21ST ANNUAL GDBBS DSAC STUDENT RESEARCH SYMPOSIUM

Wednesday, March 27th, 2024

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Wednesday, March 27th, 2024

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Oral Presentation Abstracts

Session 1:

Cellular Development and Pathways

9:00AM

Katherine Westover, GMB **9:00AM**

Genome-wide dysregulation of R-loops in Ataxia Telangiectasia neurological pathogenesis

Katherine Westover[1], Yingzi Hou[1], Yangping Li[1], and Bing Yao[1]

[1]Department of Human Genetics, Emory University, Atlanta, GA

Ataxia Telangiectasia (AT), a neurodegenerative disease characterized by cerebellar degeneration of neurons that control balance and movement, affects 1 in 40,000 to 100,000 people worldwide. A recessive early childhood onset disorder, AT is caused by mutations within the ataxia telangiectasia mutated (ATM) threonine/serine kinase which plays crucial roles within the DNA damage response (DDR). The molecular mechanisms underlying AT pathogenesis and how ATM loss-of-function leads to deficient DDR remain elusive. R-loops, three stranded RNA-DNA structures composed of a DNA-RNA hybrid and a non-template DNA strand, have emerged as key components of double strand break (DSB) DDR, playing critical roles in both causing and responding to DSBs. As DSBs and the failure of their repair play major roles in the pathology of AT, R-loop dysregulation likely contributes to AT pathogenesis. A kinase substrate of ATM, methyltransferase like 3 (METTL3) protein, a N^6-methyladenosine (m6A) methyltransferase, places m6A on the RNA strand of R-loops impacting R-loop formation. The relationship between ATM-METTL3 phosphorylation in response to DNA damage and regulation of R-loop formation through m6A deposition has yet to be defined. Our data shows that induction of DSBs results in increased accumulation of R-loops. We see a global trend towards a loss of R-loop loci compared to healthy controls. Many of these lost loci are rescued when the pathogenic mutation is corrected by genome editing and some are associated with AT symptom processes. We hypothesize that in AT, the lack of METTL3 phosphorylation by ATM could globally dysregulate R-loop formation and underly AT progression.

Tori Placentra, GMB **9:15AM**

Homeostatic control of intestinal stem cell renewal by two transcriptional coactivators

Tori Placentra[1], Shilpi Verghese[1], Ken Moberg[1]

[1]Department of Cell Biology, Emory University, Atlanta, GA

Mechanisms that integrate local and systemic cues to precisely regulate stem-cell based tissue renewal remain unclear, particularly in the rapidly cycling intestinal epithelium. A pair of transcriptional coactivator proteins that promote growth and intestinal stem cell renewal – the Hippo pathway protein YAP1 and the nuclear receptor (NR) interactor NCOA3 – are each implicated in the progression of colorectal cancers. Both factors have homologs in the common fruit fly, *D. melanogaster*, which combines robust fertility, rapid generation time, a relatively simple genome, and an extensive genetic toolbox to make an ideal setting to investigate gut renewal mechanisms. Prior studies established *Drosophila* homologs of YAP1 (Yorkie; Yki) and NCOA3 (Taiman; Tai) physically interact through respective WW (tryptophan-tryptophan) domains and PPxY (proline-proline-x-tyrosine) motifs. Genetic evidence indicates that the Yki-Tai complex results in synergy between the Hippo and NR pathways during epithelial overgrowth and inter-tissue invasion. Critically, Yki and Tai have each been shown to promote intestinal stem cell (ISC) proliferation. We hypothesize that Hippo/NR cooperativity based on the Yki-Tai complex activates a yet undefined set of target genes involved in asymmetric ISC divisions and gut maintenance. We generated a powerful tool to test this hypothesis: a CRISPR mutant fly in which the PPxY motifs of endogenous Tai are converted to PPxA, blocking Tai's binding to Yki. These viable homozygous mutants (tai^{PPxA}) allow precise and selective decoupling of the Tai/NR and Yki/Hippo pathways *in vivo*. Preliminary data suggest that tai^{PPxA} animals are sensitized to gut damage and have reduced ISC pools in the adult midgut.

Kate Hardin, BCDB **9:30AM**

Fascin1 regulates axonal development and brain wiring

***K. R. Hardin**[1], C. Ye[1], E. Bian, K. H. Moberg[1], K. R. Myers[1], and J. Q. Zheng[1,2,3]

[1]Dept. of Cell Biology, Emory Univ. School of Med., Atlanta, GA
[2]Dept. of Neurology, Emory Univ. School of Med., Atlanta, GA
[3]Center for Neurodegenerative Diseases, Emory Univ. School of Med., Atlanta, GA

Abstract

Axon guidance is a critical developmental process in which axonal projections are guided by the tips of axons, called growth cones, to their specific targets for precise wiring of the central nervous system. Growth cones are powered by two actin-rich membrane protrusions, lamellipodia and filopodia. Filopodia are finger-like protrusions supported by bundled actin filaments that sense a wide range of extracellular guidance cues. The pharmacological elimination of growth cone filopodia results in axon guidance defects without impeding axon elongation; however, the molecular and cellular mechanisms that regulate growth cone filopodia dynamics remain unknown.

Fascin1 is a ~55 kDa actin bundling protein that crosslinks actin filaments to form tight F-actin bundles in filopodia and is a known regulator of cell migration. Fascin1 is highly expressed in developing neurons and enriched in growth cone filopodia, but its role in growth cone motility and guidance has not been investigated.

Here, we use an i*n vivo* approach to investigate the role of fascin1 in axon development. We demonstrate how the loss of Singed, the *Drosophila melanogaster* ortholog of fascin1, affects *in vivo* brain wiring and function. We found that *singed* null flies exhibit marked axonal defects in the mushroom body, a center for learning and memory. We have also discovered phototaxis and negative geotaxis (climbing) defects in *singed* null flies that we speculate are due to circuitry errors. Together, our work highlights the important role of Fascin1 in actin-based axon development and brain wiring.

Madison Bangs, MSP **9:45AM**

Resolving race and sex-based heterogeneity in Alzheimer's Disease through network-based molecular subtyping of the CSF proteome

Madison C. Bangs[1,2], E. Kathleen Carter[1,3], Eric B. Dammer[1,2], Anantharaman Shantaraman[1,3], Duc M. Duong[1,2], Luming Yin[2], Caroline Watson[1,3], James J. Lah[1,2,3], Allan I. Levey[1,2,3], Nicholas T. Seyfried[1,2,3]

[1]Center for Neurodegenerative Disease, Emory University School of Medicine, Atlanta, GA
[2]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA
[3]Department of Neurology, Emory University School of Medicine, Atlanta, GA

Alzheimer's Disease (AD) is categorized by the accumulation of amyloid-beta plaques and Tau tangles within the brain, leading to dementia and cognitive decline. A wide range of genetic and demographic factors, such as race, sex, and APOE genotype intersect to drive axes of AD pathology, resulting in a molecular heterogeneity that makes AD incredibly complicated to diagnose and treat. For example, recent data has shown levels of cerebrospinal fluid (CSF) Tau, a key AD diagnostic biomarker, are lower in African Americans, which leads to later diagnosis, lower enrollment in clinical trials, and exclusion from biomedical sample collection. Thus, there is a pressing need for an effective model for characterizing the differing molecular manifestations of AD across diverse populations. To better resolve this complexity, we have established a novel integrated protein co-expression network and modularity clustering approach to resolve six distinct proteomic subtypes across 483 CSF samples, including almost 150 from African American participants. Of these subtypes, Subtype 3, which was comprised evenly of AD and control subjects and enriched with African Americans and men, was of particular interest, as it displayed CSF amyloid-beta levels consistent with AD-like subtypes, but reduced levels of Tau, consistent with control-like subtypes. Subtype 3 also had very high levels plasma enriched components such as albumin, immunoglobulins, and proteolytic enzymes, likely indicating a breakdown of the blood brain barrier and infiltration of these components into the CSF, which we hypothesize is responsible for the cleavage and depletion of CSF Tau in this population.

Session 2:

Genomics and Gene Regulation

10:15AM

Lydia Gutema, BCDB	**10:15AM**

The DNA structural landscape at the centromere and pericentromere

Lydia Gutema[1]**,** Shannon Ooi[1], Jitendra Thakur[1]

[1]Department of Biology, Emory University, Atlanta, GA

Centromeres are chromosomal loci that bind to spindles and facilitate chromosome segregation, ensuring faithful inheritance of genetic information in daughter cells. Issues during centromere formation can result in the gain or loss of genetic material in daughter cells, which can have deleterious effects on human health. DNA sequences at the endogenous centromeric locus are highly repetitive, and while there is some sequence similarity, centromeric sequences at different chromosomes are distinct from each other. Despite the lack of conserved sequences, centromeric proteins are recruited to the correct site during each round of cell division. How centromeric DNA encodes the centromere in the absence of conserved sequences is not well understood. In rare events, centromeres can form ectopically, and their ectopic loci have a non-random distribution across the genome, which raises interesting questions about how centromeres assembly at these sites. However, conserved features of DNA at ectopic centromeres and endogenous centromeres have not been discovered yet. We hypothesize that DNA structures play a role in specifying the sites of centromere formation. Our early findings show a distinct change in patterns of certain DNA structures within the active centromeric region and the surrounding inactive pericentromeric regions. This work will allow us to address longstanding questions in the field about the contribution of centromeric DNA and how unique DNA structures play a role in genome stability.

Yonina Loskove, GMB **10:30AM**

Investigating the Role of *ARID1B* in Gene Regulation and Neurodevelopmental Disorders

Yonina Loskove[1], Allen Wang[2], Kevin Peterson[3], David Gorkin[1]

[1]Department of Biology, Emory University, Atlanta, GA
[2]Center for Epigenomics, University of California San Diego, La Jolla, CA
[3]The Jackson Laboratory, Bar Harbor, ME

Neurodevelopmental Disorders (NDDs) are a group of highly heterogenous neurological conditions that present with a wide range of cognitive, social, and behavioral deficits**.** One of the most enriched genes for *de novo* coding mutations in NDDs is *ARID1B*, which encodes a subunit of the BRG1/BRM-Associated Factor (BAF) chromatin remodeling complex. Despite *ARID1B* being one of the mutated genes in NDDs, it is not well understood how *ARID1B* dysfunction impacts key gene regulatory networks essential for neuronal development. To bridge these gaps in knowledge, I will characterize *Arid1b* halpoinsufficient (*Arid1b*$^{+/-}$) and null (*Arid1b*$^{-/-}$) mice at multiple time points of embryogenesis at the single-cell level. This will allow me to identify cell type specific patterns of dysregulation to gene expression and chromatin accessibility during brain development. Currently, I have assayed six E15.5 micro-dissected forebrain samples and leveraged snMultiome data (snRNA + snATAC-seq) to profile their transcriptomes and chromatin landscapes. In preliminary analyses I have observed differences in the enrichment of functionally related gene sets in the *Arid1b* mutants relative to wildtypes. Gene sets associated with translation in the cytoplasm and at synapses are overrepresented in the *Arid1b*$^{-/-}$ mice, demonstrating alterations at the molecular level are occurring due to loss of *Arid1b*. Further analyses are underway to determine other gene sets that are affected by the loss of *Arid1b*, and in which specific cell types. Ultimately, these analyses will identify what gene pathways and regulatory networks are dysregulated during brain development in individuals with mutations in *ARID1B*, which leads to NDDs.

James Rose, GMB **10:45AM**

Clinically inactive SLE resting naïve B cells retain distinct transcriptomes and lasting alterations to the epigenome

James R. Rose[1], Yusho Ishii[2,3], Sakeenah Hicks[1], Christopher D. Scharer[1], Scott A. Jenks[2,3], Iñaki Sanz[2,3], and Jeremy M. Boss[1]

[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA
[2]Division of Rheumatology, Department of Medicine, School of Medicine, Emory University, Atlanta, GA, USA.
[3]Lowance Center for Human Immunology, School of Medicine, Emory University, Atlanta, GA, USA.

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by episodes of increased disease activity followed by periods of inactivity and remission. This study delves into the distinct molecular landscapes of B cells from active and inactive SLE patients, specifically from a cohort of adult female SLE patients of primarily black ancestry. We reveal a unique transcriptome and epigenome in B cells from patients with inactive disease, one which differs both from healthy controls and active disease patients. The transcriptional signature of all SLE rN B cells suggests abnormal activation of signaling pathways, including shared and disease activity-specific elements. Type I Interferon (IFN) response pathways are present in active and inactive states, with some genomic regions epigenetically dysregulated to a similar degree despite diminished symptoms in inactive disease. Subsets of the IFN response pathway as well as TLR signaling genes, however, are uniquely associated only with active disease. In contrast, transcriptional evidence suggests pathways like CD40, TNF/NFkB, and other activation pathways are exclusive to inactive disease rN cells. Chromatin accessibility data suggest active disease is correlated with IRF transcription factor activity, with inactive disease showing accessibility at only a subset of these regions. Analysis of inactive disease chromatin suggested activity of BHLH family factors such as MEF2C or E2A/E2-2 in promoting the unique phenotype found in these cells. Overall, this work sheds light on the intricate molecular mechanisms underlying SLE disease activity in B cell populations, providing valuable insights and potential therapeutic implications for the treatment of long-term disease.

Kyndal Goss, IMP **11:00AM**

Multiomic analysis of γMSC-suppressed alloreactive T cells

Kyndal Goss[1,2,3], Elisabetta Foppiani[2,3], Swathi Shrihari[1,2,3], Lisa Daley-Bauer[2,3], Greg Gibson[4], Ed Horwitz[2,3]

[1]Laney Graduate School, Emory University, Atlanta, GA
[2]Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA
[3]Department of Pediatrics, Emory University School of Medicine, Atlanta, GA
[4]Georgia Institute of Technology, Atlanta, GA, United States

A complication of hematopoietic cell transplantation is the development of graft-versus-host disease (GVHD), an immune-mediated disorder arising when adoptively transferred donor naive T cells become activated by host APCs and damage tissues. It is crucial to find an alternative GVHD prophylaxis that is more effective and less toxic than what is available. Mesenchymal stromal cells (MSCs), with powerful immunomodulatory properties potentiated by interferon γ priming (γMSCs), can suppress alloreactive T cells but the mechanism is not fully understood. Preliminary experiments with γMSC-suppressed alloreactive T cells revealed a deficit of reactive oxygen species within the first 24 hours which is associated with a prompt cell cycle arrest along with metabolic changes. The perturbations in redox homeostasis, cell cycle, and metabolism suggest that γMSCs induce a complex systems effect in activated T cells. We hypothesize that this effect is primarily driven by chromatin remodeling, leading to epigenetic dysregulation of gene expression of key cell cycle genes. We obtained simultaneous scRNAseq and scATACseq to identify the genetic and epigenetic changes which underlie the suppressive effects. We activated human T cells with allogeneic DCs over 6 days, with and without 48 hours of γMSC exposure. We observed remarkable differences in both genetic and epigenetic landscapes between control and suppressed T cells. Additionally, suppression is completely reversible, which is key for clinical applications as patients receiving γMSC therapy will retain normal T cell function. We are currently focusing our efforts on validating specific transcriptional perturbations in vitro and in vivo.

Elizabeth Feldman, GMB **11:15AM**

Assessment of potential loss of function variants in children with Down Syndrome-associated congenital heart defects as possible modifying factors

Elizabeth Feldman[1], David J. Cutler[1], Tracie C. Rosser[1], Stephanie B. Wechsler[1], Lauren Sanclemente[2], Angela Rachubinski[3], Karen R. Rabin[2], Michael Wagner[4], Bruce D. Gelb[5], M. Espinosa[3], Philip J. Lupo[2], Stephanie L. Sherman[1], Elizabeth J. Leslie[1]

[1]Emory University, Atlanta, GA
[2]Baylor College of Medicine, Houston, TX
[3]University of Colorado Anschutz Medical Campus, Aurora, CO
[4]Cincinnati Children's Hospital Medical Center, Cincinnati, OH
[5]Icahn School of Medicine at Mount Sinai, New York, NY

Congenital heart defects (CHDs) occur in 40-50% of children with Down syndrome (DS). Since half of infants with DS have structurally normal hearts, additional factors may modify risk for CHD in DS. We leveraged whole genome sequencing to analyze rare predicted loss of function (pLoF) variants in children with DS and atrioventricular septal defects (AVSD; n=438), atrial septal defects (ASD; n=122), ventricular septal defects (VSD; n=170), other CHDs (n=156) or normal hearts (NH; n=572). We tested for enrichment of pLoF variants exome-wide and in 611 genes from CHD clinical testing panels. Rare pLoF variants were not enriched in DS+CHD (p=0.96). However, individual DS+CHD cases (n=25) with heterozygous pLoF variants associated with autosomal dominant CHD were found in 17 genes from the gene list, including: *EP300*, *SMC3*, *ROBO1*, *EVC2*, and *LAMA4*. We also tested the hypothesis that rare, pLoF variants outside of chr21 could modify CHD by interacting with heart-expressed genes on chr21. To test for an interaction, we asked if genes with pLoF variants in DS+CHD were more directly connected to chr21 genes than genes with pLoF variants in DS+NH. Genes with pLoF variants in DS+AVSD ($p=6.22x10^{-9}$), DS+ASD ($p=2.94x10^{-31}$), and DS+VSD (p=0.006) cases had more direct interactions with heart-expressed genes on chr21 compared to those in DS+NH. Taken together, a small fraction of CHD may be caused by rare pLoF on genes outside of chr21 and being a carrier for rare pLoF variants in genes which interact with triploid chr21 genes may increase risk for developing DS-associated septal defects.

Session 3:

Molecular Mechanisms of Therapeutics

11:45AM

André Cuevas, BCDB **11:45AM**

Discovery and development of selective steroidogenic factor-1 small molecules by *in silico* screening

André R. Cuevas[1], Michael Lee Cato[1], Racheal Spurlin[2], Eric A. Ortlund[1]

[1]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA
[2]Department of Chemistry, Emory University, Atlanta, GA

The adrenal glands produce steroid hormones that regulate blood pressure, stress, and generate necessary precursors for peripheral sex steroid formation. Impaired adrenal steroidogenesis is associated with cardiometabolic, immune, and psychiatric diseases. Steroidogenic factor-1 (SF-1), a ligand-regulated transcription factor, controls expression of key cytochromes and hydroxylases in the steroidogenic pathway that support the entirety of steroidogenesis in the adrenals and gonads. Genetic reduction of SF-1 expression and activity can repress the proliferation of lethal adrenal neoplasms, but pharmaceutical approaches are largely unexplored and potent, SF-1 selective compounds are nonexistent. SF-1 is activated by phospholipids, but lipids are poor therapeutics and biochemical probes due to insolubility, rapid metabolism, and integration into membranes. Therefore, synthetic modulators of SF-1 activity are necessary to probe SF-1 biology and dynamics. Our lab has generated potent, high-affinity small molecule modulators of SF-1 activity. While these compounds are excellent scaffolds for subsequent drug design, they exhibit cross-reactivity with a close SF-1 homolog, liver receptor homolog-1 (LRH-1). Here, I employ high-throughput, *in silico* techniques to uncover novel, selective SF-1 modulators. Hits from high-throughput *in silico* screening will be modeled in the SF-1 binding pocket to predict binding poses and allostery to the coregulator interaction surface. Promising allosteric modulators will be tested for *in vitro* binding and activation of SF-1 and LRH-1. This work will produce effective biochemical tools for probing SF-1 activity in isolation from LRH-1 in live animal and tissue culture models and serve as the basis for developing novel therapeutics for treating steroidogenic organ diseases.

Jordan Silva, CB **12:00PM**

Enhancing γδ T cells to Treat Osteosarcoma

***Jordan* Silva**[1,2], Bing Yu[3], Harrison Brown[3], Brian Petrich[3], Trent Spencer[1,3]

[1]Department of Pediatrics, Aflac Cancer and Blood Disorders Center, Emory University School of Medicine, Atlanta, GA
[2]Cancer Biology Graduate Program, Graduate Division of Biological and Biomedical Sciences, Emory University School of Medicine, Atlanta, GA
[3]Expression Therapeutics, Tucker, GA

New therapies for osteosarcoma (OS) are needed as the survival rate for high-risk patients is <27%, and patient survival has not significantly changed in the past 40 years. Cellular immunotherapy for OS has focused on autologous products of αβ T cells and has been largely unsuccessful. Based on the multi-killing properties of allogeneic γδ T-cell therapies, engineering these cells to secrete a bispecific T-cell engager (sBiTE) targeting PTK7 could overcome hurdles associated with αβ cellular therapies. A second activation strategy involves introducing zoledronate (zol) to augment γδ T cell killing by inducing phosphoantigens in treated cells, recognized by the γδ TCR. Administering ifosfamide, a chemotherapy used for OS, induces stress antigens in target cells, recognized by NKG2D receptors on γδ T cells, is a third activation strategy. Therefore, we hypothesize that inducing multiple mechanisms of γδ T cell killing can provide an effective anti-OS chemoimmunotherapy. *In vitro* studies show that conditioning OS tumor cell lines with zol before co-culturing with γδ T cell, at 1:1 effector:target cell ratio, significantly increases target cell death. Similarly, PTK7-CD3 sBiTE γδ T cells kill OS cells robustly. *In vivo* studies showed non-modified and PTK7-CD3 sBiTE γδ T cells administered to zol conditioned mice increased overall survival compared to γδ T cells alone. Together, these data support our hypothesis that engaging the various targeting mechanisms of γδ T cells can provide enhanced killing of OS, which supports ongoing investigations to optimize the use of γδ T cells in a chemoimmunotherapy approach.

Saahj Gosrani, NS **12:15PM**

Investigating how inhibition of the histone demethylase LSD1 leads to neurodegeneration in Alzheimer's disease

Saahj Gosrani[1], Sonia Dalal[1], Yu Bai[1] and David Katz[1,2]

[1]Department of Cell Biology, Emory University, Atlanta, GA
[2]School of Medicine, Emory University, Atlanta, GA

H3K4me1/2 is associated with active transcription. The histone demethylase LSD1/KDM1A removes this modification to repress transcription. Extensive data from our lab suggest that pathological neurofibrillary tau tangles (NFTs) may drive neurodegeneration in Alzheimer's disease (AD) by sequestering LSD1 in the neuronal cytoplasm, interfering with continuous LSD1-dependent repression of neurodegenerative transcriptional pathways. To investigate this further, we first performed H3K4me2 chromatin immunoprecipitation (ChIP) in the hippocampus of our inducible LSD1 knockout mice to determine how LSD1 loss affects transcription. Our expectation was H3K4me2 increased at upregulated genes. Surprisingly, H3K4me2 increases not only at genes that are activated following LSD1 deletion, but at genes that are unchanged and repressed. This suggests that LSD1 maintains the terminally differentiated state of neurons by demethylating H3K4me2 and providing a thermodynamic barrier to transcription genome-wide. Required neuronal genes easily overcome this barrier. However, in AD when LSD1 is inhibited, neurodegenerative pathways vulnerable to activation are inappropriately expressed. To determine whether LSD1 is functionally inhibited by tau, we also performed ChIP in PS19 mice, a mouse model of Tauopathy. Our preliminary data suggest that, as pathological tau burden increases in the neuronal cytoplasm over time, there is a genome-wide increase in H3K4me2, compared to WT controls. This is consistent with the possibility that LSD1 is inhibited by pathological tau, as our previous data suggest.

Session 4:

Infectious Disease & Microbes

3:00PM

Sydney Bergstresser, IMP	**3:00PM**

Venetoclax treatment drives Caspase-3 expression within CD4+ T cells in a CD8α depleted, SIV-infected, ART-suppressed Rhesus macaques.

Sydney Bergstresser[1], Diane Carnathan[1], Liang Shang[2], Rebecca Shoemaker[3], Brandon Keele[3], Christine M. Fennessey[3], Hong Wang[1], Vanessa Lewis[1], Yohannes Abraham[1], Jeff Lifson[3], Mirko Paiardini[1], Guido Silvestri[1], and Deanna Kulpa[1]

[1]Department of Pathology, School of Medicine, Emory University, Atlanta, GA
[2]Center for AIDS Research, Virology and Molecular Biomarkers Core, Emory National Primate Research Center, Emory University, Atlanta, GA
[3]AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Frederick, MD

Approximately thirty-nine million people living with HIV(PLWHIV) worldwide remain without access to a cure, despite the development of suppressive anti-retroviral therapy(ART). The main obstacle to this cure is a small pool of persistent CD4+ T cells that harbor integrated, transcriptionally inactive proviral DNA known as the viral reservoir. Historically, studies found that within PLWHIV on ART these cells express high levels of the anti-apoptotic protein B cell lympoma-2 (BCL-2), particularly within CD4+ T cells that express gag versus their non gag expressing counterparts. BCL-2 overexpression could lend to enhanced resistance to CD8+ cytotoxicity and viral infection induced apoptosis, resulting in the observed persistence *in vivo.* Venetoclax is an FDA approved drug for several leukemias and functions by sequestering BCL-2 from BAK and BAX. BCL-2 regulates apoptosis by preventing mitochondrial perforation, which is a critical step the intrinsic apoptotic pathway. Here we show that in a latency reversal on ART macaque model of HIV infections, Venetoclax treatment with or without anti-SIV *env* broadly neutralizing (BnAb) monoclonal antibodies results in increased caspase-3(Csp-3) expression in the total CD4+ compartment. Mitochondrial membrane integrity (MMI), measured by a mitochondrial metabolic dye, was observed to be negatively impacted in Venetoclax treated animals and the frequency of MMI compromised, csp-3+ cells in total CD4+ cells is elevated in Venetoclax treated animals. Phenotypic changes in treated animals on the memory subsets of the CD4 compartment include elevated PD-1, CD25, CXCR5, and Ki67. This study provides a proof of concept for inducing targeted apoptosis in the viral reservoir.

Sebastian Duran-Ahumada, PBEE **3:15PM**

Wolbachia-mediated effects on the fitness and performance of *Aedes aegypti* under field-like variable temperature and initial larval densities

Sebastian Duran-Ahumada[1,2], Luiza Karrer[2,3], Chun Cheng[2,4], Isabella Roeske[2], Josie Pilchik[2], David Jimenez-Vallejo[1,2], Emily Smith[1], Kristina Roy[2,5], Oscar D. Kirstein[2,6], Abdiel Martin-Park[7], Yamili Contreras-Perera[7], Azael Che-Mendoza[7], Gabriela Gonzalez-Olvera[7], Henry N. Puerta-Guardo[7], Sandra I.Uribe-Soto[8], Pablo Manrique-Saide[7], Gonzalo Vazquez-Prokopec[2]

[1]Population Biology, Ecology, and Evolution Graduate Program. Emory University, Atlanta, Georgia, USA.
[2]Department of Environmental Sciences, Emory University, Atlanta, Georgia, USA.
[3]New York Medical College, Touro University, Valhalla, New York, USA.
[4]Rollins School of Public Health, Emory University, Atlanta, Georgia, USA.
[5]Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.
[6]Laboratory of Parasitology and Entomology, Ministry of Health, Jerusalem, Israel.
[7]Laboratory for the Biological Control of *Aedes* aegypti, Collaborative Unit for Entomological Bioassays (UCBE-LCB). Autonomous University of Yucatan, Merida, Mexico.
[8]Sciences Faculty. National University of Colombia – Medellin Campus. Medellin, Antioquia, Colombia.

Certain strains of *Wolbachia pipientis*, a ubiquitous bacterium present across the insect world, can negatively impact the fitness and capacity to transmit disease when transfected into *Aedes aegypti*. Most studies examine these impacts in a limited set of environmental regimes and under controlled conditions. Here we seek to understand the impacts of environmentally relevant conditions like larval density, temperature, and their interaction on *w*AlbB-infected *A. aegypti* using a factorial design. We measured *w*AlbB stability (post-emergence relative density in females and progeny), *w*AlbB's ability to induce cytoplasmic incompatibility, and *w*AlbB's effects on mosquito fitness (fecundity, fertility, teneral mass) and performance (adult survival, time to pupation) across two temperature regimes (fluctuating and constant) and two larval densities (low and high). We found differential effects of all treatments (*Wolbachia* infection status, temperature, larval density) across mosquito sexes and life stages. Fluctuating temperature (27-40°C) led to decreased post-emergence *w*AlbB density and increased *w*AlbB density in eggs. An increased fecundity was found in *w*AlbB-carrying females reared at fluctuating temperature cycles compared to uninfected wildtype females. *w*AlbB-carrying females showed significantly increased survival than wildtype females. Contrarily, *w*AlbB-carrying males exhibited a significantly lower survival than wildtype males. Taken together, our results indicate that realistic conditions may not impact the stability of *w*AlbB infection in *Ae. aegypti*. Nonetheless, understanding the ecological consequence of *A. aegypti*-*w*AlbB interaction is complex due to life history tradeoffs under conditions faced by natural

populations. Such complexity was evident in our study, with no universal signal of the impact of *w*AlbB throughout all experiments.

Will McFadden, BCDB	**3:30PM**

TSAR, Thermal Shift Analysis in R, identifies *in vitro* interactions between the HIV-1 capsid and endogenous molecules.

***W. M. McFadden*[1,2†],** X. Gao[1,2,†], X. Wen[1,2], S. R. Harvey[3], A. Emanuelli[1,2], Z. C. Lorson[1,2], H. Zheng[1,2], K. A. Kirby[1,2], Z. Wang,[4] V. H. Wysocki[3], S. G. Sarafianos[1,2,*]

[1]Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA
[2]Children's Healthcare of Atlanta, Atlanta, GA
[3]Department of Chemistry and Biochemistry and Resource for Native Mass Spectrometry Guided Structural Biology, The Ohio State University, Columbus, OH
[4]Center for Drug Design, College of Pharmacy, University of Minnesota, Minneapolis, MN
[†]These authors contributed equally

Thermal shift assay (TSA) is a versatile technique for studying protein-ligand interactions *in vitro* using a standard qPCR machine and purified protein samples. Here, we report a free, open-source software tool *TSAR* (Thermal Shift Analysis in R) to expedite and automate the analysis of TSA experiments. The *TSAR* package can identify T_m values (50% protein unfolding temperature) using either Boltzmann-fit or derivative modeling, which can be used for comparisons and statistical analysis. The package is written in the R coding language and available in the Bioconductor repository (V ≥3.18) at https://bioconductor.org/packages/TSAR. *TSAR* includes numerous command-line functions for experienced R users but also includes a graphic user interface (GUI) that enables easy use and interactions with the data for non-programmers. The TSAR package is a complete suite of tools from initial data processing, T_m identification, automated data comparisons, and visualization of publication-quality graphics at any library scale. To exemplify the utility of *TSAR,* we screened a chemical library of vitamins and endogenous metabolites against the capsid protein (CA) of human immunodeficiency virus type 1 (HIV-1) by TSA to identify interacting molecules. Our data suggest that hexameric CA interacts with multiple endogenous metabolites *in vitro*. Further investigation shows that select metabolites impact the rate of CA assembly, impair reverse transcription, and decrease viral infectivity. The role and relevance of these molecules in HIV-1 biology remains an ongoing topic of investigation, but the findings of our screen reported here show the utility of the *TSAR* package to identify hit molecules of interest.

Dormarie Rivera Rodriguez, IMP **3:45PM**

Impact of antibiotic treatment on Lactobacillus population dynamics and intestinal immune homeostasis

Dormarie E. Rivera Rodriguez[1,2], Naomi Rodriguez Marino[1], Emma Seto[1], Walter M. Avila[1,3], Charlotte J. Royer[1,4], Isabelle Gracien[1], David S. Weiss[2#], Luisa Cervantes-Barragan[1#]

[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA
[2]Division of Infectious Diseases, Emory Vaccine Center, Emory School of Medicine, Atlanta, GA
[3]Present address: Data Science, School of Graduate Studies, Fisk University, Nashville, TN
[4]Present address: Computational Biology and Bioinformatics, School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA
[#]co-corresponding author

Antibiotics are our most important tool for treating bacterial infections. Many broad-spectrum antibiotics, like oral ciprofloxacin, are commonly prescribed for gastrointestinal infection treatment. However, antibiotic overuse promotes resistance in many bacteria, leading to a global crisis in infection treatment. During treatment, the microbiota is affected by antibiotics too. Previous studies showed that changes in the microbiota alter mucosal immunity. In this study, we aim to understand how clinically relevant doses of broad-spectrum antibiotics impact the diversity of microbiota species and change the intestinal regulatory immune system. We examined if oral ciprofloxacin affects intestinal immune homeostasis, especially in IL-10 production. We treated an IL-10 GFP reporter mice with ciprofloxacin for two weeks. Our results show that IL-10 production from small intestinal T cells increases during ciprofloxacin treatment. Analyses of the microbiota composition by 16S rRNA sequencing showed an expansion of Lactobacilli in the ileum. We hypothesize that some Lactobacilli species have different resistance levels to ciprofloxacin. Our results show that *Lactobacillus reuteri* and *Lactobacillus johnsonii* are more resistant to ciprofloxacin in comparison to *Lactobacillus murinus*. Interestingly, we observed that oral ciprofloxacin decreases *L. murinus* and expands *L. johnsonii* and *L. reuteri*. We conclude that oral ciprofloxacin enriches resistant *L. reuteri* and *L. johnsonii* species, which may alter species population dynamics in the microbiota. Additionally, we conclude that ciprofloxacin increases the IL-10 production from small intestinal T cells. Future experiments will focus on if Lactobacilli expansion during ciprofloxacin treatment increases IL-10 production in small intestinal T cells.

Brooke Talbot, PBEE **4:00PM**

Metagenome-wide characterization of shared antimicrobial resistance genes in sympatric people and lemurs in rural Madagascar

Brooke M. Talbot,[1,2], Julie A. Clennon[3], Fara Rakotoarison[4], Lydia Rautman[3], Sarah Durry[5], Leo J. Ragazzo[3], Patricia C. Wright[4,6], Thomas R. Gillespie[3,4,5], Timothy D. Read[2]

[1]Graduate Division of Biology and Biomedical Sciences, Emory University, USA
[2]Division of Infectious Diseases, School of Medicine, Emory University, USA
[3]Department of Environmental Sciences, Emory University, USA
[4]Centre ValBio, Ranomafana, Madagascar
[5]Department of Environmental Health, Emory University, USA
[6]Institute for the Conservation of Tropical Ecosystems, Stony Brook University, USA

Tracking the spread of antibiotic resistant bacteria is critical for reducing the global morbidity and mortality of humans and animals. Antibiotic resistance genes (ARGs) can be carried on mobile genetic elements, and these elements may be shared from resistant to susceptible bacteria. The scope of ARGs in bacterial microbiomes of wild animal hosts and how they facilitate antibiotic resistance transfer to humans is not well characterized. Using metagenomics, we identified and compared the abundance of bacterial species and ARGs detected in gut microbiomes from sympatric humans and wild mouse lemurs in a forest-dominated, roadless region of Madagascar at the interface of Ranomafana National Park. We examined the contribution of demographic factors among human residents to differences in ARG abundance. Finally, we compared the genomic similarity of ARGs shared between human and lemur microbiomes. Bacterial species and ARG diversity between human and lemur microbiomes were distinct in both alpha and beta diversity measure, with human microbiomes having a higher burden of ARG abundance but a similar number of detectable alleles to lemur microbiomes. Human and lemur microbiomes shared 14 distinct ARGs which were highly conserved in nucleotide identity between sample source pairwise comparisons. Synteny of ARG-associated assemblies revealed that there is a distinct multidrug-resistant gene cassette carrying *dfrA1* and *aadA1* present in human and lemur microbiomes without evidence of geographic overlap, suggesting that these resistance genes could be widespread in this ecosystem. This study highlights the need to investigate intermediary processes of ARG spread that could maintain populations of drug-resistant bacteria.

Session 5:

Metabolic Pathways and Immune Function

4:30PM

Julia Bazzano, IMP **4:30PM**

Ganglioside metabolism functionally regulates the spectrum of CD8$^+$ T cell alloreactivity

Julia M. R. Bazzano[1], Kirsten Baecher[1], Danya Liu[1], Miguel Fribourg[2], Paolo Cravedi[2], Peter Heeger[2], Mandy L. Ford[2].

[1]Emory Transplant Center, Department of Surgery, Emory University, Atlanta, GA.
[2]Center for Translational Transplant Research, Icahn School of Medicine at Mount Sinai, New York NY.

A critical step in alloreactive T cell activation is the recognition of donor antigen. However, the pathways governing strength of T cell allorecognition are incompletely understood. Here, we identified a novel regulator of TCR expression that controls the extent of the allogeneic response. Using data from the CTOT-09 clinical trial in which kidney transplant recipients were weaned from immunosuppression, CD8$^+$ T cells from stable patients were found to exhibit increased transcript levels of the glycosphingolipid-catabolizing protein *Gm2a* compared to patients who went on to reject. To investigate the role of Gm2a in alloimmunity, we performed skin graft surgery in wildtype recipients of wildtype *vs* *Gm2a*$^{-/-}$ CD8$^+$ T cells. Results indicate that recipients of *Gm2a*$^{-/-}$ CD8$^+$ T cells exhibited accelerated allograft rejection along with increased accumulation of donor-reactive CD8$^+$ T cells *vs* recipients of wildtype cells, illuminating a CD8$^+$ T cell-intrinsic role for Gm2a in regulating alloreactivity. Mechanistically, *Gm2a*$^{-/-}$ CD8$^+$ T cells exhibited sustained TCR expression following activation compared to wildtype cells, conferring increased responsiveness to low-affinity alloantigen. Moreover, an in vivo mixed lymphocyte reaction showed 50% augmentation in the frequency of alloreactive precursors among *Gm2a*$^{-/-}$ *vs* wildtype T cells. Finally, a TCR sequencing analysis demonstrates that Gm2a deficiency increases the number and diversity of alloreactive CD8$^+$ T cell clones. Therefore, we show that Gm2a regulates the spectrum of CD8$^+$ T cell alloreactivity by reducing TCR expression, thus increasing the T cell threshold of activation and limiting the number of alloreactive ligands to which a given T cell can respond.

Dejah Blake, CB **4:45PM**

Heme attenuates T cell exhaustion and drives effector function: implications for immune and adoptive T cell therapy

Dejah Blake*[1], Pulkit Gupta*[1], Remya Nair[1], Heather Lin[1], Ruby Freeman[1], Kiran Lakhani[1], Doris R. Powell[1], Sagar Lonial[1], Benjamin G. Barwick[1], Ajay K. Nooka[1], Jean Koff[1], Sarwish Rafiq[1], and Mala Shanmugam[1]
[1]Department of Hematology and Medical Oncology, Winship Cancer Institute, School of Medicine, Emory University, Atlanta, GA, USA

Chronic Lymphocytic Leukemia is the most common B cell malignancy in adults. Treatment often consists of targeted therapies such as the BTK inhibitor ibrutinib or chemotherapy; however, patients who relapse or are refractory to these treatments may benefit from CAR T cell therapy. Unfortunately, efficacy of CAR T therapy in CLL is significantly decreased when compared to other B cell malignancies. With the autologous nature of CAR T cells, one hypothesis is that this discrepancy is due to chronic dysfunction of CLL T cells. CAR T cells with less exhausted phenotypes and robust killing is an active area of research. Mitochondrial function and energetics play a central role in regulating T cell fate and function, and heme (iron-protoporphyrin IX) is critical for maintaining electron transport chain activity and oxidative phosphorylation. In the present study, we investigate the effects of heme on CAR T cell efficacy in the context of CLL. Human healthy donor and CLL patient CAR T cells were activated and expanded with α-CD3/CD28 and IL-2 for 7 days +/- hemin during CAR manufacturing, after which immunostaining and metabolic assays were conducted. We show that heme supplementation increases effector T and CAR T cell function and decreases T cell exhaustion. Inquiry of cellular energetics of heme-supplemented CAR T cells revealed increased spare respiratory capacity. Ongoing *in vivo* studies aim to characterize the efficacy of heme-supplemented CAR T cells in a CLL xenograft model.

Naomi Rodriguez-Marino, MMG **5:00PM**

Dietary fiber and segmented filamentous bacteria support the development of intraepithelial CD4+CD8αα+ T cells via epithelial MHCII

Naomi Rodriguez-Marino[1], Dormarie E. Rivera-Rodriguez[1], Charlotte J Royer[2], Adam Gracz[3] and Luisa Cervantes-Barragan[1]

[1]Department of Microbiology and Immunology, Emory University, Atlanta, GA
[2]Computational Biology and Bioinformatics, Georgia Institute of Technology, Atlanta, GA
[3]Department of Medicine, Division of Digestive Diseases, Emory University, Atlanta, GA

Double positive intraepithelial lymphocytes (DP IELs) are microbiota induced $CD4^{+}CD8\alpha\alpha^{+}$ anti-inflammatory T cells that help maintain homeostasis in the small intestine. It is poorly understood how changes in dietary fiber intake impact intestinal immune cell development through changes in the microbiota. Here we show that a diet low fermentable fiber (LFF) impairs development of DP IELs. 16s rRNA sequencing of the microbiota of LFF mice showed that segmented filamentous bacteria (SFB) is reduced in these mice. SFB closely interacts with intestinal epithelial cells (IECs). Bulk RNA sequencing of IECs of LFF-fed mice revealed lower expression of MHCII. Colonizing mice with SFB restored DP IEL development and increased expression of IEC MHCII and ileal IFNγ. IEC-specific deletion of MHCII and complete deletion of IFNγ receptor impaired SFB's ability to induce DP IELs. Using $Rag1^{-/-}$ mice we show that T cell derived IFNγ is dispensable for SFB-induced IEC MHCII expression and that type 1 innate lymphoid cells (ILC1s) express higher IFNγ in response to SFB colonization. Depleting ILCs resulted in lower expression of IEC MHCII on $SFB^{+}Rag1^{-/-}$ mice. Finally, we demonstrate that inducing MHCII on IEC with recombinant IFNγ is sufficient to support DP IEL development in SFB^{-} mice. Together, these results show that SFB colonization supported by dietary fiber is required for DP IEL development by inducing IFNγ expression from ILC1s and consequently increasing MHCII expression on IECs.

Maegan Brockman, MSP **5:15PM**

Investigating the Cardioprotective Role of Neutrophil-Specific STING in Myocardial Ischemia/Reperfusion Injury

Maegan L. Brockman[1,2]***,*** Lanfang Wang[1], Triniti A. Scruggs[1], John W. Calvert[2,3], Rebecca D. Levit[1,2]

[1]Division of Cardiology, Emory University School of Medicine, Atlanta, GA
[2]Molecular and Systems Pharmacology Program, Emory University, Atlanta, GA
[3]Department of Surgery, Emory University School of Medicine, Atlanta, GA

Inflammation following myocardial ischemia/reperfusion injury (MI/R) plays a significant role in damaging cardiomyocytes and influencing infarct size, with neutrophils being the most rapid and numerous cells recruited to the myocardium post reperfusion. Single-cell RNA sequencing data identified neutrophils with upregulated type I interferon (IFN) signaling in the hearts of mice 24 hours post-MI/R. This study aims to address how the type I IFN neutrophil phenotype identified affects inflammation in MI/R.
Male neutrophil-specific STING knockout (KO) mice underwent 60 minutes of ischemia followed by reperfusion for 24 hours. Immune cells were isolated from the left ventricle for flow cytometry. Subsequently, mice underwent 60 minutes of ischemia followed by reperfusion, and cardiac function was assessed two weeks-post reperfusion.
Flow cytometry revealed a significant decrease in the number of neutrophils in the myocardium of STING KO mice compared to wildtype mice after 24 hours of reperfusion ($5.7x10^4 \pm 1.0x10^4$; $8.7x10^4 \pm 1.9x10^4$, Two-way ANOVA, N=5-6, p=0.0006). Neutrophil-specific STING KO mice showed significant reduction in ejection fraction (29.2% ± 6.4) compared to wildtype mice (38.3% ± 6.7; Two-way ANOVA, N=4-5, p=0.036), and significantly increased left ventricular end systolic volume (4.39mm ± 0.48) compared to wildtype mice (3.59 ± 0.62; Two-way ANOVA, N=4-5, p=0.017), indicating impaired cardiac function in knockout mice.
These data suggest that neutrophil STING plays a cardioprotective role in MI/R. These studies provide the foundation to further investigate the cardioprotective mechanisms behind neutrophil-specific STING. Identifying a novel cardioprotective mechanism could be a clinically feasible point of intervention to improve recovery after MI/R.

Poster Presentation Abstracts

Poster Presentations

Session 1: 1:15 – 2:00PM - Odd-numbered posters

Session 2: 2:15 – 3:00PM - Even-numbered posters

Poster	Name		Program	Poster	Name		Program
1	Carly	Lancaster	BCDB	**31**	Mina	Henes	BCDB
2	Brandon	Wehmiller	BCDB	**32**	Dariana	Torres-Rivera	BCDB
3	Mohamed	Barmada	BCDB	**33**	Sarah	Webster	BCDB
4	Grace	Neilsen	BCDB	**34**	Enoch	Ayamga	BCDB
6	Alejandro	Oviedo	BCDB	**35**	Ziben	Zhou	BCDB
7	Delaney	Geitgey	CB	**36**	Vanessa	Avalos	CB
8	Anna	Cole	CB	**37**	Eleanor	Wettstein	CB
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10	Megen	Wittling	CB	**39**	Robert	Chavez	GMB
11	Isabel	Petrescu	GMB	**40**	Sisi	Falcone	GMB
12	Lauryn	Cureton	GMB	**41**	Jonathan	Owen	MMG
13	Taylor	Shue	MMG	**42**	Sushma	Timalsina	MMG
14	Yasmine	Bassil	NS	**43**	William	McCallum	NS
15	Tana	Pottorf	NS	**44**	Betty	Bekele	NS
16	Viviana	Valentin	NS	**45**	Ben	Dykstra	NS
17	Yu	Bai	NS	**46**	Hyma	Balasubramanian	NS
18	Swathi	Shrihari	IMP	**47**	Katie	Alexander	IMP
19	Nahara	Vargas-Maldonado	IMP	**48**	Melissa	Gutierrez	IMP
20	Rachel	Sutton	IMP	**49**	Megan	Phillips	PBEE
22	Nadia	Raytselis	PBEE	**50**	Ally	Su	MSP
23	Monica	Reeves	GMB	**51**	Roy	Mulpur	GMB
24	Alexander	Gulka	GMB	**52**	Nicole	Roos	GMB
25	Keenan	Wiggins	GMB	**53**	Ranjit	Pelia	GMB
26	Kristy	Wen	MSP	**54**	Madison	Schwab	PBEE
27	Winston	Li	MSP	**55**	Diane	Choi	MSP
28	Shakshi	Patel	MSP	**56**	Edu	Usoro	MSP
29	Joyce	Kariuki	MSP	**57**	Lester	Manly	MSP
30	Jordan	Goldy	BCDB	**58**	Eden	Zhu	NS

Carly Lancaster, BCDB **Poster #1**

The RNA binding protein Nab2 regulates m^{6}A levels and splicing of the RhoGEF *trio* transcript to govern axon development

Carly L. Lancaster[1,2,3], Pranav Yalamanchili, Anita H. Corbett[1], Kenneth H. Moberg[2]

[1]Department of Biology, Emory University, Atlanta, GA
[2]Department of Cell Biology, Emory University, Atlanta, GA
[3]Graduate Program in Biochemistry, Cell, and Developmental Biology, Emory University, Atlanta, GA

Intellectual disabilities (ID) are common in the general population and are linked to lesions in >700 genes. Emerging evidence suggests that this diverse group of genes converge on a limited set of neurodevelopmental pathways, including those that rely on RNA binding proteins (RBPs) to guide spatiotemporal patterns of neuronal mRNA expression. Our labs co-discovered a monogenic form of ID caused by loss-of-function mutations in the ubiquitously expressed RBP ZC3H14. Functional analysis of the conserved ZC3H14 ortholog in *Drosophila*, Nab2, illustrates that Nab2 localizes to neuronal nuclei and cytoplasmic ribonucleoprotein granules and is required specifically within brain neurons for olfactory memory and proper axonal patterning. However, neuronal signaling pathways regulated by Nab2, as well as mechanisms that elevate ZC3H14/Nab2 function in neurons, remain elusive. We will present evidence that Nab2 controls neuronal expression of a well-conserved guanine-nucleotide exchange factor (GEF), Trio that mediates growth cone guidance and axon projection. Nab2 controls Trio levels by modulating an intron-retention event within the 5' UTR of *trio* mRNA isoforms, and this mechanism appears to be dependent on N^6-methyladenosine (m^{6}A) deposition on the *trio* pre-mRNA. Data will be presented on the role of m^{6}A and Nab2 in controlling Trio splicing and expression, along with Nab2-Trio coregulation of axonal development in the CNS. Given that human TRIO is mutated in a dominant form of ID, this link between Nab2 and Trio in *Drosophila* could suggest that Nab2/ZC3H14 and Trio/TRIO act in a conserved ID pathway required to pattern neuronal processes in the developing nervous system.

Brandon Wehmiller, BCDB **Poster #2**

Regulation of transposition by the essential ribosome biogenesis factor Bcd1.

Brandon Wehmiller[1,2] and Homa Ghalei[2]

[1]Biochemistry, Cell and Developmental Biology (BCDB) Graduate Program, Emory University, Atlanta, GA
[2]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA

Dysregulation of transposable element (TE) genome integration, known as transposition, accelerates aging and contributes to the development of neurodegenerative diseases. Class I TEs, or retrotransposons, are capable of “copy-and-paste” insertions into the cellular genome that alter gene expression, leading to potential genome instability. Regulation of TE genome integration is therefore critical to combat the development of NDs. Saccharomyces cerevisiae serves as an ideal system to study retrotransposition because its genome contains self-encoded TEs known as Ty elements. Among these, Ty1 is the most active and best studied, providing a robust model for studying the mechanisms that control its insertion into the genome. Although studies have elucidated several steps needed for Ty1 integration, the roles of many host factors during transposition remain unclear. A key gap in knowledge remains in understanding the mechanisms by which host factors influence transposition pathways. Using immunoprecipitation coupled with mass spectrometry, our lab has identified a novel interaction network between the conserved ribosome biogenesis factor Bcd1, Ty1-encoded proteins and RNA Pol III subunits. Based on these data, I hypothesize Bcd1 limits TE genome integration through interactions with Ty1 Gag-Pol polyproteins and Pol III-transcribed RNAs. My data based on in vivo transposition assays show that Bcd1 plays a prominent role in regulating transposition. Future studies will address the role of Bcd1 within the retrotransposition pathway through in vitro binding assays, co-localization studies, retromobility assays, and unbiased sequencing approaches. Completion of this study will provide a foundation for designing therapeutics to restrict undesired genome integrations in NDs.

Mohamed Barmada, BCDB **Poster #3**

Intrinsic ribosomal decoding center methylation and the bacterial antibiotic response

Mohamed Barmada[1,2], Natalia Zelinskaya[2], Graeme L. Conn[2]

[1]Graduate Program in Biochemistry, Cell, and Developmental Biology (BCDB), Laney Graduate School, Emory University, Atlanta, GA
[2]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA

As the threat of bacterial antibiotic resistance continues to expand globally, it is imperative that we design antibiotics with new targets and/or find ways to resuscitate the activity of existing drugs. A relatively unexplored avenue in the fight against antibiotic resistance is the role that intrinsic bacterial factors play. For instance, loss of the intrinsic 16S rRNA methyltransferases RsmH and RsmI causes increased susceptibility to aminoglycoside antibiotics. RsmH and RsmI methylate nucleotide C1402 near the ribosome 30S subunit decoding center, where aminoglycosides bind, and targeting them might be a promising approach to counter resistance against these antibiotics. However, we currently do not know how RsmH and RsmI recognize the 30S subunit to methylate C1402 and the full extent of how they influence aminoglycoside susceptibility. I hypothesize that loss of C1402 methylation by RsmH and RsmI induces rRNA structural rearrangements that increase aminoglycoside susceptibility. To test this, I will determine the mechanism of C1402 methylation by RsmH and RsmI and the molecular basis behind how loss of C1402 methylation alters aminoglycoside susceptibility. I have generated initial cryo-EM maps of each 30S-enzyme complex and tested the interdependency of RsmH and RsmI using an *in-vitro* methylation assay. Additionally, I have subjected bacterial strains lacking one or both of *rsmH/rsmI* to a panel of aminoglycosides and determined the associated antibiotic minimum inhibitory concentration values. Defining how RsmI and RsmH function and how they influence drug susceptibility will enhance our understanding of decoding center modifications and their role in bacterial the aminoglycoside response.

Grace Neilsen, BCDB **Poster #4**

Overcoming drug resistant in the SARS-CoV-2 main protease

***Grace Neilsen*[1,2],** Shuiyun Lan[1,2], Ryan L. Slack[1,2], Zachary C. Lorson[1,2], Andres Emanuelli Castaner[1,2], Rachel Lee[1,2], Huanchun Zhang[1,2], Jasper Lee[1,2], William A. Cantara[1,2], Maria E. Cilento[1,2], Philip R. Tedbury[1,2], Karen A. Kirby[1,2], Stefan G. Sarafianos[1,2]

[1]Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA
[2]Children's Healthcare of Atlanta, Atlanta, GA

The antiviral component of Paxlovid, nirmatrelvir (NIR), covalently binds Cys145 of the SARS-CoV-2 nsp5 protease. In 16 Omicron (BA.1) and WA1 SARS-CoV-2 replicons with mutations designed to impair NIR binding, E166V imparted high NIR resistance (~130-fold in WA1; ~65-fold in BA.1). E166V significantly decreased WA1 (~20-fold), but not BA.1 fitness (~2-fold) indicating this mutation will require less of a fitness cost in current Omicron strains. E166V has become increasingly prevalent since the introduction of Paxlovid. NIR-resistant replicons remained susceptible to GC376 and PF-00835231. Crystal structures and molecular dynamics simulations show steric clashes between the rigid and bulky NIR t-butyl and β-branched V166 that may distance the NIR warhead from its Cys145 target resulting in a weaker covalent bond. In contrast, GC376, through "wiggling and jiggling," accommodates V166 and can still covalently bind Cys145. The strategically positioned methoxy-indole of PF-00835231 forms β-sheet-like interactions with nsp5 thus evading V166. These crystal structures also confirm the reduced fitness of E166V enzymes stems from impaired dimer formation although E166V can still undergo substrate induced dimerization. Looking to the future, strategic flexibility and compensating interactions should help design second-generation antivirals against NIR-resistant viruses.

Alejandro Oviedo, BCDB **Poster #6**

Mechanism of activation by a conserved SARS-CoV-2 region

Alejandro Oviedo[1,2], Camden Bair[3] and Graeme L. Conn[2]

[1]Graduate Program in Biochemistry, Cell and Developmental Biology, Emory University, Atlanta GA.
[2]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.
[3]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA.

The innate immune system is a critical line of cellular defense against pathogens, comprising a diverse set of pattern recognition receptors (PRRs) that are responsible for sensing specific pathogen-associated molecular patterns (PAMPs). Cytosolic double-stranded RNA (dsRNA) is a potent PAMP and its accumulation is an indication of viral infection that leads to downstream antiviral responses once detected by PRRs. As a result, viruses have evolved various strategies to evade detection of dsRNA. SARS-CoV2, the virus responsible for the on-going COVID-19 pandemic, uses membranous replication organelles to prevent detection of its dsRNA by cytosolic PRRs. Recently, however, reduced COVID-19 disease severity in certain individuals was attributed to a single nucleotide polymorphism (SNP) that leads to expression of an alternative form of the dsRNA sensor 2',5'-oligoadenylate synthetase 1 (OAS1-p46 instead of the more common OAS1-p42), of the OAS/RNase L anti-viral pathway. The C-terminal sequence of OAS1-p46 encodes a canonical CAAX-box prenylation signal, unique among known OAS1 isoforms. The proposed model is that OAS1-p46 is recruited to the SARS-CoV2 viral replication organelles, where it recognizes a dsRNA region of the SARS-CoV2 RNA, leading to activation of the OAS/RNase L pathway and blocking viral replication. Although the 5'-end structured elements of the SARS-CoV2 genome have been implicated in binding and activation of OAS1, the exact region has not been confirmed. As part of our broader efforts to understand regulation of OAS1 activation by specific RNA sequences and structures, my focus has been on identifying the precise region responsible for OAS1 activation.

Delaney Geitgey, CB **Poster #7**

Improving immunotherapies for pancreatic cancer using CD4+ T cells

Delaney K. Geitgey[1], Megan M. Wyatt[1], Anna C. Cole[1], Stephanie R. Bailey[2], Michelle H. Nelson[2], Christopher R. Funk[1], Shuhua Wang[1], Edmund K. Waller[1], Carl H. June[3], Chrystal M. Paulos[1], Gregory B. Lesinski[1]

[1]Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, GA.
[2]Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC.
[3]Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Pancreatic ductal adenocarcinoma (PDAC) remains a formidable challenge in oncology, with limited success both in traditional therapies and immunotherapies. Although adoptive T cell therapy (ACT) has succeeded in many hematologic malignancies, ACT thus far has failed to adequately combat aggressive solid tumors such as PDAC. Here, we outline a groundbreaking approach to overcome the barriers hindering its effectiveness against PDAC. We seek to translate a chimeric antigen receptor (CAR) T cell therapy targeting mesothelin (Meso), a highly relevant antigen overexpressed in PDAC, into a transformative treatment. Our approach centers around the strategic selection and engineering of CD4+ T cells expressing CD26, a costimulatory molecule known for its ability to enzymatically degrade immunosuppressive peptides. Additionally, we employ duvelisib, an FDA-approved PI3K inhibitor, in the expansion protocol to enhance CAR T cell function.

Our preliminary data substantiates the hypothesis that duvelisib-expanded, CD26+CD4+ T cells constitute an ideal foundation for CAR T therapy in PDAC. In murine models, these cells have demonstrated robust anti-PDAC activity, with duvelisib not only increasing CD26 expression on T cells but also preventing their exhaustion. This project seeks to unravel the interactions between duvelisib-expanded CD26+CD4+ CAR T cells and the endogenous immune system *in vivo*, elucidating the role of CD26 enzymatic activity in immunotherapy efficacy. By combining different modalities to improve the T cell response against PDAC, we expect to shed light on methods to improve the treatment of this formidable disease and set the stage for broader applications of our immunotherapy approach across various cancer types.

Anna Cole, CB **Poster #8**

B cells drive anti-tumor responses of adoptively transferred Th17 cells

Anna C. Cole[1], Hannah M. Knochelmann[2], Megan M Wyatt[1], Aubrey S. Smith[2], Guillermo O. Rangel Rivera[2], Megen C. Wittling[1], Ayana T. Ruffin[1], Soundharya Kumaresan[1], Gregory B. Lesinski[3], Chrystal M. Paulos[1]

[1]Department of Surgery, Emory University, Atlanta, GA
[2]Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, United States
[3]Department of Hematology & Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA

Adoptive T cell transfer therapy mediates potent immunity in some patients with aggressive malignancies, but many individuals do not respond, or may relapse. Therefore, our team aimed to create a therapy that can sustain long term anti-tumor immunity. We reported that Th17 cells, a subset of $CD4^+$ T cells, can eradicate melanoma when infused into mice, but the mechanism behind this enhanced immunity is unclear. To understand how Th17 cells elicit robust antitumor activity, we performed an unbiased analysis of RNA transcripts on tumor-draining lymph nodes of mice treated with Th17 cells. Surprisingly, we found that mice infused with anti-tumor Th17 cells have increased transcripts associated with B cells, and factors that trigger B cell maturation, antibody-secretion, and enhanced antigen presentation. Furthermore, host B cells, but not host T cells, were surprisingly critical in sustaining long-term immunity, as their depletion significantly impaired survival. B cells enhance Th17 cell persistence and promote the ability of Th17 cells to produce multiple cytokines, including IL-21 which was critical for this sustained anti-tumor immunity. Th17 cells induce B cell activation and maturation, causing the production of class switched tumor specific antibodies which can alone partially protect against tumor challenge. These data suggest that transferred Th17 cells and host B cells harmonize to sustain immunity against melanoma. Our findings highlight Th17 cell ACT as a novel way to engage B cell responses to cancer. Ongoing experiments are investigating the mechanism behind these cells' cooperation.

Austre Schiaffino, CB **Poster #9**

Strategies to target T-cell acute lymphoblastic leukemia using γδ T cells

Austre Y. Schiaffino Bustamante[1,2], Katie Skinner[1,2], Brian G. Petrich[3], Christopher B. Doering[2], H. Trent Spencer[2]

[1]Cancer Biology Program, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA, USA
[2]Aflac Cancer and Blood Disorders Center, Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, USA
[3]Expression Therapeutics LLC, Tucker, GA, USA

T-cell acute lymphoblastic leukemia (T-ALL) poses a significant therapeutic challenge due the absence of T-ALL specific antigens, making it difficult to develop targeted therapies. Our recent studies have highlighted the potential of γδ T cells, an innate T cell, as a possible cell therapy approach for T-ALL. Using RNA-sequencing, the gene-expression profile of eight T-ALL samples and two clinically processed γδ T cell products were compared and used to generate a principal component analysis (PCA) plot, providing a measure of the gene expression differences among both groups. Likewise, a volcano plot was used to show differentially expressed genes between T-ALL and γδ T cells. The results demonstrate high variability between T-ALL and γδ T cells, indicating significant mRNA expression variation. In addition, ~14000 genes were found to be upregulated in the T-ALL samples. Genes not encoding transmembrane proteins were filtered, providing a list of possible membrane proteins upregulated in T-ALL and downregulated in γδ T cells. Interestingly, protein tyrosine kinase (PTK7) was found to be highly expressed in T-ALL and lowly expressed in γδ T cells. Chimeric antigen receptors (CAR) and bispecific T-cell engager (BITE) were designed, lentiviral vectors were generated, and used to genetically engineer γδ T cells. PTK7-targeting demonstrated a remarkable 88% cytotoxicity against T-ALL cells at a low 2.5:1 effector to target ratio, surpassing the 76% observed with mock modified γδ T cells. Ongoing research aims to optimize PTK7-BITE γδ T cells and further elucidate the specific mechanisms underlying γδ T cell-mediated killing of T-ALL cells.

Megen Wittling, CB **Poster #10**

Inducible costimulatory molecule is important in the efficacy of Th17 Therapy

Megen Wittling[1,2,3], Hannah Knochelmann[4], Anna Cole[1,2], Soundharya Kumarasan[1,2], Megan Wyatt[1,2], Ayana Ruffin[1,2], Frances Bennet[1,2], Shannon Swisher[1,2], Chrystal Paulos[1,2]

[1]Department of Microbiology and Immunology, Emory University, Atlanta, GA
[2]Department of Surgery, Emory University, Atlanta, GA
[3]MSTP Program, Emory University, Atlanta, GA
[4]Department of Medicine, Stanford University, Stanford, CA

Adoptive T cell therapy is a very promising approach for the treatment of cancer, with many FDA-approved CAR T cell therapies for hematologic malignancies as well as promising approaches for the treatment of solid tumors. Our lab has found that antigen specific Th17 cells are potent regressors of melanoma tumors in our mouse model, and they regress tumors better than Th1 or other CD4 subsets. Due to the high expression of Inducible Costimulatory Molecule (ICOS) on these cells, I wanted to explore the role of ICOS in this therapy. Antigen-specific TRP-1 $CD4^+$ T cells were polarized to the Th17 phenotype and were adoptively transferred into B16F10 melanoma bearing mice. ICOS signaling was then diminished by either antibody blockade or genetic deletion and compared to mice given an isotype control. RNA-Sequencing, flow cytometry, and tumor growth measurements were additionally used to assess differences when ICOS was blocked or not. ICOS was found to be important in the success of Th17 therapy for the treatment of melanoma as when blocking this costimulatory pathway, both survival and antitumor activity was negatively impacted. Additionally, this signaling pathway appears to be important early on after Th17 cell transfer into mice as blocking this pathway early but not at later time points compromised antitumor immunity. I also assessed which cells express the binding partner to ICOS (ICOS-Ligand), which has important implications for therapeutic success. In summary, ICOS has an important role for the antitumor activity of Th17 cell therapy targeting melanoma.

Isabel Petrescu, GMB **Poster #11**

Characterizing the role of the glycosyltransferase GALNT14 in osteosarcoma tumorigenesis

Isabel Petrescu[1,2], Jason T. Yustein[2]

[1]Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA
[2]Aflac Cancer and Blood Disorders Center, Emory University, Atlanta, GA

Osteosarcoma (OS) is the most common primary malignant bone tumor with bimodal inheritance by predominantly affecting adolescents and adults 60 years of age and older. OS treatment commonly involves surgical resection and adjuvant and neoadjuvant chemotherapy. However, refractory or relapsed OS significantly reduces patient survival even following treatment. OS presents a challenge for the discovery of novel therapeutic targets due to its genetic heterogeneity. Previous results demonstrated that altered protein glycosylation patterns due to increased α-GalNAc transferase (GALNT) activity decreases patient survival and chemotherapeutic efficiency. High expression of the GALNT family member *GALNT14* in patient tumors significantly reduces survival likelihood and chemosensitivity compared to patient tumors with low expression of *GALNT14*. To gain further insights into the role of *GALNT14* in OS tumorigenesis and chemosensitivity, we are characterizing *in vitro* and *in vivo* OS models of *GALNT14* high and low expression. Towards this goal, we have established *GALNT14* knock-out (KO) models in high-expressing *GALNT14* OS cell lines with CRISPR editing. Conversely, we have established *GALNT14* overexpression (OE) models in low-expressing *GALNT14* OS cell lines using lentiviral transfection. Characterization of these models confirmed decreased *GALNT14* expression in KO models and increased *GALNT14* expression in OE models, supporting the validity of these models. Furthermore, KO models demonstrated reduced cell proliferation with the opposite result seen for OE models. Immunodeficient mice injected with *GALNT14* KO cell lines displayed decreased tumor growth and metastasis in preliminary studies. These results indicate a role for GALNT14 in OS tumorigenesis dependent on high versus low expression.

Lauryn Cureton, GMB **Poster #12**

Disease-associated variants of the structural subunits of RNA exosome complex cause distinct translation defects

Lauryn A. Cureton[1], Milo B. Fasken[2], Maria C. Sterrett[2,3], Sohail Khoshnevis[1], Anita H. Corbett[2] and Homa Ghalei[1]

[1] Department of Biochemistry, Emory University School of Medicine
[2] Department of Biology, Emory University
[3] IRACDA Postdoctoral Scholar, Tufts University School of Medicine

The RNA exosome is an evolutionarily conserved ribonuclease complex required for processing and degradation of many cellular coding and non-coding RNAs, including ribosomal RNA (rRNA). The multi-subunit RNA exosome consists of a 3-subunit cap, a 6-subunit barrel-like core, and a catalytically active base. Missense mutations in genes encoding several structural subunits of the RNA exosome have been linked to human diseases, collectively termed “RNA exosomopathies”. Symptoms of these diseases vary amongst patients and range from neurological defects to developmental disorders. Using biochemical approaches, here, we compare the impact of four disease-causing missense variants in two cap exosome subunits (Rrp4 and Rrp40) and two core exosome subunits (Rrp41 and Rrp46), modeled in the budding yeast, *S. cerevisiae*, on ribosome biogenesis and translation. Our results show that all four variants cause rRNA processing defects and an overall significant decrease in translation. However, translation is perturbed in distinct ways in cap vs core subunit variants. Our findings suggest that different RNA exosomopathy mutations can result in *in vivo* consequences that are both unique and shared amongst the variants and could provide new insights into the molecular defects underlying each distinct pathology.

Taylor Shue, MMG **Poster #13**

Identification of a novel host-dependency factor for human coronaviruses

Taylor Shue[1,2,3], Dar-Yin Li[2,3], Jeremie LePen[4], Yee Ong[2,3], Antonis Athanasiadis[1,2,3], Stefan G. Sarafianos[2,3], Charles M. Rice[4], Eleftherios Michailidis[2,3]

[1]Microbiology and Molecular Genetics Graduate Program, Emory University
[2]Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory School of Medicine
[3]Children's Healthcare of Atlanta, Atlanta, Georgia
[4]Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, NY, USA

Human coronaviruses are positive-sense, single-stranded RNA viruses that are a major global public health threat. Over the last twenty years, three highly pathogenic coronaviruses have spilled over into humans causing severe human health and economic impacts. SARS-CoV-2 alone has resulted in over six million deaths worldwide. This impact indicates a need for a better understanding of how coronaviruses interact with the host innate immune system which may lead to novel therapeutics. Coronavirus infections trigger the expression of type I interferons (IFNs) and downstream effectors, interferon-stimulated genes (ISGs). Our goal was to identify and validate ISGs that function as host-restriction or dependency factors during coronavirus infections. To identify potential genes of interest, we performed a transient genome-wide CRISPR knockout (KO) screen in Huh7.5 cells with SARS-CoV-2 infection. This screen showed that a KO of the ISG, Shiftless (SHFL), reduces SARS-CoV-2 infection. To validate this finding, we used recombinant Cas9 protein to make stable single-cell KO clones of SHFL in Huh7.5 cells (Huh7.5-SHFL KO). Experiments using wild-type Huh7.5 cells and Huh7.5-SHFL KO cells validated the screening phenotype that SHFL acts as a host-dependency factor across multiple coronaviruses of different subgenera. Titration of IFNα pre-treatment during infection experiments revealed a reduction in IC_{50} for the compound in Huh7.5-SHFL KO cells compared to wild-type indicating an effect on IFN signaling for SHFL. Ongoing experiments will further identify the mechanism of action for SHFL during coronavirus infections. This knowledge will expand our understanding of virus-host interactions, coronavirus biology, and the innate immune system.

Yasmine Bassil, NS **Poster #14**

Reference frame utilization as a potential marker of aging-related deficits in human spatial navigation

Yasmine Bassil[1], Anisha Kanukolanu[2], Emily Cui[3], Michael Borich, PT, DPT, PhD[4]

[1]Neuroscience Graduate Program, Emory University, Atlanta, GA
[2]College of Sciences, Georgia Institute of Technology, Atlanta, GA
[3]Department of Rehabilitation Medicine, Emory University, Atlanta, GA
[4]Department of Rehabilitation Medicine, Emory University, Atlanta, GA, Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

With advancing age, older adults (OAs) report impaired spatial navigation, one of the earliest indicators of aging-related cognitive decline. Specifically, OAs demonstrate deficits in utilizing allocentric (world-centered) information and rely on egocentric (body-centered) cues during navigation, resulting in 'reference frame (RF) bias'. While traditional navigational assessments have characterized aging-effects on RF bias, RF utilization during naturalistic, real-world-like navigation remains unclear. This study characterized interactions between RF bias utilizing a traditional Y-Maze task and navigation in a novel, city-like, virtual reality environment (NavCity), with an associated NavCity Allocentric Representation Assessment (NARA). We hypothesized that OAs with egocentric bias would exhibit greater deficits in navigation performance and allocentric RF formation, compared to OAs who primarily utilized allocentric RFs or younger adults (YAs).

To test this hypothesis, YAs (N = 12; 18-35 years) and OAs (N = 12; 60+ years) completed 3 exposures in NavCity, NARA, and the Y-Maze. Independent t-tests and chi-squared tests evaluated group differences.

Compared to YAs, OAs demonstrated higher mean completion times and distances traveled in NavCity (both $p < .01$) and lower NARA scores ($p < .001$) that were strongly correlated across groups (all $p < .001$). However, the rate of navigation improvement across exposures was similar between groups ($p > .05$). Additionally, OAs exhibited greater Y-Maze egocentric RF utilization, compared to YAs ($\chi 2 = 14.96$, $p < .001$). Findings suggest that RF characterization may serve as a marker of aging-related navigational deficits. Next steps include recruiting additional participants to compute interactions between aging and RF groups.

Tana Pottorf, NS **Poster #15**

TREM2's role in spinal cord microglia morphology and function following Peripheral Nerve Injury

Tana S. Pottorf[1], Veronica Amores Sanchez[1], Zoë Haley-Johnson[1], Elizabeth Lane[1], Francisco J. Alvarez[1*]

[1]Emory University, Atlanta, GA

Motoneuron (MN) degeneration is a common hallmark of many neuropathologies including but not limited to Amyotrophic Lateral Sclerosis, Spinal Muscle Atrophy, and Peripheral Nerve Injuries (PNI). PNI can induce MN death depending on age, the location of the injury, and delays between injury and repair and regeneration. Permanent loss of MNs limits functional recovery. Therefore, identification of mechanisms that govern selective MN loss following PNI is crucial for therapeutic advancement and improved patient prognosis and may have implications for other MN pathologies. We utilize a PNI mouse model to investigate diverse microglia interactions with MNs of varying health states. Following PNI, microglia proliferate, migrate, and extend processes towards MNs and thereafter, adhere to and scan the MN surface with dynamic filopodia. Microglia that associate with regenerating MNs remain in this "sampling" state, whereas microglia associated with degenerating MNs transform into ameboid-like cells with minimal processes. They tightly associate in groups we denoted as "death clusters." The differentiating signals between regenerating and dying motoneurons that microglia respond to while changing morphology and potential functionality are currently unknown. Our evidence suggests that Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) is differentially upregulated in microglia that associate with regenerating MNs compared to microglia in death clusters around dying MNs. TREM2 levels correlate with increases in the phagocytic marker, CD68. Our results provide insights into signaling cascades that regulate microglia morphology, dynamics, and function around injured MNs destined to survive or die and suggest possible therapeutics to increase MN survival in pathological conditions.

Viviana Valentin, NS **Poster #16**

Early habits: how the young brain supports routinized behaviors.

Viviana P. Valentín-Valentín & Shannon L. Gourley

Habits are routinized behavioral patterns that can be elicited by environmental cues and occur almost automatically. Young mammals are experts in forming habits and routines for daily life (e.g. feeding schedules, bedtime, etc.). Nevertheless, the neurobiology supporting habit formation has been mostly studied in adult individuals, leaving the neural mechanisms underlying habits early in life unclear. Here, we sought to identify the neural underpinnings of habit formation and execution early in life. Using a contingency updating task, we found that young mice are biased towards habitual actions under conditions in which adult mice engage in goal-directed, flexible strategies. Next, we quantified cFOS levels as a proxy for neuronal excitation in the ventral hippocampus (vHC), a brain region necessary for habit learning in adult mice and found similar levels across ages.

Next, we induced inhibitory chemogenetic constructs in vHC neurons active during habit execution early in life to test whether these neurons were necessary for the expression of habits later in adulthood. Inhibiting the activity of these neuronal ensembles blocked habit-like actions in adulthood. Thus, the vHC may form ensembles controlling habit-like behavior that are stable across development. Further research will investigate the neural systems important for habit formation early in life, and the development of the circuits necessary to engage in more flexible strategies with time.

Yu Bai, NS **Poster #17**

The mechanism underlying neurodegeneration induced by loss of LSD1

Yu Bai[1], Saahj Gosrani[1] and David Katz[1,2]

[1]Department of Cell Biology, Emory University, Atlanta, GA
[2]School of Medicine, Emory University, Atlanta, GA

Our lab found that the histone demethylase LSD1 mislocalizes to pathological tau aggregates in human Alzheimer's disease (AD) cases and altering LSD1 modulates neurodegeneration in Tau P301S mice. We also showed that inducible deletion of LSD1 in adult mice leads to severe neurodegeneration with transcriptional alternations that overlap with the transcriptional alternations in late onsite AD cases. As a result, understanding how loss of LSD1 triggers pathways that leads to neurodegeneration in mice is of particular interest.

The neurodegeneration in Lsd1 inducible deletion mice is associated with sever paralysis. At terminal paralysis stage, many transcriptional alternations could be secondary responses. Therefore, to identify primary responses, we examined early transcriptional changes in the hippocampus of Lsd1 inducibly deletion mice at onset of paralysis.

At the onset of paralysis, Lsd1 inducible deletion mice already have severe transcriptional alternations compared to WT animals. Although the fold change of those altered genes is generally less in animals with early onset paralysis compared to terminal paralysis, gene set enrichment analysis demonstrated that similar pathways are up regulated. Interestingly, one of the Lsd1 inducible deletion mice was analyzed at a stage when the paralysis was less severe than the other early onset animals. Consistent with this, the transcriptional changes in this mouse were more like WT controls, with only 47 genes significantly upregulated, and half of them remain significantly altered in more severe stage. This raises the possibility that these genes represent the first genes that are affected by loss of LSD1 in neurodegeneration.

Swathi Shrihari, IMP **Poster #18**

IFNγ Primed MSCs suppress stimulated T Cells by inducing perturbations of redox homeostasis

S. Shrihari[1], **E. Foppiani**[1], **K. Goss**[1], G. Gafford[1], L. Daley-Bauer[1] and E. Horwitz[1]

[1]Department of Pediatrics, Emory University, Atlanta, USA

Acute Graft *versus* host disease (aGVHD) remains the primary cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). Despite the current standard prophylaxis, approximately 50% of patients will develop aGVHD. The outcome of patients with steroid-refractory and severe GVHD is poor; hence, more effective prophylaxis is greatly needed. In recent years, considerable attention has been directed toward mesenchymal stromal cells (MSCs) as prophylaxis and therapy for aGVHD. Even though the immunosuppressive and immunomodulatory properties of MSCs are enhanced by interferon γ (γMSCs), less attention has been focused on these cells. We sought to understand the mechanism(s) of γMSC suppression of alloreactive T cells in this specific clinical and biological setting. Our lab showed that in an In vitro setting, the reversible T cell suppressive activity was entirely contact-independent, while cell-to-cell contact could induce T cell death. After 24 hours of T cell activation or γMSC suppression in vitro, we found the T cells were arrested in G0. In scRNA Seq, we identified 16,478 expressed genes, but only 98 genes were significantly differentially expressed. ROS response pathways were significantly enriched in the suppressed cells, and flow cytometric analysis of T cells revealed *less ROS* in suppressed cells than in controls, suggesting reductive stress. Adding Trp to γMSC CM (conditioned media) abolished suppression. Trp supplementation of γMSC CM showed the expected level of ROS in activated T cells, suggesting the Trp deficiency caused the reduced ROS. Our data indicate that IDO1-mediated Trp deficiency perturbs redox homeostasis, suppressing activated T cells.

Nahara Vargas-Maldonado, IMP **Poster #19**

Detection of infectious influenza A virus in exhaled air from experimentally infected humans

Nahara Vargas-Maldonado[1], Nishit Shetty[2], Kayle Patatanian[1], Shamika Danzy[1], Michelle N. Vu[1], Matthew Pauly[1], A.J. Campbell[1], Meredith J. Shephard[1], Hollie Macenczak[1], Jessica Traenkner[1], Ralph Tainos[1], Colleen S. Kraft[1], Nadine Rouphael[1], Seema S. Lakdawala[1], Linsey Marr[2], Anice C. Lowen[1]

[1]Emory University, School of Medicine, Atlanta, Georgia
[2]Virginia Tech, Department of Civil & Environmental Engineering, Blacksburg, Virginia

Transmission of influenza viruses initiates with expulsion of virus to the environment. Due to technical challenges with detecting infectious virus in the air, it is unclear how viral expulsion is shaped by different expiratory activities and the anatomical source of released virus. Here, we examine infectious viral expulsion from eight individuals intranasally infected with influenza A/Perth/16/2009 (H3N2) virus. All individuals were confirmed positive for infection via qPCR of nasopharyngeal swabs. To evaluate respiratory aerosol production and infectious viral content therein, we developed the Modular Influenza Sampling Tunnel (MIST). The MIST is an enclosed device 3 feet long with an opening at one end for the nose and mouth. Infectious virus within coarse aerosols produced during talking, coughing, and sneezing are collected in six cell culture plates situated along the length of the tunnel. MIST samples were collected from day 1 to 6 post-inoculation along with nasal and oral samples. Viral titers from nasal and oral specimens were determined by plaque assay. Seven out of eight individuals shed virus in the MIST between 2 to 5 days post-inoculation. MIST positivity ranged from 1 to >2366 plaque forming units.The extent of infectious virus production varied by individual and was higher upon coughing and sneezing. Additionally, individuals with virus in oral samples emitted more infectious virus compared to participants with viral positivity only in their nasal cavity. These data suggest that expulsion of infectious influenza virus occurs over several days and that the oral cavity is an important source of emitted virus.

Rachel Sutton, IMP **Poster #20**

The crucial role of A20 in human B cell function: implications for immunoregulation and autoimmunity

Rachel E. Sutton[1], Mansi Gupta[1], Christopher D. Scharer[1]

[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA

Autoimmune diseases, such as systemic lupus erythematosus (SLE), are underscored by dysregulated B cell function including the production of autoantibodies, skewed population ratios, and aberrant signaling. Given that the family of nuclear factor kappa B (NF-kB) transcription factors govern responses to stimuli, survival, differentiation, and so forth understanding the intricate regulatory network of NF-kB in B cell biology is paramount for unraveling treatments for B cell-linked autoimmune diseases. Here, we focus on a negative regulator of NF-kB signaling, A20 (*TNFAIP3*), that deactivates NF-kB transcription factor translocation through the ubiquitination and deubiquitination of target proteins. Haploinsufficiency in A20 results in an autoimmune phenotype and mutations to A20 have been associated with SLE, suggesting implications to B cell function. To investigate the role of A20 in B cell signaling, we generated a *TNFAIP3* knockout (KO) human B cell line. We then stimulated KO and WT cells with agonists such as B-cell activating factor (BAFF), CD40L, anti-IgM, TNFa, Resiquimod (R848) that activated distinct modes of NF-kB signaling. Using a combination of qRT-PCR, western blotting, and flow cytometry, the differences in gene expression patterns, protein production, and activation of NF-kB transcription factors were evaluated. Together, these findings provide a framework to understand how A20 controls B cell signaling and contributes to B cell-regulated autoimmune diseases.

Nadia Raytselis, PBEE **Poster #22**

Dynamic connectivity of water bodies may facilitate alternate Guinea worm transmission pathway

Nadia Raytselis[1], David J. Civitello[1]

[1]Biology Department, Emory University, Atlanta, GA

Guinea worm disease (GWD) eradication is complicated by a newly hypothesized alternate transmission pathway to humans involving a paratenic fish host. In the lab, fish have been shown to consume GW-infected copepods and viable GW larvae can be recovered from them. Our field data on copepod densities and water turbidities from Chad suggests that fish and copepods typically do not co-occur in natural settings: copepod densities are much higher in turbid ponds than in clear ponds and rivers, indicative of low predation by visual fish predators. Testimonies from locals also indicate that fish are in lower abundance in turbid ponds. To determine if and when copepods and fish co-occur in the landscape, we assessed water body connectivity over time in Chad. We hypothesized that discrete waterbodies form at the beginning of the wet season (in ~April), and then merge with rivers, ponds, and reservoirs over time (until ~October), leading to the dynamic connectivity of fish and copepods. To test this hypothesis, we quantified flood plain merging across space and time using on-the-ground data on water body presence from ten Chadian villages from 2022 provided by our Carter Center collaborators. We then used Generalized Additive Mixed Models (GAMM) to assess patterns in floodplain merging. Results from our GAMM support our hypothesis that water body connectivity is dynamic. Our findings identify a period during which these typically separate species may potentially interact, allowing for fish-dependent transmission to occur. Understanding when fish-dependent GW transmission is occurring can allow for more targeted control measures.

Understanding how the maternal epigenetic reprogramming function of LSD1/KDM1A contributes to neurodevelopmental disease.

Monica Reeves[1], Eilleen Falkenberry[1], Juan Rodriguez[1], Sindy Chavez[1], David Katz[1]

[1]Department of Cell Biology, Emory University, Atlanta, GA

Human *de novo* mutations in the H3K4 demethylase LSD1/SPR-5/KDM1A cause neurodevelopmental disease, characterized by developmental delay, craniofacial defects and intellectual disability. It is assumed that these defects occur developmentally due to zygotic loss of LSD1. However, our work challenges these assumptions. Loss of both LSD1 and the H3K9 methyltransferase MET-2/SETDB1 in *C. elegans* results in a severe chemotaxis defect in the progeny caused by ectopic expression of germline genes in somatic tissues. Surprisingly, using lineage tracing we find no developmental defects in these worms. This suggests that the altered behavior may be due to ongoing defects in terminally differentiated cells rather than a developmental defect. To test this, we shut off the ectopic expression of germline genes in *spr-5; met-2* larvae. Strikingly, we see rescued chemotaxis in the same adult worms that previously had a chemotaxis defect at the L2 stage. This suggests that ongoing ectopic transcription can block normal behavior in a fully intact nervous system. If the human LSD1 mutations also result in ongoing defects, it may be possible to rescue some of these defects, even in adult patients. In mice, we find that partial loss of LSD1 maternally results in inherited phenotypes, such as developmental delay and craniofacial abnormalities, that overlap with the human patients. This raises the possibility that there may be a maternal contribution to the neurodevelopmental defects in these patients. To test this possibility directly, we are determining whether the human *de novo* mutations preferentially occur on the chromosome inherited from the mother.

Alexander Gulka, GMB **Poster #24**

A role for BAF complexes in enabling cellular responses to stimuli

Alexander O. D. Gulka[1,2], David U. Gorkin[2]

[1]Graduate Program in Genetics & Molecular Biology, Emory University, Atlanta, GA
[2]Department of Biology, Emory University, Atlanta, GA

Chromatin remodeling plays a central role in transcriptional regulation, and mutations in genes encoding chromatin remodeling proteins cause neurodevelopmental disorders (NDDs) and cancer. BRG1/BRM-Associated Factors (BAF) complexes remodel chromatin at enhancers and other *cis*-regulatory elements (cREs). This remodeling generates “accessible” states that allow binding by regulatory proteins such as transcription factors (TFs). Acute loss of BAF complex activity leads to abrogated accessibility at thousands of enhancers, while many other loci are unaffected or only modestly perturbed. We hypothesized that BAF-dependent and BAF-independent enhancers represent functionally distinct and molecularly distinguishable cRE classes. To characterize the features of BAF-dependent chromatin, we employed small molecule inhibitors to disrupt BAF function and generated time-resolved maps of chromatin accessibility changes in GM12878 cells. Importantly, the genomic distributions of histone modifications and TFs have been extensively profiled in this system. Strikingly, we find that poised enhancers lose accessibility more rapidly and to a greater extent than any other cRE class following BAF inhibition. Moreover, poised enhancers are enriched for AP-1 TF binding, which is associated with an even more pronounced treatment response. AP-1 motifs are enriched at poised enhancers in HAP-1 and HMEC cells, respectively, suggesting the existence of a generalizable BAF/AP-1 axis that maintains accessibility at these cREs. This may be important for enabling cellular responses to environmental or developmental cues. Heterozygous inactivating mutations in BAF subunits can cause NDDs, and it is interesting to speculate that compromised poised enhancer function due to reduced BAF activity may contribute to disease phenotypes in these cases.

Keenan Wiggins, GMB **Poster #25**

EZH2 Unleashed - The Power of Repression in Shaping Memory B Cell Development and Function

Keenan J. Wiggins[1], Mark E. Williams[1], Bagdeser Akdogan-Ozdilek[1], Sakeenah L. Hicks[1], Jeremy M. Boss[1,2], and Christopher D. Scharer[1],

[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA
[2]Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA 30322, USA

Memory B cells (MBC) are a heterogenous population that consists of immunoglobin class switched and non-class switched MBC, and these populations arise via germinal center departments or germinal center independent processes. These different populations of MBC are regulated by cell signaling and epigenetic mechanisms. EZH2 is an important histone methyltransferase that catalyzes H3K27me3 resulting in gene repression. EZH2 has been shown to regulate different stages of B cell differentiation; however, it is unknown if EZH2 regulates MBC development. To study EZH2 role, a knockout model has been established where EZH2 is conditionally deleted once CD19 (a B cell specific factor) is expressed. Here we used the influenza (PR8) model to ascertain the kinetics of MBC differentiation following an live infection in wild-type and conditional EZH2 knockout aged and sex matched mice. Splenic MBC development can be seen as early as 14 days post infection, with long lived MBC captured 39 days post infection. In EZH2-KO mice, class switched, $CCR6^+$ and $CD73^+$ MBC development was significantly reduced. Transcriptome analysis revealed that deletion of EZH2 results in dysregulation of proliferation, cell migration and the viral response pathways. The chromatin landscape of wildtype MBC was established and used to display H3K27me3 repression of B cell genes needed for proper MBC development such as *Ncor2*. Overall, this model defines the early kinetics of MBC development and highlights the heterogenicity of MBC. With the ongoing advances in vaccine developments, these data share insights, and promising avenues to design safe and effective MBC populations.

Supported by grants from NIH/NIAID to CDS (R01 AI148471) and NIH F31 (1F31AI172377-01A1)

Kristy Wen, MSP **Poster #26**

Inhibition of HIV-1 Reverse Transcription by a Novel Antiviral, JT-4-173, *via* a Unique Mechanism of Action.

Xin Wen[1,2], Mary C. Casey-Moore[3,4], Cole Smith[5], Maria E. Cilento[1,2], Karen A. Kirby[1-4], Michael A. Parniak[6], Michael Malim[7], Darja Pollpeter[8], Zhengqiang Wang[8], Philip R. Tedbury[1-4], Stefan G. Sarafianos[1-4]

[1]Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University, Atlanta, GA
[2]Children's Healthcare of Atlanta, Atlanta, GA
[3]Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO
[4]Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO
[5]Department of Chemistry, Emory University, Atlanta, GA
[6]Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA
[7]Department of Infectious Diseases, King's College London, London, UK
[8]Center for Drug Design, University of Minnesota, Minneapolis, MN

Highly active antiretroviral therapy treatment regimens have been successful in controlling viral loads and preventing the onset of AIDS in individuals living with HIV-1. However, the emergence of drug-resistance mutations threatens its long-term effectiveness. Therefore, there is a continuous need to discover and develop novel antiretrovirals with unique mechanisms of action (MOA) to improve therapeutic options. The Sarafianos lab previously identified a compound, JT-4-173 (JT), with a potent antiviral activity against HIV-1 (EC_{50} = 0.22 μM). Cellular and structural studies revealed JT is a HIV-1 reverse transcriptase (RT) inhibitor that binds the non-nucleoside RT inhibitor (NNRTI) binding pocket (NNIBP) but does not inhibit reverse transcription like other NNRTIs. qPCR analysis showed JT does not impact early, intermediate, nor late RT products, yet integrated provirus and 2-LTR circles were significantly reduced without impacting 1-LTR circles. These data suggest JT binds to the NNIBP but does not inhibit reverse transcription until the very late stage, a phenotype not reported in other RT inhibitors. We hypothesize JT inhibits strand displacement synthesis at the 3'- and 5'-ends and potentially at the central polypurine track (cPPT). *In vitro* primer-extension assays with relevant DNA substrates showed JT inhibits PPT strand displacement in a dose-dependent manner. In the absence of a PPT to be displaced, JT does not impact RT polymerization. These results indicate JT may specifically inhibit strand displacement during late-stage reverse transcription. Ongoing experiments aim to further characterize this unique MOA and address the molecular details of how JT inhibits strand displacement syntheses.

Winston Li, MSP **Poster #27**

Enhancement of polymer thermoresponsiveness and drug delivery across biological barriers by addition of small molecules

Zipei Zhang[1], ***Winston Xiyu Li***[1, 2], Changwoo Do[3], Daniel S. Kohane[1]

[1]Laboratory for Biomaterials and Drug Delivery, Department of Anesthesiology, Division of Critical Care Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA
[2]Department of Medicine, Division of Cardiology, School of Medicine, Emory University, Atlanta, GA
[3]Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN

Thermoresponsive polymers that undergo sol–gel transitions in the physiological temperature range have been widely used in biomedical applications. However, some commercially and clinically available thermoresponsive materials, particularly poloxamer 407 (P407), have the significant drawback of insufficient gel strength, which limit their performance. Furthermore, co-delivery with some small molecules, including chemical permeation enhancers (CPEs) can further impair the physical properties of P407. Here, we have developed a thermoresponsive platform by combination of CPEs with the poloxamer P188 to enable gelation at physiological temperatures and enhance gel strength. P188 gels at 60 °C, which is far above the physiological range. In combination with limonene (LIM) and sodium dodecyl sulfate (SDS), P188 gels at ~25 °C, a temperature that in useful for biomedical applications. Gelation behavior was studied by small angle neutron scattering (SANS) experiments, which identified micelle-to-cubic mesophase transitions with increasing temperature. Analysis of the SANS intensities revealed that P188 micelles became larger as LIM or SDS molecules were incorporated, making it easier to form a micellar gel structure. P188-3CPE (i.e., 2% LIM, 1% SDS and 0.5% bupivacaine (BUP)) had low viscosity at room temperature, facilitating administration, but rapidly gelled at body temperature. P188-3CPE enabled the flux of the antibiotic ciprofloxacin across the TM and completely eradicated otitis media from nontypable Haemophilus influenzae (NTHi) in chinchillas after a single administration.

Shakshi Patel, MSP **Poster #28**

Antagonist-induced changes in the structure of the nuclear receptor, liver receptor homolog-1

***Shakshi Patel*[1]**, Michael Lee Cato[1], Racheal Spurlin[2], Eric A Ortlund[1]

[1]Department of Biochemistry, Emory University, Atlanta, GA
[2]Department of Chemistry, Emory University, Atlanta, GA

Breast cancer (BC) is the second most common form of cancer diagnosed in women. Of these diagnoses, 15-20% are estrogen receptor-α negative (ERα), progesterone receptor negative, and human epidermal growth factor receptor 2 negative, known as triple-negative breast cancer (TNBC). Most TNBC therapeutics target ERα, PR, or HER2; therefore, discovery of new, non-invasive treatments is necessary. Liver receptor homolog-1 (LRH-1) is a ligand-regulated transcription factor that controls BC cell proliferation and invasion. Phospholipids bind at the ligand binding domain (LBD) to induce conformational changes in helices 3, 4, and 12, which comprise the activation function surface-2 (AF-2) resulting in the binding of coregulators. Therefore, LRH-1 antagonists present a novel treatment option for BC and TNBC by driving repression of BC proliferation genes. Our lab previously developed high-affinity, potent, agonists that were leveraged to design antagonists. The lead antagonist, ANT3, has low nanomolar affinity for the LBD of LRH-1 and significantly reduces expression of LRH-1 target genes, NR0B2 and CYP7A1. Additionally, ANT3 reduces interactions between LRH-1 and co-activators PGC1α and SRC3. However, the structural changes driving LRH-1 repression are poorly understood. Therefore, I hypothesize that ANT3 induces conformational changes in the AF-2 of LRH-1 LBD. Using structural probing techniques like x-ray crystallography structural changes in the LBD with ANT3 binding will be determined. Thus, a crystal structure of LRH-1 LBD bound to ANT3 will allow for the development of more potent and efficacious antagonists and eventually, to the targeted treatment of breast cancer.

Joyce Kariuki, MSP **Poster #29**

Developing pharmacological agents that target tumor-specific protein-protein interactions

***Joyce Kariuki*[1,3]**, Yuhong Du[1,2], Qiankun Niu[1], Min Qiu[2], Dacheng Fan[1], Haian Fu[1,2,3]

[1]Department of Pharmacology, Emory University School of Medicine
[2]Emory Chemical Biology Discovery Center, Emory University School of Medicine
[3]Molecular and Systems Pharmacology Program, Laney Graduate School

Small molecule inhibitors to attenuate $BRAF^{V600E}$-mediated oncogenic activity are crucial to therapeutically treat $BRAF^{V600E}$ driven cancers. However, patients often exhibit intrinsic or acquired resistance to these drugs. Therefore, there is a need to develop innovative, and precise strategies that could be used to treat $BRAF^{V600E}$ driven cancers. We have previously discovered that $BRAF^{V600E}$ has an increased interaction with the tumor suppressor KEAP1, forming a mutation-created neo-protein-protein interaction (neoPPI). This neoPPI leads to NRF2 upregulation even in the absence of oxidative stress, possibly exerting a cytoprotective effect on cancer cells. We hypothesize that inhibiting $BRAF^{V600E}$ and KEAP1 interaction with small molecules would provide an avenue to understand the role of $BRAF^{V600E}$ and KEAP1 PPI in tumorigenesis and therefore offer novel therapeutics for $BRAF^{V600E}$ driven cancers. In order to discover $BRAF^{V600E}$/ KEAP1 PPI inhibitors, we developed a time resolved fluorescence resonance energy transfer (TR-FRET) assay in a 1536-well ultrahigh-throughput screening format (uHTS) to monitor the $BRAF^{V600E}$-KEAP1 PPI. A screen with 12,483 FDA approved and bioactive compounds identified several primary hits. The inhibitory effect of one of the positive hits was further confirmed in an orthogonal anti-flag immunoprecipitation assay and thermal shift assay. This study has not only validated the TR-FRET assay for uHTS, but also provided the proof-of-concept that the $BRAF^{V600E}$ and KEAP1 PPI can be disrupted by small molecules, representing the first generation of neoPPI-targeting agents. This study set the stage for the discovery of novel $BRAF^{V600E}$/KEAP1 disrupting agents for therapeutics development and precision oncology.

Jordan Goldy, BCDB **Poster #30**

A conserved RNA binding protein regulates lipid storage and metabolic transcripts

Jordan Goldy[1], Anita Corbett[2], Ken Moberg[1]
[1]Department of Cell Biology, Emory University, Atlanta, GA
[2]Department of Biology, Emory University, Atlanta, GA

The brain plays a critical role in regulating metabolism by sensing metabolic status and modulating release of metabolic regulators. This is evident in clinical data as an elevated risk among individuals with intellectual disability(ID) for metabolic defects. A group of ID are caused by mutations in genes encoding RNA binding proteins(RBPs), which control polyadenylation, splicing, localization, and turnover. RBPs with conserved roles in development of the central nervous system can also modulate metabolic status. Loss of function mutations in the RBP *ZC3H14* are linked to a form of non-syndromic, autosomal recessive human intellectual disability. Studies of the ZC3H14 ortholog Nab2 in *D. melanogaster* show that loss of Nab2 results in behavioral impairments, short-term memory deficits, and altered brain morphology. Previous studies reveal a role for Nab2 in modulating levels of N^6-methyladenosine(m^6A) deposition by Mettl3, the catalytic component of m^6A methyltransferase complex, on specific mRNAs. RNA-seq data indicate that two transcripts altered in *Nab2*^{null} females are *dilp2* and *dilp5*, which regulate *Drosophila* metabolism and lipid storage. dilp2/dilp5 are synthesized in IPCs(insulin-producing cells), analogs of pancreatic beta cells in humans, and signal to cells in the fat body(FB) to promote lipid storage. Consistent with female-specific *dilp2/dilp5* data, analysis of *Nab2*^{null} larvae reveals increased size of lipid droplets in female FBs. Interestingly, a loss of Mettl3 in females results in increased *dilp2* transcript levels and increased average lipid droplet size comparable to *Nab2*^{null} females. Thus, we hypothesize that Nab2 regulates mRNA transcripts critical for proper metabolic function and lipid storage.

Mina Henes, BCDB **Poster #31**

Structural Basis of Multiprotein Recognition by the *P. aeruginosa* Outer Membrane Protein OprM

Mina Henes[1,2] and Graeme L. Conn[2]

[1]Graduate Program in Biochemistry, Cell & Developmental Biology (BCDB), Emory University, Atlanta, GA.
[2]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA

Pseudomonas aeruginosa is a growing threat to the healthcare system, especially to immunocompromised patients, with infections that are challenging to treat due to intrinsic resistance to many clinically relevant antibiotics. Expression of multiple members of the **R**esistance-**N**odulation-cell **D**ivision (RND) superfamily of multidrug efflux pumps contributes significantly to this intrinsic resistance. RND efflux systems are tripartite protein complexes, e.g. MexAB-OprM, comprising an inner membrane-embedded transporter protein (e.g. MexB), a periplasmic adaptor protein (PAP; e.g. MexA) and an outer membrane factor (OMF; e.g. OprM). OprM forms functional complexes with different PAPs, including MexA and MexX, whereas other OMFs, such as OprJ and OprN, have single, specific partners. The basis of this specificity or promiscuity in PAP/OMP interaction is currently not known. Using published crystal structures, cryoEM structures, and homology models, this work has three goals: (1) establish an appropriate molecular dynamics (MD) simulation protocol for membrane-embedded PAP/OMP complexes, (2) develop the computational tools to analyze these simulations, and (3) use these procedures to simulate individual OMFs and their complexes with PAPs to define the basis of OMF selection in RND efflux pump assembly. Energy plots from MD simulations show that we successfully developed a protocol for membrane-bound proteins. All systems were simulated using this protocol and the analysis program is currently in development and publicly available through GitHub. While outer membrane fusion proteins share a high sequence similarity, initial results suggest that differences in protein conformation and electrostatic landscape contribute to the observed differences in OMP interacting partner selectivity.

Dariana Torres-Rivera, BCDB **Poster #32**

Imaging HIV-1 restriction by MX2

Dariana Torres-Rivera[1], Gilberto Betancor[2], Michael H. Malim[2], and Gregory B. Melikian[1,3]

[1]Department of Pediatrics, Emory University, Atlanta, GA
[2]Department of Infectious Diseases, King's College London, London, UK
[3]Children's Healthcare of Atlanta, Atlanta, GA

HIV-1 capsid core plays a major role in infection by interacting with multiple host factors that facilitate its nuclear import and integration into the host genome. The myxovirus resistance protein 2 (MX2) is an interferon alpha-inducible GTPase that restricts HIV-1 infection. MX2 directly binds the HIV-1 capsid and interacts with the nuclear pore complex (NPC). MX2 is thought to restrict infection by inhibiting capsid core uncoating (disassembly of capsid) and nuclear entry, as measured by *in vitro* capsid tube uncoating assay and 2-Long Terminal Repeats (2-LTR) circle assay, respectively. However, it is not well understood how and where MX2 interacts with HIV-1 cores in cells and how these interactions lead to inhibition of infection. Our goal is to elucidate the spatial and temporal HIV-1 restriction by MX2 using single virus imaging. Recently, we have shown that MX2 phosphorylation at the residues S28 and T151 enhances its ability to restrict HIV-1 infection (Betancor et al., 2022). By single virus imaging, we found that the phosphomimetic MX2-S28D/T151D mutant potently inhibits nuclear import of fluorescently labeled HIV-1. Stimulated Emission Depletion (STED) superresolution microscopy revealed that MX2-S28D/T151D forms a streak-like pattern that colocalizes with the NPCs and viruses. Colocalization of viruses with MX2-S28D/T151D is higher than with WT MX2 or MX2-11R-13A mutant, which is unable to restrict infection. Live cell imaging data show engulfment of viruses by MX2-S28D/T151D condensates, co-trafficking in the cytoplasm and subsequent release of the core-entrapped fluid face marker, iGFP, suggesting loss of core integrity and onset of uncoating.

Sarah Webster, BCDB **Poster #33**

The influence of snoRNAs on non-small cell lung cancer invasion

Sarah F Webster[1,2,3,4], Tala O. Khatib[2,3,4], Lutfur Rahman[1], Virginie Marchand[5], Yuri Motorin[6], Adam I Marcus[2,3,4], Homa Ghalei[1,4].

[1]Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322, USA
[2]Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, Georgia, USA
[3]Winship Cancer Institute of Emory University, Atlanta, Georgia, USA
[4]Graduate Program in Biochemistry, Cell, and Developmental Biology, Emory University, Atlanta, Georgia, USA
[5]Université de Lorraine, UAR2008/US40 IBSLor, CNRS-INSERM, Biopôle, 9 Avenue de la Forêt de Haye, 54505 Vandoeuvre-les-Nancy, France
[6]Université de Lorraine, UMR7365 IMoPA, CNRS- Biopôle, 9 Avenue de la Forêt de Haye, 54505 Vandoeuvre-les-Nancy, France

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases globally and has a 6% survival rate once it has metastasized. My research explores translation regulation in NSCLC invasion. Recent studies have revealed a novel layer of translation regulation in cancer at the level of ribosomes and show that changes in ribosome quality or quantity can impact the aggressiveness of tumor cells. A fundamental aspect of translation regulation occurs at the level of ribosomal RNA (rRNA), which is the bulk of the ribosomal mass and is modified at functionally important sites. Emerging data indicate that changes in the modification pattern of rRNAs can tune the mRNA preference of ribosomes. How variation in rRNA modifications contribute to the invasiveness of cancer cells is currently unknown. In eukaryotes, most rRNA modifications are guided by a conserved class of non-coding RNAs called small nucleolar RNAs (snoRNAs). This project exploits a cell culture-based model of NSCLC collective invasion to characterize contributions of snoRNAs and their guided modifications to translation regulation. This work benefits from NSCLC subpopulations isolated using Spatiotemporal Genomic and Cellular Analysis (SaGA), an image-guided technique that identified leader and follower cells within the collective invasion pack. Here we show that subpopulations within NSCLC have distinct snoRNA expression patterns and exhibit variability in the 2'-O-methylation status of their ribosomes. Results from this work will reveal whether changes in snoRNA expression and their guided modifications can be exploited to selectively block tumor cell growth and invasion in NSCLC.

Defining the allosteric activation network in human OAS1

***Enoch A. Ayamga*[1]**, Debayan Dey[1], Kurt B. Miller[1], and Graeme L. Conn[1]

[1]Department of Biochemistry, Emory University, Atlanta, GA

Pattern recognition receptors (PRRs) of the innate immune system are an important class of proteins involved in the detection and elimination of infection by recognizing and binding to specific molecular structures associated with these pathogens. Dysregulation of these host responses can lead to heightened susceptibility to infection or autoimmune diseases. PRRs such as 2'-5' oligoadenylate synthetases (OAS) play a pivotal role in antiviral defense by sensing cytosolic double-stranded RNA (dsRNA), a hallmark of viral infection.

OAS1, a member of the OAS family, synthesizes 2'5'-oligoadenylates (2-5As) upon activation by dsRNA binding, initiating an antiviral state through RNase L-mediated degradation of viral and host RNAs. Despite its key role as an antiviral protein, the pathways of allosteric communication from the dsRNA binding surface to the distant catalytic center that lead to appropriate OAS1 activation, and how this becomes dysregulated in human disease are still poorly understood.

This project, therefore, aims to unravel the allosteric protein residue networks governing OAS1 activation by dsRNA using loss- and gain-of-function (LOF and GOF, respectively) variants. *I hypothesize that OAS1 activity is regulated by an allosteric protein residue network that maintains an "off" state in the free protein and facilitates enzyme activation ("on" state) upon dsRNA binding.* This will be tested through site-directed mutagenesis, OAS activity assays, molecular dynamics simulations, and Hydrogen Deuterium Exchange coupled with Mass Spectrometry (HDX-MS).

Beyond increasing our understanding of the OAS/RNase L pathway, the studies proposed here provide a firm foundation for developing novel therapeutics for treating conditions associated with OAS1 dysregulation.

Understanding the Functions of Chromatin Remodeling Factor BICRA

***Ziben Zhou*[1]**, David Gorkin[1]

[1]Department of Biology, Emory University, Atlanta, GA

Neurodevelopmental disorders (**NDDs**) are a group of conditions associated with disrupted nervous system development and severely impaired brain function. Mutations in genes encoding subunits of BRG1/BRM-Associated Factor (**BAF)** chromatin remodeling complexes (which are also known as “SWI/SNF” complexes) cause a subtype of NDDs known as SWI/SNF-related intellectual disability disorders (**SSRIDDs**). Recently, putative loss-of-function mutations in the *BICRA* gene were reported in SSRIDDs patients. The *BICRA* gene codes for a protein found exclusively in a newly discovered subtype of BAF complexes known as non-canonical BAF (**ncBAF**) complexes. Moreover, disruption of the *BICRA* gene orthologs in zebrafish and *Drosophila* causes phenotypes that mimic symptoms of human SSRIDDs patients. Taken together, these findings demonstrate an essential role for *BICRA* in neuronal development. However, we do not yet understand the molecular mechanisms that link BICRA and ncBAF to neuronal development. To bridge these gaps of knowledge, I will generate iPSC cell lines with disrupted BICRA function to model the disease. I will differentiate these cell lines into neural progenitor cells and conduct single-cell multi-omics profiling to identify gene networks that are affected in the absence of proper BICRA function, and the dysregulation of chromatin accessibility due to disruption of ncBAF function. Currently, we have generated 6 iPSC lines including 2 $BICRA^{+/+}$, $BICRA^{+/-,}$ and $BICRA^{-/-}$ cell lines. The protein expression level was confirmed by immunoblotting. For the next steps, I will do bulk transcriptomic profiling for initial checking and then conduct single-cell multiomic profiling at different time points during the neural differentiation process.

Vanessa Avalos, CB **Poster #36**

Deciphering the activation mechanism of the chemoresistant driver MAST1 in human cancer

Vanessa Avalos[1, 2], Jungseok Hwang[1], Jihoon Kang[1], and Sumin Kang[1]

[1]Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory, Emory University School of Medicine, Atlanta, GA
[2]Cancer Biology Program, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA

Cisplatin resistance is the process by which cancer cells no longer die after treatment with the chemotherapeutic agent cisplatin, and there is an unmet need for further elucidation of the cisplatin resistance mechanism, including a complete understanding of new therapeutic targets and the development of effective therapy against these targets. Kinases phosphorylate proteins essential for cellular processes and are often dysregulated in human cancers. Through a kinome-based RNAi screen, we identified a microtubule-associated serine/threonine kinase 1 (MAST1) as a critical synthetic lethal target partner for cisplatin and identified lestaurtinib as a MAST1 inhibitor. However, how MAST1 is activated in cancer cells is unknown. We identified aurora kinase B (AURKB), a key enzyme in the mitotic spindle checkpoint, as a MAST1 binding partner that promotes MAST1 activity. MAST1 loss and cisplatin treatment resulted in G2/M cell cycle arrest. Through a phospho-proteomics analysis and in vitro kinase assay, we found that AURKB directly phosphorylates MAST1 and AURKB activation leads to MAST1 phosphorylation at S789 and S1310 in cancer cells. The combination of MAST1 inhibitor lestaurtinib and AURKB inhibitor barasertib enhanced cisplatin response. Mutating potential phosphorylation sites on MAST1 led to decreased MAST1 activity, and combining cisplatin treatment with AURKB or MAST1 knockdown led to decreased cisplatin-resistant cell viability. These data suggest that the phosphorylation of MAST1 by AURKB enhances its activity and promotes cell cycle progression and cisplatin-resistant cancer cell proliferation. Co-targeting of MAST1 and AURKB could be a promising combinatorial therapy to improve survival outcomes for patients affected by chemoresistance.

Eleanor Wettstein, CB **Poster #37**

Characterization of brain metastases-infiltrating neutrophils

Eleanor Wettstein[1,2,3], Muna Ahmad[1,2], Alaina Waters[1,2], Kimberly Hoang[1,4], Jeffrey Olson[1,4], Edjah Nduom[1,4], and Lisa Sudmeier[1,2,3]

[1]Emory University School of Medicine, Atlanta, GA
[2]Department of Radiation Oncology, Division of Cancer Biology, Emory University, Atlanta, GA
[3]Cancer Biology Graduate Program, Emory University, Atlanta, GA
[4]Department of Neurosurgery, Emory University, Atlanta, GA

Brain metastases affect approximately 20% of cancer patients and are associated with poor clinical outcomes. Although immunotherapy has revolutionized cancer treatment, many brain metastases patients fail to respond or experience disease recurrence. Current immunotherapies are generally directed at the adaptive immune system, targeting and restoring the function of exhausted T cells. However, many receptors through which immunotherapies exert their effects are also expressed by innate immune cells, of which neutrophils are the most abundant. Neutrophils also play a key role in regulating levels of arginine, an amino acid critical for T cell function. The goal of my research is to elucidate mechanisms of neutrophil recruitment and activation in the tumor microenvironment and their influence on T cell function and tumor control in the brain. I have developed a high-parameter flow cytometry panel and have applied it to characterize neutrophils in the context of human brain metastasis. My work thus far demonstrates that many human brain metastases are infiltrated by neutrophils with diverse phenotypes, some of which appear to have released the enzyme arginase – which degrades arginine – into the tumor microenvironment. Future experiments will use human samples and mouse models to further assess the function of brain metastases-infiltrating neutrophils in regulating arginine availability and orchestrating a pro- or anti-tumor immune response. Ultimately, this research has the potential to identify opportunities for therapeutic intervention that counteract the immunosuppressive functions of tumor-associated neutrophils to achieve improved clinical outcomes for patients with brain metastases.

Joseph Doherty, CB **Poster #38**

Role of the UFMylation pathway in the pathogenesis and therapeutic response of lung adenocarcinoma

Joseph P. Doherty[1], Md Al Nayem Chowdhury[2], Mithila Sawant[2], Alexandre Orthwein[2], Aparna H. Kesarwala[2]

[1]Cancer Biology Program, Graduate Division of Biological and Biomedical Sciences, James T. Laney School of Graduate Studies, Emory University, Atlanta, Georgia, USA.
[2]Department of Radiation Oncology, Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia, USA.

Lung cancer is the leading cause of cancer death in the United States, and encompasses a series of histologically distinct subtypes, including lung adenocarcinoma (LUAD). Despite significant improvement in outcomes, patients frequently experience relapse from and resistance to chemo- and radiotherapy, which represents a major unmet clinical need.

The UFMylation pathway is a new post-translational modification pathway which has recently been shown to play a role in cell proliferation and DNA damage repair. Still, little is known about the role of this pathway in LUAD. The UFMylation pathway includes the ubiquitin like protein UFM1, which is actively conjugated to substrate proteins by the E1 (UBA5), E2 (UFC1), and E3 (UFL1) enzymes.

Utilizing a CRISPR based genome-wide screening, we previously identified several members of the UFMylation pathway as sensitizers to a series of clinically relevant DNA damaging agents, such as cisplatin. Pan-cancer analysis showed that members of the UFMylation pathway are overexpressed in LUAD compared to corresponding normal lung tissue. RNA expression of multiple members of the UFMylation pathway is associated with poor prognosis in a cohort of patients with LUAD.

Future directions will investigate the role of the UFMylation pathway in the proliferation and therapeutic response of both H460 and A549 lung adenocarcinoma cell lines *in vitro*, using a small molecule based pharmacological approach to inhibit UBA5 and an siRNA based genetic approach to knockdown UBA5. This work will define a novel contribution of the UFMylation pathway in the pathogenesis and therapeutic response of LUAD.

Robert Chavez, GMB **Poster #39**

NSD2 expression driven by the t(4;14) translocation disrupts the DNA methylation landscape in multiple myeloma

***Robert M. Chavez*[1,2]**, Doris R. Powell[1], Kiran Lakhani[1], Mark Hamilton[3], George Mulligan[3], Daniel Auclair[3], Jonathan J. Keats[4], Paula M. Vertino[5], Lawrence H. Boise[1], Sagar Lonial[1], Karen N. Conneely[2], Benjamin G. Barwick[1]

[1]Department of Hematology and Medical Oncology, Winship Cancer Institute, Atlanta, GA
[2]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA
[3]Mulitple Myeloma Research Foundation, New Haven, CT
[4]Translational Genomics Research Institute, Phoenix, AZ
[5]Department of Biomedical Genetics, Wilmot Cancer Institute, Rochester, NY

The t(4;14) translocation, which places the lysine methyltransferase gene *NSD2* under the regulatory control of an enhancer of *IGH*, is the primary genetic event in 15-19% of multiple myeloma patients and is associated with poor prognosis. The alteration gives rise to a transcriptionally distinct subtype of myeloma, but the mechanisms by which overexpression of *NSD2* alters gene expression and leads to high-risk disease are poorly understood. Dimethylation of lysine 36 on H3 (H3K36me2), which is deposited by NSD2, is widely distributed at high levels in t(4;14) myeloma. To better understand how increased NSD2 activity and widespread H3K36me2 lead to epigenetic and transcriptional dysregulation, we interrogated DNA methylation (DNAm) and H3K36me2 at the genomic level in a t(4;14) myeloma cell line in which *NSD2* is ablated (KMS11-TKO). A differential methylation analysis between these cells and an isogenic control revealed that high *NSD2* activity is associated with high levels of DNAm. Furthermore, hypermethylated loci ($p < 1e-9$) were enriched within areas of elevated H3K36me2. We similarly generated DNAm data for 415 patient samples from the MMRF CoMMpass study and integrated these with existing transcriptional and clinical data. Analysis of these data indicated t(4;14) was the most epigenetically and transcriptionally distinct form of the disease and identified 2,075,489 differentially methylated loci ($p < 1e-9$). Similarly to results in cell lines, the majority (92%) of these loci were hypermethylated in t(4;14) samples. These results indicate that t(4;14)-driven *NSD2* expression and resulting widespread H3K36me2 disrupts the DNAm landscape leading to a transcriptionally distinct and high-risk form of multiple myeloma.

Sierra Falcone, GMB **Poster #40**

Investigating mechanisms of histone gene expression using multiple *Drosophila* species

Sierra Falcone[1], Leila Rieder[1]
[1]Department of Biology, Emory University, Atlanta, GA

Histone proteins are essential for organization of the genome. Misregulation of these genes can cause aberrations in cellular divisions, leading to embryonic lethality. Canonical histone proteins in most metazoans are encoded by multigene families, but it is unclear how these genes are co-regulated to carefully control histone levels. Other multigene families, such as the rRNA genes, can provide clues. Understanding how similar genes are differentially regulated is critical to our understanding of the relationship between transcriptional regulation and gene dosage.

I am investigating how expression of multiple histone genes is controlled using the powerful genetic model *Drosophila*. I am leveraging existing tools available in *Drosophila melanogaster*, as well as the novel histone locus organization found in other *Drosophila* species to investigate mechanisms of histone gene expression. I will utilize a parallel approach to profile dynamic developmental histone gene expression from a single locus using RNA sequencing. I will be utilizing the natural system of *Drosophila simulans*, in which there exists natural sequence variation in histone coding regions, and a transgenic system in *Drosophila melanogaster.* I will also profile locus-specific expression of histone genes in tissues with varying histone requirements. I will be using SNV FISH to determine relative abundance of histone transcripts using *Drosophila virilis*, which carries two asymmetrical histone loci, and *D. melanogaster-D. simulans* hybrids. These experiments will elucidate critical mechanisms in gene family regulation by leveraging transgenic tools in *D. melanogaster* and underutilized genomes of non-*melanogaster* species.

Jonathan Owen, MMG **Poster #41**

Membrane tethering by the host GTPase Atlastin-2 is critical for flavivirus replication

Authors: *Jonathan Einterz Owen*[1], Cheyanne L. Bemis[1], Christopher J. Neufeldt[1]

[1]Department of Microbiology and Immunology, Emory School of Medicine, Emory University, Atlanta, GA, USA

Flaviviruses are a globally distributed group of arboviruses which infect over 400 million people each year. All flaviviruses replicate their genome inside a membranous structure known as the viral replication organelle, or vRO, which is formed from the endoplasmic reticulum (ER) of host cells. Several recent studies have demonstrated that disruption of vROs has potent antiviral activity; thus, detailed knowledge of vRO formation could help identify broad-spectrum therapeutic targets.

Because vROs are derived from cellular ER membranes, cellular proteins have been implicated in vRO biogenesis. We have previously identified that the ER resident protein atlastin-2 (ATL2) influences vRO formation. The goal of our research was therefore to determine how ATL2 is involved in the formation of vROs during flavivirus infection.

We used cell culture models to investigate vRO formation by dengue virus (DENV) and Zika virus (ZIKV). Fluorescence microscopy showed a significant redistribution of ATL2 during infection and enrichment at sites of viral genome replication. Taking advantage of a novel plasmid-inducible replication organelle system, we used electron microscopy to show that ATL2 depletion alters vRO size, shape, and distribution. Mutational analysis demonstrated that the role of ATL2 in viral replication is linked to its ability to tether ER membranes, as ATL2 mutants which could not tether membranes were unable to rescue viral replication in ATL2-knockout cells. Our results indicate that the tethering function of ATL2 plays a critical role in flavivirus infection, potentially by mediating the formation of vROs; importantly, this function is conserved for different flavivirus infections.

Sushma Timalsina, MMG **Poster #42**

NDM1 mediated heteroresistance to B-lactams

Sushma Timalsina, **Jacob Choby, David S. Weiss**

Abstract –

The emergence of antibiotic resistance poses a significant threat to public health, necessitating a deeper understanding of the underlying mechanisms. Heteroresistance (HR) is a form of resistance to antibiotics where a minority subpopulation of resistant bacterial cells exists with a majority susceptible population of cells. HR has been observed in both gram-negative and gram-positive pathogens and has been shown to cause treatment failure in the clinical setting. This study focuses on clinical isolates of gram-negative *Enterobacterales* carrying the resistance enzyme New-Delhi metallo beta lactamase 1 (NDM1). We observed heteroresistance of a NDM1-encoding *Klebsiella pneumoniae* isolate to a beta-lactam antibiotic, meropenem. RNA sequencing of the isolate in the presence of meropenem revealed heterogeneity in expression of the metal importer gene, *mntB*, which is present in various copy numbers in *Enterobacterales* strains. In this study, the clinical *Enterobacter* isolate Mu208 serves as a model to investigate the role of *mntB* expression in the development of HR to meropenem. We hypothesize that the source of heterogeneity which creates a meropenem resistant subpopulation is enhanced import of metal cofactor via MntB to support the function of NDM1. Our data suggests that insertion of *ndm1* to a meropenem susceptible Mu208 isolate makes the isolate HR to meropenem, depending on the availability of Zn in media. This study will help in better understanding the mechanism of heteroresistance to β-lactams and can help develop novel diagnostics and therapeutics by targeting metal import pathways.

William McCallum, NS **Poster #43**

Tools for high resolution electromyography in neonatal mice.

William M. McCallum[1], Olivia Mistretta[1], Kyle Thomas[2], Matthew Williams[2], Francisco J. Alvarez[1], Samuel J. Sober[1,2,3].

[1]Department of Cell Biology, Emory University, Atlanta, GA
[2]Coulter Department of Biomedical Engineering, Georgia Tech, Atlanta, GA
[3]Department of Biology, Emory University, Atlanta, GA

Very little is understood about the functional organization of spinal circuits during the postnatal maturation of the motor system. This is in large part due to the lack of methods for the interrogation of motor function *in vivo* and in awake mouse pups. To study motor output during postnatal development we have developed minimally invasive, implantable high-density microelectrode arrays (MEAs) for flexible and high-resolution electromyography (EMG) recordings of single motor units in awake behaving P7 to P12 mouse pups. These devices allow recording from multiple muscles and collect high-quality bulk EMG recordings that can be leveraged for analysis of single-unit motoneuron firing patterns. We have applied this technology to interrogate the postnatal development of the motor unit in mice, by use in combination with a model of neonatal limb coordination in which mouse pups engage in vigorous stepping bouts after receiving a subcutaneous injection of L-DOPA. In this behavior, the animals display the full extent of the intra- and inter-limb coordination patterns controlled by spinal cord premotor circuits, while allowing separation of limb coordination from weight-bearing and postural adjustments that occur during overground locomotion. L-DOPA induced air-stepping in neonatal mice enables the study of the evolution of neonatal motor unit properties and their control from spinal circuits during dynamic behaviors more complex than previously possible. Using this behavior in combination with novel EMG device design, we can now interrogate developing motor units and motor circuits at a level of resolution higher than ever previously available.

Bethlehem Bekele, NS **Poster #44**

Molecular and behavioral characterization of a brain-specific, tetracycline-controlled CUG repeat expansion mouse model of myotonic dystrophy type 1

Bethlehem A. Bekele[1], Juan Arboleda[2], Liang Shi, Luke Knudson[1], Jingsheng Gu[1], Eric T. Wang[2], Jie Jiang[1], Gary J. Bassell[1]

[1]Emory University School of Medicine, Atlanta, GA 30322
[2]Center for Neurogenetics, University of Florida, Gainesville, FL 32610

In myotonic dystrophy type 1 (DM1), transcription of a trinucleotide cytosine-thymine-guanine (CTG) repeat expansion in the 3' UTR of the *dystrophia myotonica protein kinase* (DMPK) gene results in intranuclear RNA foci and sequestration of Muscleblind-like (MBNL2) family of RNA-binding proteins. Loss of MBNL function causes dysregulated splicing of target RNAs and likely causes CNS disease symptoms, although underlying mechanisms are unclear. We generated double-transgenic mice by crossing Camk2a-tTA mice with TREDT960I mice (containing a human genomic DMPK segment with exon 11-15 gene and 960 interrupted CTG repeats under direction of the tet-responsive element promoter). We confirmed the expression of CUG_{960} in heterozygous and homozygous double-transgenic mice and tested the reversibility of transgene expression by the administration of doxycycline at varying durations. We also performed qRT-PCR, Western Blot analysis, fluorescence *in-situ* hybridization and immunofluorescence on different brain regions of control, single-transgenic and double-transgenic mice. Lastly, we performed a range of assays to determine if CUG_{960} display behavioral phenotypes consistent with CNS symptoms in DM1. We found that our mouse model recapitulates features of the DM1 brain, including the formation of nuclear RNA foci, co-localization of MBNL protein with CUG foci and mis-splicing of target RNAs including *Mbnl1* exon 5 and *Gabrg2* exon 9 as well as increased MBNL protein in nuclear fraction of cortical lysates. Our behavioral analysis revealed no significant difference in open-field, grip and rotarod assays but showed significant difference in contextual fear conditioning. Our results highlight the translational relevance of our mouse model for therapeutic studies.

Benjamin Dykstra, NS **Poster #45**

The ventral hippocampus and lateral septum encode social recognition information

Benjamin Dykstra[1], Paul Kim[2], Gordon Berman[1], Malavika Murugan[1]

[1]Emory Laney Graduate School
[2]Emory College of Arts and Sciences, Biology Department

One of the most critical behavioral decisions that animals make is whether to approach or ignore a conspecific based on previous interactions. For example, a mouse will readily approach and investigate a novel conspecific while ignoring familiar conspecifics. The neural substrates that allow animals to discriminate between novel and familiar conspecifics and the neural substrates that promote social approach towards novel conspecifics remain poorly understood. The ventral CA1 (vCA1) subfield of the hippocampus and the lateral septum (LS) have been identified as key brain regions that are necessary for differentiating novel and familiar conspecifics in mice. Yet, it remains unclear if the vCA1 and the LS encode social recognition information. Cellular resolution calcium imaging was used to record endogenous activity in the vCA1 or the LS in two separate populations of mice. Mice completed a novel social recognition paradigm in which they investigated one novel and one familiar conspecific. Logistic regressions were performed to decode conspecific identity from population neural activity. Both the vCA1 and the LS significantly decoded conspecific identity above chance levels. Additionally, individual LS neurons significantly preferred novel conspecifics over familiar conspecifics. A future direction is to determine if vCA1-LS projection neurons encode social recognition information. Another future direction is to determine if vCA1 and LS neurons encode motivated social behaviors such as social approach, allogrooming, and olfactory investigation.

Hymavathy Balasubramanian, NS **Poster #46**

Sex differences in neural representations of social and nonsocial reward in the medial prefrontal cortex

Jennifer Isaac[1], ***Sonia Karkare***[1], ***Hymavathy Balasubramanian***[1], Nicholas Schappaugh[2], Jarildy Javier[1], Maha Rashid[1], Malavika Murugan[1,2,3]

[1]Emory Neuroscience Graduate Program, Emory University, Atlanta, GA 30322
[2]Department of Biology, Emory University, Atlanta, GA 30322
[3]Center for Translational Social Neuroscience, Emory University, Atlanta, GA 30322

The perception of social interactions as rewarding is necessary for appropriate social behavior. While there has been progress made towards understanding the neural circuits underlying social behavior, it remains unknown if social rewards are processed by the same or different neural populations as nonsocial rewards. It is also unclear how these neural representations might differ based on the sex and internal state of the animal. We developed a fully automated, novel two choice (social-sucrose) operant assay to directly compare the neural representations underlying social and nonsocial reward-related behaviors. We performed cellular resolution calcium imaging in the medial prefrontal cortex (mPFC) of male and female mice during the two choice assay across varying internal states. We found that mPFC neurons (n = 459 neurons, 9 male mice; 570 neurons, 6 female mice) maintain sex-dependent, largely non-overlapping and flexible representations of social and nonsocial reward. Furthermore, optogenetic manipulation (both excitation and inhibition) of mPFC neurons during the reward period of the assay disrupted reward-seeking behavior across both sexes. Thus, using a novel operant assay, we identified non-overlapping neural representations of social and nonsocial reward in the mPFC that vary by sex and internal state of the animal and that are essential for appropriate reward-seeking behavior.

Katie Alexander, IMP **Poster #47**

CD154:CD11b Blockade: A novel costimulatory target that maintains protective immunity while attenuating allograft rejection

Katie Alexander[1], Danya Liu[1], Kelsey Bennion[1], and Mandy Ford[1]

[1]Department of Surgery and the Emory Transplant Center, Emory University, Atlanta, GA

CD154 pathway antagonism induces long-term graft survival, demonstrating superior efficacy compared to anti-CD40, likely due in part to its interactions with the alternate receptor CD11b. Recently, we showed that CD154:CD11b blockade improved long-term graft survival, indicating therapeutic potential for transplantation. However, the impact on protective immunity is unknown. The goal of this study was to determine the impact of a CD154:CD11b-specific peptide inhibitor on protective T cell immunity to a murine EBV homolog (MHV68) and compare this to its effect on alloimmunity using murine skin graft models. During transplantation, CD154:CD11b blockade significantly *decreased* graft-specific $CD8^+$ T cells in the spleen and allograft 10 days post-transplantation. In contrast, CD154:CD11b blockade *increased* virus-specific $CD8^+$ T cells 10 days post-infection. CD154:CD11b blockade during viral infection also significantly increased the frequency of $CD127^{hi}KLRG1^{lo}$ $CD8^+$ memory precursor effector cells (MPEC), with a commensurate increase in phosphorylation of S6 downstream of mTOR signaling and the transcription factor cJun. In contrast, MPEC differentiation was not altered by CD154:CD11b blockade during transplantation. Direct comparison of antigen-specific $CD8^+$ T cells elicted via infection or transplantation showed significant differential induction of transcription factors known to determine MPEC differentiation, Eomes and T-bet. These data demonstrate that while CD154:CD11b blockade suppresses alloimmunity, it paradoxically enhances the quantity and quality of virus-specific $CD8^+$ T cells. These disparate outcomes are underpinned by differential activation of mTOR signaling and key transcription factors in graft- vs. virus-elicited antigen-specific $CD8^+$ T cells. Targeting CD154:CD11b interactions therefore holds promise for inhibiting alloreactivity while maintaining protective immunity following transplantation.

Melissa Gutierrez, IMP **Poster #48**

Chronic ethanol exposure qualitatively alters regulatory T cell subsets in septic mice

Melissa B. Gutierrez[1], Craig M. Coopersmith[1], Mandy L. Ford[1]

[1]Emory Transplant Center, Department of Surgery, Emory University School of Medicine, Atlanta, GA, USA.

Chronic alcohol consumption has been associated with increased mortality in human septic patients. Our lab has shown in murine models that chronic alcohol consumption directly increases sepsis mortality and increases the frequency of $CD4^{+}FoxP3^{+}$ regulatory T cells (Treg). To better understand the phenotype of Tregs in alcohol-fed versus water-fed septic mice, C57/BL6 mice received 5% ethanol, increasing 5% every 5d to 20%. Control mice received water. After 3 months, both groups underwent cecal ligation and puncture (CLP). Splenocytes were stained for flow cytometry at 24h. FCS files were pre-gated on the CD4+FoxP3+ T_{reg} population using FlowJo and concatenated for CITRUS analysis using the Cytobank platform. Using LASSO via GLMNET, four Treg populations were significantly different in abundance between alcohol-fed versus water-fed septic mice. Cluster A is $KLRG1^{hi}Helios^{lo}CTLA\text{-}4^{med}CD28^{hi}Ki67^{lo}CD25^{hi}CD103^{hi}CCR4^{hi}Ly6C^{lo}GITR^{med}ICOS^{med}CD69^{hi}CD44^{hi}$ $CD62L^{lo}$ and Cluster B is $KLRG1^{lo}Helios^{lo}CTLA\text{-}4^{lo}CD28^{lo}Ki67^{med}CD25^{lo}CD103^{lo}CCR4^{lo}Ly6C^{hi}$ $GITR^{lo}ICOS^{lo}CD69^{lo}CD44^{lo}CD62L^{hi}$ showed a higher abundance in alcohol-fed mice while Clusters C is $KLRG1^{lo}Helios^{hi}CTLA\text{-}4^{lo}CD28^{lo}Ki67^{hi}CD25^{lo}CD103^{hi}CCR4^{lo}Ly6C^{lo}GITR^{hi}ICOS^{hi}CD69^{hi}CD44^{hi}$ $CD62L^{lo}$ and Cluster D is $KLRG1^{lo}Helios^{lo}CTLA\text{-}4^{lo}CD28^{lo}Ki67^{lo}CD25^{lo}CD103^{lo}CCR4^{lo}Ly6C^{lo}$ $GITR^{lo}ICOS^{lo}CD69^{lo}CD44^{lo}CD62L^{hi}$ showed a lower abundance in alcohol-fed mice compared to water-fed. This study identified significant changes in Treg populations in the setting of sepsis in ethanol- vs water- fed mice. These findings are consistent with previous results in non-septic mice which identified an increase in a $Helios^{lo}$ cluster and a decrease in a $KLRG1^{lo}LyC6^{lo}$ Treg cluster in ethanol- vs water-fed mice. These qualitative differences in Tregs in ethanol- vs water-fed mice could mechanistically underlie the increased mortality observed in ethanol-fed septic mice.

Megan Phillips, PBEE **Poster #49**

The little plasmid that could: spread of a small antimicrobial resistance plasmid across *Staphylococcus aureus*

Megan A. Phillips[1], Daniel B. Weissman[2,3], Timothy D. Read[4]

[1]Population Biology, Ecology, and Evolution Program, Graduate Division of Biological and Biomedical Sciences, Laney Graduate School, Emory University, Atlanta, GA
[2]Department of Physics, Emory University, Atlanta, GA
[3]Department of Biology, Emory University, Atlanta, GA
[4]Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, GA

Mobile genetic elements (MGEs) can be inherited vertically or transferred horizontally between bacteria. These elements frequently confer antibiotic resistance, contributing to the spread of antimicrobial resistance in a population. One of these MGEs, the small plasmid pT181, confers tetracycline resistance in bacterial pathogen *Staphylococcus aureus*. Bacterial genome analysis tools and high-quality sequences with metadata are publicly available, but these resources remain underleveraged for examining historical data, especially when studying the spread of MGEs across a species and overtime. Using publicly available genomic data, we show that the history of pT181 is characterized by spread across clonal complexes (CCs) over time and sequence evolution since its introduction into *S. aureus*. pT181 emerged in CC8 and became highly prevalent soon after its detection, though spread to other CCs did not occur until approximately 30 years later. pT181 remains unequally distributed across the phylogeny of *S. aureus*. While instances of pT181 detection have increased in number with increased sampling, prevalence of the plasmid has gone down over time. The original methicillin-resistant *S. aureus* (MRSA) clone arose in CC8 and was also resistant to tetracycline; we find that nearly all early isolates containing pT181 also contained SCC*mec* type I, suggesting that pT181 may have increased in frequency alongside other resistance mechanisms. Further study into the spread of pT181 offers a greater understanding of MGE dissemination on a population level, which has important implications for tracking and managing antibiotic resistance.

Ally Su, MSP **Poster #50**

Utilizing ultrahigh-throughput screening technology to identify 14-3-3/YAP interaction modulators

Ally Su[1,2], Dacheng Fan[2], Min Qui[2,3], Yuhong Du[2,3], Haian Fu[1,2,3]

[1]Molecular and Systems Pharmacology, Emory University, Atlanta, GA
[2]Department Pharmacology and Chemical Biology, Emory University, Atlanta, GA
[3]Emory Chemical Biology Discovery Center, Emory University, Atlanta, GA

Yes-associated protein (YAP) is commonly over-expressed in human cancers. The nuclear transcriptional co-activator is predominantly regulated by the Hippo pathway. An inactive Hippo pathway allows YAP to translocate to the nucleus and initiate transcription for pro-tumorigenic genes. An active Hippo pathway will phosphorylate YAP to generate a 14-3-3 binding motif. The resulting 14-3-3 and phosphorylated YAP (pYAP) complex remains sequestered in the cytoplasm until YAP degradation. However, no pharmacological agents are available that can be used to modulate the 14-3-3/YAP interaction and to probe the functional consequences of YAP cytoplasmic sequestration. One approach is to identify compounds that stabilize the 14-3-3/YAP interaction, preventing its entry into nucleus. To achieve this goal, we developed a fluorescent polarization (FP) assay for 14-3-3ζ and pYAP in a 1536-well ultrahigh-throughput screening format (uHTS). From the screen with a library of bioactive compounds, we identified several primary hits that stabilized 14-3-3/YAP interaction . We then validated the positive hits using thermal shift and GST pull-down assays. Given that rapid YAP shuttling is required for its transcription activity, compounds that keep YAP away from nucleus could promote anti-tumor effects in human cancer. This study set the foundation to explore the functional consequences of YAP cytoplasmic retention using small molecules.

Roy Mulpur, GMB **Poster #51**

Genetic screens reveal mechanisms of transcriptional regulation in B cell differentiation

Roy N Mulpur[1], Herbey O. Padilla-Quirarte[1], Christopher D. Scharer[1], and Jeremy M. Boss[1]

[1]Emory University School of Medicine

Naïve B cells (NBCs) differentiate into effector states such as antibody secreting cells (ASC) and memory B cells (MBC) to provide immunity against pathogens. Yet, the mechanism responsible for programming naïve B cells to adopt a particular cell fate is not fully understood. During differentiation, B cells undergo changes in gene expression and transcription factor networks that are influenced by alterations in the epigenome to correspond to their effector functions. Our previous work shows that NBCs undergo a hierarchy of reprogramming after exposure to T independent (TI) antigens, including an ASC and MBC cell fate bifurcation at division 3-4. To identify factors controlling each cell fate, RNA-seq of ASC and MBC committed B cells revealed 87 differentially expressed transcription factor/epigenetic modifier genes that were targeted in a CRISPR/Cas9 dropout screen. NBCs were transduced with a gRNA library and differentiated into ASCs in-vitro using a T cell Independent (TI) and dependent (TD) like approach in which they were co-cultured with feeder cells expressing CD40L+ BAFF. This screen revealed that *Runx1* and the histone variant *H1f0* play a role in positive regulation and *Msh5* and *Arid5a* as negative regulators of ASC differentiation in the TI condition. These hits along with the hits from the TD ASC differentiation screen reveal mechanisms of transcriptional regulation in ASC differentiation.

Nicole Roos, GMB **Poster #52**

Investigating mechanisms of *D. melanogaster* histone locus body initiation and maintenance.

Nicole Roos[1], Greg Kimmerer[1], Leila Rieder[1]

[1]Department of Biology, Emory University, Atlanta, GA

Histone proteins are essential for compaction and regulation of the eukaryotic genome. Dysregulation of histone gene expression leads to aberrant development and lethality. In animals, genes encoding the histone proteins are commonly clustered at loci. The histone locus body (HLB) is a collection of factors that localize to histone loci and regulate histone gene expression. By studying initiation and maintenance of the HLB, we gain insight into how unique genes are targeted by specialized transcription factors for specialized regulation. We are employing the powerful model system *Drosophila melanogaster* in which we leverage histone transgenes that recruit HLB factors, allowing us to manipulate *cis* regulatory elements and determine the effects on HLB formation and histone expression. In flies, the HLB is initiated during early embryogenesis. The HLB is then maintained at the single locus in mature tissues and throughout cell divisions. To test whether the *cis* elements that are critical for HLB initiation are also important for maintenance, we employ a transgenic system where we will detect HLB maintenance using a combined DNA FISH and immunofluorescence protocol. To test whether HLB initiation can occur in non-embryonic cells, we will combine transgenes and mitotic recombination in wing discs. Overall, studying HLB initiation and maintenance allows us to illustrate how and when histone proteins are regulated and in what contexts factors target these crucial genes for regulation.

Ranjit Pelia, GMB

Characterizing Protein interactions with H3K27me3 using Polycomb Chromodomain Fusion Proteins

***Ranjit Pelia*[1], Seong Hu Kim[3], Karmella Haynes[1,2,3]**

[1]Genetics and Molecular Biology, Emory University School of Medicine, Atlanta, GA
[2]Department of Cancer Biology, Emory University, Atlanta, GA
[3]Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine, Atlanta, GA 30322, USA

Polycomb group proteins are essential regulators of chromatin, yet the mechanisms that govern their spatial organization along chromosomes remain elusive. Current approaches to investigate the behavior of regulators that bind H3K27me3, a modification that is central to cell development and disease, are limited due to off-target interactions of tools such as chromatin protein inhibitors, and the redundancy of H3K27me3-binding protein orthologs. We built a fusion protein that includes a human CBX8 H3K27me3-binding polycomb chromodomain (PCD) and a red fluorescent protein (RFP) to identify PCD binding sites within the genome[1]. We engineered MCF7 cells where PCD-RFP expression was controlled by a doxycycline (dox) inducible promoter. Cells treated with low (0.5µg/ml, n=2), and high (1.0µg/ml, n=2) concentrations of dox were analyzed by ChIP-seq. PCD-binding sites were identified as those where ChIP signals increased with PCD-RFP levels.
Many H3K27me3-enriched sites are transcriptionally repressed and are primed for increased transcription. To determine transcriptional plasticity within these sites, we built a PCD-RFP-VP64 fusion construct, SRA (Synthetic Reader Actuator)-PcTF (Polycomb Transcription Factor) [1]. Previously we showed that in MCF7 cells, 125 genes, mostly H3K27me3-enriched, were activated by this SRA [2]. We engineered MCF7 cells that expressed PCD-RFP-VP64 in response to dox. Time course RNA-seq n=19 PcTF activatable genes, and ChIP-qPCR verified accumulation of PCD-RFP-VP64 at known H3K27me3 loci such as HOXB9. Next, we will use public MCF7 ChIP-seq data to determine the overlap of PCD fusion binding sites with H3K27me3, versus other histone modifications. Thus, synthetic biology provides an alternative for investigating protein-protein interactions in chromatin.

Strain-level temporal analyses of antimicrobial resistant *E. Coli* colonization in the infant gut microbiome

Madison A. Schwab[1], Maya L. Nadimpalli[2]

[1]Program in Population Biology, Ecology, and Evolution, Emory University, Atlanta, GA
[2]Gangarosa Department of Environmental Health, Emory University, Atlanta, GA

Antimicrobial resistant (AMR) infections represent a significant health and economic burden, particularly in lower- and middle- income countries, and an understanding of how antimicrobial resistant infections arise is necessary to combat this threat. While *E. coli* is a commensal microbe commonly found in the human gut, it is also an opportunistic pathogen that is linked to more antimicrobial resistant infections than any other bacterial species. As colonization with AMR strains increases risk of subsequent AMR infections, gut-colonization can be an important indicator for future health outcomes. Developments in "culture-free" sequencing methods and bioinformatic analysis tools present an opportunity to generate higher-resolution insights into gut-colonization patterns and temporal dynamics than traditional culture-based methods, which are limited in their ability to detect strains that are present at low abundance. By leveraging metagenomes and sequenced *E. coli* isolates collected from sequential infant stool samples collected in Lima, Peru, we employ strain tracking tools to identify strain-resolved *E. coli* colonization patterns. First, by comparing the results of strain-tracking tools applied to compare metagenomic sequence data to sequences of cultured isolates from the same stool samples, we demonstrate the reliability of strain-tracking tools to correctly identify the presence of bacterial strains identified through culture. Next, we characterize the diversity of *E. coli* strains colonizing individual infants between sampling points and compare the sensitivity of strain-tracking tools and culture-based sequencing methods to detect temporal changes in strain diversity. Our findings advance the overall understanding of the dynamics of infant gut-colonization with AMR *E. coli*.

Diane Choi, MSP **Poster #55**

Ultrastructural localization of glutamate delta receptor 1 in the lateral habenula

Diane Choi[1,3]**,** Jean-Francois Pare[2,3], Shashank Dravid[4], and Yoland Smith[1,2,3]

[1]Graduate Program in Molecular & Systems Pharmacology, Emory University, Atlanta, GA
[2]Department of Neurology, Emory University, Atlanta, GA
[3]Emory National Primate Research Center, Atlanta, GA
[4]Departments of Pharmacology and Neuroscience, Creighton University, Omaha, NE

Glutamate delta receptors (GluD) are synaptogenic molecules that form trans-synaptic complexes with presynaptic neurexin and cerebellin to regulate synapse formation and maintenance. Recent studies demonstrate synaptogenic functions of GluD1 in the striatum, hippocampus, and amygdala. Despite its strong expression, nothing is known about the function of GluD1 in the lateral habenula (LHb), a subcortical structure that processes reward-related behaviors and regulates monoaminergic systems. Mutations of *GRID1*, the gene that encodes for GluD1, and LHb dysregulation are associated with psychiatric disorders such as depression and schizophrenia suggesting that disruption of GluD1 signaling in the LHb may contribute to the pathobiology of these disorders. A deeper understanding of GluD1 function in LHb requires a detailed map of its subcellular localization at the ultrastructural level. To address this, we used immuno-electron microscopy (EM) in LHb tissue of wild-type mice and primates. We found GluD1 is preferentially bound to the plasma membrane of postsynaptic dendritic elements where it mainly aggregates at axo-dendritic symmetric synapses. Post-embedding GABA studies are in progress to determine if GluD1 expression at symmetric synapses is associated with inhibitory GABAergic neurotransmission. Additionally, we used anterograde tracing methods in rats to label terminals from the lateral hypothalamus (LH), medial prefrontal cortex (mPFC), and entopeduncular nucleus (EPN) with AAV-GFP. Consistent with previous literature, both EPN and LH provide much stronger inputs to LHb than mPFC. Double-immuno-EM studies indicate stronger postsynaptic GluD1 labeling at EPN than LH inputs. To our knowledge, these studies are the first to explore GluD1's synaptic organization in the LHb.

Edu Usoro, MSP **Poster #56**

Inhibition of *trans*-translation as a novel antibiotic strategy to combat *M. tuberculosis*

***Edu Usoro*[1,2]**, Pooja Srinivas, Ph.D.[1], Christine M. Dunham, Ph.D[1].

[1]Department of Chemistry, Emory University, Atlanta, GA
[2]Molecular and Systems Pharmacology Graduate Program, Emory University, Atlanta, GA

Mycobacterium tuberculosis (Mtb) infects about 1.8 billion people worldwide, leading to approximately 1.5 million deaths annually. Tuberculosis treatment involves lengthy and often incomplete regimens, contributing to the emergence of multidrug-resistant strains of Mtb. The atypical dormant phase of Mtb further hinders antibiotic effectiveness, necessitating the identification of antibiotics that effectively counteract resistance and latent mechanisms. Researchers are intensively enhancing the antibiotic arsenal to address tuberculosis complexities, focusing on new antibiotics that only target bacterial ribosomes. Existing antibiotics that inhibit protein synthesis are excellent antibiotics but interact with conserved regions of both bacterial and human ribosomes and cause toxic side effects in humans. Despite their effectiveness in killing bacteria, these antibiotics are considered last-resort options due to their toxicity. To identify novel antibiotics that are bacterial-specific and non-toxic to humans, a specific step in translation called *trans*-translation is targeted. This pathway is unique and essential to bacteria, making it a promising new antibiotic target. Furthermore, these new *trans*-translation inhibitors efficiently inhibit the growth of both actively growing and latent Mtb, which impairs the effectiveness of antibiotics. The lead inhibitor prevents *trans*-translation *in vivo* and *in vitro* without affecting regular translation; however, the molecular mechanism of how the antibiotic interacts with the Mtb ribosome and its mechanism of action is unknown. My research seeks to uncover this mechanism using biochemical and structural biology techniques including binding and activity assays and cryogenic electron microscopy (cryo-EM). These results will provide valuable insights into this fundamental bacterial survival process and further the development of a potential new class of antibiotics for Tuberculosis treatment.

Lester Manly, MSP **Poster #57**

"Proteomic network analysis revealed that late-stage progression of hSOD1G37R ALS mouse model is characterized by an upregulation of co-expressed proteins associated with microglial activation and function with the spinal cord."

Authors: ***Lester S. Manly***[1], Anne M. Roberts[1], Eric B. Dammer PhD[2], Ankit P. Jain PhD[1], Peter J. Crouch PhD[3], Blaine R. Roberts PhD[1]

[1]Roberts Laboratory, Department of Biochemistry, Emory University, Atlanta, GA, USA
[2]Seyfried Laboratory, Department of Biochemistry, Emory University, Atlanta, GA, USA
[3]Crouch Laboratory, School of Biomedical Sciences, University of Melbourne, VIC, AUS

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by spinal cord motor neuron degeneration, resulting in muscle control loss. Approximately 25,000 to 32,000 Americans confront ALS, often succumbing within two to five years post-diagnosis. Understanding the impact of mutant superoxide dismutase 1 (SOD1) in familial ALS cases remains incomplete. Our study investigates a prevalent ALS mouse model with hSOD1G37R mutant overexpression, mirroring ALS pathophysiology. We conducted a proteomics study utilizing high-resolution liquid chromatography tandem mass spectrometry (LC-MS/MS) on late-stage hSOD1G37R transgenic and age-matched background mice spinal cords. Tandem Mass Tag isobaric labeling and offline high pH fractionation was employed to improve throughput and proteome coverage. Data acquisition involved Thermo Scientific Orbitrap Eclipse MS coupled to an easy1200 nanoLC, and resulting RAW data searched using Proteome Discoverer. Bioinformatics included differential protein abundance, weighted correlational network analysis (WGCNA), gene ontology, and cell type matching. In hSOD1G37R and background mouse spinal cord, 7136 high-confidence proteins were identified of which were 1895 significantly downregulated and 1959 significantly upregulated. WGCNA revealed that the hSOD1G37R-containing module, M1 Turquoise, with 1039 protein members, exhibited increased co-expression of proteins related to microglia, neuroinflammation, and apoptosis. Cell type matching analysis predicted localization within astrocytes and microglia. These proteomic insights form a foundation for generating ALS pathogenesis hypotheses and provide targets for developing the next generation of ALS therapeutics.

Eden Zhu, NS **Poster #58**

Transcranial Magnetic Stimulation (TMS) Disruption of Supplementary Motor Area (SMA) Activity during Standing Balance Control.

Eden Zhu[1,2], Michael Borich[1,2,3], Lena Ting[1,2,3]

[1]Department of Neuroscience, Emory University, Atlanta, GA
[2]Emory Rehabilitation Hospital, Emory University, Atlanta, GA
[3]Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

With aging-related neural and biomechanics declines in balance control, there is a higher incidence of falling for ageing people. Understanding the neural mechanism of balance control will benefit the balance rehabilitation for them. We focus on studying a supplementary motor area (SMA) event-evoked potential during balance control, and ask the questions - what neural information this SMA activity carries to maintain balance, and what the changes of this signal mean during ageing and disease. SMA functions in elaborating complicated and challenging movement, and is a transition from cognition function in prefrontal cortex to the motor cortex. Therefore, we conduct research on healthy young adults with a hypothesis that SMA activity generates extra muscle patterns to sustain good balance control. Next, we will study any mechanism differences with the population of neurotypical older adults. Finally, we will study whether the executive function is the source of this SMA activity, and whether the executive function decline potentially causes freezing of gait (FoG) in Parkinson's disease (PD). We combine surface electromyography (EMG), platform balance perturbation tasks, and single-pulse inhibitory TMS to determine the effect of this SMA activity on balance control. Currently, we have collected data from three subjects. With refinement on the experiment design, results from the later two participants suggest the SMA activity might inhibit and adjust long-latency muscle responses (LLR) in balance control. In conclusion, we have shown the possibility of conducting experiments to study the causal relation of a cortical activity with human movement for translational clinical research purposes.