressed genes, and detect transient splicing intermediates.

Poster 24A | Immunology

Hepatic CD9 regulates adipose tissue inflammation and metabolic dysfunction during obesity

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Rationale: The prevalence of obesity has been increasing at alarming rates leading to significant morbidity, mortality, and healthcare costs. During obesity, adipose tissue must undergo extensive remodeling in response to pathologic levels of lipids, leading to adipose expansion, inflammation, and fibrosis. Adipose tissue inflammation has been shown to play both regulatory and pathologic roles in the development of obesity. However, the mechanisms regulating adipose tissue inflammation are not fully understood. Recent evidence suggests that communication between metabolic organs such as liver and adipose tissue can contribute to both physiologic and pathologic changes during obesity. Extracellular vesicles (EVs) are nanometer-sized lipid particles containing miRNAs, proteins, and lipids, among other molecules.[1,2] EVs have recently been appreciated as a mode of interorgan communication during obesity though the mechanisms and functions of this communication network are understudied. CD9 is a tetraspanin family member that regulates EV biogenesis, release, and uptake by target cells.

Methods: We hypothesized that hepatic CD9 expression regulates adipose tissue inflammation and metabolic dysfunction during obesity. To test this hypothesis, we developed a conditional knock-out mouse targeting the tetraspanin CD9 in hepatic cells. Mice were given either normal chow diet or high fat diet for 12 weeks. At the end of the 12 weeks, mice were subjected to a glucose tolerance test and adipose tissue immune cells was analyzed using flow cytometry.

Results: We found that hepatic CD9 KO mice gained 29% more body weight after 12 weeks of high fat diet ($p=0.0001$). In addition, they had increased glucose intolerance ($p<0.01$). Additionally, we found that loss of hepatic CD9 expression led to increased accumulation of neutrophils, monocytes, and adipose tissue macrophages (ATMs) in visceral epididymal adipose tissue. Neutrophil and monocyte numbers increased by 9 fold and 3.5 fold respectively ($p<0.01$). Adipose tissue macrophage frequency was doubled and numbers were 10 fold higher in hepatic CD9 KO mice ($p<0.001$). Furthermore, monocytes and ATMs displayed increased accumulation of lipids in hepatic CD9 KO mice ($p<0.01$).

Conclusions: Future studies will aim to understand the effects of deletion of CD9 on EV production and composition. In addition, we will further investigate the effect of hepatic CD9 on adipose tissue inflammation and remodeling. These studies will improve our understanding of the role of liver- adipose tissue communication on the metabolic and inflammatory changes seen during obesity.

Poster 25A | Immunology

Are commensal-induced Tregs protective in a gnotobiotic model of type 1 diabetes?

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While type 1 diabetes (T1D) results from a complex combination of genetic and environmental risk factors, the early-life gut microbiota represents a likely source of protection. Studies in the non-obese diabetic (NOD) mouse model reveal a strong influence of the microbiota on diabetes incidence, with germ-free (GF) NOD mice experiencing an almost 100% incidence of diabetes. However, understanding of how commensal bacteria protect from T1D remains limited. Many immunomodulatory effects of the gut microbiota occur due to commensal promotion of $Foxp3^+$ regulatory T cells (Tregs). Some Tregs develop following T cell receptor (TCR) recognition of self peptides presented in the thymus (tTregs), while others differentiate from naïve $CD4^+$ T cells in peripheral tissues (pTregs). Commensal bacteria promote the development of pTregs through both non-antigen-specific and antigen-specific mechanisms. pTregs also contribute to protection from T1D, with pTreg-deficient NOD.CNS1$^{-/-}$ mice experiencing an increased incidence of diabetes. Colonization of NOD mice with PedsCom, a defined consortium of nine early-life commensals, induces pTregs and significantly reduces diabetes incidence compared to GF NOD mice. Preliminary bioinformatics analysis of PedsCom members identifies several potential insulin peptide mimics, provoking the novel hypothesis that commensal-induced Tregs protect from T1D through a mechanism of tolerogenic molecular mimicry. We will test this hypothesis through several complementary approaches: 1) experiments using GF and PedsCom-colonized NOD and NOD.CNS1$^{-/-}$ mice to assess whether commensal-induced pTregs are necessary and sufficient to confer protection from diabetes; 2) experiments using insulin-peptide tetramers to assess whether commensals induce protective Tregs with TCRs that recognize islet self peptides; 3) paired single-cell RNA sequencing (scRNA-seq) and TCR sequencing (scTCR-seq) to test the hypothesis that commensal colonization yields shared TCR clonotypes between intestinal lamina propria and pancreatic islet Tregs; 4) generation of T cell hybridomas expressing identified TCRs and *in vitro* screening to detect cross-reactive recognition of commensal antigens and islet autoantigens; 5) finally, generation of CD45.2 TCR-transgenic NOD mice expressing *in vitro* cross-reactive TCRs and adoptive transfers of naïve transgenic T cells to assess *in vivo* differentiation to pTregs and protection of recipient NOD mice from diabetes.

Poster 26A | Immunology

Fate induction in chimeric antigen receptor T cells through asymmetric cell division

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Early expansion and long-term persistence predict efficacy of chimeric antigen receptor (CAR) T cells. This is thought to reflect the induction of both effector and memory T cell populations to provide short-term target clearance and long-lasting remission. Despite these subsets' roles in therapeutic success, the cellular mechanisms of fate induction after CAR T cell activation are unknown.

Here, we show that activated human CAR T cells undergo asymmetric cell division to impose distinct fates upon first-division daughter cells. We use a novel protein-protein interaction dependent molecular labeling technique to label target-engaged CAR molecules and sort first division proximal and distal daughter CAR T cells for single-cell surface proteomic and transcriptional profiling; metabolic profiling; and assessment of *in vitro* and *in vivo* cytotoxic function. Target-engaged CAR molecules aggregate on proximal first-division daughter cells and induce asymmetry between proximal and distal daughter cells in surface proteome, transcriptional profile, and metabolic program. Proximal daughter cells enrich in the surface protein levels for CD25 and Notch1; upregulate *MYC* and *MTORC1* target genes; exhibit increased activity in the *TBX21* regulon; and demonstrate increased metabolic activity largely supported by glycolysis, consistent with proximal daughter cell activation and differentiation toward a terminal effector cell fate. In contrast, distal daughter cells enrich in the surface protein levels for CD45RA and CD5; upregulate genes such as *CCR7*, *IL7R*, and *KLF2*; enrich in the activity of *TCF7*, *BACH2*, and *FOXP1* regulons; and employ an oxidative phosphorylation-predominant metabolic profile indicative of distal daughter cell differentiation toward a memory cell fate. In line with their memory T cell fate, distal daughter T cells exhibit greater *in vivo* persistence and clearance of target tumor cells compared to proximal daughter T cells. However, despite their memory phenotypes and *in vivo* functional longevity, first-division distal daughter cells demonstrate potent cytolytic activity similar to proximal daughter cells for up to 48 hours after first cell division. This period of 'target readiness' is followed by a substantial decrease in cytotoxicity in distal, but not proximal, daughter cells. Together, these phenotypes and transcriptional profiles reveal that distal daughter cells assume a transient effector-like state along their differentiation trajectory towards becoming memory T cells. These studies establish asymmetric cell division as a framework for studying mechanisms of human CAR T cell differentiation and improving therapeutic outcomes.

Poster 27A | Immunology

Immunometabolic reprogramming of pulmonary macrophages in obesity

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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 immune response to infectious and allergic stimuli, and prior work has found that obesity shapes macrophage activation states outside the lung. However, the mechanisms by which obesity alters lung macrophage functions, and the potential consequences of these effects on lung inflammatory disease, 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. Flow cytometric analysis found that obesity expands lung macrophage populations with features of obesity-associated activation including cell-surface CD9 and accumulation of intracellular lipid. Additionally, we found that obesity increased lung macrophage production of IL-1b, a cytokine implicated in the development of OAA. Lipidomics analysis revealed increased abundance of the saturated long chain fatty acid stearate (stearic acid, SA) in obese lung macrophages. *In vitro*, SA induces priming of the macrophage NLRP3 inflammasome. *In vivo*, we found that SA causes expansion of IL-1b producing, CD9+ lung macrophages. 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 studies identify a component of high fat diets that prime the lung macrophage inflammasome and potentiate inflammasome activation and IL-1b production in the context of an allergic stimulus. These observations have broad implications for understanding the etiology and refractory nature of OAA and will inform future treatment efforts through dietary modification or inflammasome-directed therapeutics. Ongoing studies will investigate the molecular and metabolic mechanisms by which stearate alters macrophage activation and determine the relative contribution of this metabolic signaling axis to a model of OAA.

Poster 28A | Neuroscience

Ndnf-IN dysfunction in a mouse model of Dravet Syndrome

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Neurodevelopmental disorders, such as autism spectrum disorder (ASD), schizophrenia, and childhood epilepsy, have been linked to dysfunction of forebrain GABAergic inhibitory interneurons (INs). Dravet Syndrome (DS) is a neurodevelopmental disorder characterized by severe epilepsy and features of ASD due to variants in *SCN1A* encoding the voltage-gated sodium channel α subunit Nav1.1. DS pathology is attributed to IN dysfunction, given that cerebral cortex INs preferentially rely on Nav1.1 for action potential generation and propagation. INs exhibit a broad diversity of electrophysiological, anatomical, and molecular properties; understanding the contribution of different classes of INs to microcircuit function in normal brain, and dysfunction in the setting of pathology, is important for elucidating the mechanisms of neurodevelopmental disorders. Three of the major subtypes of GABAergic INs, namely those expressing parvalbumin, somatostatin, and vasoactive intestinal peptide, show impaired action potential generation in an *Scn1a+/-* mouse model of DS. Here, we attempt to determine if a fourth major subtype of IN – those expressing Neuron-Derived Neurotrophic Factor (Ndnf) – are also dysfunctional in DS. We performed current clamp recordings of Ndnf-INs in layer 1 primary somatosensory cortex under fluorescent guidance in acute brain slices prepared from Ndnf-Cre.*Scn1a+/-* mice and age-matched littermate Ndnf-Cre.*Scn1a+/+* controls. We found that Ndnf-INs display abnormalities in sodium channel-dependent properties of individual action potentials and of repetitive firing in *Scn1a+/-* mice relative to wild type, but do not show major differences in passive membrane properties which are not directly dependent on sodium channel function. In summary, Ndnf-INs are a recently identified and understudied subclass of INs whose intrinsic firing is impaired in the setting of heterozygous loss of function of *Scn1a*, suggesting that Ndnf-INs also rely on Nav1.1, are dysfunctional in DS, and may contribute to DS pathophysiology.

Poster 29A | Neuroscience

Refinement of Functional Connectivity in Development Aligns with the Sensorimotor to Association Axis

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A recent neurodevelopmental hypothesis posits that cortical maturation progresses along the sensorimotor-association (S-A) axis, which spans from unimodal sensorimotor to transmodal association cortices. Prior work suggests that patterns of functional network development vary across this cortical hierarchy. In this pre-registered analysis, we will use multiple large-scale datasets to replicate and extend prior work. We hypothesize that integration of unimodal networks increases whereas segregation of transmodal networks increases during functional development in youth.

We will use resting-state functional MRI in youth ages 5-22 from four large datasets. The Philadelphia Neurodevelopmental Cohort (N=1621) was used for discovery and feature selection; these findings will be replicated prospectively in other large-scale developmental datasets including the Human Connectome Project: Development (N= 652), Healthy Brain Network (N=5,290), and Nathan Kline Institute-Rockland (N=505). Data will be processed with fMRIPrep and eXtensible Connectivity Pipeline; all results will be evaluated across multiple parcellations. To characterize functional development, we will quantify multiple facets of connectivity, including global connectivity strength and within- and between-network coupling. To model both linear and non-linear associations between functional connectivity metrics and age, we will fit generalized additive models with penalized splines and adjust for sex and in-scanner motion. The developmental relationship effect size will be quantified by the change in adjusted R^2 between a full model and reduced model with no age term. Correspondence of developmental effects to the S-A axis will be assessed with Spearman's rank correlation, with significance testing done with spin-based spatial permutation tests. Taken together, results from this study will robustly establish how patterns of functional brain development align with a major axis of brain organization.

Poster Session B

Biochemistry & Molecular Biophysics

Poster 1B
Plasma Focused Ion Beam (FIB) Milling to Reveal Biological Structures in situ
Presenter: Katie Kixmoeller | Advisor: Dr. Ben Black

Poster 2B
From CoA to CoQ: Acetyl-CoA Sensing and the Mevalonate Pathway
Presenter: Joyce Liu | Advisor: Dr. Kathryn Wellen

Bioengineering

Poster 3B
High-throughput and ultra-sensitive extracellular vesicle isolation via electroformed inverse-opal nanomaterials
Presenter: Andrew Lin | Advisor: Dr. David Issadore

Poster 4B
Resting State fMRI of Bilateral Temporal Lobe Epilepsy
Presenter: Alfredo Lucas | Advisor: Dr. Kathryn Davis

Poster 5B
Translational ionizable lipid nanoparticle base editing platform for treatment of congenital brain disease
Presenter: Rohan Palanki | Advisor: Drs. Michael J. Mitchell and William H. Peranteau

Poster 6B
Defining Dose-Dependent Impacts of Exercise on Rat Achilles Tendon Fatigue Function
Presenter: Maggie Tamburro | Advisor: Dr. Louis J Soslowsky

Cell and Molecular Biology

Cancer Biology

Poster 7B
Glutamine metabolism regulates dendritic cell activity systemically and in the soft tissue sarcoma microenvironment
Presenter: Graham Lobel | Advisor: Drs. Malay Haldar and Celeste Simon

Poster 8B
Targeting YAP signaling to overcome MAPK/MEK inhibitor resistance in melanoma
Presenter: Raymond Ng | Advisor: Dr. Sydney Shaffer

Cell Biology, Physiology and Metabolism

Poster 9B
The Impact of Iron on Lysosomal Function in the Retinal Pigment Epithelium
Presenter: Kevin Zhang | Advisor: Dr. Joshua Dunaief

Gene Therapy and Vaccines

Poster 10B
Large-scale in vivo Comparison of Recombinant and Wild-Type AAV Integrations in Macaques and Humans
Presenter: Kelly Martins | Advisor: Dr. James Wilson

Genetics and Epigenetics

Poster 11B
PAQR8 Promotes Breast Cancer Recurrence and Confers Resistance to Multiple Therapies
Presenter: Saisai Chen | Advisor: Dr. Lewis Chodosh

Poster 12B
Increasing branched-chain amino acid metabolism reduces growth of clear cell renal cell carcinoma
Presenter: Nate Coffey | Advisor: Drs. Celeste Simon and Zoltan Arany

Poster 13B
Disrupted X chromosome inactivation (XCI) maintenance in B lymphocytes predisposes female mice to lupus-like disease
Presenter: Claudia Lovell | Advisor: Dr. Montserrat Anguera

Poster 14B
De Novo Mutations in Replication-Independent Histone Genes Elude Diagnosis by Exome/Genome Sequencing
Presenter: Emily Lubin | Advisor: Dr. Elizabeth Bhoj

Microbiology, Virology and Parasitology

Poster 15B
Infection of primary nasal epithelial cells differentiates SARS-CoV-2, MERS-CoV, and HCoV-NL63
Presenter: Clayton Otter | Advisor: Dr. Susan Weiss

Poster 16B
Host Regulation of Ebola Virus Egress and Spread: Role of Cytoskeletal Filamin Proteins
Presenter: Ariel Shepley-McTaggart | Advisor: Dr. Ronald N. Harty

Poster 17B
Non-pathogenic variation in mitochondrial DNA modulates murine SARS-CoV-2 pathogenesis
Presenter: Yentli Soto Albrecht | Advisor: Dr. Douglas C. Wallace

Genomics & Computational Biology

Poster 18B
Exploiting cell cycle dynamics to interrogate YY1's role in spatiotemporal chromatin organization
Presenter: Jessica Lam | Advisor: Dr. Gerd Blobel

Poster 19B
Interactions between aging, dietary restriction, and the gut microbiome
Presenter: Lev Litichevskiy | Advisor: Drs. Mingyao Li and Christoph Thaiss

Poster 20B
Progenitor Populations in Treatment Resistant T-Lineage ALL
Presenter: Jason Xu | Advisor: Dr. Kai Tan

Immunology

Poster 21B
Proteogenomic immune signatures delineate the landscape of pediatric acquired demyelinating syndromes
Presenter: Diego Espinoza | Advisor: Dr. Amit Bar-Or

Poster 22B
Do early life commensal microbes prevent type 1 diabetes?
Presenter: Jamal Green | Advisor: Dr. Michael Silverman

Poster 23B
Predictors of Nonseroconversion after SARS-CoV-2 Infection
Presenter: Ashwin Skelly | Advisor: Drs. Beatrice Hahn and Amelia Escolano

Poster 24B
Liver macrophage subsets differentially regulate metastasis in pancreatic cancer
Presenter: Stacy Thomas | Advisor: Dr. Gregory L. Beatty

Neuroscience

Poster 25B
Uncovering seizure etiology in a model of CDKL5 deficiency disorder with single-nucleus transcriptomic profiling
Presenter: Dayne Martinez | Advisor: Dr. Zhaolan (Joe) Zhou

Poster 26B
The Aged Microbiome Drives Cognitive Decline via Vagal Inhibition
Presenter: Tim Cox | Advisor: Drs. Virginia Lee and Christoph Thaiss

Poster 27B
Single nucleus transcriptome analysis of human reactive astrocytes
Presenter: David Dai | Advisor: Dr. Edward B. Lee

Poster 28B

Distinct pathogenic mutations in LRRK2 disrupt axonal transport of autophagosomes
Presenter: Dan Dou | Advisor: Dr. Erika Holzbaur

Poster 1B | Biochemistry and Molecular Biophysics

Plasma Focused Ion Beam (FIB) Milling to Reveal Biological Structures *in situ*

Kathryn Kixmoeller, Yi-Wei Chang, and Ben Black

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Cryo-electron microscopy (cryo-EM) makes it possible to study the structures of vitreous frozen biological structures. However, cryo-EM is limited by the depth to which electrons can penetrate, so only thin samples can be imaged using this technique. This limitation makes it difficult to study structures *in situ*, in their native context within cells, using standard cryo-EM techniques. Focused ion beam (FIB) milling uses a concentrated ion beam to ablate cellular material to isolate a thin lamella of material from within vitreous frozen cells. Thin lamellae can then be imaged by transmission electron microscopy (TEM) to study intracellular structures. FIB-milling of vitreous biological samples has traditionally been accomplished with a Gallium (Ga) ion beam. I have developed a workflow for using an alternate ion source, a plasma Xenon (Xe) beam. Xe plasma FIB (PFIB) offers several advantages over traditional Ga FIB including avoiding Ga deposition on milled samples and enabling faster milling for increased throughput in lamella generation.

Poster 2B | Biochemistry & Molecular Biophysics

From CoA to CoQ: Acetyl-CoA Sensing and the Mevalonate Pathway

Joyce Y. Liu, Steven Zhao, Sophie Trefely, Jimmy P. Xu, Clementina Mesaros, Nathaniel W. Snyder, Kathryn E. Wellen

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The ability to sense and adapt to nutrient availability is essential for metabolic homeostasis and often dysregulated in disease. Acetyl-CoA is a metabolite at the intersection of catabolic and anabolic pathways and thus, may be uniquely suited to report on cellular nutrient status. Substantial evidence suggests that acetyl-CoA availability is monitored and that compensatory responses are triggered when its production pathways are compromised. Specifically, our lab has previously shown that loss of ATP-citrate lyase (ACLY) leads to upregulation of acetyl-CoA synthetase 2 (ACSS2), allowing for maintenance of nuclear-cytosolic acetyl-CoA from acetate. However, the mechanisms mediating this compensatory upregulation are not known. Here, we investigate how cells detect the loss of ACLY-derived acetyl-CoA in order to upregulate ACSS2 and the functional consequences of this adaptive response. Using liquid chromatography mass spectrometry, we find that the mevalonate pathway is particularly sensitive to acetyl-CoA availability. The mevalonate pathway feeds into the synthesis of two branches of lipids - sterols and isoprenoids. Our work suggests that ACLY loss disrupts homeostasis of the sterol branch, thereby driving ACSS2 upregulation via sterol-responsive SREBP transcription factors. Using lipidomics and targeted metabolite addbacks, we further identify the isoprenoid-branch product ubiquinone (CoQ) as a limiting end fate of acetyl-CoA that is maintained by acetate in the absence of ACLY. Taken together, these results suggest a preliminary model in which the sterol branch of the mevalonate pathway acts as a gauge for ACLY-derived acetyl-CoA in order to maintain a critical isoprenoid metabolite, CoQ. Ongoing studies will investigate whether dysregulation of this sensing mechanism contributes to metabolic diseases such as non-alcoholic fatty liver disease.

Poster 3B | Bioengineering

High-throughput and ultra-sensitive extracellular vesicle isolation via electroformed inverse-opal nanomaterials

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Extracellular vesicles carry numerous protein and nucleic acid cargoes from their cells of origin which circulate peripherally in bodily fluids such as blood, saliva, and urine; as a result, clinicians and scientists have sought to leverage EV-derived biomarkers to diagnose, stage, and manage cancer. However, the nanoscale size (30-300 nm diameter) and high background in clinical samples (10^{12} EVs/mL in serum) of EVs makes the isolation of EV subpopulations especially challenging. In response to this challenge, we have developed a new approach to EV isolation which leverages billions of nanomagnetic sorters operating in parallel to perform precise and high-throughput EV sorting directly from clinical samples. The chips are fabricated via the electrodeposition of ferromagnetic materials onto a self-assembled microlattice; this yields $>10^9$ nanoscale magnetophoretic sorting devices in a postage-stamp-sized three-dimensional lattice. To demonstrate the viability of this platform, we isolated immunomagnetically-labeled EVs in a model system of pancreatic cancer and achieved a ~100% increase in yield and increase in purity compared to conventional methods. We demonstrate the ability to isolate EVs directly from plasma via multiple antibody panels including a tumor-specific panel. By expanding on the proof-of-concept device characterization and initial testing shown here, this new approach can enable low-cost, rapid, and precise sorting of EV subpopulations for clinical questions.

Poster 4B | Bioengineering

Resting State fMRI of Bilateral Temporal Lobe Epilepsy

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Background and Motivation: Temporal lobe epilepsy (TLE) is the most common type of focal epilepsy, characterized by seizures originating from the temporal lobe and adjacent structures. An increasingly identified subset of TLE patients show bilateral temporal lobe involvement during seizures. Bilateral TLE (BiTLE) remains understudied, likely due to its complex underlying pathophysiology and heterogeneous clinical presentation. Non-invasive biomarkers that distinguish BiTLE from unilateral TLE (UTLE) can aid in better management and clinical stratification of BiTLE during presurgical evaluation.

Methods: In this study, we measured whole brain functional networks of 19 BiTLE patients and compared them to those of 75 UTLE patients, using resting state functional MRI (rs-fMRI). We quantified whole brain topological properties in resting state brain networks using metrics derived from network theory, including clustering coefficient, global efficiency, participation coefficient, and modularity. For each network metric, we computed an average across all nodes (brain regions), and iterated this process across network densities ranging from 0.10-0.50. Curves of network density versus each network metric were compared between groups. Finally, by combining whole brain average clustering coefficient and global efficiency curves and applying dimensionality reduction through principal component analysis (PCA), we derived a combined metric which we term the "integration-segregation axis". In this axis, values greater than zero correspond to higher network segregation, whereas values less than zero indicate higher network integration.

Results: Compared to UTLE, BiTLE had decreased global efficiency and decreased whole brain average participation coefficient across a range of network densities ($p<0.05$, corrected). BiTLE was also found to have decreased clustering coefficient ($p<0.05$, corrected) in regions belonging to the default mode network, particularly in the posterior cingulate cortex (PCC). We also identified a larger number of communities in BiTLE than in UTLE after applying modularity maximization ($p<0.05$, corrected). Finally, we demonstrate that the differences in network properties separate BiTLE and UTLE along the integration-segregation axis. 68% (13/19) of the BiTLE patients were identified within the high segregation region, and only 41% (31/75) of the UTLE patients identified in the same region (Chi-square test, $p<0.05$)**.** Using the integration-segregation axis, UTLE patients with poor surgical outcomes were also found to be more similar to BiTLE than those with good surgical outcomes. 78% (7/9) of the poor surgical outcome patients were identified in the high segregation region, and only 35% (6/17) of the good surgical outcome patients identified in the same region (Chi-square test, $p<0.05$).

Conclusion: Our results indicate that increased interictal whole brain network segregation, as measured by rs-fMRI, is a useful biomarker of BiTLE, and may assist in non-invasively identifying this population of epilepsy patients prior to intracranial EEG implantation.

Poster 5B | Bioengineering

Translational ionizable lipid nanoparticle base editing platform for treatment of congenital brain disease

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Congenital neurologic diseases affect 1% of children at birth and account for 40% of pediatric hospitalizations worldwide. Editing the genome perinatally and correcting pathogenic mutations prior to disease onset represents a novel therapeutic strategy that could reduce neurodevelopmental symptoms associated with these disorders. A key challenge for translation of mRNA-based gene therapies is safe and effective intracellular delivery. While ionizable lipid nanoparticles (LNPs) are efficacious, biocompatible nucleic acid delivery vehicles, LNPs have not yet been engineered specifically for the delivery of nucleic acids to the perinatal brain environment. Here, we screened a diverse library of LNPs *in vivo* to target the fetal and neonatal mouse brain, optimized LNPs to deliver base editing platforms to cells of neural origin *in vitro*, engineered an LNP-base editing platform that corrected the biochemical phenotype of mucopolysaccharidosis type-1 (MPS-I) in the neonatal mouse brain, and exhibited proof-of-principle delivery and safety of LNP-mediated base editing in patient-derived brain tissues. In the future, the LNPs developed in this study can be broadly applied for perinatal mRNA therapeutics targeting the central nervous system.

Poster 6B | Bioengineering

Defining Dose-Dependent Impacts of Exercise on Rat Achilles Tendon Fatigue Function

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Clinical management of tendinopathies, which comprise >30% of musculoskeletal consultations, is challenging as contributions of exercise and ideal management protocols are not clear. Tendon is a highly mechanically responsive tissue, and exercise as a form of mechanical loading is known to modulate the tendon environment in both humans and animal models. Previous studies have utilized treadmill running of rats as a high throughput, robust model to assess effects of mechanical loading on Achilles tendons, demonstrating changes in gene expression with exercise intensity level. However, these models have not been used to elucidate the consequences of exercise-related loading responses on tendon function in fatigue loading, a highly sensitive measure of tendon function. Therefore, the objective of this study is to define specific, dose-dependent biomechanical changes in the exercised Achilles tendon. We hypothesize that increasing exercise intensity improves Achilles tendon fatigue function, corresponding with increased cycles to failure. To assess this hypothesis, sixty rats were divided into six groups (n = 10/group) corresponding to exercise chronicity (4 or 8 weeks) and activity level: low (cage activity), moderate (running 1 hour/day 5 days/week at 10 m/min, 0° incline), or high (running 1 hour/day 5 days/week at 20 m/min, 10° incline). Following sacrifice at either 4 or 8 weeks, tendons were harvested for mechanical assessment with a novel and sophisticated uniaxial tensile testing protocol consisting of preloading (0.3 N), preconditioning (10 cycles oscillating between 0.5 and 1.5% strain), viscoelastic stress relaxation testing (9% strain applied rapidly and maintained for 10 minutes) with subsequent frequency sweeps (0.1 to 10 Hz oscillations of 0.0125% strain), and fatigue testing (1 Hz oscillations between 5 and 30 N). Differences between groups at each time point will be assessed with one-way ANOVAs and post-hoc Tukey's tests (α = 0.05). To date, n=2/group tendons have been tested and testing will be completed in the next 30 days. This study will be the first to define the dose-dependent effects of exercise on Achilles tendon fatigue mechanical properties which will provide a crucial foundation for future investigations of the mechanistic impacts of exercise on tendon. Moreover, understanding dose-dependent, exercise-related tendon changes will begin to indicate the therapeutic potential of exercise for tendon health.

Poster 7B | CAMB - Cancer Biology

Glutamine metabolism regulates dendritic cell activity systemically and in the soft tissue sarcoma microenvironment

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Soft-tissue sarcomas (STS) are diverse malignancies with an overall 16% five-year survival rate for metastatic disease, reflecting the need for novel therapeutic strategies to treat advanced disease. Immune checkpoint blockade has shown promise against multiple cancers, but has shown only modest efficacy as a treatment for STS. Recent work has also shown that blocking glutamine metabolism modulates the anti-tumor activity of tumor-infiltrating immune cells, but this work has focused mainly on T cells. To investigate the effects of glutamine blockade on other intratumoral immune cell populations, we treated mice with a model of STS with a glutamine antagonist and found that the population of intratumoral dendritic cells (DCs) was significantly reduced. DCs are "professional" antigen-presenting cells that are specialized for the generation of potent anti-tumor responses. The majority of DCs present at homeostasis and in the tumor microenvironment are type 1 and type 2 "classical" DCs (cDC1s and cDC2s, respectively). cDC1s are especially crucial for immune surveillance against tumors due to their ability to cross-present antigens directly to CD8 T cells. We found that all DCs were depleted from the STS microenvironment after glutamine blockade, but cDC1s in particular were almost entirely absent. DCs and especially cDC1s were also depleted from the lungs and liver after glutamine blockade. Additionally, treatment with glutamine blockade before transplantation with an immunogenic fibrosarcoma cell line limited the ability of mice to reject that line. In an *in vitro* model of cDC1 and 2 differentiation, glutamine deprivation or blockade limited the generation of all cDCs, but cDC1s were particularly impacted. cDC1 differentiation is known to be regulated by mTORC1 signaling, which is promoted by glutamine availability. In these cultures, we found that glutamine deprivation or blockade inhibited mTORC1 to a similar extent and had similar effects on cDC1 and 2 population as rapamycin. Collectively, these findings suggest that glutamine availability regulates the population and trafficking of mature systemic and tumor-associated cDCs, and especially cDC1s, via an mTORC1-dependent mechanism.

Poster 8B | CAMB - Cancer Biology

Targeting YAP signaling to overcome MAPK/MEK inhibitor resistance in melanoma

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Malignant melanoma is a common and lethal form of skin cancer. Around 50% of melanomas are driven by a *BRAF* mutation, which hyperactivates the MAPK/MEK signaling pathway. Patients with this aberration are often treated with MAPK/MEK inhibitors such as "CombiDT", which is a combination of dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor). However, over 50% of patients treated with CombiDT develop drug resistance and progress within 9-10 months. There is thus a pressing need to determine ways to overcome resistance to CombiDT in melanoma. Our laboratory has pioneered the identification of transcriptional pathways that confer CombiDT resistance in melanoma. We have found that melanoma cells that persist in CombiDT arise from a rare subpopulation of cells that expresses unique transcriptional programs prior to drug treatment; we term these as "primed cells". Our data also indicate that the primed cell state is not stable. Rather, the transcriptional state of tumor cells undergoes back-and-forth switching between a primed state and a drug-susceptible state. But under CombiDT treatment, primed cells undergo epigenetic changes that turn the transient primed state into a stable drug-resistant state. The transient nature of the primed state is significant because it is reversible. Thus, targeting primed cells introduces a novel method to combat drug resistance: we could prevent resistance from emerging by eliminating primed cells or by pushing primed cells towards a drug-susceptible state. We propose that the key to tackling drug resistance is to understand the signaling pathways that drive the primed cell state and that can turn primed cells into resistant cells. We find that YAP, a transcriptional coactivator, plays a key role in primed cell state. Primed cells and drug-resistant cells both upregulate YAP transcriptional targets that are implicated in drug resistance and cancer progression. YAP is regulated by several signaling pathways – canonically by the Hippo pathway. Previously, we have shown that knocking out *LATS2*, a member of the Hippo pathway, increases the abundance of primed cells in a cell population; this knockout also increases the ability of a tumor to become resistant to BRAF inhibition *in vitro* and *in vivo*. This data leads us to hypothesize that YAP signaling drives the expression of the primed cell transcriptional state and the emergence of drug resistance to MAPK/MEK inhibition (MAPK/MEKi) in melanoma. We propose two aims to test this hypothesis. Aim 1, we will identify the regulators of YAP that drive the primed cell state. In Aim 2, we will determine whether pharmacological YAP inhibition can synergize with CombiDT. Through these aims, we will explore the role of the YAP pathway in drug resistance to MAPK/MEKi, with the goal of identifying new therapeutic targets in melanoma. Overall, these studies will help pave the path to dramatically improve the prognosis for cancer patients.

Poster 9B | CAMB - Cell Biology, Physiology and Metabolism

The Impact of Iron on Lysosomal Function in the Retinal Pigment Epithelium

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Age-related macular degeneration (AMD) is the most common cause of blindness in individuals older than 50 years, but its pathogenesis is poorly defined. Patients with AMD have been shown to accumulate retinal iron, but the link between iron and AMD is unknown. By catalyzing free radical formation via the Fenton reaction, excess iron could cause organelle dysfunction.

To investigate the role iron may have on lysosomal function, we studied a hepcidin-knockout ($Hepc^{KO}$) mouse model that accumulates retinal iron. The RPE of $hepc^{KO}$ mice is hypertrophic and autofluorescent. Electron microscopy (EM) with a lipid-sparing technique demonstrated that $hepc^{KO}$ RPE was filled with numerous electron dense vesicles. These vesicles were revealed to be lysosomes based on immunohistochemistry (IHC) for the lysosomal markers Lamp1 and cathepsin D. Numerous other lysosomal proteins were found to be elevated in hepcKO RPE by proteomics, and Western blot analysis revealed an accumulation of incompletely processed cathepsin D. Additional IHC studies showed that RPE lysosomes accumulate neutral lipids and the lipid peroxidation products CEP and MDA, with mass spectrometry further finding an accumulation of ceramides. These studies on $hepc^{KO}$ RPE demonstrate that iron induces lysosomal accumulation and dysfunction, along with intralysosomal accumulation of lipids and lipid peroxidation products.

We also studied an induced pluripotent stem cell derived-RPE (iPS-RPE) model to determine whether iron induces a cell autonomous phenotype. Iron-loaded iPS-RPE accumulated incompletely processed lysosomal cathepsins D and B, indicating both lysosomal accumulation and dysfunction. Enzyme activity assays showed a decrease in lysosomal acid lipase activity. Iron increased the number of lysosomes per cell but reduced proteolytic activity, suggesting accumulation of dysfunctional lysosomes in the setting of unhampered lysosomal biogenesis.

Together, our studies show that iron induces lysosomal accumulation and impairs lysosomal function. This phenotype is likely due to iron-mediated formation of lipid peroxidation products, which have been shown to bind to the active site of lysosomal enzymes. As a result, lysosomes become impaired yet cannot be degraded, leading to their accumulation. Iron may similarly contribute to AMD pathogenesis by causing lysosomal dysfunction with consequent intralysosomal lipid accumulation.

Poster 10B | CAMB - Gene Therapy and Vaccines

Large-scale *in vivo* Comparison of Recombinant and Wild-Type AAV Integrations in Macaques and Humans

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Naturally occurring adeno-associated virus (AAV) (wild type/wtAAV) is an endemic, non-pathogenic virus in the human population that naturally integrates into the host genome. Recombinant AAV (rAAV) vectors are produced by removing the wtAAV genome and replacing it with a therapeutic transgene of interest. Unexpectedly, rAAV can still integrate into the host genome and concerns for insertional mutagenesis and clonal expansion have been raised in mouse and dog studies. It remains unknown whether rAAV integration increases the risk for insertional mutagenesis in primates or if there is any increased risk compared with natural wtAAV integration. The goal of this study was to characterize wtAAV and rAAV integrations in the macaque and human genomes following in vivo exposures. Using a modified NGS-based unbiased technique, we identified the location, number, and distribution of genome-wide AAV integration loci from 126 non-human primates (NHPs) and 70 humans naïve to rAAV exposure, and 64 NHPs treated with rAAVs as part of gene therapy studies. Our results suggest that rAAV and wtAAV integrations exhibit similar integration distribution patterns within the primate genome. Furthermore, analysis of liver samples indicated that AAV integration (rAAV or wtAAV) occurred at a significantly higher frequency in genomic regions vulnerable to DNA damage. These results support the notion that exposure to wtAAV or rAAV carries a similar chance for integration at a given location in the primate genome, but that treatment with rAAV results in a higher number of integration loci. This detailed characterization of AAV integration in primates has important translational implications for the safety of rAAV as a gene therapy vector and highlights the clinical translatability of NHP AAV integration data.

Poster 11B | CAMB - Genetics and Epigenetics

PAQR8 Promotes Breast Cancer Recurrence and Confers Resistance to Multiple Therapies

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Breast cancer mortality is mainly due to recurrent disease that is resistant to therapy. A recent analysis of primary and recurrent breast cancers from patients identified copy number (CN) gain of the putative membrane progesterone receptor *PAQR8* as one of four focal CN alterations enriched in recurrent tumors compared to primary tumors. PAQR8 has not previously been shown to play a functional role in cancer. Interestingly, CN gain of *PAQR8* was found in 56% of patients treated with endocrine therapy, and it was mutually exclusive with activating *ESR1* mutations in those patients. Strikingly, however, *PAQR8* gain was also found to be equally common in patients not treated with endocrine therapy but instead with other targeted therapies or chemotherapy. Not only was *PAQR8* CN gain enriched in recurrences but it has subsequently been found to be associated with earlier recurrences and poor survival in patients.

Experimentally, we found that PAQR8 is both necessary and sufficient for breast cancer recurrence, and that PAQR8 promotes resistance to multiple therapies, including anti-estrogen therapy, anti-Her2 therapy, and chemotherapy. We found that PAQR8 enhances tumor cell survival following estrogen receptor pathway blockade by fulvestrant or estrogen deprivation, Her2 pathway blockade by lapatinib or Her2 downregulation, and treatment with chemotherapies such as doxorubicin and docetaxel. The pro-survival effects of PAQR8 did not require progesterone and were mediated by a G_i protein-dependent reduction in cAMP levels. Moreover, PAQR8 also decreased ceramide levels and increased sphingosine-1-phosphate levels, effects that favor cell survival and are consistent with PAQR8 functioning as an alkaline ceramidase. Altogether, our data provide the first functional evidence that PAQR8 plays a role in cancer progression and identify a novel mechanism of resistance to multiple therapies that result in cancer recurrence.

Poster 12B | CAMB - Genetics and Epigenetics

Increasing branched-chain amino acid metabolism reduces growth of clear cell renal cell carcinoma

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North America has the highest incidence of renal cancer in the world with clear cell renal cell carcinoma (ccRCC) being the most common subtype accounting for greater than 75% of cases. Metastatic ccRCC has a five-year survival rate of only ~10% and is in dire need of new therapies. One such approach is to target dysregulated metabolic pathways in this setting. Metabolic dysfunction is a hallmark of ccRCC as demonstrated by its clear cell histologic appearance due to significant cytoplasmic accumulation of lipid droplets and glycogen. Using publicly available ccRCC RNA-seq and proteomics datasets, I found reduced expression of all subunits of BCKDH, the rate-limiting enzyme in branched-chain amino acid (BCAA) metabolism, in ccRCC tumors compared to normal adjacent kidney tissue (NAT). BCKDH is a heteromeric protein complex consisting of multiple subunits called DBT, BCKDHA/B, and DLD and regulates the catabolism of all three BCAA metabolites (leucine, isoleucine, and valine). I then validated that BCKDH expression was reduced in our own cohort of ccRCC tumors and cell lines, and performed immunohistochemistry on ccRCC tumor microarrays to demonstrate that downregulation of BCKDH occurs as early as stage one and is associated with reduced overall survival in patients. Furthermore, I found that reduced expression of BCKDH was reinforced at the genome level by copy number loss of BCKDH subunits *DBT* and *BCKDHB*, underscoring the selection pressure for suppression of this pathway. Lastly, metabolomics performed with LC-MS revealed decreased abundance of BCAAs and their catabolic metabolites in ccRCC tumors. These multiple observations have led me to the hypothesis that reduced BCAA catabolism promotes ccRCC tumorigenesis. I then wanted to determine if increasing BCAA metabolism would reduce ccRCC tumor growth. I first demonstrated that ccRCC cell lines have reduced BCAA catabolism. To enhance BCAA catabolism, short hairpin RNAs were used to knockdown BCKDK, a kinase that inhibits BCAA metabolism by phosphorylating BCKDH. Excitingly, increasing BCAA metabolism genetically with short hairpin RNAs targeting BCKDK reduced the proliferation of multiple ccRCC cell lines *in vitro* by inducing apoptosis but did not kill renal epithelial cells. This suggests that targeting BCAA metabolism could be a novel way to selectively kill ccRCC. My next steps will be to validate these results in mouse models of ccRCC and determine the mechanism of how increased BCAA catabolism reduces ccRCC tumor growth.

Poster 13B | CAMB - Genetics and Epigenetics

Disrupted X chromosome inactivation (XCI) maintenance in B lymphocytes predisposes female mice to lupus-like disease

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90% of individuals with systemic lupus erythematosus (SLE) are women, but the mechanisms that govern sex bias in SLE are not understood. Susceptibility to SLE increases with the number of X chromosomes carried by an individual. This suggests that having more than one X chromosome, which contains immune-regulatory genes, may contribute to sex-biased risk for developing SLE. The dosage of X-linked gene expression in mammals with more than one X chromosome is balanced by X-Chromosome Inactivation (XCI). XCI is maintained in somatic cells by association of the long non-coding RNA Xist with the inactive X chromosome (Xi), along with repressive histone marks. Our lab discovered that naïve B cells contain no visible Xist RNA in the nucleus. Upon B cell stimulation, Xist RNA and heterochromatic modifications re-localize to the Xi. Dynamic XCI maintenance is disrupted in B cells from SLE patients, with incomplete re-localization of silencing marks to the Xi. Furthermore, B cells from SLE patients exhibit dysregulation of X-linked immune genes, likely due to incomplete XCI maintenance. To determine whether impaired localization of Xist RNA to the Xi impacts B cell biology and increases predisposition to autoimmune disease, we leverage a B-cell specific conditional knockout of *Xist*. Female *mb1-cre +/- ; Xist fl/fl* ("Xist cKO") mice are viable, have no survival deficits, and exhibit minimal changes in B cell subsets compared to age-matched wild type mice. Strikingly, in a chemically induced model of SLE, female Xist cKO mice develop and maintain higher levels of autoantibodies than wildtype counterparts. Female Xist cKO mice with chemically induced SLE also have a higher percentage of class switched B cells and germinal center B cells compared to wildtype mice. These findings suggest that B-cell specific perturbed XCI maintenance contributes to sex biased development of SLE, and further investigation into this model will demonstrate how contributions from the X chromosome are impact B cell biology and will reveal an X-chromosome based mechanism that contributes to female-biased SLE.

Poster 14B | CAMB - Genetics and Epigenetics

De Novo Mutations in Replication-Independent Histone Genes Elude Diagnosis by Exome/Genome Sequencing

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Mutated replication-independent (RI) histone genes are an emerging cause of rare pediatric Mendelian syndromes. Recurrent "hot spot" *somatic* mutations in members of the histone H3.3 family were first identified in large cohorts of patients with pediatric gliomas. *Germline* mutations in multiple histone genes encoding distinct proteins have subsequently been found to cause neurodevelopmental and neurodegenerative disorders that may act through a targetable dominant-negative mechanism.

These RI histone mutations eluded detection by exome and genome sequencing methods, despite patients' profound phenotypes, because of the high sequence homology between histone genes. Thus, we employed an innovative approach using population-level data, specifically gnomAD constraint metrics and GTEx expression data, to predict that five previously unreported histone-encoding genes (*H2AFV, H2AFY, H2AFY2, H2AFZ* and *H1F0)* are most intolerant to mutations, rendering them putative disease candidate genes. Working with our international network of collaborators and GeneMatcher, we have subsequently validated our approach by identifying cohorts of patients with mutations in three of those five disease candidate genes (*H2AFY*, *H2AFY2*, *H2AFV*). These patients share overlapping neurodevelopmental phenotypic traits, including intellectual disability and developmental delay.

To delineate the pathogenesis of these mutations in a neurodevelopmental context, we are developing a pipeline in which we generate isogenic human induced pluripotent stem cell lines harboring the patients' mutations and then quantify the resulting epigenetic dysregulation. Combining our innovative candidate gene prediction project with our patient-driven approach to interrogating disease-relevant mutations allows us to both overcome the limitations of traditional sequencing methods and build evidence for a potential unifying model by which *de novo* heterozygous missense mutations in RI histone genes act through a shared, therapeutically-targetable mechanism that converges on conserved cellular pathways.

Poster 15B | CAMB - Microbiology, Virology and Parasitology

Infection of primary nasal epithelial cells differentiates SARS-CoV-2, MERS-CoV, and HCoV-NL63

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SARS-CoV-2 (SARS-2) and MERS-CoV (MERS) are pathogenic coronaviruses (CoVs) that have caused public health emergencies in the past 20 years, and HCoV-NL63 (NL63) is another CoV that causes a common cold in humans. Few studies have characterized these viruses in the nasal epithelium, the primary barrier to infection by all respiratory pathogens. Studying viral replication, host cell tropism, and cytotoxicity in the nasal airway is essential to understanding pathogenesis and transmission of these viruses. We utilize a primary epithelial culture system in which patient-derived sinonasal samples are differentiated at an air-liquid interface (ALI). This nasal ALI system provides an optimal context in which to compare CoVs in the upper airway, as it recapitulates many features of the *in vivo* airway epithelium, including cell types represented, ciliary function, and mucus production. SARS-2, MERS, and NL63 all replicate productively in these primary nasal cultures; however, SARS-2 replication surpasses that of MERS and NL63 by ~2 $\log_{10}$ PFU/mL. We compare viral replication at 33ºC vs 37ºC and note that SARS-2 and NL63 both replicate more efficiently at 33ºC, closer to the temperature in the nasal airway, whereas MERS replication is not dependent on temperature. In accordance with the receptor for each virus, SARS-2 and NL63 infect primarily ciliated cells (which express receptor ACE2), while MERS infects mucus-producing goblet cells (which express receptor DPP4). To characterize virus-induced cytotoxicity in the nasal epithelium, we measure trans-epithelial electrical resistance (TEER) to quantify epithelial barrier integrity as well as apical lactate dehydrogenase (LDH) release as a readout for cell death. While SARS-2 and MERS infection do not result in a significant defect in TEER, NL63 infection results in significant loss of epithelial barrier integrity at late time points. LDH assays show that SARS-2 and NL63 cause robust cytotoxicity in nasal ALI cultures, while MERS-infected cultures shows minimal LDH release even at late time points. Treatment of nasal ALI cultures with IL-13 to induce goblet cell hyperplasia (a prominent feature in asthmatic or inflamed airways) further highlights differences between SARS-2, MERS, and NL63. IL-13 treatment causes robust changes in receptor availability (ACE2 expression decreases while DPP4 expression drastically increases). In agreement with these effects of IL-13 on receptors, viral replication in IL-13-treated cultures increases for MERS, decreases for SARS-2, and is almost completely ablated for NL63 compared to sham-treated cultures. Work is ongoing to determine how SARS-2, MERS, and NL63 differentially induce cytokines and innate immune pathways in the nasal epithelium. Further understanding of host-virus interactions in this immune sentinel site have the potential to inform nasal-targeted therapies for pathogenic CoVs and respiratory viruses that emerge in the future.

Poster 16B | CAMB - Microbiology, Virology and Parasitology

Host Regulation of Ebola Virus Egress and Spread: Role of Cytoskeletal Filamin Proteins

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Ebola virus (EBOV) causes severe hemorrhagic fever associated with high mortality rates in humans. EBOV and other filoviruses are emerging BSL-4 pathogens for which the development of novel and effective therapeutics is urgently needed. Egress and spread of EBOV is mediated by the VP40 matrix protein (eVP40), whereby expression of eVP40 alone directs the production and egress of virus-like particles (VLPs) that accurately mimics the budding process of live infectious virus. We have shown that eVP40 hijacks or recruits select host proteins to facilitate the budding process. On the other hand, we have also identified several host proteins that interact with eVP40 to negatively regulated eVP40-mediated budding. Thus, a better understanding of the eVP40-host interactome will provide important insights into the mechanisms of virus egress and spread, as well as inform about potential targets for the development of novel antiviral therapeutics. Here, we investigated the possible role for host filamin A and B proteins (actin crosslinking proteins that function in cell morphology and migration at the plasma membrane) in regulating both entry and egress of EBOV. Toward this end, we employed filamin A (FLNaKD) and filamin B knockdown cells (FLNbKD) to assess EBOV entry by using live EBOV, as well as recombinant viruses and pseudotypes expressing the EBOV surface glycoprotein (GP). We also used the KD cells to assess virus egress by using our well-established eVP40 VLP budding assay. In sum, our results indicate that expression of host filamin proteins appears to be important for EBOV to efficiently complete different stages of its lifecycle.

Poster 17B | CAMB - Microbiology, Virology and Parasitology

Non-pathogenic variation in mitochondrial DNA modulates murine SARS-CoV-2 pathogenesis

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Host factors that influence outcomes of SARS-CoV-2 infection are of increasing interest, as this pathogen has caused over 500 million cases and 6 million deaths from COVID-19. ***The contribution of host mitochondrial genetics to the severity of SARS-CoV-2 infection remains under studied.*** Mitochondrial variants (haplogroups) are ubiquitous in the population and represent differences in mitochondrial DNA (mtDNA). To model human haplogroup variation in mice, our lab generated strains containing murine mitochondrial haplogroups 129 or NZB mtDNA on the C57BL/6J (B6) nuclear background. The total mtDNA variance between groups is less than 100 base-pairs, mirroring the range in human haplogroups. To determine the effect of mtDNA variation on SARS-CoV-2 outcome, we used a murine model expressing the human ACE2 viral receptor under the keratin 18 promoter (k18-hACE2) and harboring B6 (wild-type), 129 or NZB mtDNA. Consistent with the literature, we found that k18-hACE2 mice with wild-type mtDNA exhibited 100% fatality by 14 days post infection (DPI) when challenged intranasally with SARS-CoV-2. Remarkably, mice with 129 or NZB mtDNA both showed increased survival (40%). Furthermore, mice with NZB mtDNA showed decreased clinical disease at 6 DPI compared to mice with 129 ($p = 0.0444$) and B6 ($p = 0.0027$) mtDNA, paired with increased inflammatory lung infiltrates and a trending increase in lung cytokines at 7 DPI. Remarkably, no difference in lung viral replication was observed across the three genotypes at any timepoint examined. Similarly improved survival of the mtDNA NZB mice was obtained using Stanley Perlman's mouse-adapted SARS-CoV-2 virus. Cumulatively, these data demonstrate that mitochondrial haplotype alone can influence clinical disease in two murine models of severe SARS-CoV-2 infection and a survival advantage is linked to a pro-inflammatory immunophenotype. Mechanistic studies on immune functional differences in these models are ongoing. Our discovery of the novel role mitochondrial haplotype plays in SARS-CoV-2 pathogenesis may contribute to increased understanding of host factors that impact the severity of COVID-19.

Poster 18B | Genomics and Computational Biology

Exploiting cell cycle dynamics to interrogate YY1's role in spatiotemporal chromatin organization

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Mitosis is marked by a global cessation of transcription, eviction of transcription factors, and the dissolution of most chromatin structure. During the mitosis-to-G1 phase transition, newly born cells must therefore address the challenge of rapidly re-establishing 3D genome organization that faithfully reflects that of the mother cell. However, much remains unknown about how cells transition from a relatively disorganized chromatin state to a cell type-specific conformation. While CTCF and cohesin-mediated loop extrusion has been shown to forge some chromatin loops, many observed architectural features cannot be explained by this mechanism. Here we examine the architectural functions of the transcription factor YY1, which has been implicated in enhancer-promoter loops in studies in interphase cells.

Using an erythroblast cell line we profiled YY1 chromatin occupancy and discovered minimal overlap with the cohesin subunit RAD21, arguing against YY1 serving as a loop extrusion blocker. To dissect YY1's role in chromatin architecture, we exploited its occupancy dynamics during the mitosis-to-G1 phase progression. We observed that YY1 was retained at a subset of binding sites during pro-metaphase, an interval during which most transcription factors are evicted from chromatin. These sites as well as those that display rapid post-mitotic chromatin occupancy showed a strong preference towards active promoters. In contrast, sites at which YY1 chromatin occupancy occurred later in G1 phase did not show promoter versus enhancer bias. These binding characteristics were partially informed by the underlying YY1 motif.

Prometaphase-specific degradation of YY1 enabled examining its role during establishment vs maintenance of YY1-regulated chromatin loops. YY1 depletion during the mitosis-to-G1 phase transition surprisingly affected not only the formation of YY1-anchored loops, but also structural loops anchored by both CTCF and cohesin. We will present and discuss results that inform YY1's influence on these and additional architectural features in relation to postmitotic transcription reactivation.

Poster 19B | Interactions between aging, dietary restriction, and the gut microbiome

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One of the most promising interventions for extending lifespan and improving health is dietary restriction (DR), such as fasting and caloric restriction. In many different animals, DR extends lifespan and healthspan, but how DR does this remains incompletely understood. One intriguing hypothesis is that the gut microbiome plays a role. For example, transferring the microbiome from an organism on DR to a microbiome-depleted recipient improves health in the recipient. In order to unravel the interactions between aging, DR, and the gut microbiome, a large, longitudinal experiment was initiated in mice. In this experiment, 960 mice were randomized at 6 months of age to one of five dietary groups: control (*ad libitum* diet), two caloric restriction groups (20% or 40% fewer calories), and two fasting groups (one-day fast or two-day fast). Every six months until death, the gut microbiome was profiled by metagenomic sequencing of stool samples, and extensive aging-associated phenotypes were collected. We found that all dietary interventions significantly increase lifespan, with the 40% caloric restriction group increasing lifespan the most. We were able to use the microbiome to predict the age of a mouse with mean absolute error of 15.6 weeks. Furthermore, we discovered that microbiome "uniqueness" increases with age. Finally, we found through microbiome-phenotype association analysis that body weight remains significantly associated with the microbiome after controlling for age and diet, suggesting that the microbiome influences body composition.

Poster 20B | Genomics and Computational Biology

Progenitor Populations in Treatment Resistant T-Lineage ALL

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T-cell acute lymphoblastic leukemia (T-ALL) is a lymphoid malignancy that accounts for 10-15% of pediatric and ~25% of adult acute lymphoblastic leukemia cases. In comparison to its B-lineage counterpart, T-ALL has historically been considered an aggressive subtype of ALL, with few options for relapsed/refractory disease, lack of genetic and genomic markers for upfront risk stratification, and no unifying framework for targeted therapy. Initial genomic profiling of a medium sized T-ALL cohort (n=264 patients) revealed genomic drivers in the disease, but was not powered to associate specific drivers with response to therapy.

To address this problem, comprehensive, large-cohort (n=1300+ patients) bulk-level genomic profiling of T-ALL is currently underway with the promise to reveal novel drivers and associate genetics with response. Yet, these studies lack the resolution to make observations of intra-tumoral biology, which may be paramount to understand the causes of resistant disease. To synergize with bulk-level genomic studies, we designed a single-cell multi-omic study powered to comprehensively characterize intra-tumoral arrest patterns in T-ALL. Using single cell genomic analysis on 40 T-ALL cases alongside a novel, single cell atlas of pediatric-T-cell development, we identify and characterize a bone-marrow progenitor-like (BMP-like) tumor sub-population associated with resistance to conventional therapy across distinct immunophenotypic subtypes of T-ALL. Molecular signatures of tumor sub-populations obtained from single cell data were used to stratify outcome in larger T-ALL cohorts and to perform computational drug target screening, nominating a number of novel surface markers and intracellular targets. When joined with bulk-level genomic phenotypes, we discovered a distinct set of driving mutations associated with high-risk and low-risk phenotypes, with an enrichment of AML-like signaling and TF mutations associated with the high-risk progenitor like state.

Our work comprehensively defines the arrest state of T-ALL in reference to human early T-cell development. By doing so, we reveal a significant degree of sub-population level overlap between patients with distinct subtypes of T-ALL that correlates with treatment response. Together with large-cohort bulk genomic studies, our study provides a framework and patient-specific model systems for targeted therapeutics and upfront-risk-stratification to be realized in treatment-refractory T-ALL.

Poster 21B | Immunology

Proteogenomic immune signatures delineate the landscape of pediatric acquired demyelinating syndromes

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Introduction: Approximately 20-30% of children presenting with acquired inflammatory demyelinating syndromes (ADS) have multiple sclerosis (MS). Another 30% harbor serum antibodies against myelin oligodendrocyte glycoprotein and are referred to as having MOG-associated disease (MOGAD). While MS and MOGAD can have similar features, differences in response to immune therapies point to distinct underlying immune mechanisms.

Objectives, aims and methods: To assess potentially distinct immune mechanisms underlying MS and MOGAD, we applied proteogenomics to high quality cryopreserved peripheral blood mononuclear cells collected from patients with ADS prior to institution of immune therapy, as well as from healthy controls. CITE-Seq profiling recovered a total of 92,716 single cells with equal contribution from 24 children (6 healthy donors; 6 with ADS but neither MS or MOGAD; 6 with MOGAD; and 6 with MS, ascertained with long-term follow-up).

Results: Cluster-based and cluster-free comparative analyses revealed features within the T-cell compartment that differed between children with MS and MOGAD. Specifically, the CD4 T-cell compartment in children with MS (when compared to MOGAD) was enriched for a memory population with a Th1-like (*STAT4+* and CXCR3+/CCR6-) phenotype and enriched for a Th17-like (*RORA+/KLRB1+*) memory population expressing surface components for VLA-4. Within the CD8 compartment, CD8 effector memory T cells in children with MS (when compared to MOGAD) were enriched for the checkpoint-molecule TIGIT and the activation marker *CD137 (4-1BB)*. Furthermore, CD8 effector memory T cells in children with MS preferentially expressed *GZMM*, while CD8 effector memory T cells in children with MOGAD preferentially expressed *GZMB* and *GZMH*.

Conclusions: Overall, our study points to distinct features of circulating cellular immune profiles in children with MS and MOGAD and provides novel insights into early immune mechanisms that may be involved in each of these conditions.

Poster 22B | Immunology

Do early life commensal microbes prevent type 1 diabetes?

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Early-life microbes are critical for the development of the immune system and disruption of the microbiome in early life can lead to autoimmunity, yet the specific mechanisms that support healthy immune system development remain largely unknown. To rigorously study the role of early-life microbiota in autoimmune diabetes (T1D), we developed a novel consortium of 9 culturable bacteria that dominate the early life microbiome (PedsCom) and colonized germ-free, non-obese diabetic (NOD) mice. The PedsCom consortia represent over 90% of the bacteria in pre-weaning NOD mice. We find that early life colonization with the PedsCom consortia protects NOD mice from developing diabetes compared to germ-free mice. PedsCom colonization of NOD mice increases peripheral regulatory T cells in the intestinal and lymphatic tissue suggesting a potential mechanism by which early-life commensal microbes may prevent system autoimmunity. Additionally, we demonstrated that specific early life microbes drive distinct adaptive mucosal and systemic immune responses. We aim to leverage PedsCom NOD mice to identify specific mechanism by which commensal bacteria educate the developing immune system and inform preventive therapies for T1D.

Poster 23B | Immunology

Predictors of Nonseroconversion after SARS-CoV-2 Infection

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Coronavirus disease (COVID-19) is typically diagnosed by reverse transcription PCR (RT-PCR) amplification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA from nasopharyngeal fluids. RT-PCR yields cycle threshold (Ct) values that are inversely correlated with viral loads and thus provide an estimate of the number of SARS-CoV-2 RNA copies in the sample. Serologic assays complement COVID-19 diagnosis by documenting past infections. In most persons, binding and neutralizing antibodies develop within 1-3 weeks after onset of symptoms, and titers correlate with disease severity. Initial serosurveys identified antibodies in nearly 100% of persons with RT-PCR–confirmed SARS- CoV-2 infection. However, more recent studies have shown that seroconversion rates are surprisingly variable. For example, a multicenter study from Israel reported that 5% of participants remained seronegative despite a positive test result on a nasal swab specimen. In contrast, a seroprevalence study from New York found that 20% of persons with a positive RT-PCR test result did not seroconvert. Another study from Germany reported that 85% of confirmed infected COVID-19 contacts failed to develop antibodies. To examine the reasons for these differences, we investigated the relationship between seroconversion and demographic, clinical, and laboratory data in a convenience sample of convalescent persons recruited at the University of Alabama at Birmingham (Birmingham, Alabama, USA). Additionally, we sorted RBD^+ memory B cells from one patient of interest and performed BCR sequencing. We then produced and characterized several monoclonal antibodies and assayed their neutralization capabilities against a panel of SARS2 variants of concern.

Poster 24B | Immunology

Liver macrophage subsets differentially regulate metastasis in pancreatic cancer

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Metastasis is the main cause of morbidity and mortality in patients with cancer. In pancreatic ductal adenocarcinoma (PDA), the liver is the most common site of metastasis. During PDA progression, the liver microenvironment becomes altered to support metastatic seeding and colonization by disseminated tumor cells (DTC). Macrophages (MΦ), including liver-resident Clec4f+ Kupffer cells (KCs) and bone marrow derived MΦ (BMDMs), are the dominant immunological components of this niche, yet their precise roles in regulating metastasis remain poorly defined. Here, we sought to understand the interaction of DTCs with liver MΦ as they seed and colonize the liver. To model liver metastasis, mice received intraportal (iPo) injection of murine PDA cells with analysis 2 or 14 days later to examine metastatic seeding and outgrowth, respectively. Liver metastatic burden and immune cell populations were quantified by flow cytometry and immunohistochemistry. Liver MΦ were depleted using clodronate encapsulated liposomes (CEL) and KCs were depleted using Clec4fDTR mice administered diphtheria toxin. DTCs were found to interact with BMDMs more often than KCs during the seeding phase of metastasis. During colonization, KCs became excluded from metastatic colonies whereas BMDMs actively infiltrated. Although depletion of liver MΦ and KCs specifically did not affect metastatic seeding, MΦ depletion during the colonization phase inhibited metastatic colony outgrowth. Interestingly, BMDM infiltration into lesions was largely unaffected by CEL treatment, whereas KCs were uniformly depleted. This finding suggests that MΦ residing outside of metastatic lesions may be critical to supporting metastatic outgrowth.

Poster 25B | Neuroscience

Uncovering seizure etiology in a model of CDKL5 deficiency disorder with single-nucleus transcriptomic profiling

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CDKL5 deficiency disorder (CDD) is one of the most common forms of genetic epilepsy and is a debilitating childhood disorder characterized by infantile-onset seizures, global neurodevelopmental delay, motor and intellectual impairments, and autistic features. CDD is caused by loss-of-function mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene, which encodes a largely uncharacterized serine-threonine kinase that is highly expressed in neurons of the brain. Currently, there are no effective treatments for CDD.

Recent studies in the Zhou lab reported that heterozygous loss of CDKL5, but not hemizygous or homozygous loss, leads to epileptic spasms and handling-associated behavioral seizures in mouse models of CDD, which suggests that mosaic expression of CDKL5 could contribute to seizure development. The brain is composed by a multitude of cell types, and the effects of losing CDKL5 function are likely cell-type-specific, with the dysregulation of certain types being more or less consequential. This diversity, along with the finding that X-linked mosaic expression of CDKL5 appears to contribute to seizure development, necessitates molecular profiling at the single-cell level. Thus, a part of my thesis project is to perform single-nucleus RNA-seq of mouse cortical tissue using an F1 hybrid cross strategy that will allow me to identify wild type (WT) CDKL5-expressing or mutant CDKL5-expressing cells from heterozygous females using allele-specific single-nucleotide polymorphisms (SNPs). The goal of my transcriptomic profiling is to identify pathogenic signaling pathways and vulnerable cell populations that might underly seizure development in mouse models of CDD.

Poster 26B | Neuroscience

The Aged Microbiome Drives Cognitive Decline via Vagal Inhibition

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Aging is a complex process in which the body loses fitness and resiliency. Alzheimer's disease and age-associated cognitive decline are devastating diseases experienced by tens of millions of people worldwide. The collection of microorganisms living in or on the body, known as the microbiome, also changes with age, becoming less diverse and accumulating pathogenic species over time. The mammalian microbiome resides primarily in the gut, with the well-established gut-brain axis mediating bidirectional communication between the enteric and central nervous systems. While it is known that the gut microbiome can modulate brain function based on studies showing causal effects of the microbiome on psychiatric and neurological conditions including depression and autism, it remains an open question whether age-associated changes in cognition and microbiome composition are causally linked. Studies have shown that performing fecal microbiome transplant (FMT) of stool from old into young mice is sufficient to induce cognitive deficits, suggesting that age-related changes in the microbiome can impair cognition, yet the underlying mechanisms remain largely unclear. The identification and mechanistic understanding of a causal relationship would open the possibility of entirely novel therapeutics for the highly prevalent, incurable, and devastating cognitive decline associated with aging.

To investigate the effect of the aged microbiome on cognition, I developed a cohousing paradigm in which young (2-month-old) mice are cohoused with old (18-month-old) mice. After 1 month, cohoused young mice exhibit impaired performance compared to control young mice in novel object recognition (NOR) and the Barnes Maze test, two tasks of learning and memory. This effect is not seen in antibiotics-treated mice or germ-free mice and is recreated upon FMT of stool from old mice into young germ-free mice. When exposed to a novel object, the hippocampus in young and old mice with an aged microbiome show reduced neuronal activation. Thus, the aged microbiome impairs cognition and response to novelty. Using 16S rDNA sequencing, I identified a bacterial species, *Parabacteroides goldsteinii*, which is increased in aging and is sufficient to cause cognitive impairment when colonized in young mice.

Given the dependence of the phenotype on the aged microbiome, we hypothesized it was mediated by the vagus nerve, which anatomically connects the gut and the brain. The nucleus tractus solitarius, where the afferent vagus synapses in the brainstem, is inhibited by the aged microbiome. Ablating or inhibiting the vagus nerve impaired cognition while stimulating it rescued cognition in cohoused mice, establishing the vagus nerve as a key mediator of the phenotype.

Poster 27B | Neuroscience

Single nucleus transcriptome analysis of human reactive astrocytes

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Reactive astrocytes are a feature of many neurodegenerative diseases, including Alzheimer's disease and frontotemporal dementia. These pathologic glia are canonically associated with increases in cytoskeletal proteins. However, it is not fully understood how astrocytes are changed in disease. Data from normal, pathologic aging, and Alzheimer's disease single nucleus RNA sequencing datasets were analyzed to identify the transcriptomic changes associated with reactive astrocytes. Deep learning algorithms denoised gene expression data and clustered astrocytic nuclei. RNA trajectory, linear regressions, and Gene Ontology analyses were used to characterize reactive astrocytes. Deep learning-based clustering algorithms denoised gene expression data for 17,012 genes and clustered 15,529 astrocyte nuclei, enabling identification of gray matter (GM) and white matter (WM) astrocyte clusters. RNA trajectory analyses revealed distinct transcriptional differences between GM and WM astrocytes as well as a spectrum within the GM astrocytes. Reactive astrocyte markers (GFAP, VIM) increased along this spectrum, while homeostatic markers (LSAMP, NRXN1) decreased, consistent with graded amounts of reactivity of GM astrocytes. To identify reactivity-associated genes, linear regressions of gene expression versus reactivity were used to identify 52 upregulated and 144 downregulated genes. Gene Ontology analysis revealed that upregulated genes were associated with responses to metal ions, immune responses, and protein refolding. Downregulated genes were involved in cellular/neuronal development and maintenance. Transcription factors were significantly enriched among the downregulated genes ($p = 0.013$). Thus, in neurodegenerative disease, GM astrocytes exist within a spectrum of reactivity that is marked by a modest upregulation of reactive genes and a strong downregulation of homeostatic genes.

Poster 28B | Neuroscience

Distinct pathogenic mutations in LRRK2 disrupt axonal transport of autophagosomes

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Dysfunctional autophagy has been repeatedly implicated in Parkinson's disease (PD) pathogenesis. Leucine-rich repeat kinase 2 (*LRRK2)* mutations are the most common genetic cause of PD and result in hyperactive phosphorylation of the Rab family of GTPases. We hypothesized that LRRK2 hyperactivity contributes to autophagic disruption by causing deficits in autophagosome transport in neuronal axons. To investigate, we live-imaged the autophagosome marker LC3B in axons of human iPSC-derived neurons with knock-in (KI) of the most common LRRK2 mutation, p.G2019S. In wild-type neurons, we observed smooth retrograde transport of autophagosomes, characteristic of physiologic axonal autophagy. In contrast, imaging p.G2019S KI neurons revealed striking increases in autophagosome pausing. Another PD-linked mutation, *LRRK2*-p.R1441H, has been reported to induce even greater magnitude of kinase hyperactivity than p.G2019S. In p.R1441H KI neurons, we observed more severe disruption of autophagosome transport, manifesting as increased pause duration and likelihood of stationary autophagosomes. Thus, magnitude of autophagosome transport deficits may scale with LRRK2 activity. In p.R1441H KI neurons, we also found that overexpression of the small GTPase Arf6, a motor protein regulator, partially rescued the deficits in axonal transport of autophagosomes. Together, our results lead us to propose a model where interplay between Arf6 and LRRK2-phosphorylated Rabs results in an unproductive tug-of-war between molecular motors, disrupting the autophagosomal transport that is tightly linked to neuronal homeostasis. Importantly, autophagosome transport deficits were reversed by LRRK2 kinase inhibitor treatment, further reinforcing LRRK2's status as a promising therapeutic target.

Poster Session C

Biochemistry & Molecular Biophysics

Poster 1C
Dumbbell Degraders: Structure-guided design of oligonucleotide-based PROTACs targeting the genomic mutator APOBEC3A
Presenter: Juan Serrano | Advisor: Dr. Rahul M. Kohli

Poster 2C
Identifying domain-specific features of exhaustion-associated TOX activity in CD8 T cells
Presenter: Matthew Sullivan | Advisor: Dr. E. John Wherry

Poster 3C
The RNA helicase DDX39A binds to Chikungunya RNA to control viral infection
Presenter: Iulia Tapescu | Advisor: Dr. Sara Cherry

Bioengineering

Poster 22C
Entorhinal Amygdala Alpha Coherence as Biomarker of Anxiety for Closed-loop Deep Brain Stimulation: Results of First-in-human Invasive Electrophysiology
Presenter: Shreya Parchure | Advisor: Dr. Casey Halpern

Poster 4C
Injectable and Degradable Granular Materials for Meniscus Repair
Presenter: Karen Xu | Advisors: Drs. Robert L. Mauck and Jason A. Burdick

Cell and Molecular Biology

Cancer Biology

Poster 5C
Investigating the Role of ACSS2 in Dietary and Metabolic Regulation of Colorectal Cancer and Normal Colon Physiology
Presenter: Prateek Sharma | Advisor: Drs. Christoph A. Thaiss and Kathryn E. Wellen

Poster 6C
The Role of PNPLA2 in Breast Cancer Dormancy and Recurrence
Presenter: Emily Shea | Advisor: Dr. Lewis Chodosh

Gene Therapy and Vaccines

Poster 7C
RUNX1 Attenuates Toll-like Receptor and Type I Interferon Signaling in Neutrophils
Presenter: Alex Zezulin | Advisor: Dr. Nancy Speck

Genetics and Epigenetics

Poster 8C
N6-Methyladenosine (m6A) regulates the response to hypoxic stress in the intestinal epithelium
Presenter: Charles Danan | Advisor: Dr. Kate Hamilton

Poster 9C
Structural and functional neural connectome maps in hippocampus development
Presenter: Brian Franklin | Advisor: Dr. Jennifer E. Phillips-Cremins

Poster 10C
Investigating the role of BRD4 in regulating cell-state specific chromatin architecture
Presenter: Zach Gardner | Advisor: Dr. Rajan Jain

Poster 11C
Pathogenic laminopathy mutations target specific lamina-associated regions in cardiac myocytes potentially via altered mechanosensing
Presenter: Kaitlyn Shen | Advisor: Dr. Rajan Jain

Microbiology, Virology and Parasitology

Poster 12C
Human Adenovirus infection as a model to study virus-induced, cytoplasmic condensates
Presenter: R. Teddy Steinbock | Advisor: Dr. Matthew Weitzman

Poster 13C
Mining the Pig Skin Microbiome for Antimicrobial Products
Presenter: Monica Wei | Advisor: Dr. Elizabeth Grice

Poster 14C
Yersinia Type III-Secreted Effectors Subvert Caspase-4-dependent Inflammasome Activation in Intestinal Epithelial Cells
Presenter: Jenna Zhang | Advisor: Drs. Igor Brodsky and Sunny Shin

Chemistry

Poster 15C
Preparation and Application of Linked and Cyclic Collagen Mimetic Peptides
Presenter: Diane Rafizadeh | Advisor: Dr. David Chenoweth

Genomics & Computational Biology

Poster 16C
ESPRESSO-TEA: A long-read RNA sequencing workflow to study locus-specific expression of transposable elements
Presenter: Emerson Hunter | Advisor: Dr. Yi Xing

Immunology

Poster 17C
Domain-Focused, Whole-Genome Screen to Identify Fetal Hemoglobin Regulators in Human Erythroid Cells
Presenter: Chad Komar | Advisor: Drs. Gerd Blobel and Junwei Shi

Poster 18C
The role of the Type III Secretion System in presentation of Salmonella enterica epitopes on MHCII
Presenter: Kate Krauss | Advisor: Dr. Laurence Eisenlohr

Neuroscience

Poster 19C
A solution to the long-standing problem of actin expression and purification
Presenter: Rachel Ceron | Advisor: Drs. Robert O. Heuckeroth and Roberto Dominguez

Poster 20C
Creating an in vitro macrophage model for globoid cell leukodystrophy to study globoid cell formation and function
Presenter: Sai Chaluvadi | Advisor: Dr. F. Chris Bennett

Poster 21C
Interplay between DNA methylation and Polycomb silencing in mammalian brain development
Presenter: Daniel Connolly | Advisor: Dr. Zhaolan (Joe) Zhou

Poster 1C | Biochemistry and Molecular Biophysics

Dumbbell Degraders: Structure-guided design of oligonucleotide-based PROTACs targeting the genomic mutator APOBEC3A

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The APOBEC3 (A3) sub-family plays a crucial role in antiviral defense by deaminating cytosine (C) to uracil (U) in foreign ssDNA found in retroviral intermediates, primarily in the context of innate immunity. DNA deamination can also have pathological consequences, accelerating evolution of viral genomes or, when the host genome is targeted by APOBEC3A (A3A), promoting tumor evolution leading to worse patient prognosis and chemotherapeutic resistance. There has been limited success inhibiting A3A using 4-deaminocytosine analogues such as zebularine (Z) and methylzebularine (mZ) incorporated into ssDNA, as only weak micromolar-level inhibitory potency was achieved which has not translated over to cellular studies. Recently, a nanomolar-level oligonucleotide-based inhibitor of A3A was developed by our lab through incorporation of mZ onto a dumbbell scaffold, mimicking A3A's preferred substrate secondary structure. Here, we report our efforts to functionalize these dumbbell oligonucleotide inhibitors into proteolysis targeting chimeras (PROTACs) able to promote the intracellular degradation of A3A. We constructed a diverse panel of dumbbell PROTACs, varying both the class of E3 ligase ligand used (VHL- or CRBN-targeting) and the linker length between dumbbell and ligand. Using a dox-inducible cellular assay for A3A expression, preliminary studies have demonstrated the ability of dumbbell PROTACs to degrade A3A in a dose-dependent manner. Further characterization shows intracellular protein degradation is mechanism-dependent, as removal of the ligand, replacement with an inactive analogue, or competition with free ligand ablates this effect. Future work is currently focused on optimizing PROTAC design, inhibiting factors in the proteasomal degradation pathway to confirm involvement of the E3 ligase machinery, and demonstrating inhibition of A3A deamination activity in various cellular models. Overall, our work offers a blueprint for the development of inhibitors of DNA-modifying enzymes into protein degraders and potential therapeutics to circumvent APOBEC-driven viral and tumor evolution.

Poster 2C | Biochemistry and Molecular Biophysics

Identifying domain-specific features of exhaustion-associated TOX activity in CD8 T cells

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CD8 T cell responses to viral infections and tumors make critical contributions to the total adaptive immune response and can determine the clinical outcomes of such pathologies. The functional integrity of CD8 T cell activity depends on the characteristic properties of CD8 effector (Teff) and memory (Tmem) populations. During chronic viral infections and cancer, antigen persistence without clearance constrains effective Teff and Tmem development, instead biasing CD8 T cell differentiation towards the epigenetically distinct "exhausted" lineage. Exhausted CD8 T cells (Tex) exhibit progressive dysfunction, with loss of their effector properties, proliferation capacity, and memory potential, as well as sustained increases in co-expression of PD1 and multiple other inhibitory receptors (IRs). Understanding the fundamental mechanisms that initiate and maintain the Tex epigenetic state is a major barrier to identifying strategies to selectively modulate Tex function and improve the efficacy of cellular or other immunotherapies.

In models of exhaustion during chronic viral infection and of dysfunctional tumor-specific T cells, the transcription factor TOX is essential for the initiation of Tex development, repressing terminal Teff differentiation and potentiating epigenetic commitment to the Tex lineage. However, the molecular transactions TOX employs to exert its effects remain largely unknown, as do the structures and individual functions of the regions of TOX protein N- and C-terminal to its HMG-box DNA binding domain. As these N- and C-terminal domains ("NTD," "CTD") comprise ~84% of the TOX protein, they are likely prominent, but as yet unknown, determinants of TOX activity. Although TOX deficient CD8 T cells are, at least initially, phenotypically enriched for Teff-specific features and attenuated of Tex features, they characteristically exhibit time- and differentiation-dependent deficits in survival or proliferation. These deficits likely limit the therapeutic utility of complete TOX deletion for Tex modulation, necessitating alternate strategies.

We used a murine lymphocytic choriomeningitis virus model of chronic infection coupled with adoptive co-transfer of transgenic, antigen-specific *Tox*$^{-/-}$ and wild-type CD8 T cells to query (i) how the NTD and CTD contribute to global TOX activity *in vivo*, and (ii) whether NTD- or CTD-specific perturbation is sufficient *in vivo* to circumvent both Tex phenotypes and TOX-dependent deficits in survival or proliferation. With respect to expression of PD1 and other IRs, cytotoxic potential, and differentiation along select Teff and Tex lineages, the NTD, HMG, and CTD domains are all required for fully intact TOX activity. Compared to deletion of both regions, NTD *or* CTD deletion more closely approaches or exceeds, albeit variably, the numeric cellular expansion of their wild-type counterparts. These data implicate the development of smaller NTD or CTD mutations as a practical means for further interrogating or perturbing TOX mechanism.

Poster 3C | Biochemistry and Molecular Biophysics

The RNA helicase DDX39A binds to Chikungunya RNA to control viral infection

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DEAD-box (DDX) helicases play vital roles in RNA metabolism and are important for antiviral innate immunity. Here, we found that the DDX member DDX39A is antiviral against pathogenic alphaviruses such as Chikungunya (CHIKV) and Venezuelan Equine Encephalitis Virus (VEEV). Depletion of DDX39A in various cell lines increases alphavirus replication. Furthermore, the antiviral phenotype of DDX39A is independent of the canonical interferon pathway. Mechanistically, we found that under basal conditions, DDX39A is predominantly nuclear; however, alphavirus infection leads to accumulation of DDX39A in the cytoplasm, where viral RNA replication occurs. Moreover, DDX39A impacts early steps in the viral replication cycle without affecting viral entry or spread. Biochemical studies reveal that DDX39A strongly binds to CHIKV RNA. CLIP-seq demonstrates that DDX39A binds to a conserved structure in CHIKV, which is known to be important for viral replication. Thus, DDX39A is a novel antiviral factor against pathogenic alphaviruses by binding to structured viral elements to restrict viral replication.

Poster 4C | Bioengineering

Injectable and Degradable Granular Materials for Meniscus Repair

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Injectable, acellular, and degradable biomaterials have been increasingly explored as therapeutics for tissue repair, due to their ability to fill defects, recruit endogenous cells, and support extracellular matrix deposition. Cell invasion into three dimensional scaffolds is controlled through various biophysical parameters, one of which is matrix pore size. Herein, we engineer porosity into scaffolds by crosslinking microparticles into an injectable hydrogel that stabilizes *in situ*. We further demonstrate that the porosity introduced facilitates cell infiltration compared to conventional bulk hydrogels. Future directions include validating the proposed material's ability to repair tissue defects (with a focus towards meniscal tears) *in vivo*.

Poster 5C | CAMB - Cancer Biology

Investigating the Role of ACSS2 in Dietary and Metabolic Regulation of Colorectal Cancer and Normal Colon Physiology

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A critical function of cellular survival is the ability to sense and adapt to changing nutrient availability. This adaptation allows cells to sustain metabolic pathways essential for maintenance and proliferation such as fatty acid and cholesterol biosynthesis, the citric acid cycle, and energy-producing catabolic pathways. During oncogenesis and cancer progression, cancer cells must similarly adapt to ever-changing environmental pressures and optimize their metabolic functions for proliferative capacity and survival. Nutrient availability influences these aspects of cell biology and is largely dictated by diet, a significant determinant of organismal metabolic state and modulator of cancer biology. Significant effort has been made to characterize the metabolic effects of tumor-protective and tumor-enhancing dietary components, such as fiber-derived short chain fatty acids (SCFAs) and fructose, respectively. Yet, it is unclear how these divergent diet-derived metabolic signals integrate to exert a net effect and whether the interaction between them is modulated to favor one outcome. Acyl-Coenzyme A (CoA) synthetase short chain family member 2 (ACSS2) is a nucleocytosolic enzyme that converts acetate, the most abundant SCFA, to acetyl-CoA, a key metabolite used for anabolic pathways and histone modification. Due to its important role in metabolism, ACSS2 has been the subject of numerous cancer studies and targeted therapeutic development. Despite this, the role of ACSS2 in colorectal cancer (CRC) is unclear, with conflicting reports surrounding its effect on CRC. This raises the question of how ACSS2 functions in the CRC context and to what extent dietary inputs modulate ACSS2 activity. Our study seeks to address these questions using various *in vitro* and *in vivo* models to characterize the metabolic state of normal colonocytes and CRC to better inform prevention and treatment of CRC. First, we plan to characterize the role of ACSS2 in generating SCFA-CoA species, their incorporation into histone modifications, and the subsequent change in epigenetic regulation. Next, we plan to determine how ACSS2 and dietary nutrient availability impact host metabolism, colon physiology, and CRC growth. We will compare diets with varying fiber and fructose composition to understand whether nutrient availability affects CRC in an ACSS2-dependent manner. Both carcinogen and genetic mouse models will be used to address the influence of divergent metabolic inputs on CRC. Ultimately, this project will increase our understanding of the role of ACSS2 in normal colon and CRC physiology and inform future work surrounding the impact of dietary modifications on CRC prevention and treatment.

Poster 6C | CAMB - Cancer Biology

The Role of *PNPLA2* in Breast Cancer Dormancy and Recurrence

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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. The library was cloned into the lentiviral GFP vector (Addgene) and transduced into an inducible Her2 primary tumor model previously developed and validated in our laboratory. The transduced cells were sorted for GFP expression and injected orthotopically into mice. Primary tumors, multiple timepoints of residual lesions, and recurrent tumors were harvested and sequenced. The top hit by clonal enrichment was *PNPLA2*. In particular, sg*PNPLA2* was among the top 2 guides represented in 8/23 late dormancy residual lesions (day 28 and 35**).** *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 p*romotes expression of PPARα and its target genes, thus also serving as a key node for cellular signaling. In an *in vivo* competition validation study, *sgPNPLA2* was selected for in primary tumor formation and further selected for in recurrent tumor formation. I hypothesize that *PNPLA2* is a key regulator of recurrent tumor formation. I am currently pursuing further validation studies to assess *PNPLA2's* role in tumor recurrence via recurrence free survival assays *in vivo* and colony formation assays *in vitro.* I am further planning experiments to ascertain the mechanism by which *PNPLA2* may exert its control by staining for lipid droplets in residual lesions and recurrent tumors, analyzing PPARα expression, and staining for reactive oxygen species during dormancy and recurrence.

Poster 7C | CAMB - Gene Therapy and Vaccines

RUNX1 Attenuates Toll-like Receptor and Type I Interferon Signaling in Neutrophils

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The transcription factor RUNX1 is often mutated in inherited and sporadic forms of acute myeloid leukemia (AML). Familial platelet disorder with associated myeloid malignancy (FPDMM) is caused by mutations in the *RUNX1* gene and is associated with an increased lifetime risk of leukemia. In addition, many FPDMM patients have heightened inflammation. Inflammation can promote hematologic malignancies such as AML. Understanding how RUNX1 mutations increase inflammation is critical for determining how to halt leukemia progression. To understand the role RUNX1 plays in regulating inflammatory responses, we created a pan hematopoietic RUNX1 knockout (KO) mouse model. We found that RUNX1 KO neutrophils overproduced inflammatory cytokines and chemokines in response to toll-like receptor 4 (TLR4) stimulation with lipopolysaccharide (LPS). The hyper-responsiveness of RUNX1 deficient neutrophils correlated with increased expression of several genes encoding proteins in the TLR4 signaling pathway. To determine if RUNX1 functions in neutrophils to regulate TLR4 signaling, we deleted RUNX1 specifically in neutrophils. Unexpectedly, neutrophil-specific RUNX1 deletion did not cause an inflammatory phenotype, indicating that alterations in the TLR4 signaling pathway are established in a neutrophil precursor. Single cell transcriptomic data generated in our lab suggests that RUNX1 deficiency dysregulates the expression of genes that mediate TLR4 signaling in a precursor of terminally differentiated neutrophils, specifically the granulocyte-monocyte progenitor (GMP) cell population. We determined that RUNX1 loss in the GMP was sufficient to establish the inflammatory phenotype in neutrophils. We found that RUNX1 loss leads to chromatin opening in both GMPs and neutrophils at key TLR signaling genes. We determined that RUNX1 loss in GMPs was sufficient to establish an inflammatory phenotype driven by the IRF and STAT family of transcription factors. Together this data suggests that the dysregulated inflammatory response of neutrophils is a downstream consequence of RUNX1 mutations in GMPs and can be dampened by inhibiting either the TLR4 or JAK/STAT signaling pathways. By identifying novel strategies to decrease systemic inflammation, we aim to delay or prevent leukemia.

Poster 8C | CAMB - Genetics and Epigenetics

N6-Methyladenosine (m^6A) regulates the response to hypoxic stress in the intestinal epithelium

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The intestinal epithelium forms a critical barrier between foreign antigens in the intestinal lumen and immune cells in the underlying mucosa. This barrier is compromised in inflammatory bowel disease (IBD), resulting in inflammation and epithelial cell death. Improved understanding of intestinal epithelial regeneration would identify novel therapeutic targets to enhance epithelial barrier repair in diseases such as IBD. After injury, tissues such as the blood, skeletal muscle, and central nervous system activate a regenerative program that is partially regulated by N6-methyladeonsine (m^6A) modification of RNA. However, few data exist regarding the role of m^6A within the intestinal epithelium. The goal of this study was to profile m^6A-modification transcriptome-wide in the regenerating intestinal epithelium and assess the role of the m^6A methyltransferase, METTL3, in regulating regenerative pathways. Wildtype mice were given dextran sodium sulfate (DSS) in drinking water to induce intestinal inflammation and epithelial cell loss. DSS was followed by a two-week washout to allow for hyperproliferative regenerating epithelium to appear adjacent to areas of epithelial ulceration. After DSS washout, regenerating intestinal epithelium was isolated by flow cytometry, RNA was extracted, and m^6A-modified transcripts were immunoprecipitated and sequenced (m^6A-seq). For hypoxia studies, METTL3 was knocked down in murine colonic organoids using shRNA and hypoxia was simulated using a trans-well air-liquid-interface system. m^6A-seq yielded ~5500 total m^6A-modified sites within the regenerating intestinal epithelium. Pathway enrichment analysis demonstrated that these sites were enriched in transcripts involved in the response to TNF-alpha via NF-kB, as well as the response to hypoxia. Air-liquid-interface experiments in METTL3 knockdown colonoids indicated that METTL3 is required for full induction of HIF1a and the adaptive, pro-survival response to hypoxic stress. Our data support the hypothesis that m^6A modifies many transcripts with known roles in intestinal epithelial regeneration and positively regulates the adaptive response to hypoxic stress. Ongoing studies are evaluating how m^6A regulates hypoxia responsive transcripts.

Poster 9C | CAMB - Genetics and Epigenetics

Structural and functional neural connectome maps in hippocampus development

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My goal is to develop a novel, fluorescence-based method to produce synapse-level resolution maps of structural connections between neurons and use this along with imaging of neural excitation to investigate the pattern of structural and functional synaptic connections at essential early neurodevelopmental time points. Specifically, I will look at the hippocampus of the mouse brain, known to be essential in memory formation and recall, across major stages of synapse - formation or loss in early life. My **hypothesis** is that structural and functional connectivity in the hippocampus will increase in an unbiased manner during the childhood critical period, before adolescent synaptic pruning reshapes the circuit into more pronounced clusters of connectivity. To investigate these questions, I will use cultured slices of hippocampus from mice of three different ages: at birth, post-natal week 6 (onset of puberty), and post-natal week 9 (post- pubescent adulthood). I will develop a method, called SynMap-seq, using engineered synaptic proteins to localize cell-specific barcodes to both sides of neural synapses in the hippocampus. Once tissue slices are fixed tissue, synapses will be labeled specifically and sensitively using an assay based on proximity-ligation and cell-specific barcodes will be sequenced in situ to determine the location of each neuron's cell body and synaptic terminals. Synapses will be assigned to the pair of neurons with barcodes in closest spatial proximity. In cultured slices, I also will perform activity imaging in the hippocampus using a GCaMP calcium sensor upon pharmacologic activation to investigate excitatory synchrony in the hippocampus as a proxy for functional synaptic connectivity across the network. My work is **significant** along both technological and biologic axes of innovation. **Technologically**, it will develop a facile, high- throughput, sequencing and fluorescence-based method for interrogating the structural connectome at synapse-resolution that has the potential to expand connectome-mapping to labs without the need for electron microscopy (EM). Furthermore, it is compatible with spatial methods that cannot be used with EM including those investigating spatial distribution of RNA, DNA, and protein. **Biologically**, it will advance the understanding of how structural and functional connectivity in the hippocampus changes/adapts across important neurodevelopmental milestones and learning stages.

Poster 10C | CAMB - Genetics and Epigenetics

Investigating the role of BRD4 in regulating cell-state specific chromatin architecture

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Genome folding organizes mammalian chromosomes at various levels within the nucleus. At one level of organization, the ring-shaped protein complex cohesin partitions chromosomes into loops. Loss of either cohesin or the cohesin loading protein NIPBL in cell culture models attenuates progenitor cell pluripotency and impedes differentiation suggesting that chromatin architecture may play a role in mediating cell identity. While genome-wide surveys of chromatin organization demonstrate that chromatin architecture is cell-identity specific, rapid depletion of cohesin abrogates chromatin looping regardless of cell-type suggesting that cohesin alone is not sufficient to confer architectural specificity. The mechanism by which cohesin mediates cell-identity specific chromatin architecture remains unclear. Previous work by our lab identified a novel genome folding function for the bromo- and extra-terminal domain (BET) protein BRD4. A transcriptional co-activator and known reader of the cell-type and cell-state specific CRE-associated mark H3K27ac, BRD4 was found to interact with NIPBL. Loss of the BRD4-NIPBL interaction impeded cell differentiation, lowered NIPBL occupancy genome-wide, and abrogated chromatin looping.

Seeking to further define the molecular mechanism governing changes in chromatin architecture we turned to the activation of fibroblasts to myofibroblasts as a model. When starved and treated with inflammatory cytokines, quiescent fibroblasts acquire a contractile myofibroblast phenotype. Activation cooccurs with increases in H3K27ac at key genomic loci, including the *MEOX1* locus. Meox1 is a transcription factor responsible for the upregulation of pro-fibrotic gene expression programs associated with the fibroblast to myofibroblast transition. Preliminary data demonstrate that the activation of fibroblasts results in an increase in the proximity of *MEOX1* CREs consistent with chromatin loop formation. Furthermore, we have found that the loss of either NIPBL or BRD4 disrupts *MEOX1* CRE apposition and lowers *MEOX1* expression. In the future I will further leverage the inducible fibroblast to myofibroblast transition to determine if cell-state specific chromatin architecture depends on the BRD4-NIPBL interaction and if H3K27ac mediates BRD4 localization upstream of chromatin architecture changes.

Poster 11C | CAMB - Genetics and Epigenetics

Pathogenic laminopathy mutations target specific lamina-associated regions in cardiac myocytes potentially via altered mechanosensing

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The mammalian genome is organized into various regions at different scales as one mechanism regulating gene expression and mediating cellular identity. One type of well-characterized region is the lamina-associated domain (LAD), which contain areas of chromatin 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 used induced pluripotent stem cells (iPSC) to determine the impact of a DCM patient-modeled *LMNA* T10I mutation on peripheral heterochromatin organization. T10I iPSC-derived cardiomyocytes exhibited gross nuclear abnormalities and demonstrated loss of lamina-bound chromatin enriched in genes and lower LaminB1 contact frequency. These regions are also enriched in genes related to non-myocyte identity and mutant myocytes expressed these genes associated with non-myocyte lineages. These effects were myocyte-specific, as the LMNA variants did not disrupt lamina-chromatin interactions in iPSC-derived hepatocytes or adipocytes. Our data indicate that lamina network functions to maintain silencing of undesired cellular identities. Additionally, evidence from mouse models and human genetic studies have also suggested a potential role for the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex in mediating genome organization. However, while a LINC-LMNA-gene positioning axis has been suggested, the mechanism of how this may occur remains elusive. Using a combination of population-based genomics analyses and single-cell microscopy, we will test the hypothesis that LMNA and the LINC complex function in concert across the nuclear membrane to mediate genome organization in cardiomyocytes. These studies will provide mechanistic insights into how the nuclear lamina and LINC complex are cooperating to maintain LAD organization in cardiomyocytes, which will begin to provide novel understanding of the molecular basis of laminopathy phenotypes.

Poster 12C | CAMB - Microbiology, Virology and Parasitology

Human Adenovirus infection as a model to study virus-induced, cytoplasmic condensates

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Eukaryotic cells restrict translation in response to diverse stresses including heat shock, oxidative damage, and viral infection. Stress-activated kinases phosphorylate the translation initiation factor eIF2α to halt cap-dependent translation. The double-stranded RNA (dsRNA) sensor Protein Kinase R (PKR) is a key eIF2α kinase activated during viral infection. Under stress conditions, translationally repressed mRNAs and associated proteins coalesce into cytoplasmic assemblies called stress granules (SGs). Growing evidence suggests SGs have antiviral functions since they recruit pattern recognition receptors to augment antiviral responses, and many viruses inhibit SG formation. Recent work characterizing the SG proteome and transcriptome have mostly relied on arsenite-induced oxidative stress to provoke *PKR-independent* SG formation. However, up to 20% of SG protein diversity is stress-dependent, and no work to date has characterized the global SG proteome during viral infection.

Activation of another antiviral dsRNA pathway, OAS/RNase L, was recently found to restrict translation independently of eIF2α and lead to formation of related granules termed RNase L-dependent bodies (RLBs). Little is known about the mechanisms underpinning RLB assembly, composition, and function. Studies characterizing RLBs have also relied on non-physiologic transfection with the dsRNA-mimic poly(I:C). Critically, no system has been identified to compare SGs and RLBs formed during viral infection.

Using human adenovirus (AdV) as a model system, we uncovered two viral mutants that differentially activate the dsRNA pathways of PKR and OAS3/RNase L to induce SGs and RLBs, respectively. All human AdVs express a non-coding, virus-associated (VA) RNA that binds PKR, and mutants lacking VA RNA (ΔVA) activate PKR. Our lab recently discovered that mutants lacking a virus-directed ubiquitin ligase (ΔE4) also activate PKR as well as RNase L. Using immunofluorescence and knockout cell lines, we have found that ΔVA induces PKR-dependent SGs while ΔE4 provokes PKR-independent RLBs. Furthermore, wildtype (WT) AdV infection reduces expression of the core SG components G3BP1 and 2, and WT-infected cells are resistant to arsenite-induced SG formation. These physiologically relevant viral models provide an ideal system to study similarities and differences between virus-induced SGs and RLBs. Our studies will provide insights into regulation of these cellular structures, and how viruses counter their formation.

Poster 13C | CAMB - Microbiology, Virology and Parasitology

Mining the Pig Skin Microbiome for Antimicrobial Products

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a leading cause of infections by antibiotic resistant organisms in the US, with the skin being the most frequent site of infection. MRSA colonization of skin and nasal passages further contributes to spread of MRSA within the community. There is a therefore a growing need for new antibiotics against drug resistant pathogens such as MRSA. One potential source of novel antibiotics against MRSA is the skin microbiome, which can be colonized by MRSA and is an unmined source of natural products. The increasing availability of whole genome sequencing of bacterial genomes has renewed interest in genomics-driven approaches to finding novel antimicrobials. Using pigs as a model organism, our lab screened over 10,000 culturable isolates from the skin microbiome for inhibition of MRSA by disk diffusion assay. We identified 31 unique bacterial isolates that inhibited the growth of MRSA. One of these, *D.* incerta, secretes a heat and protease-sensitive molecule that inhibits MRSA *in vitro*. Analysis of the *D. incerta* genome shows little homology to known antimicrobial genes clusters. The identity of the antimicrobial molecule and whether its antimicrobial activity persists in the context of MRSA skin infection thus remain unclear. We investigate the identity of this antimicrobial molecule via parallel genomic and biochemical approaches. We further find that *D. incerta* supernatant inhibits *Staphylococcal* species via a non-lytic mechanism of action. *D. incerta* exhibits temperature-dependent cell and colony morphologies when grown on blood agar, and we are currently examining how this might influence production of the anti-MRSA activity. Finally, we propose to test ability of *D. incerta* to improve MRSA colonization and improve healing of MRSA-mediated skin infection, two clinically important skin conditions.

Poster 14C | CAMB - Microbiology, Virology and Parasitology

Yersinia Type III-Secreted Effectors Subvert Caspase-4-dependent Inflammasome Activation in Intestinal Epithelial Cells

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Yersinia are gram-negative zoonotic bacteria responsible for significant disease burden in humans, ranging from recurrent disease outbreaks (yersiniosis) to pandemics (*Yersinia pestis* plague). *Y. pseudotuberculosis* (*Yptb*) is widely prevalent within global farmed livestock and causes gastrointestinal illness and mesenteric lymphadenitis in immunocompetent humans after ingestion of contaminated meat. *Yersinia* uses its type three secretion system (T3SS) to inject virulence factors termed *Yersinia* outer proteins (Yops) into the host cytoplasm in order to subvert essential components of innate immune signaling. However, T3SS activity elicits host immune activation including formation of immune complexes known as inflammasomes. Inflammasomes can cleave and activate caspases leading to inflammatory cell death and cytokine release aimed at containing infection. The requirement of different inflammasomes, as well as the role of Yops in suppressing inflammasome activation, have been studied extensively in macrophages. However, interactions between *Yersinia* and inflammasomes in intestinal epithelial cells (IECs), the primary site of gastrointestinal *Yersinia* infection remain poorly defined. We found that in human IECs, *Yptb* blocks T3SS-dependent-inflammasome activation. Notably, deletion of antiphagocytic Yops (YopE and YopH), as well as YopK, phenocopied deletion of all the secreted Yops with respect to inflammasome activation. Critically, in contrast to previous findings in macrophages, combined deletion of YopE and YopH was required to elicit inflammasome activation in IECs during *Yptb* infection. Combined deletion of YopE and YopH increased *Yptb* internalization into IECs, thereby potentially enabling cytosolic detection of bacterial LPS. Indeed, we found that the Caspase-4 inflammasome, which detects cytosolic LPS, is fully required for the inflammasome response during Yptb infection. Additionally, both caspase-1 and caspase-8 are partially required. Together, our results reveal insight into how intestinal epithelial cells employ inflammasomes to sense and respond to *Yersinia* infection and how *Yersinia* is able to evade this response.

Poster 15C | Chemistry

Preparation and Application of Linked and Cyclic Collagen Mimetic Peptides

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Collagen lays the foundation of bodily tissues, serving to strengthen, connect, and signal from the micro to the macro scale. As a permutated, triple helical polymer involved in biochemical signaling, it has potential for manipulation as both a biomaterial and a tool for modulating protein-protein interactions. These applications have been limited by its tripartite nature, which restrict its thermal and entropic stability. Linkage and/or cyclization of the three collagen strands may overcome these limitations. This work focuses on the synthesis, biophysical/structural characterization, and biological application of linked and cyclic collagen mimetic peptides (CMPs) for interrogating interactions of collagen with its protein binding partners. We aim to develop biochemical tools for modulating the collagen interactome, laying the foundation for new discoveries in collagen biology and the potential for applications in drug discovery. The first part of this work involves the preparation of double-stranded, macrocyclic CMP "hosts" capable of interacting with unlinked CMP strands, termed "guests." A series of guests were developed using novel chemistry and strategic unnatural amino acids—including aza-glycine and peptoid residues—to highly stabilize the host-guest complex. This also resulted in the serendipitous discovery of a miniaturized CMP that could represent the smallest collagen-like triple helix reported to date. The second part of this work seeks to develop a novel method for covalent capture of the triple helix via diazirine-based photocrosslinking. Early results have shown that linked dimers and trimers can be prepared by this technique; further optimization is necessary to increase yield of linked peptides. Our third goal is to employ the aforementioned molecules to modulate the interaction between collagen and the discoidin domain receptor type 2 (DDR2), a receptor tyrosine kinase implicated in multiple human cancers. This third project aims to 1) generate maximally stabilized, miniaturized linked and cyclic CMPs for specific targeting of DDR2, and 2) investigate the interaction of these CMPs with DDR2 via *in vitro* and *in cellulo* methodology. Our work represents an innovative chemical biology approach to interrogating the role of collagen in cancer biology.

Poster 16C | Genomics and Computational Biology

ESPRESSO-TEA: A long-read RNA sequencing workflow to study locus-specific expression of transposable elements

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Transposable elements (TEs) are mobile genetic elements that compose >45% of the human genome, with major implications in health and disease. The expression of TEs is notoriously challenging to study with short-read sequencing technology due to mapping ambiguity attributed to a TE's highly repetitive content, sequence length relative to read length, insertional polymorphisms, and age-related sequence divergence. Recent advances in long-read technology support more comprehensive TE detection and quantification. We developed ESPRESSO-TEA (Error Statistics Promoted Evaluator of Splice Site Options – Transposable Element Analysis), a workflow to measure locus-specific TE expression from long-read RNA-sequencing data. Using a long-read sequencing simulator, NanoSim, we assessed ESPRESSO-TEA's performance in identifying and quantifying locus-specific TE expression. We simulated reads from TEs belonging to four major TE families (Alu, L1, MIR, L2) with varying ages and sequence lengths. We demonstrate that TE read mappability varies by TE family, with mapping accuracy of a younger family (Alu) more dependent on relative age than that of older families (L1, MIR, and L2), but TE read mappability impacted by sequence lengths in all families. The young age of Alu and shorter lengths (<150-200bp) of TEs belonging to any family contributes to TE expression underestimation, but overall mapping performance is as expected and ESPRESSO-TEA captures locus-specific TE expression. Moving forward, the ESPRESSO-TEA workflow will characterize TE-transcriptome landscapes, elucidating TE contributions as independent transcriptional units, novel exonizations in chimeric transcripts, and sources for multi-exonic full-length transcripts.

Poster 17C | Immunology

Domain-Focused, Whole-Genome Screen to Identify Fetal Hemoglobin Regulators in Human Erythroid Cells

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Sickle cell disease (SCD) is an inherited blood disorder that affects over 100,000 people in the USA and more than 20 million people worldwide. Patients afflicted by SCD have a median life expectancy of 40-60 years due to complications such as infections, acute chest syndrome, and stroke. Increasing fetal hemoglobin (HbF) levels is a therapeutic goal as higher HbF levels reduce the risk of these complications and improve overall survival. Presently, hydroxyurea is the only FDA approved treatment known to induce HbF, but concerns of teratogenic effects and non-responsiveness to this pharmacological agent historically limited its widespread application.

While small-scale, focused CRISPR-Cas9 based screening approaches identified novel regulators of HbF, much of the human proteome remains unexplored. To evaluate these untested genes, some of which we hypothesize lie in druggable pathways, we conducted a domain-focused CRISPR-Cas12a based genetic screen targeting each known coding gene in the human genome (18,292 independent crRNAs representing 18,292 genes). The crRNA library was constructed to target protein domains, cloned into a lentivirus scaffold, and transduced into the erythroid cell line HUDEP-2 (human umbilical derived erythroid progenitors). These cells express low levels of HbF at baseline and stably express Cas12a. We infected HUDEP-2 cells with the lentiviral crRNA library, enriched for cells expressing crRNA by fluorescence-activated cell sorting, and induced erythroid differentiation and maturation. By sorting the top 10% and bottom 10% of HbF-expressing cells and determining the representation of guides in these populations via deep sequencing, we identified a subset of crRNAs enriched in the high-HbF population, which may represent a set of novel regulators of HbF. After analysis of the fitness scores of guides known to target genes essential for cell survival and guides known to target genes not expressed in this cell line, we observed highly reproducible gene editing between two biological replicates using this method.

Furthermore, to validate the findings of our whole-genome CRISPR-Cas12a based screening approach, we conducted a secondary validation screen using a domain-focused, CRISPR-Cas9 based approach. Preliminary analysis indicates highly reproducible recall of HbF inducers.

Ongoing studies aim to compare the results of the CRISPR-Cas9 based screening in HUDEP-2 cells to primary human hematopoietic stem and progenitor cells (HSPCs), to identify which candidate HbF regulators are most likely to be important in primary erythroid cells. We plan to individually validate and characterize the roles of several of these candidates in both HUDEP-2 and HSPCs using CRISPR-Cas9 loss-of-function approaches.

Poster 18C | Immunology

The role of the Type III Secretion System in presentation of Salmonella enterica epitopes on MHCII

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Salmonella enterica is a pathogen with an ingenious niche: a phagosome reprogrammed via bacterial effector proteins secreted into the host cytosol. CD4 T cells form the backbone of an efficient response to *S enterica* infection by activating infected macrophages. However, the role of the Salmonella containing vacuole on formation of CD4 T cell responses remains relatively unexplored. We have performed a screen of predicted Salmonella CD4 epitopes in mice and found that CD4 responses are highly skewed toward the secreted effector proteins at the expense of other bacterial proteins sequestered in the vacuole. This suggests that secreted effectors in the cytosol of infected antigen-presenting cells (APCs) are an abundant source of peptide to present to CD4 T cells, running counter to the classical paradigm of CD4 antigens originating from material outside the presenting cell. To further probe this skew, we have used an mRNA vaccine system pioneered by the Eisenlohr lab to generate epitope-specific CD4 T cell responses. Since this system allows us to quantify the presentation of individual epitopes by infected cells, it allows us to test the impact on presentation of the *S enterica* secretion system, as well as the properties of individual epitopes. In future, we would like to test whether these T cells offer differential protection from a *S enterica* infection. Understanding this skew could not only improve our understanding of the immune response to *S enterica* but could also illuminate a novel pathway for antigen presentation in bacterial infections.

Poster 19C | Neuroscience

A solution to the long-standing problem of actin expression and purification

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Actin is the most abundant protein in eukaryotic cells and plays essential roles in muscle contraction, cell motility, cytokinesis, and other cellular processes. Humans express six non-interchangeable actin isoforms, and mutations in all isoforms cause devastating diseases. Most studies of actin biochemistry use tissue-purified alpha skeletal actin, and there is currently no reliable method for producing pure recombinant human actin in its native form. Previously published purification strategies for recombinant actin failed to address folding concerns, demonstrate proper post-translational modification, and eliminate contamination with other highly similar actin isoforms. We developed a method to obtain high yields of recombinant actin in human cells that addresses the shortcomings of previous methods. We use a combination of experimental approaches to rigorously demonstrate the removal of highly homologous endogenously expressed actin and the functional integrity of recombinant actin for multiple isoforms of actin. Proteomics analysis confirms the presence of native post-translational modifications and proper affinity-tag removal. With this method, we can now study actin under fully native conditions to investigate differences among isoforms and the effects of disease-causing mutations.

Poster 20C | Neuroscience

Creating an in vitro macrophage model for globoid cell leukodystrophy to study globoid cell formation and function

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Globoid cell leukodystrophy (GLD) is a fatal neurodegenerative and lysosomal storage disease that results from the deficiency of galactosylceramidase (GALC) – a lysosomal hydrolase responsible for the catabolism of the glycolipid, galactosylceramide (galcer). A defining feature of GLD is the presence of rounded, lipid-engorged macrophages that are thought to be key drivers of disease. They are found in areas of white matter pathology, demonstrate reactive morphology, and are associated with increased levels of pro-inflammatory molecules in the brain. Importantly, when diseased macrophages are replaced with WT ones through bone marrow transplant, there are fewer globoid cells and improved clinical outcomes, corroborating their relevance to disease pathogenesis. Yet, the mechanisms underlying how these cells arise and their functional roles are unknown largely due to the lack of validated cellular models that can be genetically and pharmacologically manipulated for mechanistic study. I hypothesized that the lysosomal accumulation of galcer and its toxic byproduct, galactosylsphingosine (psychosine), sufficiently results in the formation of these lipid-laden macrophages. I treated bone marrow-derived macrophages with a range of galcer or psychosine concentrations for various time points to identify the necessary conditions to generate globoid-like cells in vitro. Using these parameters, I then found that galcer treatment is more toxic to *Galc*KO macrophages compared to *Galc*WT macrophages and induces unique morphology in *Galc*KO cells. So far, I have created a preliminary in vitro globoid cell model that will be used to assess globoid cell formation and function after further validation.

Poster 21C | Neuroscience

Interplay between DNA methylation and Polycomb silencing in mammalian brain development

Daniel Connolly and Zhaolan Zhou

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DNA methylation is a central epigenetic mark that classically occurs on cytosine nucleotides in the CG context, where cytosine is followed by a guanine. However, postmitotic neurons in the mammalian brain contain a unique methylome, with large amounts of non-CG cytosine methylation (mCH) accumulating in early postnatal life. This non-canonical methylation mark is written exclusively by the de novo DNA methyltransferase DNMT3A, and removal of *Dnmt3a* from the central nervous system results in broad transcriptional changes and early lethality in mice. Despite the importance of non-CG methylation in neuronal function, the roles of mCH have been difficult to study *in vivo* given the diversity of cell types in the mammalian brain, as mCH patterns differ significantly across neural cell types. Thus, the principles guiding how mCH patterns are established and the effects of mCH on gene expression remain poorly understood. To address this, we genetically ablated *Dnmt3a* specifically from forebrain excitatory neurons and performed cell type-specific nuclear transcriptome profiling. We uncovered many changes in gene expression, with enrichment for genes targeted by the Polycomb repressive complex 2 (PRC2). Through cell type-specific CUT&TAG profiling, we found that the PRC2-associated histone modification H3K27me3 increases exclusively at loci that lose mCG upon loss of DNMT3A, suggesting an interplay between these two repressive epigenetic pathways. In future studies, we seek to understand how DNMT3A is recruited to the neuronal genome in the developing brain and elucidate how mCH influences gene expression in postmitotic neurons.

Poster 22C | Bioengineering

Entorhinal Amygdala Alpha Coherence as Biomarker of Anxiety for Closed-loop Deep Brain Stimulation: Results of First-in-human Invasive Electrophysiology

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Mentor: Dr. Casey Halpern

Introduction: Nearly 20% of US adults suffer from anxiety-associated disorders, a hallmark symptom of which is imbalanced avoidance-biased approach-avoidance conflict (AAC) behavior. However, a third of this population are non-responders to current psycho-biological treatments, and may benefit from deep brain stimulation (DBS). Closed-loop DBS using behavior-specific biomarker signals has growing therapeutic applications for medically refractory neuropsychiatric conditions. Unfortunately, candidate DBS biomarkers underlying avoidance in anxiety have yet to be validated in humans.
Objective: Localizing signals specific to avoidance in anxiety during AAC, using invasive electrophysiology and network neuroscience.
Method: Our patient, a 55-year old man with no psychiatric conditions, underwent stereo-encephalography (sEEG) evaluation, with 130 contacts targeting corticolimbic regions that are classically associated with the neurobiology of anxiety, as detailed in Figure 1. Intracranial recordings and task-induced avoidance behavior responses were obtained while he completed a validated task for provoking AAC (Clarke et al, 2015). To characterize inter-regional neural relationships, we computed power spectrum for each channel and quantified coherence between every contact in each frequency band. Graph networks were constructed, where nodes corresponded to sEEG electrodes and edge strength was based on coherence, for all trials (31 high-conflict and 45 no-conflict). To quantify information transfer around key regions, clustering coefficient, a measure of interconnectedness surrounding each node was computed.
Result: There is significant ($p<0.05$) broadband spectral power elevation in entorhinal regions within the left amygdala during high-conflict versus no-conflict. Anxiety-induced avoidance behavior is predicted by higher clustering coefficient of the amygdala from alpha band coherence network, ($p=0.027$, $R=0.396$) using linear regression.
Conclusion: Our first-in-human invasive analyses of AAC identified the entorhinal amygdala as a candidate region for therapeutic stimulation in anxiety disorders. These biomarker signals in amygdala: increased power and network clustering, are specific to and predictive of avoidance during conflict. This research lays the foundation of personalized closed-loop DBS targeting for anxiety-associated disorders.

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Boe, Ryan	13A	Mentor: Dr. Arjun Raj *Systematic characterization and manipulation of the trade-off between proliferation and invasion in melanoma*
Bornstein, Marc	14A	Mentor: Dr. Zoltan Arany *Quantifying the Metabolic Response to Acute Cold Exposure in Mice*
Ceron, Rachel	19C	Mentors: Drs. Robert Heuckeroth & Roberto Dominguez *A solution to the long-standing problem of actin expression and purification*
Chaluvadi, Sai	20C	Mentor: Dr. F. Chris Bennett *Creating an in vitro macrophage model for globoid cell leukodystrophy to study globoid cell formation and function*
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Chen, Christina	5A	Mentor: Dr. Russell (Taki) Shinohara *LaxKAT: a more powerful method to test for association and localize signal in high-dimensional data*
Chen, Saisai	11B	Mentor: Dr. Lewis Chodosh *PAQR8 Promotes Breast Cancer Recurrence and Confers Resistance to Multiple Therapies*

Chini, Julia	24A	Mentor: Dr. David Hill *Hepatic CD9 regulates adipose tissue inflammation and metabolic dysfunction during obesity*
Coffey, Nate	12B	Mentors: Drs. Celeste Simon & Zoltan Arany *Increasing branched-chain amino acid metabolism reduces growth of clear cell renal cell carcinoma*
Cohn, Ian	18A	Mentors: Drs. Christopher Hunter & Boris Striepen *Distinct roles for cDC1s and cDC2s in CD4+ T cell responses against the intracellular parasite Cryptosporidium*
Connolly, Daniel	21C	Mentor: Dr. Zhaolan (Joe) Zhou *Interplay between DNA methylation and Polycomb silencing in mammalian brain development*
Cox, Tim	26B	Mentors: Drs. Virginia Lee & Christoph Thaiss *The Aged Microbiome Drives Cognitive Decline via Vagal Inhibition*
Creekmore, Ben	3A	Mentors: Drs. Edward B. Lee & Yi-Wei Chang *Small Molecule Activation of Valosin-containing protein (VCP)*
Dai, David	27B	Mentor: Dr. Edward B. Lee *Single nucleus transcriptome analysis of human reactive astrocytes*
Danan, Charles	8C	Mentor: Dr. Kate Hamilton *N6-Methyladenosine (m6A) regulates the response to hypoxic stress in the intestinal epithelium*
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Deshpande, Rajiv	6A	Mentor: Dr. Felix Wehrli *Quantification of bilateral renal oxygen consumption: a preliminary study*
Dou, Dan	28B	Mentor: Dr. Erika Holzbaur *Distinct pathogenic mutations in LRRK2 disrupt axonal transport of autophagosomes*

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Komar, Chad	17C	Mentors: Drs. Gerd Blobel & Junwei Shi *Domain-Focused, Whole-Genome Screen to Identify Fetal Hemoglobin Regulators in Human Erythroid Cells*
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